CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Meta-Analysis of Genetic Polymorphisms of Interleukin-1 Receptor Associated Kinase (IRAK1) with Susceptibility to Autoimmune Diseases

by

Azeeqa Gul

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
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This thesis is dedicated to my parents, family and friends who is always support, encouragement, and constant love have sustained me throughout my life.

CERTIFICATE OF APPROVAL

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Abstract

Autoimmune diseases (ADs) are diverse groups of diseases that develops and progress because of loss of tolerance resulting itself tissue destruction.Immune system is involve in damaging process of host tissues.Interleukin-1 receptor associated kinase (IRAK1) is essential mediator of innate as well as adaptive immune responses that trigger their response against invading microbes or soluble antibody by mediating release of different cytokines. IRAK1 gene positioned on X chromosome at q28 arm and identified as risk gene for autoimmune diseases (ADs). IRAK1 SNPs included rs3027898, rs1059702 and rs1059703 are considered to be associated with susceptibility for ADs. Although results about their association in different populations as well as diseases are inconsistent.Therefore, we conducted this meta-analysis to obtain more accurate findings. All literature relevant to study was searched from Google Scholar and PubMED databases. Eligible studies were further analyzed for data extraction. Review Manager 5.4 was used for the purpose of statistical analysis. Based on the heterogeneities among studies, selection of random model or fixed model was done. Our studies have 17 studies with 4375 cases (5836 controls) for rs3027898, 7 studies with 7237 cases (7551 controls) for rs1059702 and 10 studies with 3256 cases (3017 controls) for rs1059703. Overall, the results indicated no association of any genetic models for rs3027898, While one genetic models for rs1059702 and one genetic models for rs1059703 associated with ADs. In addition to, sub-group analysis based on disease category, association was noticed in one genetic model and two genetic models of rs3027898 with RA,AS and SLE respectively, one genetic model rs1059702 with RA and SSc, as well as one genetic models of rs1059703 with RA. Stratified analysis based on population represented different extent of association in different genetic models for three SNPs of IRAK1. Our findings concluded that IRAK1 SNPs (rs3027898, rs1059702, rs1059703) were associated with increased risk of ADs.

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Abbreviations

AD	Autoimmune Diseases
ADCC	Antibody-Dependent Cell-Mediated Cytotoxicity
AITD	Autoimmune Thyroid Diseases
AML	Acute Myeloid Leucocyte
AQP5	Aquaporin 5
ARM-PCR	Amplification Refractory Mutation System
AS	Ankylosing Spondylitis
CD	Complement Dependent
CI	Confidence Interval
CNS	Central Nervous System
CRP-	C-Reactive Protein
DD	Death Domain
EAE	Experimental Autoimmune Encephalomyelitis
FLS	Fibroblast-Like Synoviocytes
GCA	Giant Cell Arthritis
GWAS	Genome-Wide Association Study
HDAC	Histone Deacytlase
HLAs	Human Leucocytes Antigens
IFN	Interferon
IgG	Immunoglobulin G
IKB	Inhibitor of Nuclear Factor Kappa B
IKK	IKB kinase
IL-1	Interleukin-1
IL-10	Interleukin-10

IL-1R	Interleukin-1 Receptors
IL-6	Interleukin-6
IL-8	Interleukin-8
IRAK1	Interleukin-1 Receptor Associated Kinase
IRF7	Interferon Regulatory Factor 7
KD	Kinase Domain
LPS	Lipopolysaccharide
Mal	Myelin and Lymphocyte Protein
MAPK	Mitogen Activated Protein Kinas
MDS	Myelodysplastic Syndrome
MHC	Histocompatibility Complex
MS	Mutiple Sclerosis
MS-PCR	Methylation-Specific Polymerase Chain Reaction
MyD88	Myeloid Differentiation Primary Response 88
NFB	Transcription Factor B
NF-kB	Nuclear Factor NF-kB
NLRP3	Nod-Like Receptor Protein-3
OR	Odd Ratio
PAMPs	Pathogen-Associated Molecular Patterns
PBC	Primary Biliary Cholangitis
PCR	Polymerase Chain Reaction
PCR-SSCP	Polymerase Chain Reaction Single-Strand Conformation Polymorphism
PPRs	Pattern Recognition Receptors
PsA	Psoriatic Arthritis
RA	Rheumatoid Arthritis
RT-PCR	Real-Time Polymerase Chain Reaction
SARM	Sterile-Alpha and Armadillo Motif Containing Protein
SLE	Systemic Lupus Erythematous
SMOC	Secreted Modular Calcium-Binding Protein
SNP	Single Nucleotide Polymorphisms

SSc	Systemic Sclerosis
STAT3	Signal Transducer and Activator of Transmission 3
TAB	TGF-Beta-Activated Kinase 1 and MAP3K7-Binding Protein 1
TAK1	TGFβ -Activated Kinase-1
T-ARMS-PCR	Tetra Amplification Refractory Mutation System Polymerase Chain Reaction
TGFβ	Transforming Growth Factor β
TIR	Toll/IL-1 Receptors
TLRs	Toll-like Receptors
TNF	Tumor Necrosis Factor
TRAF6	Tumor Receptor-Associated Factor 6
TRAM	Translocating Chain-Associated Membrane Protein
TRIF	TIR-Domain-Containing Adapter-Inducing Interferon-β
UC	Ulcerative Colitis
WT	Wild Type

Chapter 1

Introduction

1.1 Background

Autoimmune diseases (ADs) are defined as tissue that undergoes destruction by the immune system. Autoimmune diseases are characterized as the tissues damages by T cells or antibody reaction to itself. Autoimmunity plays dual role in autoimmune diseases either precisely causative or prominently conducive role. Damaging of host is initiated and recruited by immune effector mechanisms. The effector processes include traditional immune pathways against attacking pathogen likewise CD4+ and CD8+ T cells, soluble antibody, macrophages and other phagocytic as well as mast cells. Damage is induced by direct attachment of antibody with cellular antigen and in turn this binding leads to triggering of complement, by preventing or activating a receptor, or by production of disruptive immune complexes [1].

Autoimmune diseases (ADs) are categorized in two groups; involved single organ and other involve in more than one organ. Such as type I diabetes is a type of autoimmune disease (ADs) of specifically one organ while systemic lupus erythematous (SLE) is a type autoimmune diseases in which multiple organs are affected [2,3]. In case of organ-specific autoimmune diseases, auto-reactive T cells or antibodies specific for antigens are only present in that specific target organ while in

case of systemic autoimmune diseases antigen distributed to multiple tissues. The example of organ-specific autoimmune diseases is Hashimoto's disease in which auto-antibodies interact with thymus tissue resulting in formation of localized lesions. While in case of non-organ-specific diseases in which multiple organs are involved because of either distributing nature of a single auto-antigen or to the identification of multiple auto-antigens as in case of systemic lupus erythematous (SLE) [4].

The host immune system has two essential components named innate as well as adaptive immunity. The innate immunity is non- specific in response to antigens whereas adaptive immunity or specific immunity involves profoundly specific defensive response [5]. Innate immune response provides initial primary protection and use immune cells like monocytes, macrophages, dendritic cells and neutrophils for maintenance of homeostasis and adaptive immune response involves recruitment of T, B cells for secondary protection. Identification of pathogenic microbe by special receptors known as pattern recognition receptors that utilize by pathogen associated molecular patterns (PAMPs) for detection, leading to activation of innate immune cascade. one of the PPRs is the Toll-like receptor (TLRs) which are described as having Toll/IL-1Receptor (TIR) domain found on cytoplasmic site and has extracellularly leucine rich repeats (LRRs). The attachment of TLRs to PAMPs results in receptor dimerization leading to activation of TIR domain having adaptor protein such as Mal, TRIF, SARM, MyD88 and TRAM. The regulation of connection between death domain of MyD88 and interleukin-1 receptor-associated kinases (IRAK) members, which are considered to be mandatory regulators in IL-1R signaling mechanisms [6].

The family of interleukin-1 receptor (IL-1R) associated kinase (IRAK) [7] has important role in defensive immune responses against pathogenic microbes enter in human body by activation of additional adaptive immune responses. In humans, four members of IRAK family are found named: IRAK1, IRAKM, IRAK2 as well as IRAK4. There are two conserved regions of the IRAK1 include; N-terminal death region and a central kinase region. IRAKs have ability to stimulate signaling gene called NF-κB. IRAK1 and IRAK4 are two active kinases while IRAK2

and IRAK-M are inactive but all members are necessary for modulation of nuclear factor-kB (NF-kB) as well as mitogen-activated protein kinase (MAPK) functioning [8]. IRAKs are important component of interleukin-1 receptor signaling pathway as well as in mechanisms of Toll-like receptor signaling cascades. Toll-like receptors (TLRs) identify pathogens by using pathogen-associated molecular patterns (PAMPs) for recognition and IRAKs reaction to cytokines of interleukin-1 (IL-1) family [9].

The gene of Interleukin-1 receptor (IL1R)-associated kinase (IRAK1) belongs to non-HLA gene category that takes part in RA. The interleukin-1 receptor-related kinase (IRAK1) gene is positioned on q arm of X chromosome (Xq28) that binds to interleukin-1 receptor (IL1R) with intracellular domain. IRAK1 is a serine/threonine kinase that is fundamental element of Toll/IL1R signaling pathway, which is responsible for inflammatory responses including C-reactive protein (CRP)[10].

The regulation of Toll-like receptor (TLR)/IL1R (TIR) relying on signal conduction with the help of MYD88 is mediated by IRAK1. Moreover, IRAK1 is involved in inflammatory processes. IRAK1 is important among other family members because of above described processes and important in innate as well as adaptive immune interactions. Likewise, specific cytokines synthesis is regulated by the binding of MYD88 with interferon regulatory factors (IRFs) and this formation is supported by different TIR inducing signaling cascade [11]. Different SNPs of IRAK1 influence the expression of gene as well as their function.

IRAK1 functions by connecting sequence of events started by attachment of ligands to IL-1R and TLRs [12,13]. Diverse stimuli like detection of microbial pathogens/products reactive oxygen species presence, DNA damage detection, tissue matrix problems due to chronic inflammatory and genetic varables are participated in modulation of IL-1 as well as TLR signaling cascades [14,15]. IL-1R/TLR signaling through SMOC [16] called myddosome is essential for innate immunity [17] and regulates various cellular mechanisms. Dysregulation of this signaling cascade results in different variety of diseases. The attachment of IL-1 to its agnate receptor or TLRA to lipid component of pathogen-associated molecular patterns (PAMPs)

such as lipopolysaccharides induces immune along inflammatory responses regulated with the help of myddosome complex [18]. Functionally activated TIR receptors provide position on cystolic side for attachment of activated MyD88 protein results in releasing free MyD88 death domain (DD) for other connections. Next, IRAK4 reach DD, also an oligomer that leading to recruitment of IRAK1 and or IRAK2 [19,20]. The resulting structural complex induces IRAK4 for phosphorylation of IRAK1 results in its activation and thus hyper-phosphorylation, release from myddosome, and ultimately collaboration with E3 ubiquitin ligase and TNF receptor-associated factor 6 (TRAF6). NF-kB as well as MAPK cascades are stimulated by activated TRAF6 complex which in turn leads to up-regulation of pro-inflammatory cytokines [21].

IRAK1 has a important aspect in the recruitment of interferons through IRF7 [22]. IRAK1 has also involved in stabilization of mRNA supported by IL-1 independent of TRAF-6, on the other hand this mRNA is unstable [23]. Other role played by IRAK1 is the formation of IL-1, the "master cytokines responsible for inflammation" [24]. Enhanced production of IL-1β macrophages,dendritic cells as well as leucocytes in cystolic SMOC called as inflammasome [25,26]. Among other types of inflammasome, the Nod-like receptor protein 3 (NLRP3) inflammasome reacts to different types of stress variables and is usually involve in auto-inflammatory as well as autoimmune disorders [27]. The functional IL-1β is formed by breaking of pro-IL-1β who form by its own after breaking of its pro form in the inflammasome. The cleavage is mediated by cysteine peotease caspase-1 [28]. During early phase, IRAK1 mediates simultaneous activation of inflammasome along with NLRP3 as well as TLR, essential for pyropotosis and secretion of pro-inflammatory cytokines [29]. Therefore, IRAK1 is mandatory innate immunity through IL-1β. The role of IRAK1 in NF-κB cascade as well as secretion of IL-1β propose that IRAK1 plays role in modulation of levels of other pro-inflammatory cytokines. The induction of interleukin-6 (IL-6) that is a pleotropic cytokines is mediated by IL-1β and subsequent product of the NF-κB signaling [30]. IL-6 induces changing of odd T cells to the Th1 effector cells. Transforming growth factor β (TGFβ) as well

as interleukin-8 (IL-8) secretion is mediated by Th1 effector cells. A therapeutic strategy involves inhibition of IL-6 or IL6-R through antibodies is useful for inflammatory diseases including rheumatoid arthritis [31,32,33]. Another downstream product of NF-κB cascade is IL-8 that is crucial mediator of neutrophil activation [33].

The activation of IRAK1 leads to the signaling cascade involving IL-1R and TLR signal conduction. Death domain interactions are responsible for attachment of IRAK1 with other IRAKs members to form homodimer or heterodimer [34]. Dimerization is regulated by threonine 66 found in death domain of IRAK1 [35]. IRAK1 is attached with cytosolic Tollip before receptor activation [36]. The binding TLR/IL-R as well as myeloid differentiation factor 88 (MyD88) to ligands result in recruitment of receptor through TIR domains interactions found in both of these interacting molecules. Death domain of IRAKI helps in IRAK's attachment with receptor network by interacting with MyD88 [37].

The MyD88 binding recruits IRAK1/4 to the receptor network and as a result IRAK1 undergo phosphorylation with the aid of IRAK4 at Thr-209 and Thr-387 [38]. This leads to activation of kinase activity of IRAK1 and therefore hyperphosphorylated IRAK1 is formed through auto-phosphorylation [39]. However, when IRAK1 is in non-phosphorylated state, then MyD88 and Tollip bind to it [40], after that MyD88 free hyper-phosphorylated IRAK1 and the receptor complex, a fundamental hallmark in this mechanism of signaling. The binding of IRAK1 to tumor-necrosis factor receptor-associated factor 6 (TRAF6) is facilitated by multiple regions on IRAK1 such as death domain, the undefined domain and C-terminal [41]. It is believed about these interactions considered to be essential to make TRAF6 free from the receptor and systolic complex arrangement with IRAK1 as well as other signaling molecules [40,42]. Pellino1, IRAK1, IRAK4 and TRAF6 are parts of the intermediary cytosolic signaling nettwork and their formation might be occur former to the participation of TRAF6 with subsequent signaling TGF-β activated kinase-1 (TAK1) [43]. It has been reported that IRAK1 phosphorylates transcription factor STAT3 in the nucleus and bind to promoter region of IL-10 gene for its transcription as well as expression [44]. The mechanism

of regulation of gene transcription by IRAK1 either directly localized to nucleus and regulate it or sub-cellular localization is not well understood. Further studies are required for understanding of this novel regulatory mechanism [45].

The IRAK1 phosphorylation is related to the induction of NF-kB in immune diseases and by using IRAK1 inhibitor, the functioning of NF-kB might be impeded leading to suppression of inflammatory conditions [46,47]. IRAK1 has a critical role in both ADs individuals as well as in autoimmune animal models [47, 48]. Hence, IRAK1 is identified as risk gene for susceptibility of ADs.

Single nucleotide polymorphisms (SNPs) are mostly point mutations that are present within population in a frequency higher than 1%. The main source of variability among human being is SNPs depending on their location in the DNA sequence that influence expression of function of gene. SNPs are considered to be best biological markers in association or case-control studies because these are relatively easy to be detected [49]. Modifications in genes leading to single nucleotide polymorphisms (SNPs) or mutations that effect on susceptibility of different diseases [50]. Some investigators have reported association of ADs susceptibility with three SNPs of IRAKs : IRAK1 rs3027898 C>A, IRAK1 rs1059703 T>C, as well as IRAK1 rs1059702 T>C. Majority of the researches ware performed in the developing nations for association of IRAK1 polymorphisms with susceptibility to ADs with conflicting results [48, 51, 52]. Studies showed association of IRAK1 polymorphisms in relation to susceptibility with autoimmune diseases in various ethnicities but with conflicting results.

1.2 Problem Statement

Studies showed association of IRAK1 polymorphisms with susceptibility to autoimmune diseases in various ethnicities but with conflicting and inconsistent results. These discrepancies may be due sample size differences and low statistical accuracy. Therefore, meta-analysis is conducted in that context whether IRAK1 gene polymorphisms are associated with ADs susceptibility.

1.3 Aims and Objectives

IRAK1 gene polymorphisms plays important role in autoimmune diseases by affecting innate and adaptive immune response as well as development of autoimmune diseases. Different studies reported association of three SNPs of IRAK1 with ADs susceptibility but with inconsistent results. Therefore, study was designed to reduce limitations of individual analysis, solve incompatibilties and minify false-positive or false negative association of IRAK1 with ADs susceptibility. The aim of study was to determine association of genetic polymorphisms of IRAK1 with autoimmune disease susceptibility. Following objectives were recognized to obtain current goal of the study.

1. To find association of IRAKs on ADs susceptibility from case-control studies on different populations.

2. To re-assess and update the relationship of ADs risk with polymorphisms in IRAK1 SNPs rs3027898, rs1059702 and rs1059703.

Chapter 2

Literature Review

Autoimmune diseases (ADs) are group of heterogeneous disorders with poor understanding. Pathogenesis of autoimmune diseases (ADs) is considered to be result of genetic as well as environmental variables interaction [53]. There are two major types of autoimmune diseases (ADs): tissue-specific and systemic. Pathogenic and tissue-specific auto-antibodies are involved in autoimmune diseases (ADs) leading to localized in specific targeted organs. While in case of systemic autoimmune diseases multiple organ systems are damaged. Initiation of disease in both cases is the result of disruption of self-tolerance, specifically, during recognition of well-defined pathogenic auto-antigen and disease prominent in tissue that have auto-antigen [54]. Systemic autoimmune diseases are antigen driven complex abnormalities likewise systemic lupus erythematosus (SLE) as well as rheumatoid arthritis (RA) [55]. The development of ADs shows strong sex bias ; more common in women than men [56]. Studies showed that progression of systemic autoimmune diseases is influenced by predisposition suggesting that these are genetically inherited in humans as well as in mouse models [57]. Invasion of immune tissues by its own immune system leading to inflammation, degradation, tissue assassination and organ failure among genetically predisposed individuals [58]. However, in many autoimmune diseases (AD) pathogenic antigens are not recognized such as rheumatoid arthritis (RA) [59]. This leads to the possibility that loss of self-tolerance to a

specific antigen is not always necessary for development of localized autoimmune disease (ADs). Rather, etiological trigger is involved in the target tissue [60].

Inflammatory processes commonly occur only when pathogens enter in the body. Under these conditions, immune system cells identify structures and molecular signals that are distinguishable from those against body and immune system response arise. Rarely, inflammatory processes occur in the apparent absence of pathogens and these processes may remain there. Various tissues are affected or rarely even completely damage by such events. As a result disease develop that may be chronic, lead to lifetime impairment or even death. After occurring this, no pathogen is responsible for the onset of damaging immune responses in autoimmune diseases.

Common examples of this are rheumatoid arthritis, insulin-dependent diabetes and multiple sclerosis. Inflammatory processes that result in tissue destruction, various types of cells interact and communicate with each other leading to releasing and signaling of effector molecules that respond to molecular stimuli in their surrounding [4].

Variety of effector pathways are involved in tissue destruction depending on the type of autoimmune disease. Promiscuous immune system integrate response in which multiple varieties of cell participate. This may lead to difficulty in cure of some diseases like SLE and SSc. Common attributes of autoimmune disease is the existence of auto-antibodies [61]. These are involved in tissue destruction. Cytotoxic destruction of cells is influenced by pathogenic effects of auto-antibodies through attachment and then lysis.

During this process, common pathways are used for destruction include; antibody-dependent cell-mediated cytotoxicity (ADCC) [62]. Fc receptors on Natural killer cells mediate the ADCC. This mechanism is occur in AITD that anti-thyroperoxidase antibodies [63]. Other tissue damage process is the immune complex-mediated damage that occur in SLE. Synovial damage occur in case of RA by forming rheumatoid factor-IgG complexes. Auto-antibodies also communicate with cell surface rectors that results in activation and blocking of selecting cascade [64].

2.1 Signs and Symptoms

Autoimmune diseases (ADs) show analogous symptoms in all more than eighty different types. Location and type of immune response determine the presentation and seriousness of signs and symptoms. More than one autoimmune disease (ADs) may be appear in an individual and presents symptoms of multiple diseases. Other factors including age, hormones and environmental factors can influence the signs and symptoms [65]. There are various common symptoms of autoimmune diseases (ADs) including fatigue, low grade fever, malaise, muscle aches and pain in joints as well as rash found on different locations of the skin. There may be fluctuation in displaying of signs and symptoms and reappearance of these called as flare-up [66]. These signs and symptoms may be helpful in diagnostic purposes from biological markers of autoimmune diseases [8]. There are various locations in the body where autoimmune diseases (AD) impacted. These locations are; blood vessels, underlying connective tissues, joints and muscle, red blood cells and endocrine glands likewise thyroid or pancreas glands [66].

These diseases have characteristics pathological effects that place them in the category of autoimmune diseases (ADs). These features such as damage or degradation of tissues where abnormal immune response is present, result in altered growth of organ and function of organ that alterations are relying on the areas of the disease [66]. Some ADs are organ specific while other are systemic involving multiple organs, therefore signs and symptoms depend on which category of disease individuals have [67].

2.2 Pathophysiology

T cells and B cells as elements of human immune systemt, are responsible for reaction with self-antigens but commonly these self-reactive cells are either destroyed before becoming functionally active within immune system, silently extirpated from their function because of over-activation or regulatory cells removed them

from performing their roles. Failure of any one these mechanisms, there is probability of a collection of self-reactive cells that become active to perform function within the immune system. A negative selection process occurs within thymus to prevent self-reactive T cells from being created as thymus is the site for maturation of T cells.

Some infections like *Campylobacter jejuni* having antigens that resembles to our own self-molecules.In this situation, a usual immune feedback to *C.jejuni* leads to production of antibodies that react with gangliosides of mylelin sheath enclosing peripheral nerves axons to some extent. Application of genome-wide association study is useful in understanding pathophysiology of autoimmune diseases that scans to identify role of genetic factor in autoimmune diseases [68]. As ADs are the result of disruption of immune tolerance. When immune tolerance is breakdown and inflammation occurs due to auto-antibodies and self-reactive lymphocytes, leading to development of classical or pathological autoimmunity (Fig1).

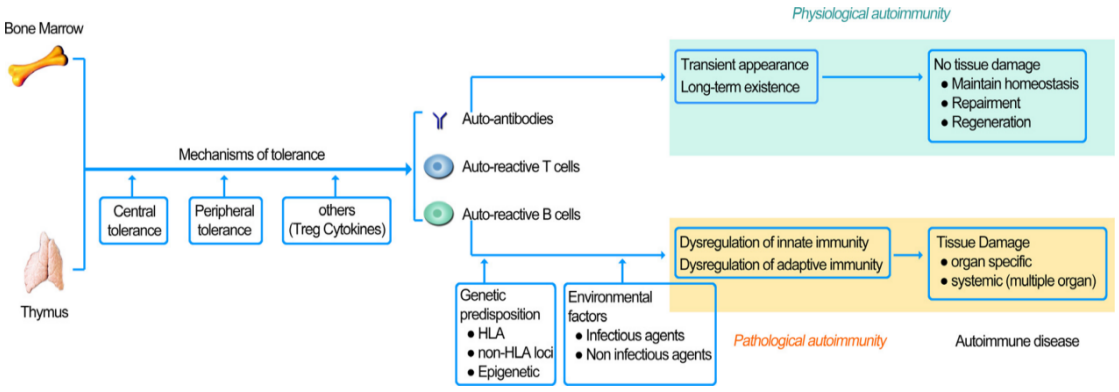


FIGURE 2.1: Summary of Development of Autoimmune Diseases. In Healthy Individuals, During Rigid Control of Central and Peripheral Tolerance, Few Self-Reactive T and B cells Drain into Outer Surface. They will Remain There Without Harming Until Genetic Predisposition Lead to Breakdown of Tolerance and Initiate Development [67].

2.3 Role of Environmental Factors

There are various environmental factors that increase the individual susceptibility for autoimmune diseases. These environmental factors are diet, the microbiota,

infections , smoking, medicines, vaccines, silica solvents, ultraviolet light,hormones and collagenous material [69,70]. Microbiota may influenced the individual's predisposition to autoimmunity in the presence of specific agents. This concept was named hygienic hypothesis in 1989 by Strachan [71]. According to this hypothesis, autoimmune diseases rise in Western world because of reduction in infectious disease exposure and in turn improvement in hygienic conditions. This hypothesis is applicable to almost all autoimmune diseases, particularly in TID and inflammatory bowel disease. Retrospesctive epidemiological studies provide evidence for this hypothesis [72].

The important subject of interest that gaining attention of researchers is to find relatedness of microbiota, autoimmunity and immune responses with each other because various environmental inter-connection such as skin, gastrointestinal tract, genital tract as well as respiratory mucosal surface. Advance sequencing and high-throughput technology described that microbiota changes in the normal hosts are essential for immune development and homeostasis. Researches showed that gut microbiome changes lead to onset of TID and also responsible for progression of disease [73]. The onset and course of the disease is influenced by changes in oral and intestinal mircobiota in case of RA. Segmential filamentous bacteria's tansformation in colonization may influenced autoimmunity even in adult life (Figure 2) [74]. Acute rheumatic fever provide good example of relationship between infection and immunity that happens by exposure of genetically susceptible hosts to Streptococcus pyogenes [75]. In acute rheumatic fever, molecular mimicry explains the mechanism of autoimmunity between bacterial M protein and lysogangliosode of human that results in disruption of tolerance of immune system and cardiac reactive T cell formation [76]. In 1964, Damian first used the word of molecular mimcry. According to him, microorganisms's preferred antigenic determinants look similar to epitopes of the host and were able to induce an autoimmune response [77].

Vitamin D level is associated with immune response. Studies showed that vitamin D is contemplated to be a natural modulator of immune responses. However, other roles of vitamin D in the body are regulation of metabolic calcium level, cell

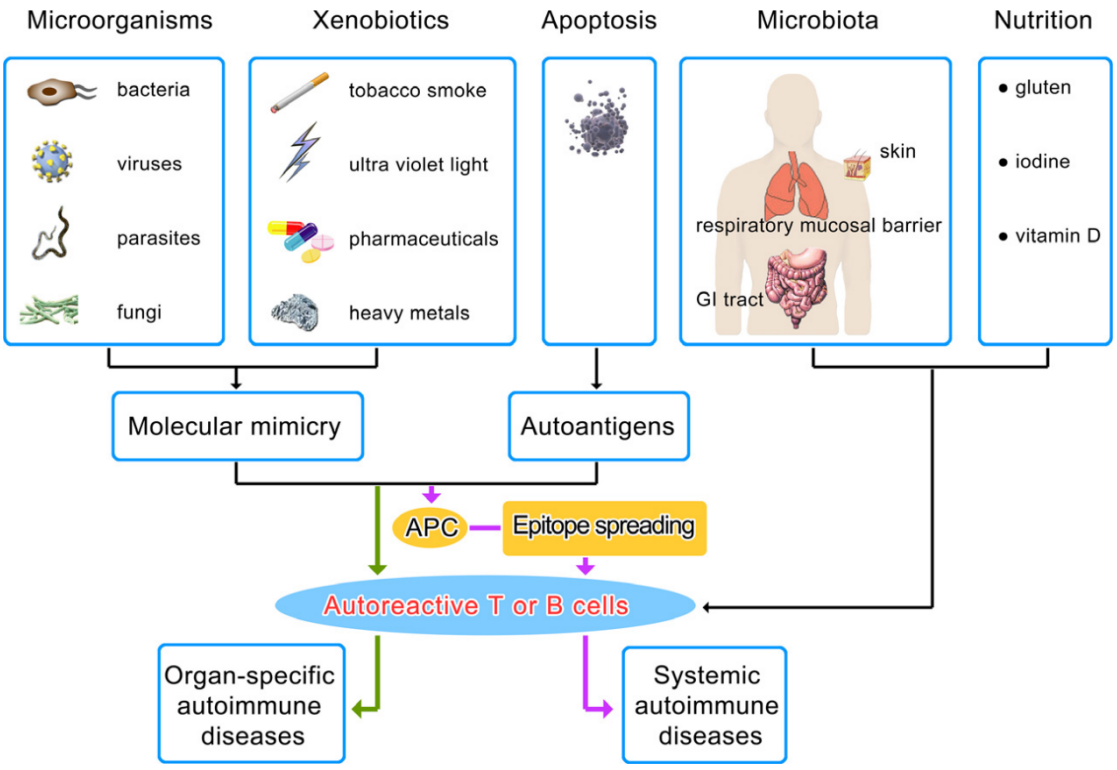


FIGURE 2.2: Role of Environmental Variables in Autoimmunity. Different Environmental Variables are Involve in Creating Implications in Autoimmunity Development. A Common Mechanism of Molecular Mimicry is Responsible for Activation of T and B Auto-Reactive Cells. Another Mechanism named Epitope Spreading is Responsible for Generation of Neoepitopes. Other Factors Such as Nutrition is Involve in Disruption of Tolerance in Addition to Normally Modulating Immune Response [67].

growth, cell proliferation and cell death (apoptosis)[78]. Disruption of tolerance is increased by reduce levels of vitamin D as epidemiological studies showed. Multiple human autoimmune diseases showed the association of reduced levels of vitamin D [79]. Individuals living in large cities and less expose to sunlight have deficiency of vitamin D that is fulfill by supplementation of vitamin D. More studies are required to show association of vitamin D with autoimmune diseases.

Tobacco smoking is risky factor for both RA [80] and SLE [81]. It involves in the pathways of disease development. The smoke of cigarette consists of many Toll-like receptors (TLRs) stimulating compounds like lipopolysaccharide that can induce innate immune response. Gene expression in the joint may change by interaction of HLA haplotypes with smoke that results in the development of RA [81]. Smoking is also considered a risky factor for other autoimmune diseases such as PBC,

SS as well as AITD [82]. Changes in apoptosis are induced by environmental factors. Apoptotic cells and autoantigens have apoptotic bodies that appear to be crucial for the promiscoous and often orchestrated response that is responsible for multitude diverse populations of cell. This leads to difficulty in treatment for some diseases including SLE and SSc [83].

2.4 Role of Genetics

Different genetic factors are involved in most of the autoimmune diseases because all are not monogenic. A number of early studies reported association autoimmune diseases (ADs) with major histocompatibility complex (MHC). The MHC is found on the chromosome 6 at short arm and have genes that encode molecule responsible for antigen presentation and therefore it is important to differentiate self from non-self.

The product of MHC gene is human leucocyte antigens (HLAs) in humans. Various studies have recognized association of these genetic variants with autoimmune disease[84]. Although massive efforts have been made in identification of genetic basis of autoimmune diseases, but results failed to determine major predictive value [85].

Involvement of mutiple of non-HLA loci has been identified in diseases such as RA, PBC, SLE, ulcerative colitis (UC), MS, autoimmune hepatitis and many other immune diseases [86]. These risky variables seem to be related with products of gene that play leading role in both non-specifc as well as specific immune responses [87].

The concordance rate of autoimmune diseases is range from 12% to 67% in monozygotic twins [88], indicating that other factors in addition to genetic factors are coexist. Although, there is increasing attention on the likelihood that epigenetic mechanisms like addition of methyl group to DNA are involved in modulation of autoimmune diseases. Various epigenetic modifications in DNA are related with

tolerance failure, such as hypermethylation of insulin DNA in TID [89], peptidylarginine deaminase 2 (PAD2) hypomethylation [90] as well as in MS Src homology region 2 domain-containing phosphatase-1 (SHP-1) [91], in PBC methylation of the promoter CD40 [92], in functional CD4 T cells in SLE involve modification of histone, histone deaceylase (HDAC) inhibitors in RA, in case of SS, addition of acetyl group to histone H4 in promoter region of gene aquaporin 5 (AQP5) [93] and microRNA (e.g. miR-21) communication in UC, SLE, T1D, SS, MS and psoriasis [94].

2.5 IRAK1 and Characteristics of IRAKs Family Members

A serine/threonine protein kinase termed Interleukin-1-receptor-associated kinase 1 (IRAK1) is involved in Toll/interleukin-1 receptor (TIR) family signaling cascade [95]. IRAK1 activated on stimulation of Toll-like receptor (TLR) and undergo phosphorylation in the TLR signaling mechanisms. There are some significant features of IRAK family members.

- IRAK-1 is important in regulation of signaling cascade leading to activation of NF-kB. Nerve growth factor (NGF) require IRAK-1 for its activation and survival [96].
- There are four isoforms of IRAK-2 named IRAK-2a to 2d. IRAK-2c and IRAK-2d are responsible for negative responses signaling processes of TLR while IRAK-2a and -2b are involve in positive activation of NF-kB/TLR pathway [97].
- IRAK-M is peculiar to macrophages as well as monocytes. It modulates TLR signaling by playing negative role leading to hampering of IRAK-1/4 complex.
- IRAK-4 is necessary for stimulation of IRAK-1 as well as its activation and degradation [98].

2.6 Discovery of IRAKs

Michael Martin and colleagues first identified IRAKs in 1994 when a protein kinase taken from T cell of human and performed co-precipitation of this kinase along type I interleukin-1 receptors (IL-1RI). They supposed that connection between transmembrane IL-1 receptor of T cells and subsequent components of cytosolic signaling pathways was because of this kinase [99]. In 1995, Zhaodan Cao and colleagues used word IRAK. IRAKs domains, DNA sequence analysis showed in *Drosphila*, the presence of various reserved amino acids rich in the threonine/serine peculiar protein kinase. Kinase activity of IRAKs associated with IL-1 receptors was confirmed by Cao's lab. They immunoprecipitated the IL-1receptors obtained from different categories of cells that undergo treatment with IL-1and some did not treated with IL-1. Even cells lack more-expression of kinase activity displayed by IL-1 receptors after exposure to IL-1 and also had ability for co-precipitation a protein kinase with IL-1 receptors of endogenous nature. Therefore, Interleukin-1 Receptor-Associated Kinase was the name given to IL-1 receptors of human [100]. In 1997, a cytosolic protein named MyD88 was identified. This protein helps in recruitment of IRAKs to site found on the cytosol of IL-1 receptors, regulating transmission of signal produced by IL-1 to cytosolic signaling pathway [101].

2.7 Structure

2.7.1 Domains of IRAKs and their Function

Members of IRAKs family are proteins having multiple domains that contains in their structure a central kinase domains as well as N-terminal Death Domain (DD) (Fig.3). The intercommunicating domain is DD site such as interaction for adaptor protein MYD88 and other IRAKs members. Kinase activity of IRAKs is due to KD that contains 12 sub-domains. ATP-binding site is positioned on this kinase domain (KD) with an constant number of residues of lysine in sub-domain II. Although, IRAK1/4 have catalytic site enrich with an aspartate residue in the

sub-domain VI which is accountable for kinase activity. While IRAK-2 and IRAK-M are non-functional catalytically due to deficiency of this aspartate residue in the KD [8].

The C-terminal domain is not similar in all IRAK family members. The C-terminal domain is mandatory for the interaction of signaling molecule named TRAF6. IRAK-1 consists of three TRAF6 elements for interaction, IRAK-2 has two and IRAK-M consists of one [6]. IRAK1 consists of a site that is abundant with serine, threonine (proST) as well as proline residues. This region is responsible for hyperphosphorylation of IRAK1. However, proST end enrich with glutamic acid (E), proline (P),threonine (T)-rich (PEST) as well as serine (S) residues that are reflected to be responsible for IRAK1 degeneration [8,102].

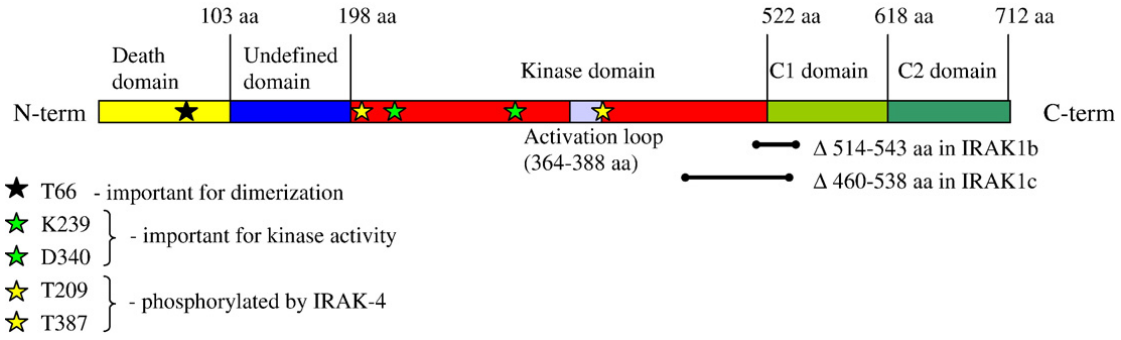


FIGURE 2.3: Functional Domains of IRAK1 [103].

2.8 Role in Immune Signaling Cascades

2.8.1 Interleukin-1 Receptor Signaling Pathway

Cytokine receptors named interleukin-1 receptors (IL-1Rs) that are involve in signaling pathway intracellularly by interacting with inflammatory cytokines such as interleukin-1 (IL-1). This signaling mechanism results in activation of transcription of different genes responsible for swelling expression. IL-1Rs being deficient of inherent kinase activity, they depend on the of adaptor molecules for communication purposes like IRAKs's interactions to transmit signals. The attachment of both IL-1 and IL-1R complex with each other initiates the participation of the

adaptor protein MyD88 via interactions along TIR site. IRAK4 attached to receptor complex with the help of MyD88. IRAK1 and Tollip are also moved to the receptor complex, cosequently binding of IRAK1 with MyD88.

The binding of IRAK1 with MyD88 recruits IRAK4 in close proximity so that IRAK4 can phosphorylate and activate IRAK1. After phosphorylation IRAK1 activates detachment of TNF receptor associated factor 6 (TRAF6) adaptor protein and IRAK1-TRAF6 complex from the IL-1R complex. This complex make interaction with a already formed complex located in the cell membrane comprising of TGF-β activated kinase 1 (TAK1), as well as two TAK binding proteins.

TAK1 is enzymatic protein also designated as mitogen-activated protein kinase kinase kinase (MAPKKK). This connection results in TAB2 and TAK1 phosphorylation which move toward cytosol with TRAF6 and TAB1. While IRAK1 is still present at the membrane and ubiquitination leads to degradation of IRAK1. Ubiquitination of TRAF6 results in stimulation of kinase activity of TAK1 after entering of complex TAK1-TRAF6-TAB1-TAB2 into the cytosol.

Two transcription mechanisms; one is nuclear factor-kB (NF-kB) cascade as well as other is mitogen-activated protein kinase (MAPK) cascade that are activated by TAK1. For activation of NF-kB, IkB kinase (IKK) complex phosphorylation, which in turn phosphorylates NF-kB inhibitor, IkB, focusing it for degeneration with the help of proteasome. After removal of IkB, the NF-kB proteins are detach and ready for entry to nucleus, where it involve in transcription activation of pro-inflammatory genes [8, 22].

2.8.2 Signaling of Toll-like Receptor

Innate immune receptors named Toll-like receptors (TLRs) that used pathogens associated molecular patterns (PAMPs) for recognition of pathogens and trigger specific immune response for elimination of a peculiar pathogen. Microorganisms likewise bacterial lipopolysaccharides, double stranded RNA viruses that are not normally found in the host, are specifically detected by these PAMPs conserved

structures. TLRs analogous to IL-1Rs in a way that lack intrinsic kinase activity as well as need adaptor molecule for their signaling. Activation of TLRs leads to transcription of NF-kB and MAPK , corresponding to the IL-1R signaling pathway [22,102]. IRAK1 is necessary for TLR9 interferon (IFN) as well as TLR7 eliciation [102].

For recruitment of interferon regulatory factor 5 (IRF5) IRAK1 is essential. IRF5 is a transcription factor that stimulates the production of IFN following activation of TLR7-9 by peculiar viruses [44]. Induction of interleukin-10 (IL-10) in TLR4 requires [104] IRAK1. Bacterial LPS are recognized by TLR4 and then transcription of IL-10, a cytokine necessary for regulation of proinflammatory response. A signal transducer and activator of transcription 3 (STAT3) activates IL-10 transcription. IRAK1 activates the transcription of IL-10 by forming a framework in the nucleus with IL-10 and STAT3 promoter element [104]

2.9 Polymorphisms in IRAK1 Gene

Polymorphism is a monogenic trait by definition but it may be polygenic relying on its extent of role in a cascade or disease [49]. Polymorphisms involve insertions and deletions of nucleotide. Polymorphisms are not cause of a disease like mutations, but it may only increase or decrease the susceptibility of disease in an individual. Likewise polymorphisms found in the hemoglobin gene (SNP rs334) are seem to be less serious development of malarial infection and there is increase chances of survival in patients carrying sickle-cell polymorphisms.

Most of the reported polymorphisms in cytokines and their receptors are found in the promoter, 3' untranslated (UTR) or intronic region of gene. Polymorphism present in the promoter region of gene may disturb the attachment of transcription factors including Janus Kinase (Jak), Nuclear Factor kappa B (NF-Kb) or Signal Transducer and Activator of Transcription (STAT) to regulatory region. Transcription factor binding elements of the gene are influenced by modification or variations in the intronic region.

2.9.1 Single Nucleotide Polymorphism (SNP)

An inheritable variation that occurs in a single nucleotide at a particular location in genome is known as single-nucleotide polymorphism (SNP), where each variation is found at particular frequency in a population [105]. The SNPs are present in a population at a frequency at least 1% in a population. SNPs are different from mutations in term of frequency because mutations are present in population at a frequency less than 1%.

Single nucleotide polymorphisms (SNPs) are commonly observed and studied in the human genetics. These SNPs affect transcriptional factor activation which in turn affects cytokine level by altering recognition site on transcription factors [106].

SNPs that are present in the coding regions of the gene influence the biological activity of the proteins by altering amino acids sequence as result structure of protein is changed, which lead to health problems or changed response to drugs target [107]. The estimated frequency of occurrence of SNPs in human genome is one in every 1900 bp [108].

Diversity within population is promoted by genetic polymorphisms. No overall advantage or disadvantages in single form over the others regarding natural selection has been observed, therefore it persists over many generations. Different types of blood groups in humans are example of different allelic forms [108].

Another type of polymorphisms is the microsatellite repeats that consist of number of di, tri, tetra, penta or hexa nucleotides. Other names used for microsatellites are Simple Sequence Repeats (SSRs) or short tandem repeats (STRs). Such repeats are placed in almost every part of the genomes [109] but are rarely present in the promoter regions.

Most of the polymorphisms are neutral, some have necessary role in regulation of gene expression or function of the protein coded by these genes. These polymorphisms could affect the variations among individuals to susceptibility and severity of disease [110].

2.9.2 Chromosomal Location and Polymorphisms of IRAK1

IRAK1 is positioned on the q arm of the X chromosome at point 28 [111].

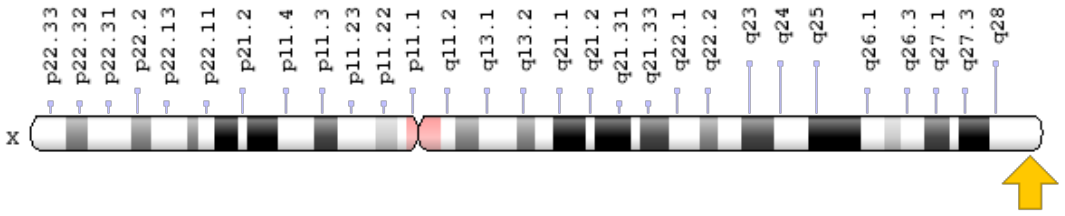


FIGURE 2.4: Location of IRAK1 on X Chromosome [111].

Polymorphisms in IRAK1 including single nucleotide polymorphism (SNP) rs 3027898 (IRAK1, rs30227889), SNP rs1059702 (IRAK1, rs1059702), and SNP rs1059703 (IRAK1, rs1059703).

2.10 Role of IRAKs in Diseases

Local and systemically functions played by Interleukin 1 (IL-1) cytokine in the non-specific immune responses. Inflammation, production of pro-inflammatory cytokines as well as fever are induced by IL-1a and IL-1ß. Due to mandatory role of IRAKs in the IL-1 receptor signaling pathway, their defect or expression beyond the limit can disturb IL-1a and IL-1ß level. Therefore,it may be considered to be therapeutic target for immunosuppressed, autoimmune as well as cancer-related abnormalities [5].

2.10.1 Cancer

Inflammatory signaling is the major factor in many cancer types and key aspect of human tumors is the inflammatory microclimate. IL-1ß, is necessary for activation of inflammatory signaling pathway consisting of IRAKs, which show direct

participation in tumor cellular growth, angiogenesis, invading to other tissues as well as metastasis [112]. In 2013, Garrett Rhyasen and his colleagues conducted a study at the University of Cincinnati showed functional role of IRAK1/4 in myelodysplastic syndrome (MDS) as well as acute myeloid leukemia (AML) in human [113].

2.10.2 Autoimmune Disorders

Autoimmune disorders and inflammatory including rehumatoid arthritis (RA), lupus, MS as well as psoriasis resulting from dysregulation of innate immune system [114]. Mostly cases in which blockage of IRAK-1/4 are supposed to be potent targets for drugs, as they play important role in the cytokine pathways necessary for chronic inflammation [115]. Some of the autoimmune and inflammatory diseases are given below.

2.10.2.1 Rheumatoid Arthritis

IRAK1 is stimulated by TLRs's ligands and detects different ligands types like pathogens, and responsible for inflammation mediated by pathogens [116]. The activation of these recptors's ligands recruit a subsequent signaling pathway that result in activation of transcription of specific genes such as costimulatory molecules as well as inflammatory cytokines. IL-6 activates IRAK1 is stimulated by IL-6 that leads to phosphorylation and stimulation of the transcriptional factors STAT3 that lead to initiation of transcription of CRP genes [117]. Innate and adaptive immune signaling pathways are triggered side by side, leading to activate function of NF-jB result in most marked event of the inflammatory response [95]. VEGF and interleukin-8 (IL-8) are produced by RA-fibroblast-like synoviocytes (FLS) with the help of IRAK1 following activation of TLR2 [118]. Interleukin-1 (IL-1) induced matrix metalloproteinase-13 for destruction of cartilage matrix with the aid of IRAK1 [119], that lead to downstream signaling of Toll-like and cytokines receptors. The expression of IRAK1 is more among cronary arteries showed by

cDNA microarray analysis. More ever, IRAK1 regulates gene expression responsible for anti-inflammatory cytokines production such as interleukin-10 (IL-10) [44]. These conclusions showed that IRAK1 activation is associated with increased level of inflammatory proteins involved in RA. Interestingly, location of IRAK1 gene is Xq28 region that harbours several SNPs associated with susceptibility to autoimmune diseases. Numerous SNPs have been found on the Xq28 locus and also recognized rs1059703 and rs1059702, encoding for pSer532Leu and pPhe196Ser, as two IRAK1 in a recent findings of case-control. These SNPs are increasingly associated with RA risk in Korean families [120]. These researchers also investigated about association of major haplotype (rs1059702 T and rs1059703 C) with enhanced IRAK1 functional activity. Eyre et al, investigated that SNP rs13397 located in upstream IRAK1 and within Xq28 locus was found to be in risk of RA among population of northern Europe[121]. This polymorphism is found on the TME187 gene which encodes a trans-membrane protein whose function is not known. The connection of these polymorphisms at Xq28 region with susceptibility to RA among different ethnicities is not well studied [122].

2.10.2.2 Systemic Lupus Erythematous

SLE is considered in category of chronic diseases of autoimmune system in which level of auto-antibodies is high and there is high level of inflammation of various organs of the body. SLE pathogenesis is influenced by innate immunity [123]. Disease initiation and its further progression is the result of aberrant immune processes. IRAK1 is seem to be involved in aberrant immune responses in SLE. IRAK1 is responsible for activation of interferon alpha and interferon gamma; in case of SLE, production of both cytokines is aberrant [124]. Moreover, NFB pathway is regulated by IRAK1. Studies reported abnormal activity of NFB in T lymphocytes in sample of SLE patients [125]. Studies reported association of IRAK1 rs1059702 SNP with enhance activity of NF-kB as well as increase risk of SLE in multiple ancestral groups [126]. The SNP of IRAK1 rs3027898 was prominently differ from healthy controls in relation to allele frequency as well as genotype and the individuals having arthritis reported enhanced frequency of

mutant allele (AC/CC) as compared to wild type (AA) [127]. In population of Chinese Han, C allele frequency in SNP rs3027898 was markedly high among patients than controls [51]. Association of IRAK1 SNP rs1059702 with susceptibility to SLE in Latian, American and United States was reported by Genome-wide association studies [128]. The risk allele in SNP rs1059702 was associated to SLE among European, Americans and Hispanics that may lead to IRAK1 substitution of amino acid S196F raising functional activity of NF-kB [126].

2.10.2.3 Multiple Sclerosis (MS)

MS is a type of long term inflammatory immune-mediated disease involving brain and spinal cord. IRAK1-/-mice demonstrated diminished activity for development of EAE after immunization in contrast to WT mice where WT mice showed a single phase of disease course, high score for EAE mean and T cells expressed proliferative response in addition to production of IFN-γ [129,130]. Culturing of splenocytes with oligonucleotide 1826 from IRAK1-/- mice demonstrated low IL-12p40 as well as TNF-α production level. Immunization of WT mice with (MOG) 35-55 peptide showed prominent infiltration of macrophages to brain leading to inflammation of CNS, while mice deficient of IRAK1 revealed diminished ability for recruitment of macrophages to CNS [130].

2.10.2.4 Ankylosing Spondylitis (AS)

Ankylosing spondylitis (AS) is fall in the category of inflammatory arthritis that showed association with IRAK1 polymorphisms [52]. AS can damage spine, sacroiliac joints as well as other parts of the body and badly affects the quality of life of patients [131]. Mice having deficiency of miR-146a lead to development of gouty arthritis in them and high levels of IRAK1 in BMMs was observed in gouty mice [132]. Inheritance, infection and other factors that results in dysregulation of immune responses are associated with onset of AS in vivo. Cytokines also take part in the pathogenesis of diseases. The SNP of IRAK1 rs3027898 was significantly related with AS [133].

2.10.2.5 Systemic Sclerosis (SSc)

Systemic sclerosis (SSc) is a type of autoimmune disease that develops among persons as a result of genetic predisposition due to exposure to certain environmental stimuli [134]. SSc characterized as the fibrotic scarring of the external skin, multiple internal organs as well as vasculopathy. Individual having SSc showed IRAK1 variants that was associated with diffuse form cutaneous SSc, anti-topoisomerase 1 antiobody and fibrosing alveolitis subsets [135]. Studies showed contribution of genetic factors for susceptibility to SSc. Antigen processing, activation of T cell and non-specific immunity is controlled by major histocompatibility complex (MHC) as well as non-MHC [136]. NF-kB as well as interleukin-1 (IL-1) are involved in pathogenesis of SSc [137]. Recent studies showed that IRAK1 SNPs found on Xq28 are associated with SSc [138].

2.10.2.6 Psoriatic Arthritis (PsA)

Psoriasis and psoriatic arthritis are interlinked disorders that develops together, having similar cytokines profiles, sharing same skin phenotype and similar response to anti-tumor necrosis factor (TNF) therapy [139]. PsA is a kind of inflammatory arthritis that showed association with Ps but negative for rheumatoid factor [140]. Monozygotic and dizygotic twin studies reported that both PsA and psoriasis are highly heritable [137]. Studies showed association of HLA genes with PsA but with conflicting results. Non-HLA genes including interleukin-1 (IL-1) reported to be associated with PsA [141]. SNP of IRAK1 rs3027898 showed association with susceptibility to PsA [52].

2.10.2.7 Giant Cell Arteritis (GCA)

Joint cell arteritis in which inflammation occur in large and medium-sized blood vessels [142]. The disease results in several complications including stroke, aortic aneurysm, myocardial infraction and sever complication is the loss of vision [143]. However, no high risk of mortality has been observed in GCA patients. GCA

develops more commonly among women with a female to male ratio range from 2-3:1 and people age cross 50 years. During eight decade of life GCA incidence rate is highest [144].

It has been hypothesized that various genes are responsible for pathology of GCA from the last few years, although, very few are closely related to GCA [144]. Most of these genes are considered to be risk factors for autoimmunity. Since GCA is a autoimmune disorders with female dominance; therefore, it is reasonable assumption that susceptibility of GCA is influenced by the genes that are found on the X-chromosome. In this regard, various genetic variants at Xq28 region having two genes responsible for regulation of autoimmunity methyl CpG binding protein 2 (MECP2) and RAK1 are associated with autoimmune diseases [145]. The SNP of IRAK rs1059702 do not show significant association with susceptibility or severity of GCA.

However, previous studies showed variable and inconclusive results. Meta-analysis is a useful tool for analysis of combined effect of individual studies, recently published meta-analysis showed association of IRAK1 with ADs in different genetic model based on the conclusion drawn from studies published before 2015 [146]. Therefore, current meta-analysis included all published results before and after 2015 that may be a comprehensive estimation of the extent of association of IRAK1 gene polymorphisms with susceptibility to ADs.

Chapter 3

Material and Methods

3.1 Literature Search

Literature was search from the PUBMED and Google Schalor for identification of all relevant studies with the following key words: interleukin-1 receptor associated kinase, or IRAK-1, rs3027898, rs1059703, rs1059702 and variation or polymorphisms or variant or mutation or SNP or case-control or genetic association study as well as their combinations and autoimmune diseases. The citations of the selected papers were also screened for inclusion criteria of the study. The articles having case-control study designs were selected for study analysis. Relevant characteristics of the studies were extracted and data were summarized in a consistent manner to help in comparison.

3.2 Selection Criteria

Firstly titles URL's and abstracts of all the concerned articles were saved in .doc file. After that full texts were reviewed and analyzed. The studies which fulfill the set criteria for analysis were eventually picked up to perform analysis.

- The study design was a case-control.

- The IRAK1 gene polymorphisms should be included.
- The articles have enough data for computational analysis of odds ratio with 95% confidence intervals and P-value that could be used for comparison to infer the results.
- Only publications in English language were included.
- If more than one population was studied in a selected article, results of the study were considered as separate results.

3.3 Exclusion Criteria

Publications were considered ineligible; I. if not report anything about associations of polymorphisms in IRAK1 with autoimmune diseases; II. Narrative and systematic reviews or comments; III. Papers having only ADs population due to lack of control group for comparison (Figure 3.1).

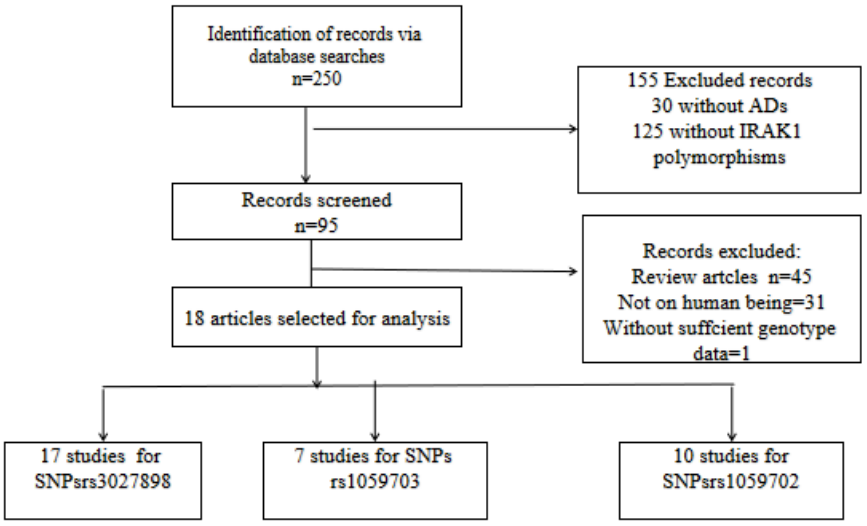


FIGURE 3.1: Flow Diagram Depicting Process of Literature Selection.

3.4 Data Extraction

The author retrieved following essential particulars from selected publications

- Name of the first author
- Year of publication;
- First author's country
- State of studied populations
- Numbers of patients having ADs and control among all studies
- Analyze association of IRAK1 polymorphisms between ADs patients and controls
- Data of SNPs (Table 3.1)
- Distribution of IRAK1 genotype allele frequencies among both cases and controls

Frequencies of recessive model as well as homozygote model were computed from genotype frequencies. For computational analysis, numerical data for events and total in both cases and control among allele, homozygote, heterozygote, dominant as well as recessive model was enumerated by using values of allele and genotype frequencies. For rs3027898, C allele is dominant than A allele. For rs1059702 as well as rs1059703 T allele was dominant than C allele (Table 3.2).

3.5 Statistical Analysis

Statistical analysis were computed with Review Manager Version 5.4. Results of individual studies were combine by using this software. For assessment of association of IRAK1 genetic polymorphisms with ADs susceptibility, calculation of pooled OR ratio along with 95% confidence interval was done based on dominant model (CA+AA vs CC), recessive model (AA vs CC+CA), heterozygote model (CA vs CC), homozygote model (AA vs CC) and allele model (A vs C) for SNP rs3027898 and allele model (C vs T), dominant model (TC+CC vs TT), recessive model (CC vs TT +TC), heterozygote model (TC+TT) and homozygote model

TABLE 3.1: Included Studies for Meta-Analysis.

Sr. No	**Authors**	**Population**	**Disease**	**Sample Size**		**Polymorphisms**
				Patients	**Controls**	
1	Chatzikyriakidou, 2010	Europe	RA	136	147	rs3027898 rs1059703
2	Zhang, 2013	Asian	RA	211	478	rs3027898
3	Han, 2013	Asian	RA	1318	1016	rs3027898 rs1059702 rs1059703
4	Chatzikyriakidou, 2010a	Europe	AS	49	66	rs3027898
5	Gao, 2012	Asian	RA	123	220	rs3027898 rs1059703
6	Atabaki, 2017	Asian	RA	120	120	rs3027898
7	Yang, 2017	Asian	RA	386	576	rs3027898
8	Hassine, 2017	African	RA	172	224	rs3027898
9	Marquez et.,2014	Europe	GCA	627	1520	rs1059702
10	Chatzikyriakidou, 2010b	Europe	PsA	29	66	rs3027898 rs1059703
11	Shaker, 2018	African	RA	104	112	rs3027898
12	Najme, 2020	Europe	RA	200	200	rs1059703

TABLE 3.1: Included Studies for Meta-Analysis.

Sr. No	**Authors**	**Population**	**Disease**	**Sample Size**		**Polymorphisms**
				Patients	**Controls**	
13	Labib et al. ,2019a	African	SLE	80	120	rs3027898
14	Labib et al. ,2019b	African	MS	70	120	rs3027898
15	Zhai et al.,2013	Asian	SLE	661	663	rs3027898 rs1059702
16	Carmona. 2013	Europe	SSc	2415	2361	rs1059702
17	Dieude, 2011	Europe	SSc	1808	2217	rs1059702
18	Rong et al.,2015	Asian	AITD	1042	897	rs3027898 rs1059703
19	Wang, 2020	Asian	AS	200	200	rs3027898
20	Khalifa, et al., 2017a	African	RA	119	131	rs1059702 rs1059703
21	Khalifa ,et al., 2017b	Europe	RA	289	340	rs1059702 rs1059703

(CC vs TT) for SNP rs1059702 as well as for SNP rs1059703. Z test was applied to calculate association of IRAK1 polymorphisms with disease susceptibility.

Threshold of p value was set at 0.05. I_2 that was applied to assess heterogeneity of the studies that value considered to be statistical significant.

Association between IRAK1 SNP rs3027898, rs1059702, rs1059703 and various different autoimmune disorders were analyzed by re-calculation of combing crude ORs along their subsequent 95% CIs.

If the P value of the heterogeneity test was ≥ 0.05, fixed effect model was considered to be applicable to calculate the combined OR (Mantel-Haenszel method).

The fixed effect model supposed to be similar homogeneity of effect size among all studies otherwise the choice was random effect model. P-value was used for test of association and p value less than 0.05 was regarded as statistical significant and it represented the association.

Odd ratio values with 95% confidence interval for all allele frequencies were used to analyze the strength of association between IRAK1 SNPs and its associated diseases. The heterogeneity was not regarded as statistically significant if values were P>0.10 and I_2 <50%.

Heterogeneity check the variability found in studies, in other words it identifies how comparable literature found in the meta-analysis. I_2 is used as a most reliable test for heterogeneity.

Studies are considered to be homogeneous if the CIs of all the studies overlap. I_2 ranges between 0 to 100%. A rough guidelines for clarification of I_2 are as follows;

- 0% to 40% > might not be important
- 30% to 60%> may depict moderate heterogeneity
- 50% to 90%> may depict meaningful heterogeneity
- 75% to 100 %> Extreme heterogeneity

The stability of study was checked by eliminating one study each time and combining together the results of rest studies.

TABLE 3.2: Distribution of Allele and Genotype Frequency of IRAK1 Gene Polymorphism with ADs

Sr.No	**Authors**	**Disease**	**Patients**			**Controls**		
	rs3027898		AA	AC	CC	AA	AC	CC
1	Chatzikyriakido ,2010	RA	71	45	20	91	47	9
2	Chatzikyriakido ,2010a	AS	39	1	9	40	22	4
3	Chatzikyriakido, 2010b	PsA	18	3	8	40	22	4
4	Zhang, 2013	RA	28	42	141	35	103	337
5	Han, 2013	RA	56	383	719	50	321	478
6	Atabaki, 2017	RA	23	51	30	19	40	17
7	Yang, 2017	RA	17	79	290	26	171	379
8	Hassine, 2017	RA	83	66	23	116	83	25
9	Shaker, 2018	RA	22	40	42	28	10	74
10	Labib et al., 2019a	SLE	28	24	28	88	10	22
11	Labib et al., 2019b	MS	30	32	14	88	10	22
12	Zhai et al., 2013	SLE	21	167	473	40	202	421
13	Gao, 2012	RA	4	33	86	10	54	156
14	Wang, 2020	AS	79	85	36	45	113	42

15	Rong et al.,2015a	AITD	45	260	479	25	155	412
16	Rong et al.,2015b	GD	29	155	291	25	155	412
17	Rong et al.,2015c	HT	16	188	105	25	155	412
	rs1059702		CC	TC	TT	CC	TC	TT
1	Dieude, 2011	SSc	1240	490	78	1587	561	69
2	Han, 2013	RA	62	393	707	59	336	465
3	Marquez et.,2014	GCA	466	128	23	1066	344	39
4	Zhai et al., 2013	SLE	24	185	456	41	220	404
5	Carmona, 2013	SSc	1729	605	81	1746	548	67
6	Khalifa,et al., 2017a	RA	63	43	14	15	27	89
7	Khalifa,et al., 2017b	RA	157	100	32	237	83	20
	rs1059703		CC	TC	TT	CC	TC	TT
1	Chatzikyriakidou, 2010	RA	7	52	77	7	46	94
2	Chatzikyriakidou, 2010	PsA	5	4	20	4	22	40
3	Gao, 2012	RA	85	34	4	152	58	10
4	Han, 2013	RA	59	389	715	52	337	468
5	Rong et al.,2015	AITD	456	279	49	400	164	27
6	Rong et al.,2015	GD	277	167	31	400	164	27
7	Rong et al.,2015	HT	179	112	18	400	164	27

8	Khalifa,et al., 2017a	RA	9	51	59	15	27	89
9	Khalifa,et al., 2017b	RA	34	133	122	27	85	228
10	Najme, 2020	RA	51	99	50	110	62	28

Sr.No	**Authors**	**Disease**	**Patients**		**Controls**	
	rs3027898		A	C	A	C
1	Chatzikyriakido ,2010	RA	187	85	229	65
2	Chatzikyriakido ,2010a	AS	79	19	102	30
3	Chatzikyriakido, 2010b	PsA	39	19	102	30
4	Zhang, 2013	RA	98	324	173	777
5	Han, 2013	RA	495	1821	421	1277
6	Atabaki, 2017	RA	97	111	78	74
7	Yang, 2017	RA	113	659	223	929
8	Hassine, 2017	RA	232	112	315	133
9	Shaker, 2018	RA	84	124	66	158
10	Labib et al., 2019a	SLE	80	80	186	54
11	Labib et al., 2019b	MS	92	60	186	54
12	Zhai et al., 2013	SLE	209	1113	282	1044

13	Gao, 2012	RA	41	205	74	366
14	Wang, 2020	AS	243	157	203	197
15	Rong et al.,2015a	AITD	350	1218	205	979
16	Rong et al.,2015b	GD	213	737	205	979
17	Rong et al.,2015c	HT	137	481	205	979
	rs1059702		C	T	C	T
1	Dieude, 2011	SSc	2970	646	3735	699
2	Han, 2013	RA	517	1807	302	1028
3	Marquez et.,2014	GCA	1060	174	2476	422
4	Zhai et al., 2013	SLE	233	1097	302	1028
5	Carmona, 2013	SSc	4063	767	4040	682
6	Khalifa,et al., 2017a	RA	196	71	167	262
7	Khalifa,et al., 2017b	RA	414	164	557	123
	rs1059703		C	T	C	T
1	Chatzikyriakidou, 2010	RA	66	206	60	234
2	Chatzikyriakidou, 2010	PsA	14	44	30	102
3	Gao, 2012	RA	204	42	362	78
4	Han, 2013	RA	507	1819	441	1273

5	Rong et al.,2015	AITD	1191	377	964	218
6	Rong et al.,2015	GD	721	229	964	218
7	Rong et al.,2015	HT	470	148	964	218
8	Khalifa,et al., 2017a	RA	69	169	57	205
9	Khalifa,et al., 2017b	RA	201	377	139	541
10	Najme, 2020	RA	201	199	282	118

Chapter 4

Result and Analysis

4.1 Characteristics of Study

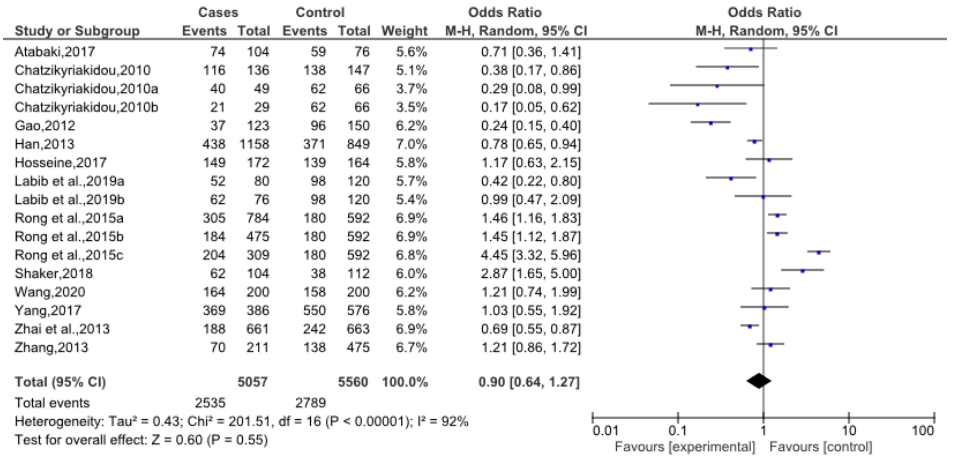
There were 250 articles that were accordance with the searching terms. After viewing of titles, abstracts as well as full texts of articles, there were 18 papers (Chatzikyriakidou, 2010, Chatzikyriakidou, 2010 a,b; Zhang, 2013; Han, 2013; Atabaki, 2017; Yang, 2017; Hossiene, 2017; Shaker, 2018; Labib et al., 2019; Zhai et al., 2013; Gao, 2012; Wang, 2020; Rong et al., 2015; Dieude, 2011; Carmona, 2013; Khalifa et al., 2017; Najme, 2020, Marquez, 2014) suitable for meta-analysis, which involved 17 studies of rs3027898, 7 studies of rs1059702, 10 studies of rs1059703 with 10159 patients (cases) and 11000 healthy (control) individuals by excluding remaining studies due to lack case-control study, without genotype data for analysis.

One study investigated IRAK1 SNPs in two different populations and three studies investigated different autoimmune diseases simultaneously that were treated as separate study in meta-analysis. Various types of genotyping methods were used in the selected articles for association finding such as real-time polymerase chain reaction (RT-PCR), polymerase chain reaction (PCR), MS-PCR, PCR-SSCP, T-ARMS-PCR, TaqMan and ARM-PCR. The data search range from 2010 to 2020.

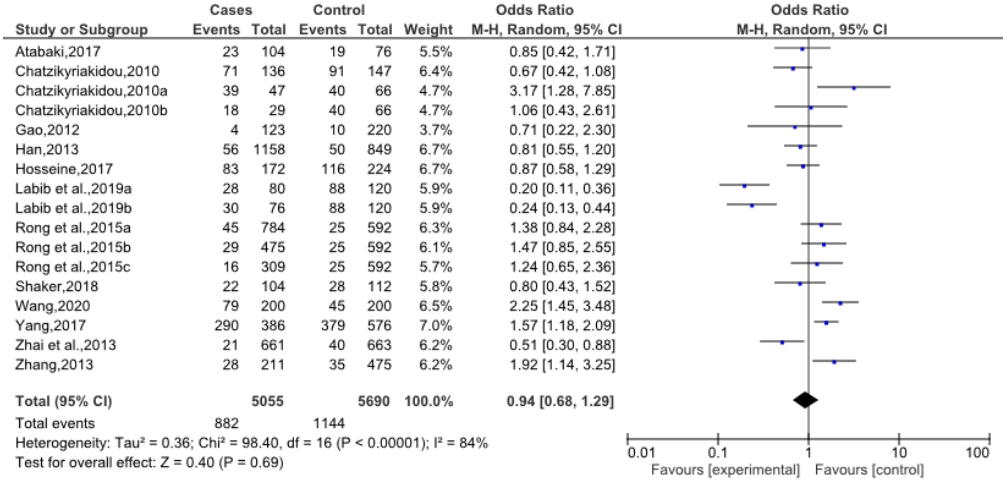
4.2 Association of IRAK1 rs3027898 C>A Polymorphism with Susceptibility to ADs

First, meta-analysis was performed 17 included studies to analyze association of rs3027898 C>A with susceptibility to ADs for five genetic models. No association was depicted among five genetic models based on p-value, OR as well as position of diamond; allele model (A versus C: OR=1.07, 95% CI=0.83-1.39, P=0.60,I_2=93%), heterozygote model (CA versus CC: OR=1.11, 95%CI=0.77-1.59, P=0.58, I_2=91%), homozygote model (CC versus AA: OR=0.82, 95% CI= 0.58-1.18, P=0.29), recessive model (AA versus CC+CA; OR=0.94, 95% CI=0.68-1.29, P=0.69) and dominant model (CA +AA versus CC:OR=0.90, 95%CI= 0.64-1.27, P=0.55). The heterogeneity was 93%, 91%, 80%, 84%, 92% respectively, therefore random model was applied (Figure 4.1,A-E).

A



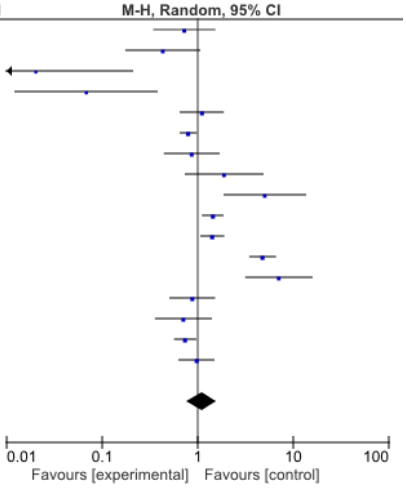
B



C

Study or Subgroup	Cases Events	Cases Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Atabaki,2017	51	81	40	57	5.8%	0.72 [0.35, 1.49]
Chatzikyriakidou,2010	45	65	47	56	5.2%	0.43 [0.18, 1.05]
Chatzikyriakidou,2010a	1	10	22	26	1.8%	0.02 [0.00, 0.21]
Chatzikyriakidou,2010b	3	11	22	26	2.8%	0.07 [0.01, 0.37]
Gao,2012	33	119	54	210	6.6%	1.11 [0.67, 1.84]
Han,2013	383	1102	321	799	7.4%	0.79 [0.66, 0.96]
Hosseine,2017	66	89	83	108	6.1%	0.86 [0.45, 1.66]
Labib et al.,2019a	24	52	10	32	5.1%	1.89 [0.75, 4.76]
Labibi ar al.,2019b	32	46	10	32	4.9%	5.03 [1.89, 13.35]
Rong et al.,2015a	260	739	155	567	7.4%	1.44 [1.14, 1.83]
Rong et al.,2015b	155	446	155	567	7.3%	1.42 [1.08, 1.85]
Rong et al.,2015c	188	293	155	567	7.2%	4.76 [3.52, 6.44]
Shaker,2018	40	82	10	84	5.6%	7.05 [3.20, 15.52]
Wang,2020	85	121	113	155	6.5%	0.88 [0.52, 1.49]
Yang,2017	79	96	171	197	6.0%	0.71 [0.36, 1.38]
Zhai et al.,2013	167	640	202	623	7.3%	0.74 [0.58, 0.94]
Zhang,2013	42	183	103	440	6.9%	0.97 [0.65, 1.47]
Total (95% CI)		**4175**		**4546**	**100.0%**	**1.11 [0.77, 1.59]**
Total events	1654		1673			

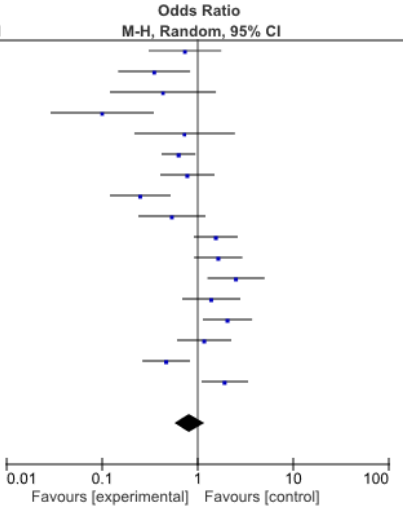
Heterogeneity: Tau² = 0.44; Chi² = 181.69, df = 16 (P < 0.00001); I² = 91%
Test for overall effect: Z = 0.56 (P = 0.58)



D

Study or Subgroup	Cases Events	Cases Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Atabaki,2017	23	51	19	36	5.4%	0.73 [0.31, 1.73]
Chatzikyriakidou,2010	71	91	91	100	5.4%	0.35 [0.15, 0.82]
Chatzikyriakidou,2010a	39	48	40	44	4.0%	0.43 [0.12, 1.52]
Chatzikyriakidou,2010b	18	36	40	44	4.1%	0.10 [0.03, 0.34]
Gao,2012	4	90	10	166	4.2%	0.73 [0.22, 2.38]
Han,2013	56	775	58	528	7.2%	0.63 [0.43, 0.93]
Hosseine,2017	83	106	116	141	6.3%	0.78 [0.41, 1.46]
Labib et al.,2019a	28	56	88	110	6.0%	0.25 [0.12, 0.50]
Labib et al.,2019b	30	44	88	110	5.7%	0.54 [0.24, 1.18]
Rong et al.,2015a	45	524	25	437	6.8%	1.55 [0.93, 2.57]
Rong et al.,2015b	29	320	25	437	6.6%	1.64 [0.94, 2.86]
Rong et al.,2015c	16	121	25	437	6.2%	2.51 [1.29, 4.87]
Shaker,2018	22	64	28	102	6.1%	1.38 [0.71, 2.72]
Wang,2020	79	115	45	87	6.5%	2.05 [1.15, 3.64]
Yang,2017	290	307	379	405	6.3%	1.17 [0.62, 2.20]
Zhai et al.,2013	21	494	40	461	6.6%	0.47 [0.27, 0.81]
Zhang,2013	28	169	35	372	6.7%	1.91 [1.12, 3.26]
Total (95% CI)		**3411**		**4017**	**100.0%**	**0.82 [0.58, 1.18]**
Total events	882		1152			

Heterogeneity: Tau² = 0.42; Chi² = 78.19, df = 16 (P < 0.00001); I² = 80%
Test for overall effect: Z = 1.07 (P = 0.29)



E

Study or Subgroup	Cases Events	Cases Total	Control Events	Control Total	Weight	Odds Ratio M-H, Random, 95% CI
Atabaki,2017	111	208	74	152	5.7%	1.21 [0.79, 1.83]
Chatzikyriakidou,2010	85	272	27	294	5.5%	4.49 [2.80, 7.20]
Chatzikyriakidou,2010a	19	58	30	132	4.5%	1.66 [0.84, 3.28]
Chatzikyriakidou,2010b	19	98	30	132	4.7%	0.82 [0.43, 1.56]
Gao,2012	205	246	366	440	5.7%	1.01 [0.67, 1.54]
Han,2013	1821	2316	1277	1698	6.5%	1.21 [1.05, 1.41]
Hosseine,2017	112	344	133	448	6.1%	1.14 [0.84, 1.55]
Labib et al.,2019a	80	160	54	240	5.6%	3.44 [2.23, 5.31]
Labib et al.,2019b	60	152	54	240	5.6%	2.25 [1.44, 3.50]
Rong et al.,2015a	1218	1568	979	1184	6.4%	0.73 [0.60, 0.88]
Rong et al.,2015b	737	950	979	1184	6.4%	0.72 [0.58, 0.90]
Rong et al.,2015c	398	618	979	1184	6.4%	0.38 [0.30, 0.47]
Shaker,2018	124	208	158	224	5.8%	0.62 [0.41, 0.92]
Wang,2020	157	400	197	400	6.2%	0.67 [0.50, 0.88]
Yang,2017	113	772	223	1152	6.3%	0.71 [0.56, 0.91]
Zhai et al.,2013	1113	1322	1044	1326	6.4%	1.44 [1.18, 1.75]
Zhang,2013	324	422	777	950	6.2%	0.74 [0.56, 0.97]
Total (95% CI)		**10114**		**11380**	**100.0%**	**1.07 [0.83, 1.39]**
Total events	6696		7381			

Heterogeneity: Tau² = 0.26; Chi² = 220.56, df = 16 (P < 0.00001); I² = 93%
Test for overall effect: Z = 0.53 (P = 0.60)

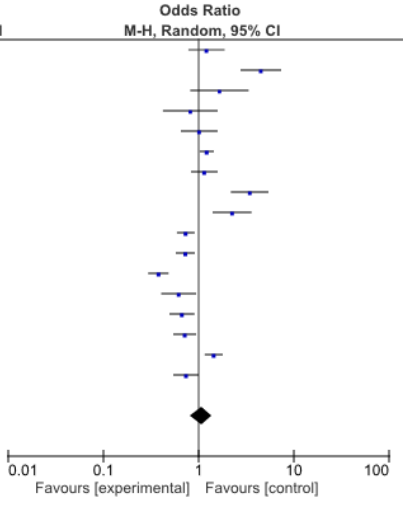
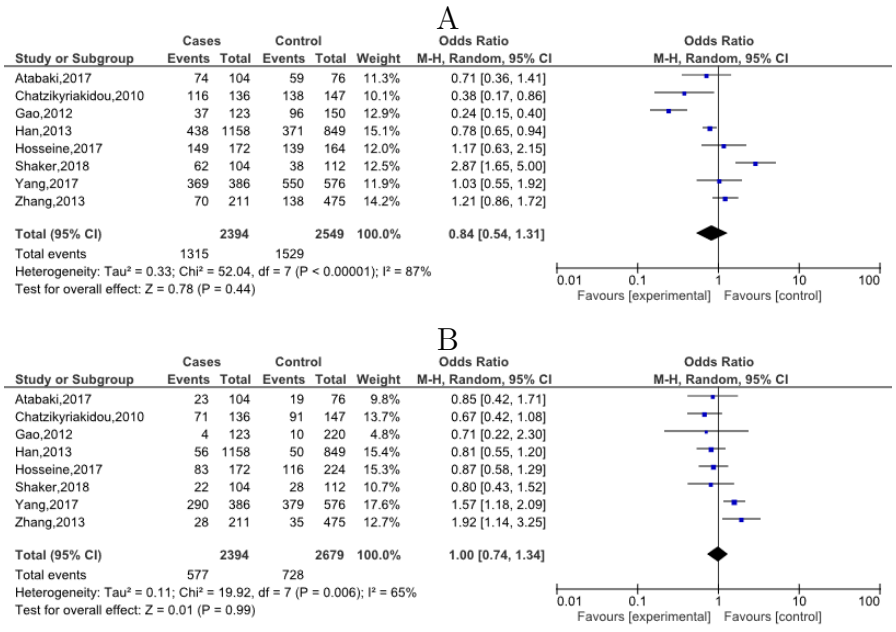


FIGURE 4.1: Meta-Analysis of Association IRAK1 rs3027898 C>A Polymorphisms with Susceptibility to ADs. A)Dominant Model (CA+AC vs CC), B) Recessive Model (AA vs CC+AC), C) Heterozygote model (CA vs CC), D) Homozygote Model (AA vs CC), E) Allele Model (A vs C).

Sub-group analysis was accomplished by stratifying included studies on the basis of autoimmune diseases (ADs). The findings of meta-analysis of rs3027989 C>A with susceptibility to rheumatoid arthritis (RA) indicated slight association of allele model (A versus C: OR=1.10, 95% CI=0.80-1.50, P=0.57, I_2=89%) based on OR as well as position of diamond but not statistical significant association due to greater p-value. This model having heterogeneity 89% so random effect model was preferred.

Recessive model (AA versus CC+CA; OR=1.00, 95% CI=0.74-1.34, P=0.99, I_2= 65%) and heterozygote model (CA versus CC: OR=1.00,95%CI=0.68-1.48, P=1.00, I_2=78%) showed no significant association between disease and controls as supported by p-value, OR as well as position of diamond. Homozygote model (CC versus AA: OR=0.89, 95%CI=0.61-1.30, P=0.05, I_2=62%) and dominant model (CA +AA versus CC: OR=0.84, 95% CI=0.54-1.31, P=0.44, I_2=87%) were high among controls than cases.

No association was noticed with RA in four model except allele model that indicated slight association based on position of diamond and OR ratio but not statistical significant association due to p-value higher than statistical value (0.05). The heterogeneity was more than 50% among these models, therefore, random effect model was applied (Figure 4.2,A-E).



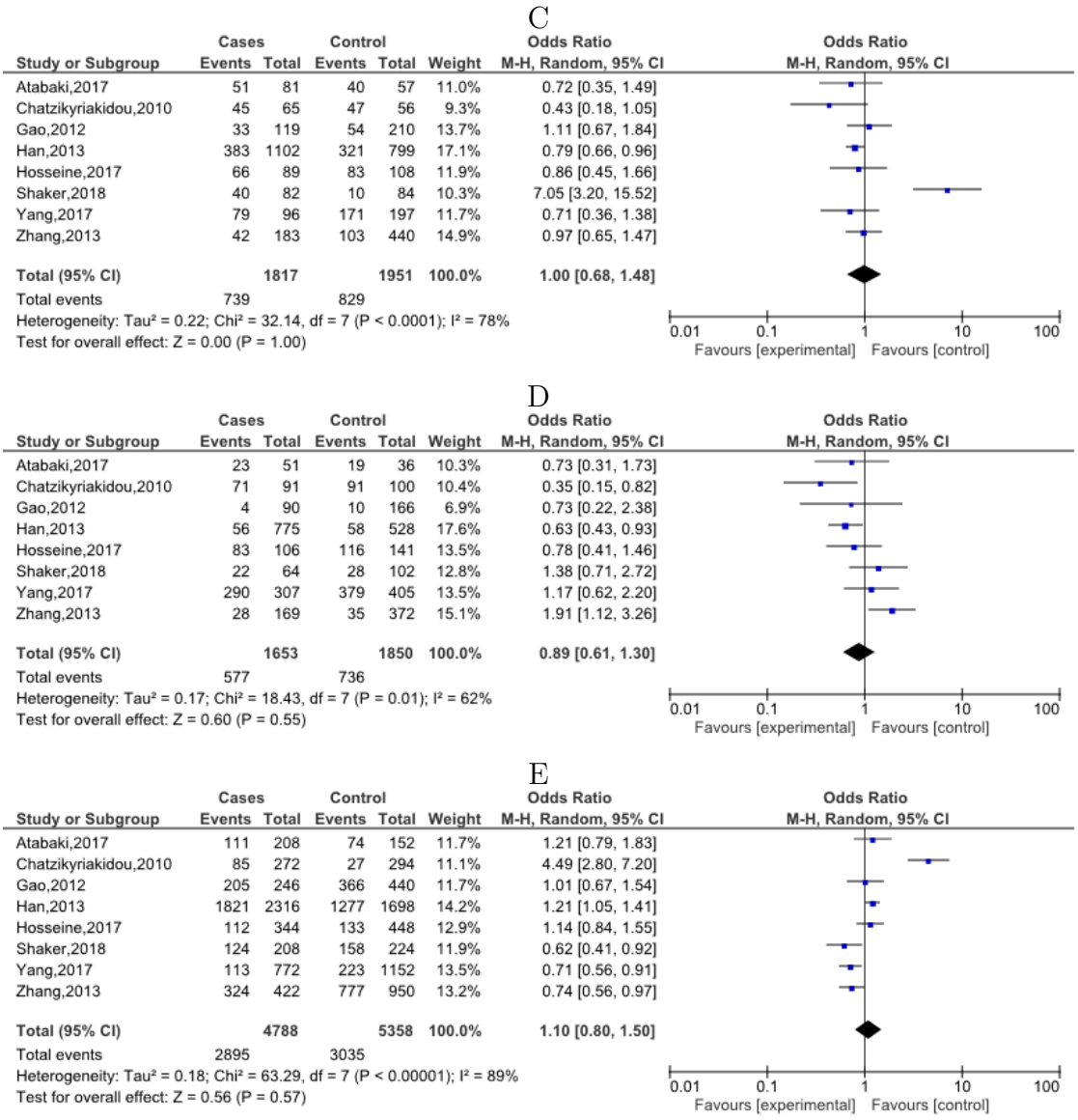


FIGURE 4.2: Meta-Analysis of Association Between IRAK rs3027898 C>A Polymorphisms and RA. A) Dominant Model (CA+AA vs CC), B) Recessive Model (AA vs CC+CA), C) Heterozygote Model (CA vs CC), D) Homozygote Model (CC vs AA), E) Allele Model (C vs A).

In case of SLE two studies were included for analysis. Allele model (A versus C; OR=2.18, 95% CI=0.93-5.12, P=0.07, I_2=92%) showed slight association with SLE based on their OR as well as position of diamond but this association was not found to be statistical significant due to p-value 0.07. No association was noticed in heterozygote model (CA versus CC; OR=1.06, 95% CI=0.43-2.59, P=0.91, I_2=73%) to SLE based on value of p and OR as well as position of diamond. P-value and position of diamond indicated that dominant model (CA +AA versus CC; OR=0.59, 95% CI=0.37-0.93, P=0.02, I_2=51%), recessive model (AA versus

CC+CA; OR=0.32, 95% CI=0.12-0.82, P=0.02, I_2=81%) and homozygote model (CC versus AA; OR=0.37, 95% CI=0.24-0.58, P<0.00001, I_2=48%) were significantly high among controls than SLE. No association was also found with susceptibility to SLE in heterozygote model (CA versus CC; OR=1.06, 95% CI=0.43-2.59, P=0.91, I_2=73%) based on p-value, OR as well as position of diamond. Random model was applied in all comparison models (Figure 4.3,A-E).

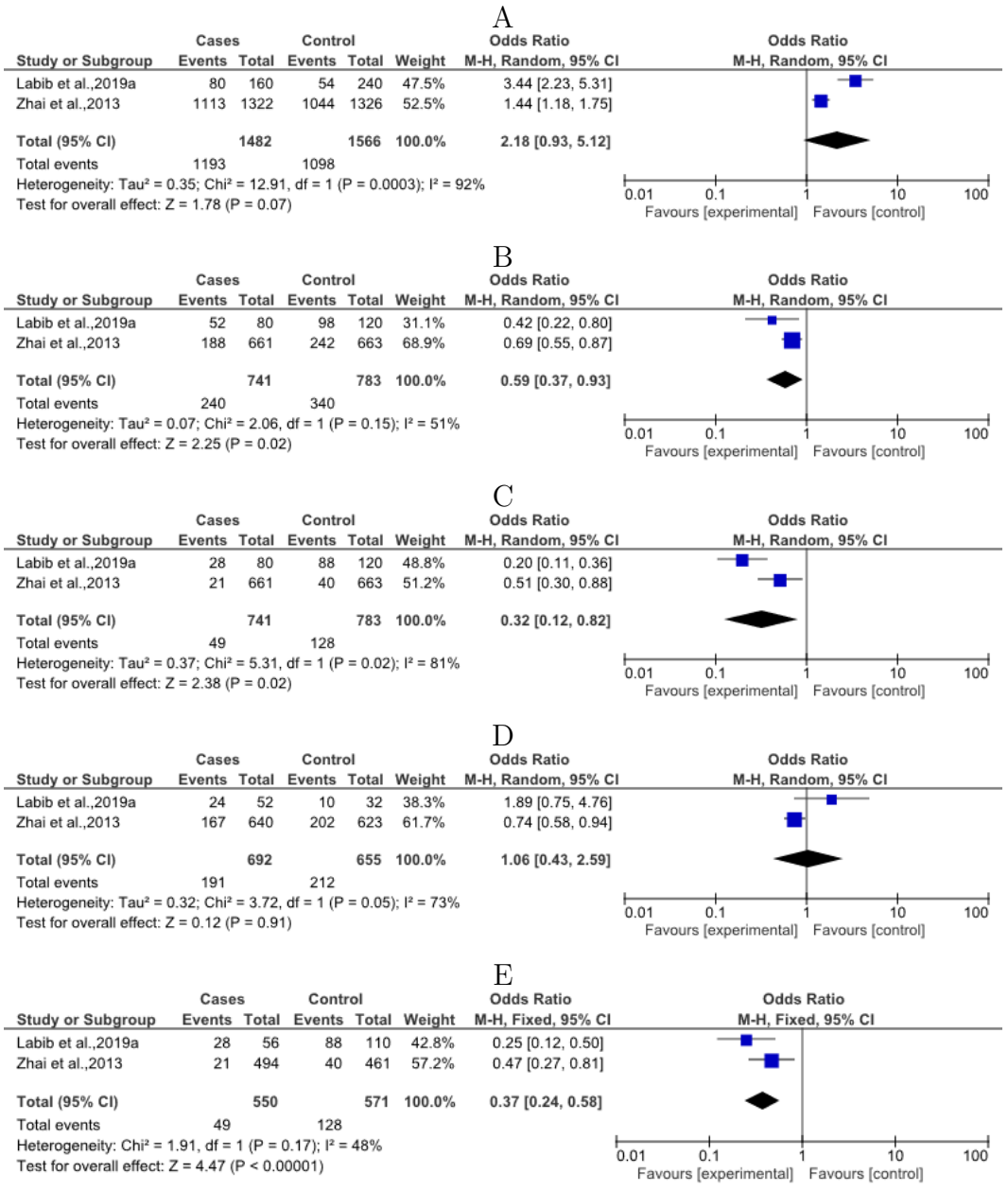


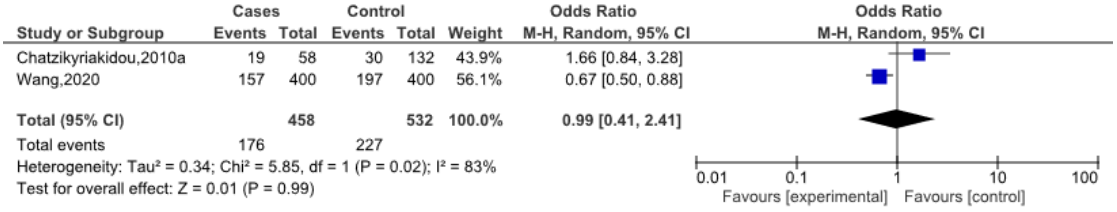
FIGURE 4.3: Meta-Analysis of Association of IRAK1rs3027898 C>A Polymorphisms with SLE. A) Allele Model (C vs A), B) Dominant Model (CA+AA vs CC), C) Recessive Model (AA vs CC+CA), D) Heterozygote Model (CA vs CC), E) Homozygote Model (CC vs AA).

Two studies were included for analysis of association of rs3027898 C>A with AS. No association was found in allele model (A versus C; OR=0.99, 95% CI=0.41-2.41, P=0.99, I_2=83%).

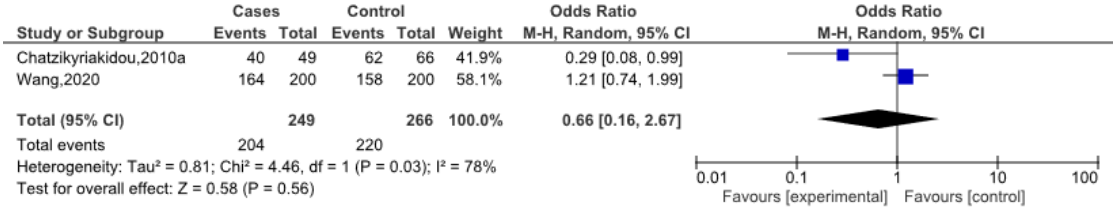
Recessive model (AA versus CC+CA; OR=2.41, 95% CI=1.63-3.58, P<0.0001, I_2=0%) showed increased association with susceptibility to AS that was supported by p and OR value as well as position of diamond. Dominant model (CA +AA versus CC; OR=0.66, 95% CI=0.16-2.67, P=0.56, I_2=78%) and heterozygote model (CA versus CC; OR=0.16, 95% CI=0.00-6.55, P=0.33, I_2=90%) were slightly high among control than AS as indicated by their p-value.

No association was depicted in homozygote model (CC versus AA; OR=1.05, 95% CI=0.23-4.73, P=0.95, I_2=79%). Fixed model was only applied in recessive model while in others random model was used (Figure 4.4 A-E).

A



B



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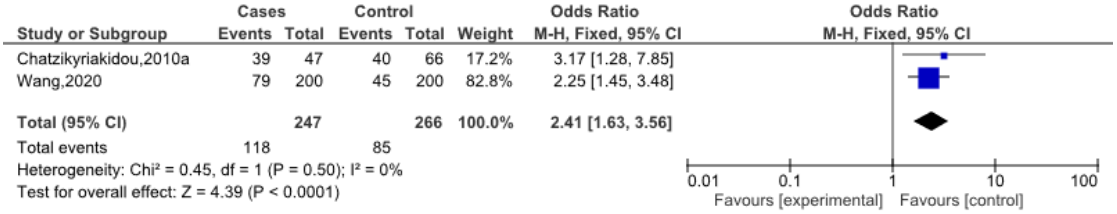

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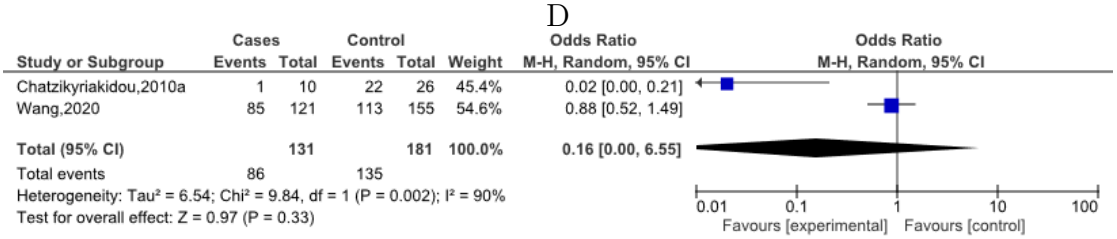


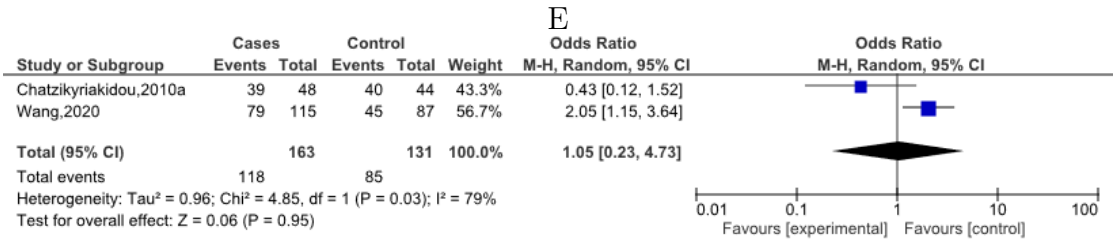
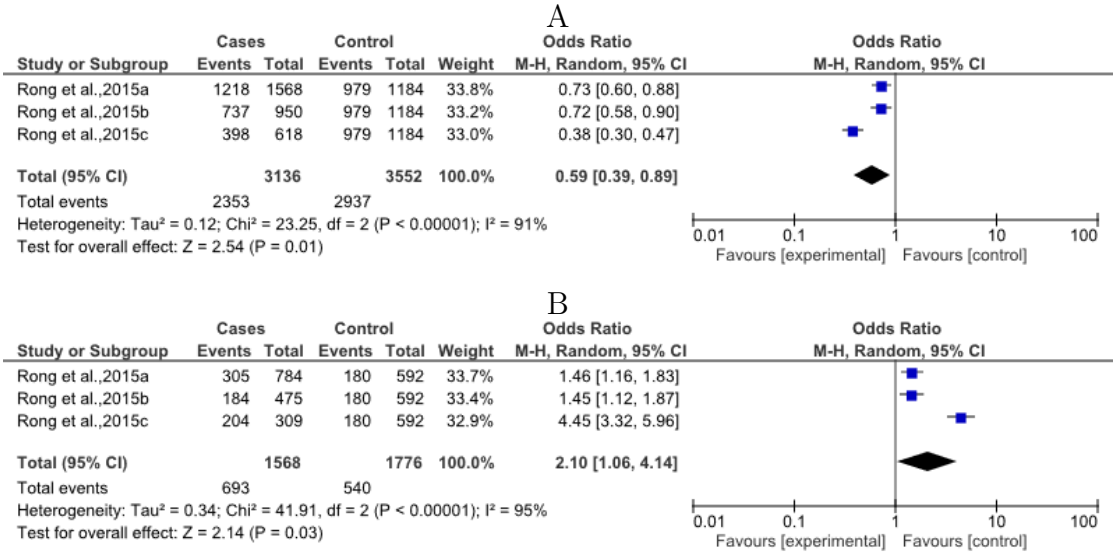


FIGURE 4.4: Meta-Analysis of Association of IRAK1rs3027898 C>A Polymorphisms with AS. A) Allele Model (C vs A), B) Dominant Model (CA+AA vs CC), C) Recessive Model (AA vs CC+CA), D)Heterozygote Model (CA vs CC), E) Homozygote model (CC vs AA).

Three studies were included for analysis of AITDs. Four models showed increased susceptibility to AITDs; P and OR value as well as position of diamond provide evidence for heterozygote model (OR=2.13, 95% CI=1.02-4.44, P=0.04, I_2=96%), homozygote model (OR=1.75, 95% CI=1.26-2.43, P=0.0008, I_2=0%), dominant model (OR=2.10, 95% CI=1.06-4.14, P=0.03, I_2=95%) and recessive model (OR=1.38, 95% CI=1.00-1.89, P=0.05, I_2=0%) increased risk for AITD susceptibility.

While allele model (OR=0.59, 95% CI=0.39-0.89, P=0.01, I_2=91%) was significantly high among controls than patients as supported by p-value as well as position of diamond. Fixed model was applied in homozygote and recessive model because of low heterogeneity while in other three models, heterogeneity was extremely high, hence random model was applied (Figure 4.5,A-E).



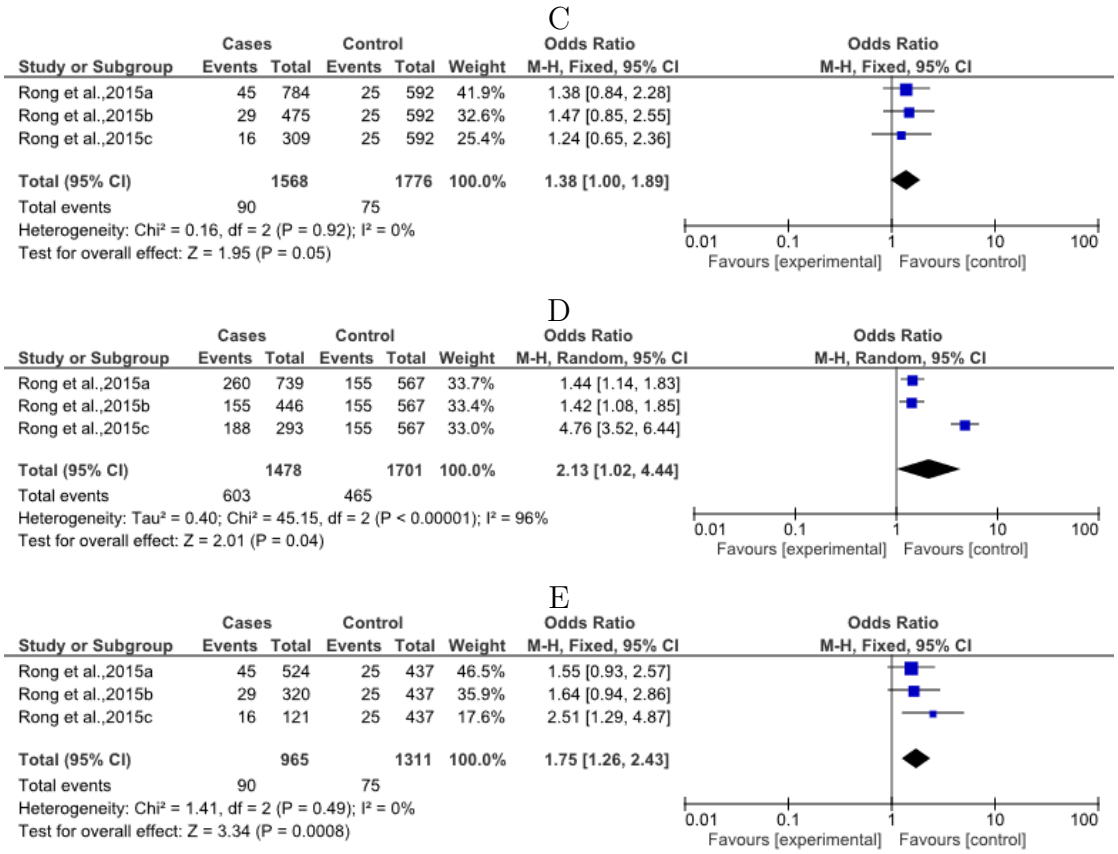
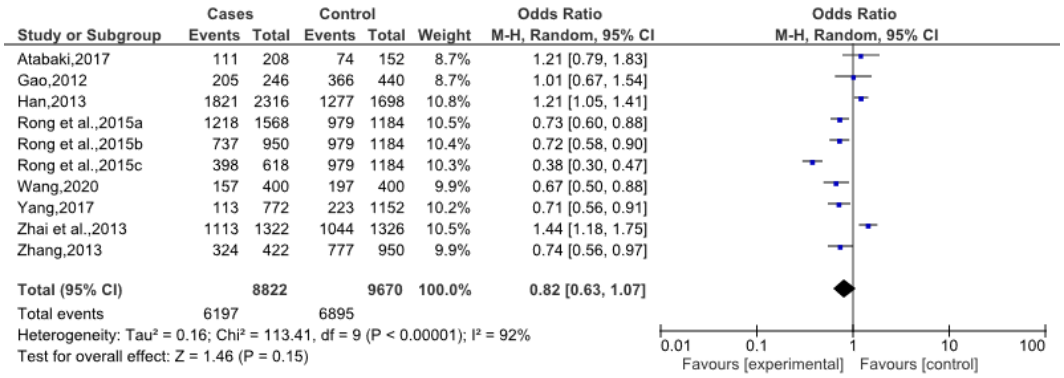


FIGURE 4.5: Meta-Analysis Depicted Association Between IRAK1rs3027898 C>A Polymorphisms and AITDs. A) Allele Model (C vs A), B) Dominant Model (CA+AA vs CC),C) Recessive Model (AA vs CC+CA), E) Heterozygote Model (CA vs CC), E) Homozygote Model (CC vs AA).

We also analyzed association of rs3027898 C>A with ADs by stratified subgroup analysis based on populations and different results was observed in different populations. Ten studies were included for analysis in Asian population. Homozygote model (CC versus AA; OR=1.19, 95% CI=0.81-1.74, P=0.38, I_2=76%), heterozygote model (CA versus CC; OR=1.13, 95% CI=0.77-1.66, P=0.53, I_2=93%) and recessive model (AA versus CC+CA; OR=1.21, 95% CI=0.90-1.63, P=0.20, I_2=70%) showed slight association with ADs based on OR value as well as position of diamond but not statistical significant association due to high p-value. Dominant model (CA +AA versus CC; OR=1.05 95% CI=0.69-1.59, P=0.82, I_2=94%) and allele model (C versus A; OR=0.82, 95% CI=0.63-1.07, P=0.15, I_2=92%) indicated no association with both groups as supported by position of diamond as well as p-value. Random effect model was applied due to greater than 50% value of heterogeneity among all models (Figure 4.6, A-E, Table 4.1).

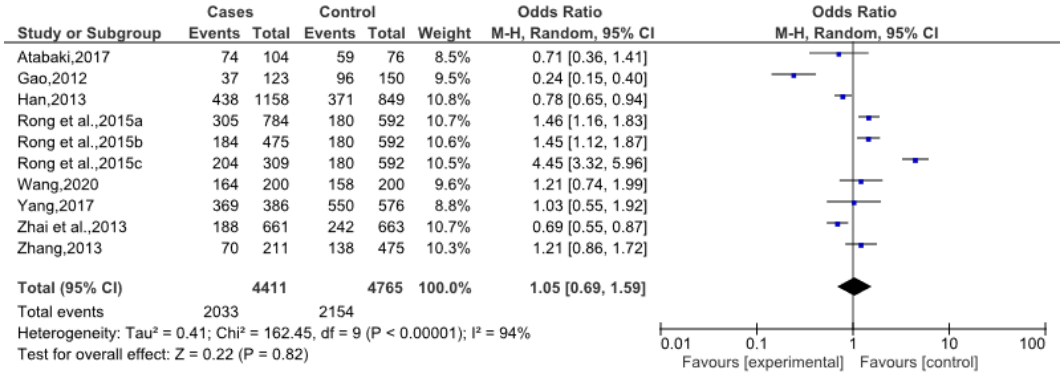
A



Study or Subgroup	Cases Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Atabaki,2017	111	208	74	152	8.7%	1.21 [0.79, 1.83]
Gao,2012	205	246	366	440	8.7%	1.01 [0.67, 1.54]
Han,2013	1821	2316	1277	1698	10.8%	1.21 [1.05, 1.41]
Rong et al.,2015a	1218	1568	979	1184	10.5%	0.73 [0.60, 0.88]
Rong et al.,2015b	737	950	979	1184	10.4%	0.72 [0.58, 0.90]
Rong et al.,2015c	398	618	979	1184	10.3%	0.38 [0.30, 0.47]
Wang,2020	157	400	197	400	9.9%	0.67 [0.50, 0.88]
Yang,2017	113	772	223	1152	10.2%	0.71 [0.56, 0.91]
Zhai et al.,2013	1113	1322	1044	1326	10.5%	1.44 [1.18, 1.75]
Zhang,2013	324	422	777	950	9.9%	0.74 [0.56, 0.97]
Total (95% CI)		**8822**		**9670**	**100.0%**	**0.82 [0.63, 1.07]**
Total events	6197		6895			

Heterogeneity: Tau² = 0.16; Chi² = 113.41, df = 9 (P < 0.00001); I² = 92%
Test for overall effect: Z = 1.46 (P = 0.15)

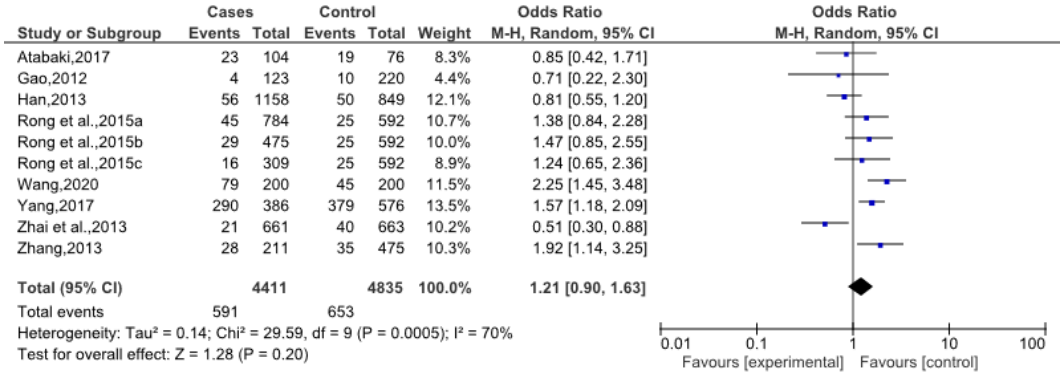
B



Study or Subgroup	Cases Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Atabaki,2017	74	104	59	76	8.5%	0.71 [0.36, 1.41]
Gao,2012	37	123	96	150	9.5%	0.24 [0.15, 0.40]
Han,2013	438	1158	371	849	10.8%	0.78 [0.65, 0.94]
Rong et al.,2015a	305	784	180	592	10.7%	1.46 [1.16, 1.83]
Rong et al.,2015b	184	475	180	592	10.6%	1.45 [1.12, 1.87]
Rong et al.,2015c	204	309	180	592	10.5%	4.45 [3.32, 5.96]
Wang,2020	164	200	158	200	9.6%	1.21 [0.74, 1.99]
Yang,2017	369	386	550	576	8.8%	1.03 [0.55, 1.92]
Zhai et al.,2013	188	661	242	663	10.7%	0.69 [0.55, 0.87]
Zhang,2013	70	211	138	475	10.3%	1.21 [0.86, 1.72]
Total (95% CI)		**4411**		**4765**	**100.0%**	**1.05 [0.69, 1.59]**
Total events	2033		2154			

Heterogeneity: Tau² = 0.41; Chi² = 162.45, df = 9 (P < 0.00001); I² = 94%
Test for overall effect: Z = 0.22 (P = 0.82)

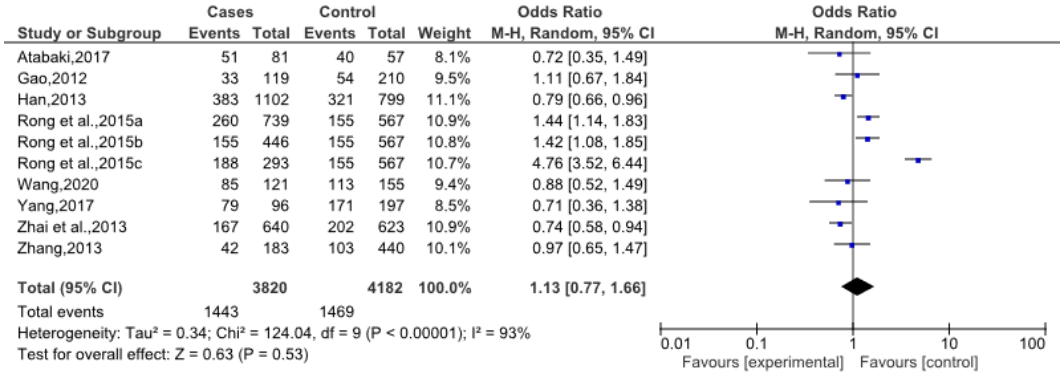
C



Study or Subgroup	Cases Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Atabaki,2017	23	104	19	76	8.3%	0.85 [0.42, 1.71]
Gao,2012	4	123	10	220	4.4%	0.71 [0.22, 2.30]
Han,2013	56	1158	50	849	12.1%	0.81 [0.55, 1.20]
Rong et al.,2015a	45	784	25	592	10.7%	1.38 [0.84, 2.28]
Rong et al.,2015b	29	475	25	592	10.0%	1.47 [0.85, 2.55]
Rong et al.,2015c	16	309	25	592	8.9%	1.24 [0.65, 2.36]
Wang,2020	79	200	45	200	11.5%	2.25 [1.45, 3.48]
Yang,2017	290	386	379	576	13.5%	1.57 [1.18, 2.09]
Zhai et al.,2013	21	661	40	663	10.2%	0.51 [0.30, 0.88]
Zhang,2013	28	211	35	475	10.3%	1.92 [1.14, 3.25]
Total (95% CI)		**4411**		**4835**	**100.0%**	**1.21 [0.90, 1.63]**
Total events	591		653			

Heterogeneity: Tau² = 0.14; Chi² = 29.59, df = 9 (P = 0.0005); I² = 70%
Test for overall effect: Z = 1.28 (P = 0.20)

D



Study or Subgroup	Cases Events	Total	Control Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Atabaki,2017	51	81	40	57	8.1%	0.72 [0.35, 1.49]
Gao,2012	33	119	54	210	9.5%	1.11 [0.67, 1.84]
Han,2013	383	1102	321	799	11.1%	0.79 [0.66, 0.96]
Rong et al.,2015a	260	739	155	567	10.9%	1.44 [1.14, 1.83]
Rong et al.,2015b	155	446	155	567	10.8%	1.42 [1.08, 1.85]
Rong et al.,2015c	188	293	155	567	10.7%	4.76 [3.52, 6.44]
Wang,2020	85	121	113	155	9.4%	0.88 [0.52, 1.49]
Yang,2017	79	96	171	197	8.5%	0.71 [0.36, 1.38]
Zhai et al.,2013	167	640	202	623	10.9%	0.74 [0.58, 0.94]
Zhang,2013	42	183	103	440	10.1%	0.97 [0.65, 1.47]
Total (95% CI)		**3820**		**4182**	**100.0%**	**1.13 [0.77, 1.66]**
Total events	1443		1469			

Heterogeneity: Tau² = 0.34; Chi² = 124.04, df = 9 (P < 0.00001); I² = 93%
Test for overall effect: Z = 0.63 (P = 0.53)

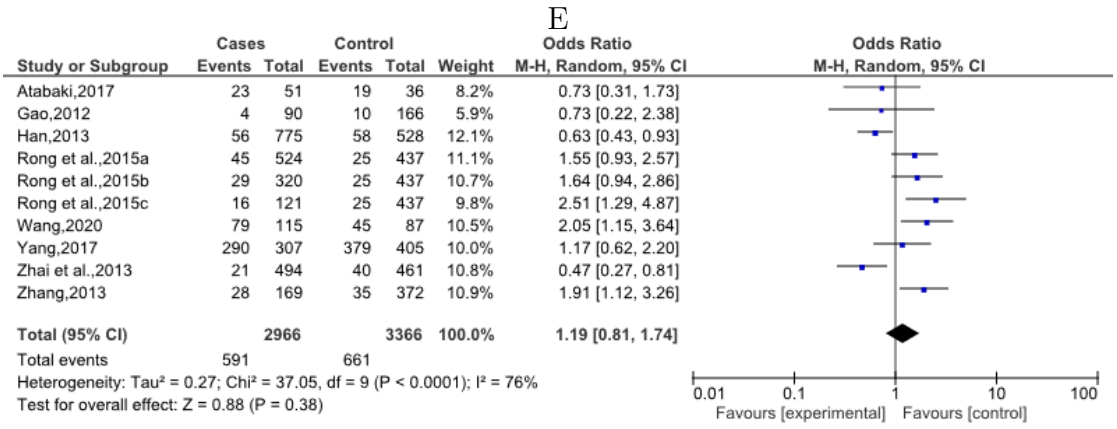


FIGURE 4.6: Meta-Analysis of Association of IRAK1rs3027898 C>A Polymorphisms Among Asian Population. A)Allele Model (C vs A), B) Dominant Model (CA+AA vs CC),C) Recessive Model (AA vs CC+CA), E) Heterozygote Model (CA vs CC), E) Homozygote Model (CC vs AA).

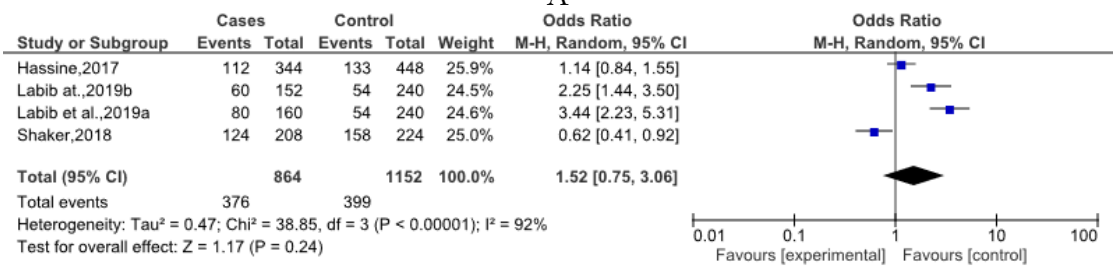
Four studies were included for analysis of rs3027898 C>A with ADs in African population. Dominant model (CA +AA versus CC; OR=1.10, 95% CI=0.49-2.49, P=0.82, I_2=85%) revealed no association with ADs as well as control as depicted by position of diamond as well as OR and p-value.

Position of diamond as well as OR and P-value depicted that recessive model (AA versus CC+CA; OR=0.43, 95% CI=0.20-0.95, P=0.04, I_2=88%) was significantly high among control. Slight association was found in allele model (C versus A; OR=1.52, 95% CI=0.75-3.06, P=0.24, I_2=92%) with ADs risk as supported by position of diamond as well as OR but not significant association due to high p-value.

OR and position of diamond revealed slight association of heterozygote model (CA versus CC; OR=2.70, 95%=0.96-7.62, P=0.06, I_2=84%) with ADs risk but not significant due to high p-value.

Homozygote model (CC versus AA; OR=0.62, 95% CI=0.31-1.26, P=0.19, I_2=76%) had showed no association with ADs because p-value was not significant to depict their association but it was slightly high among control supported by diamond position as well as OR. Random effect model was applicable in all models because heterogeneity was more than 50% (Figure 4.7, A-E, Table 4.1).

A



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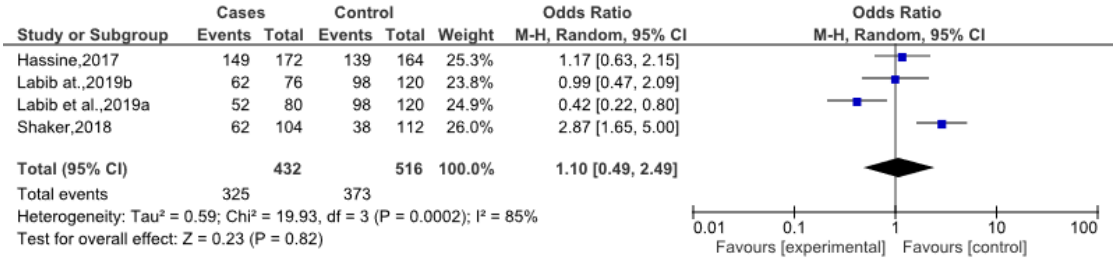

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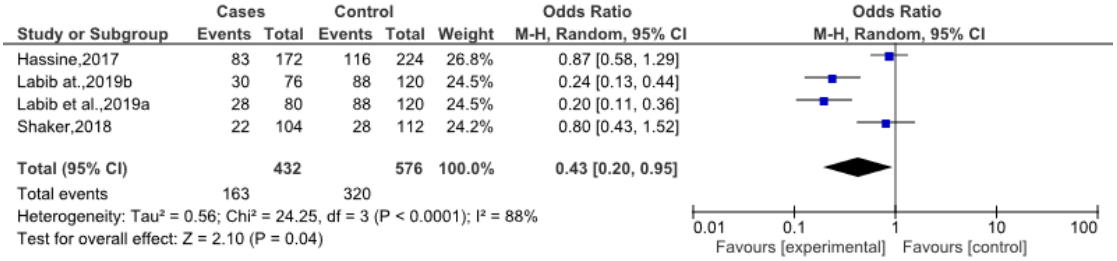

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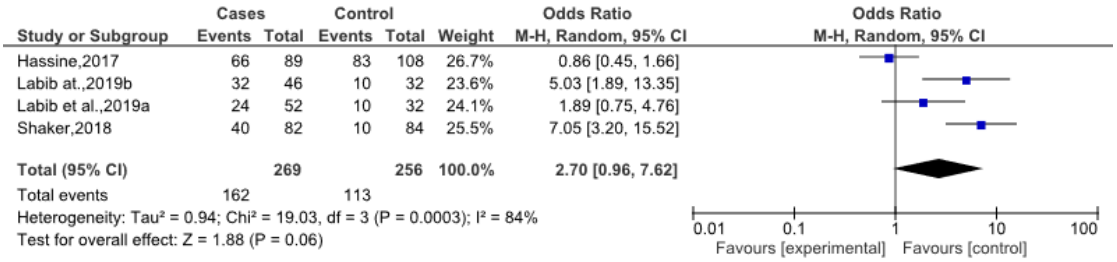


E

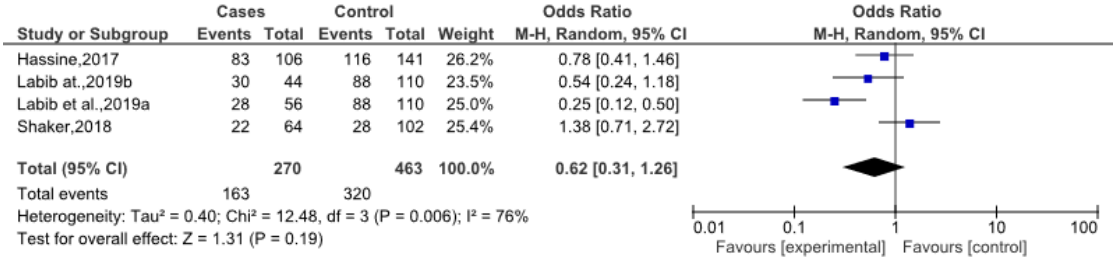
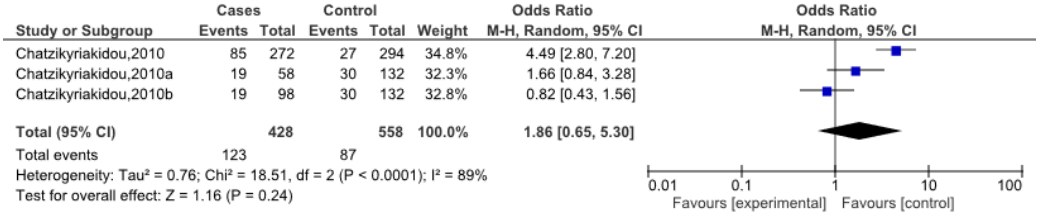


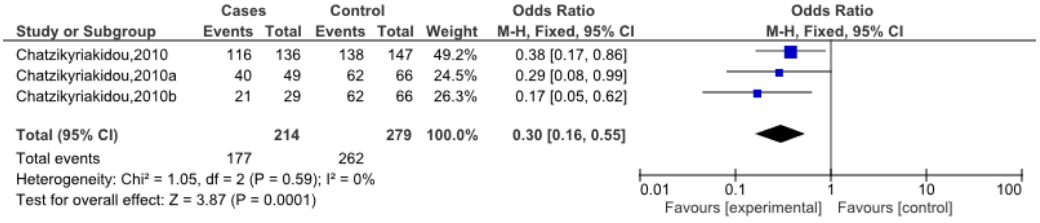
FIGURE 4.7: Meta-Analysis Showed Association of IRAK1rs3027898 C>A Polymorphims in African Population. A) Allele Model (C vs A), B) Dominant Model (CA+AA vs CC), C) Recessive Model (AA vs CC+CA), D) Heterozygote Model (CA vs CC), E) Homozygote Model (CC vs AA).

Three studies were included in European population for analysis of rs3027898 C>A with ADs. Dominant model (CA +AA versus CC; OR=0.30, 95% CI=0.16-0.55, P=0.0001, I_2=0%), heterozygote model (CA versus CC; OR=0.11, 95% CI=0.02-0.66, P=0.02, I_2=75%) and homozygote model (CC versus AA; OR=0.26, 95% CI=0.15-0.48, P<0.0001, I_2=42%) were significantly high among control based on p-value, OR as well as position of diamond. In allele model (C versus A;OR=1.86, 95% CI=0.65-5.30, P=0.24, I_2=89%) OR and diamond position indicated slight association with ADs but not statistical significant due to greater p-value. Diamond position as well as p-value depicted no association of recessive model (AA versus CC+CA; OR=1.24, 95% CI=0.50-3.09, P=0.64, I_2=78%) with both groups. Fixed model was applied in homozygote and dominant models due to low value of heterogeneity while in other three models random model was applied (Figure 4.8, A-E, Table 4.1).

A



B



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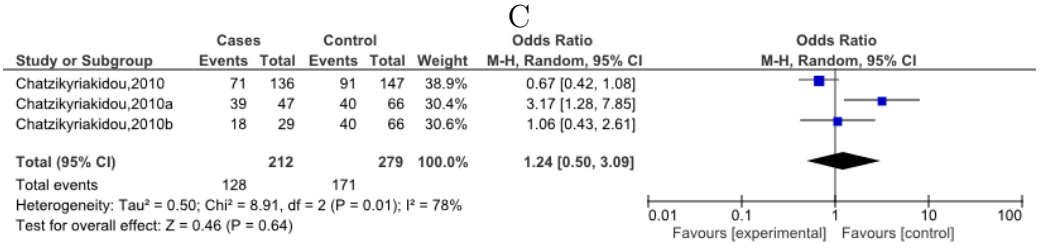

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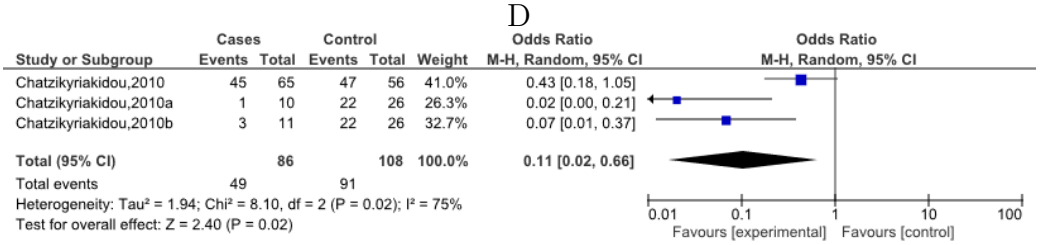


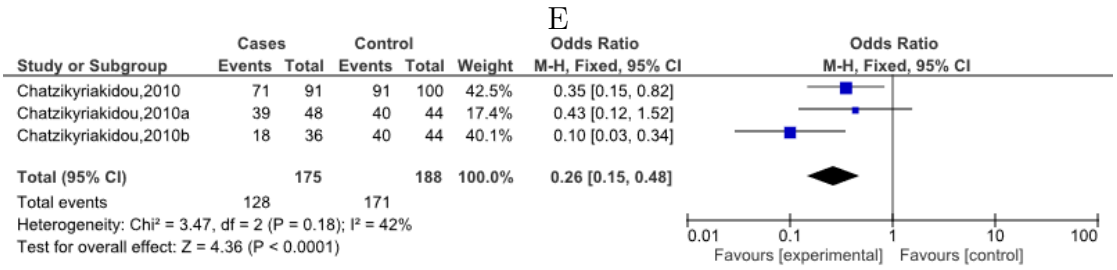
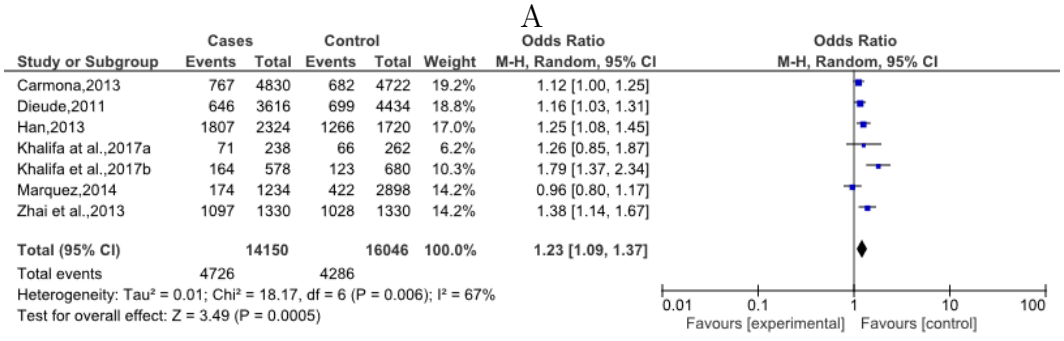


FIGURE 4.8: Meta-Analysis Results Showed Association of IRAK1 Polymorphims in European Population. A)Allele Model (C vs A), B) Dominant Model (CA+AA vs CC),C) Recessive Model (AA vs CC+CA), E) Heterozygote Model (CA vs CC), E) Homozygote Model (CC vs AA).

4.3 Association of IRAK1 rs1059702 T>C Polymorphism with Susceptibility to ADs

Seven studies were included for association finding of rs1059702 T>C with susceptibility to ADs.First, we checked overall association of rs1059702 T>C with susceptibility to ADs. Allel model (C vs T; OR=1.23, 95% CI=1.09-1.37, P=0.0005. I_2=67%) showed increased association with ADs susceptibility supported by diamond position, p-value as well as OR. No association was found dominant model (TC+CC vs TT; OR=1, 95% CI=0.62-1.61, P=0.99, I_2=93%), heterozygote model (TC+TT; OR=1, 95% CI=0.69-1.45, P=0.99, I_2=87%), recessive model (CC vs TT+TC; OR=0.99, 95% CI=0.73-1.34, P=0.93, I_2=91%) and homozygote model (CC vs TT;OR=1.02, 95% CI=0.54-1.92, P=0.98, I_2=93%) in both groups as supported by diamond position, p-value as well as OR. In all models, heterogeneity was higher and random model was used (Figure 4.9, A-E).



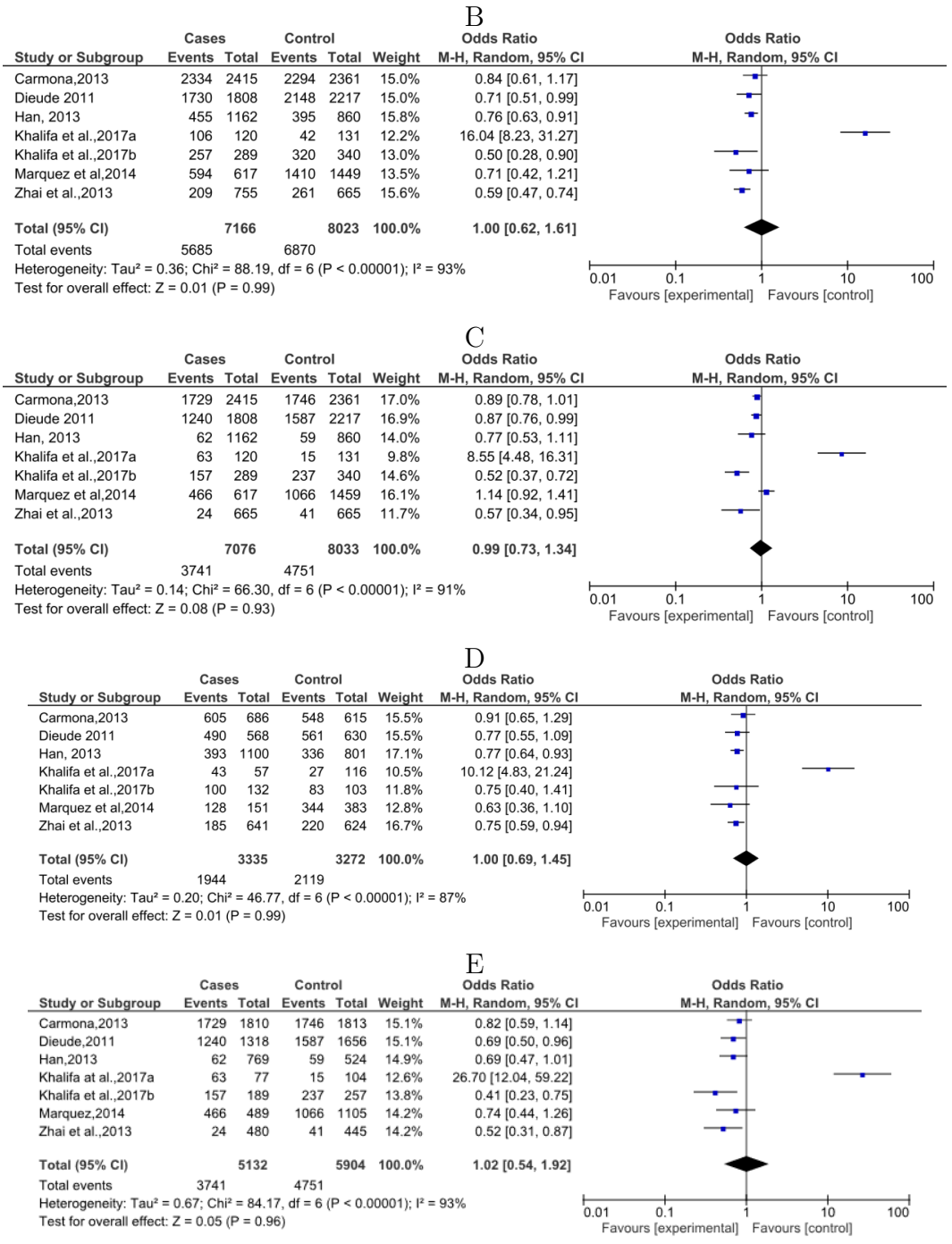


FIGURE 4.9: Meta-Analysis Depicted Association of IRAK1 rs1059702 T>C Polymorphisms and Susceptibility to ADs. A) Allele Model (C vs T), B) Dominant Model (TC+CC vs TT), C) Recessive Model (CC vs TT +TC), D)Heterozygote Model (TC+TT), E) Homozygote model (CC vs TT).

We performed meta-analysis on sub-group by stratifying the included studies on the diseases. First analysis was performed on RA. Three studies were included to find association of rs1059702 T>C with RA. Based on diamond position, p-value as well as OR, allele model (C vs T; OR=1.41, 95% CI=1.11-1.80, P=0.005,

I_2=63%) showed increased association with RA risk. While based on diamond position as well as OR dominant model (TC+CC vs TT; OR=1.76, 95% CI=0.28-0.90, P=0.55, I_2=97%), recessive model (CC vs TT+TC; OR=1.47, 95% CI=0.54-3.98, P=0.45, I_2=97%) and heterozygote model (TC vs TT;OR=1.74, 95% CI=0.44-6.93, P=0.43, I_2=95%) showed slight association with RA but not statistical significant association due to greater p-value. 100% heterogeneity in homozygote model makes its interpretation difficult, hence not shown here. In all models heterogeneity was high therefore random effect model was selected (Figure 4.10, A-D).

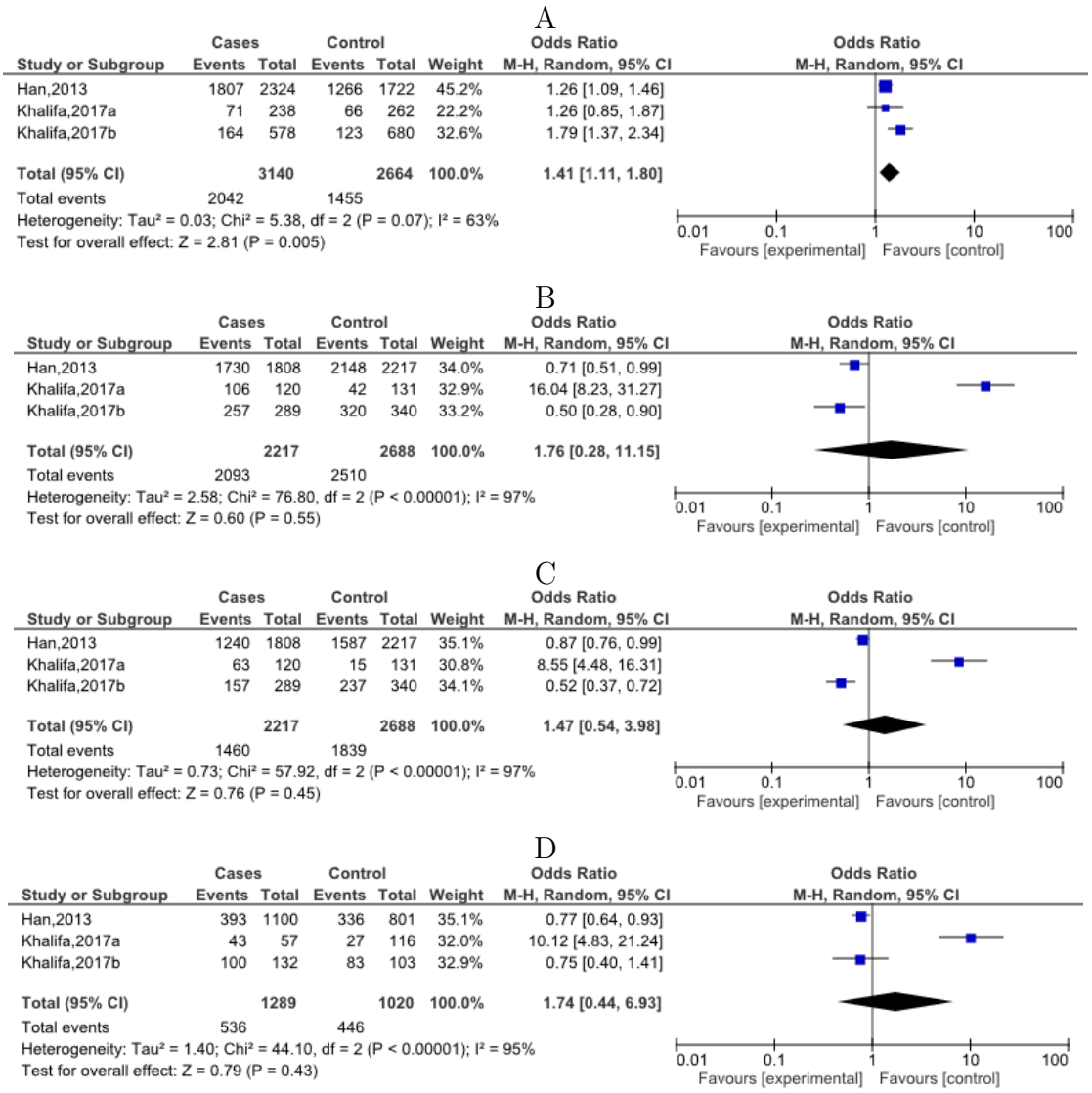
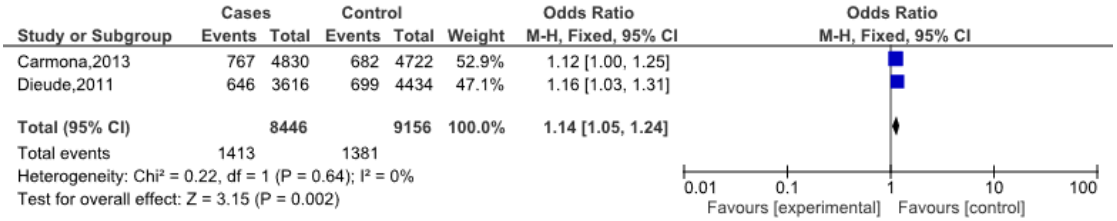


FIGURE 4.10: Meta-Analysis Depicted Association IRAK1 rs1059702 T>C with Susceptibility to RA. A) Allele Model (C vs T), B)Dominant Model (TC+CC vs TT), C) Recessive Model (CC vs TT+TC), D) Heterozygote Model (TC vs TT),

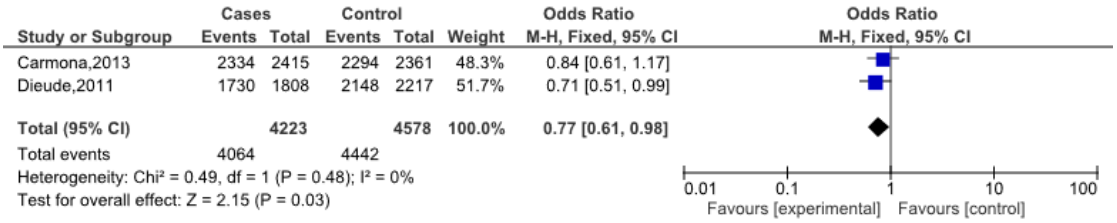
Two studies were included for analysis of SSc. Significant p-value, OR as well as diamond position indicated significant increased association of allele model (C vs T; OR=1.14, 95% CI=1.05-1.24, P=0.002, I_2=0%) with SSc susceptibility.

Dominant model (TC+CC vs TT; OR=0.77,95% CI=0.61-0.98, P=0.03, I_2=0%), recessive model (CC vs TT+TC; OR=0.88,95% CI=0.80-0.96, P=0.006, I_2=0%), and homozygote model (CC vs TT; OR=0.75, 95% CI=0.60-0.95, P=0.02, I_2=0%) were significantly high among control as supported by significant p-value as well as position of diamond while heterozygote model (TC vs TT; OR=0.84, 95% CI=0.66-1.07, P=0.16, I_2=0%) was slightly high in control but not significantly due to greater p-value. Fixed model was used due to absence of heterogeneity (Figure 4.11, A-E).

A



B



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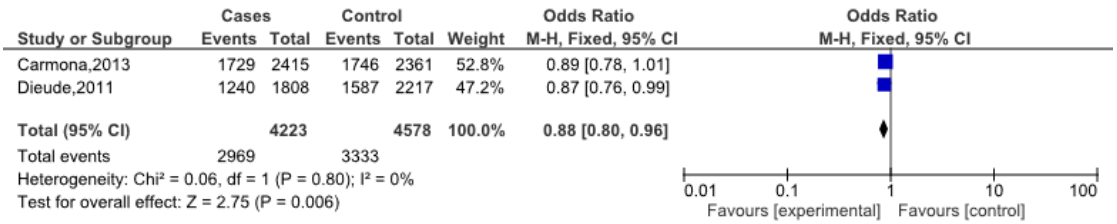

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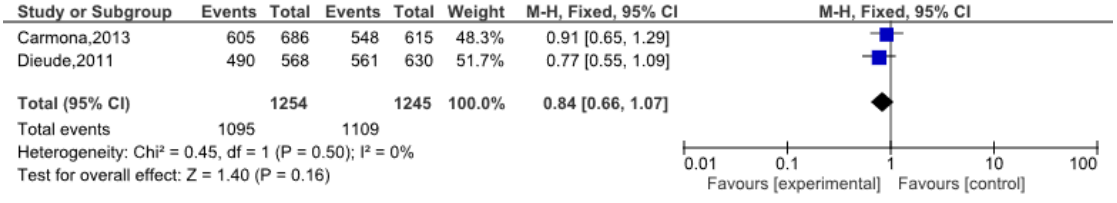

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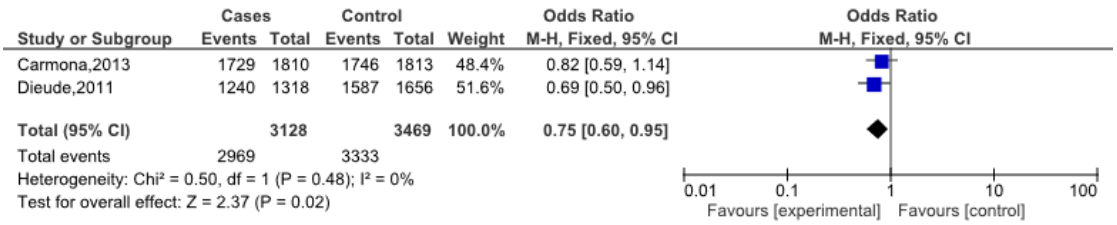


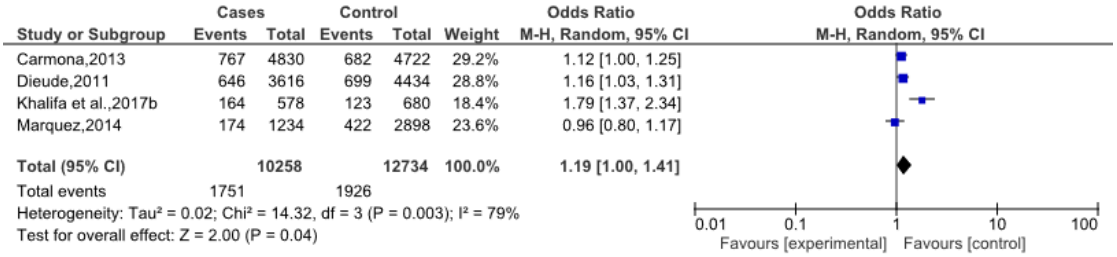
FIGURE 4.11: Meta-Analysis Showed Association of IRAK1 rs1059702 T>C with Susceptibility to SSc. A) Allele Model (C vs T), B)Dominant Model (TC+CC vs TT), C) Recessive Model (CC vs TT+TC), D) Heterozygote Model (TC vs TT), E) Homozygote Model (CC vs TT).

Stratified analysis was performed in different populations to check association of rs1059702 T>C in different populations. Four studies were included in European population, allele model (C vs T; OR=1.19, 95% CI=1.00-1.41, P=0.04, I_2=79%) showed increased association with susceptibility to ADs based on significant p-value, OR as well as position of diamond.

While position of diamond,OR as well as significant p-value evidenced that dominant model (TC+CC vs TT;OR=0.73, 95% CI=0.60-0.89, P=0.002, I_2=0%), heterozygote model (TC vs TT; OR=0.80, 95% CI=0.65-0.99, P=0.04, I_2=0%) and homozygote model (CC vs TT; OR=0.70, 95% CI=0.57-0.86, P=0.0005, I_2=24%) were high among control.

No significant association was noticed in recessive model (CC vs TT+TC; OR=0.85, 95% CI=0.69-1.04, P=0.12, I_2=81%) in both groups due to p-value. Random model was implemented for allele model and recessive model due to high heterogeneity while fixed model was applied for heterozygote model, homozygote model and dominant model due to low heterogeneity (Figure 4.12, A-E, Table 4.2).

A



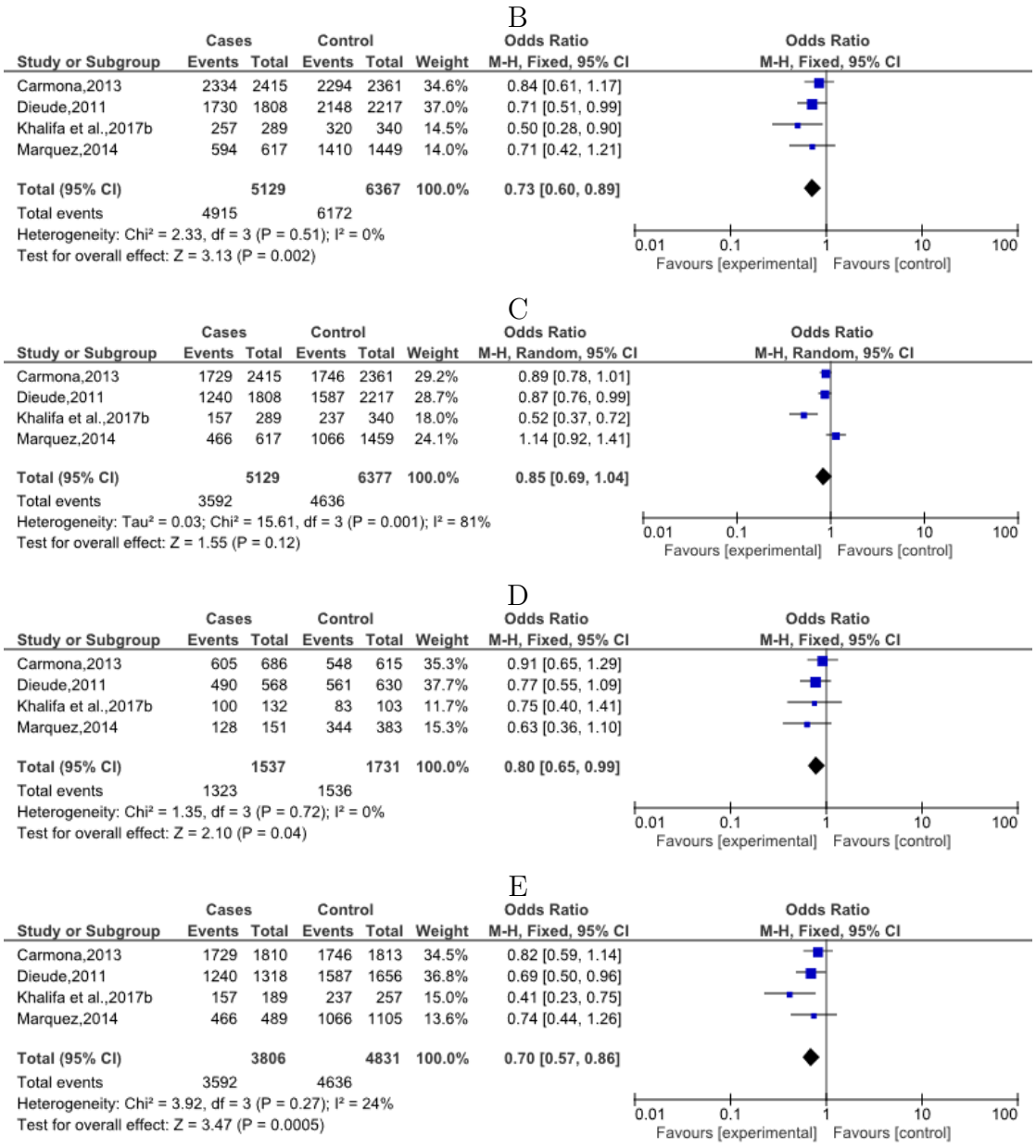


FIGURE 4.12: Meta-Analysis Depicted Association of IRAK1 rs1059702 T>C Susceptibility in Asian. A) Allele Model (C vs T), B)Dominant Model (TC+CC vs TT), C) Recessive Model (CC vs TT+TC), D) Heterozygote model (TC vs TT),E) Homozygote Model (CC vs TT).

In Asian, two studies were found for analysis. Allele model (C vs T; OR=1.30, 95% CI=1.16-1.46, P<0.00001, I_2 =0%) showed significant association with ADs susceptibility based on significant p-value, OR as well as position of diamond. While dominant model (TC+CC vs TT; OR=0.68, 95% CI=0.68-0.86, P=0.001, I_2=65%), heterozygote model (TC vs TT; OR=0.76, 95% CI=0.66-0.88, P=0.0002, I_2=0%), recessive model (CC vs TT+TC; OR=0.69, 95% CI=0.51-0.93, P=0.02,

I_2=0%) and homozygote model (CC vs TT; OR=0.62, 95% CI=0.46-0.85, P=0.002, I_2=0%) were significantly high among control than ADs as supported by significant p-value, OR as well as position of diamond. Fixed model was used in four models except dominant model in which heterogeneity was moderate and random model was used (Figure 4.13,A-E, Table 4.2).

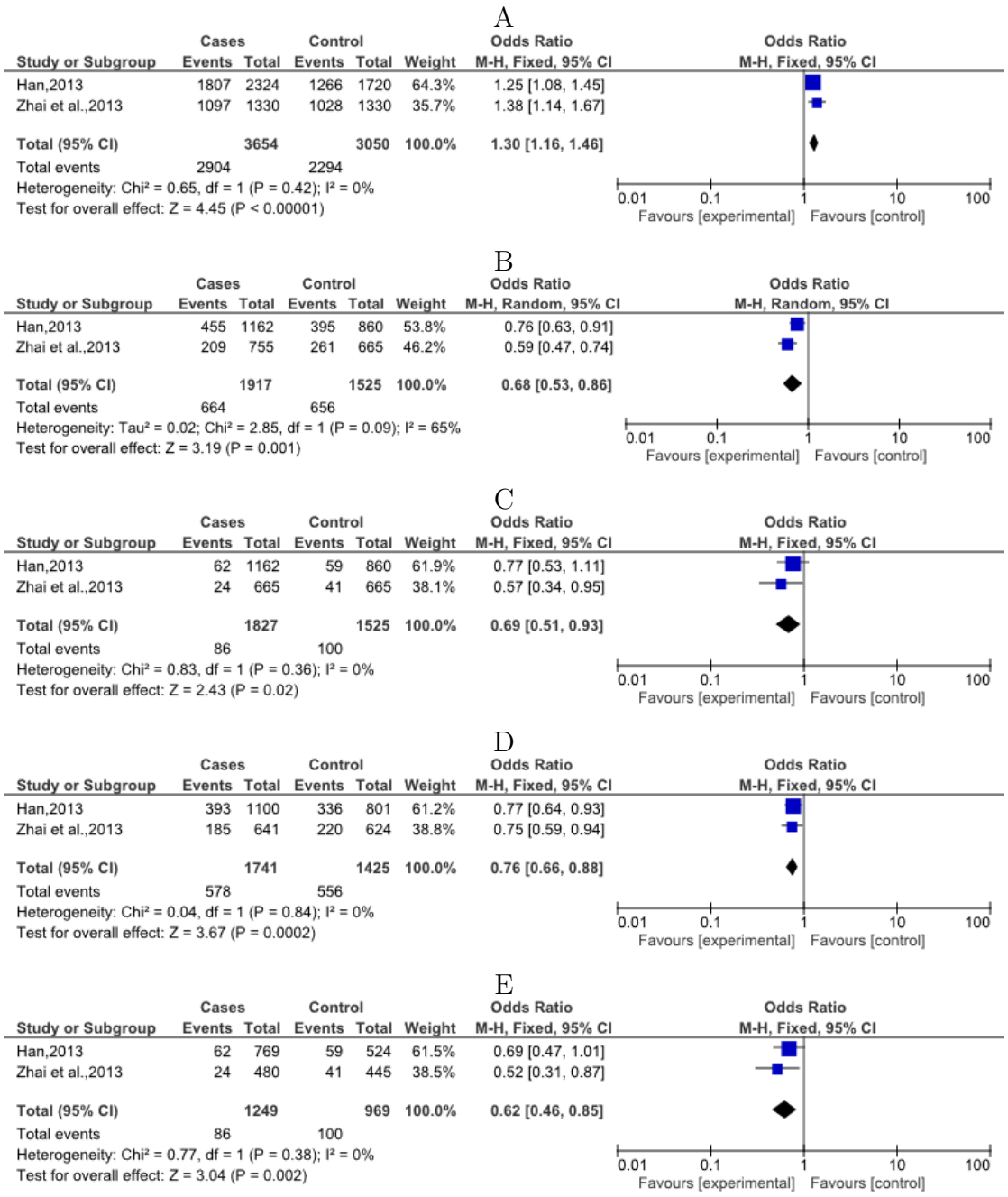


FIGURE 4.13: Meta-Analysis Depicted Association of IRAK1 rs1059702 T>C Susceptibility in Asian. A) Allele Model (C vs T), B)Dominant Model (TC+CC vs TT), C) Recessive Model (CC vs TT+TC), D) Heterozygote Model (TC vs TT), E) Homozygote Model (CC vs TT).

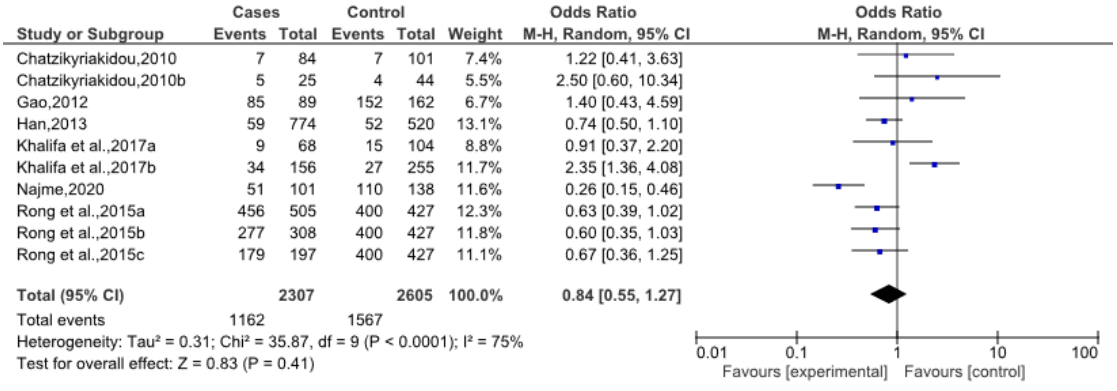
In African, only one study was present and heterogeneity was not applicable. For allele model (C vs T; OR=1.26, 95% CI=0.87-1.85, P=0.25), dominant model (TC+CC vs TT; OR=16.0, 95% CI=8.23-31.2, P=0.00001).

Recessive model (CC vs TT+TC; OR=8.55, 95% CI=4.48-16.3, P=0.00001), heterozygote model (TC vs TT; OR=10.1, 95% CI=4.83-21.24, P=0.0001), homozygote model (CC vs TT; OR=26.1, 95% CI=12.04-59.2, P=0.00001, Table 4.2).

4.4 Association of IRAK1 rs1059703 T>C Polymorphism with Susceptibility to ADs

The overall association of rs1059703 T>C polymorphisms was analyzed by including ten studies. Allele model (C vs T; OR=1.37, 95% CI=1.17-1.60, P=0.0001, I_2=70%) revealed high risk for T allele as well as TT genotype in ADs as supported by significant p-value, OR and position of diamond. Heterozygote model (TC vs TT; OR=1.18, 95% CI=0.79-1.78, P=0.42, I_2=85%) depicted slight association with ADs susceptibility based on OR as well as position of diamond but not statistical significant due to greater p-value. Dominant model (TC+CC vs TT; OR=1.02, 95% CI=0.68-1.52, P=0.84, I_2=87%) showed no association in both groups as depicted by non-significant p-value, OR as well as position of diamond. Homozygote model (CC vs TT; OR= 0.84, 95% CI=0.55-1.27, P=0.41, I_2=75%) was slightly but not significantly high in controls based on p-value. Recessive model (CC versus TT+TC ; OR=0.73, 95% CI=0.64-0.82, P=0.08, I_2=43)

A



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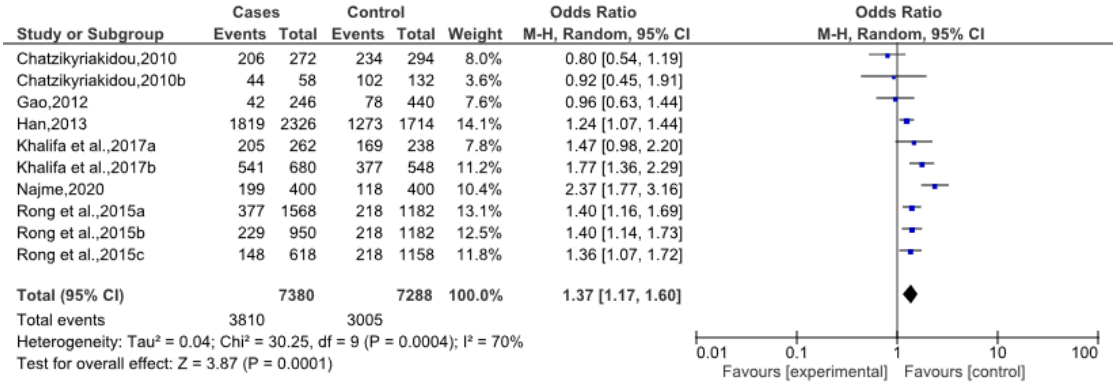

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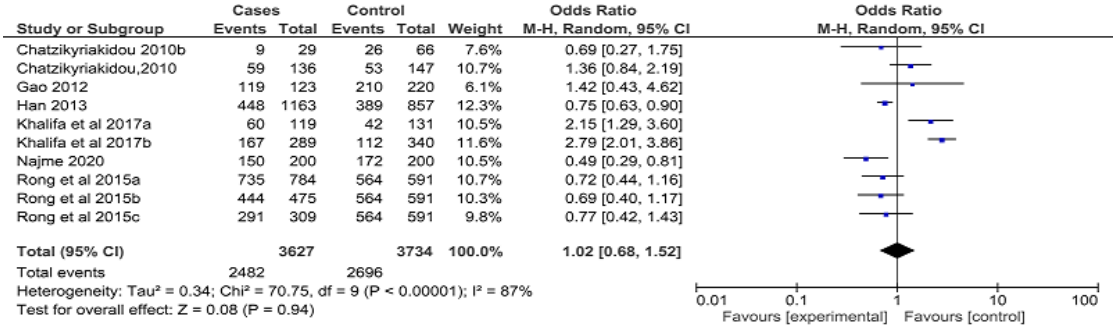

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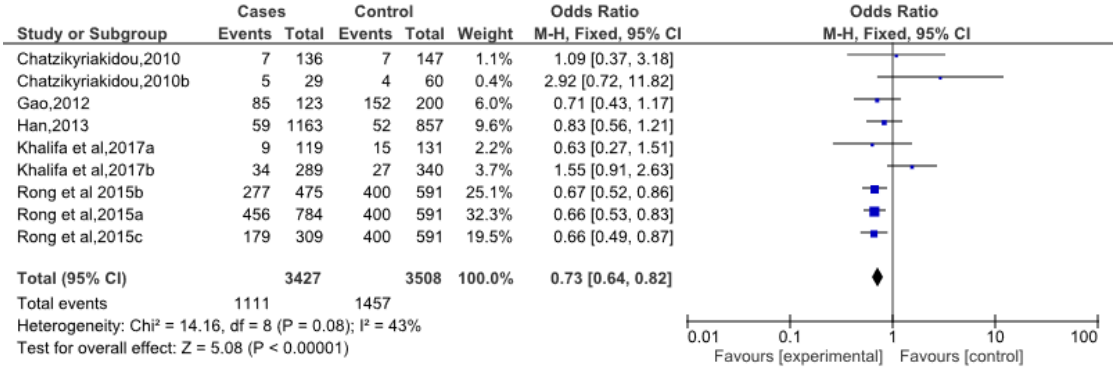

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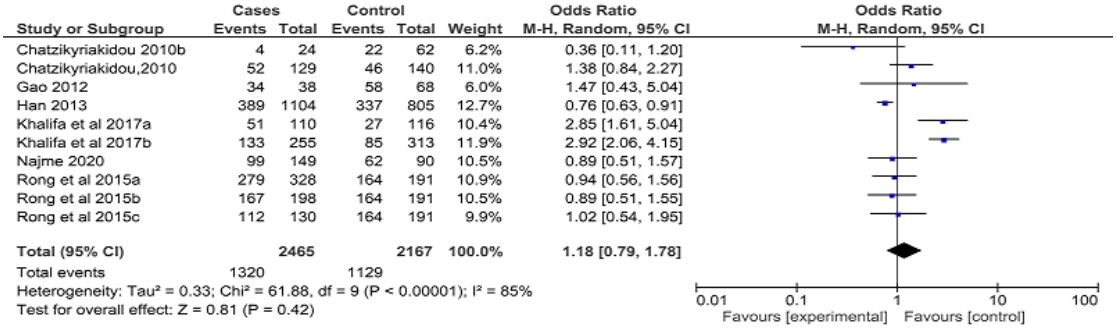


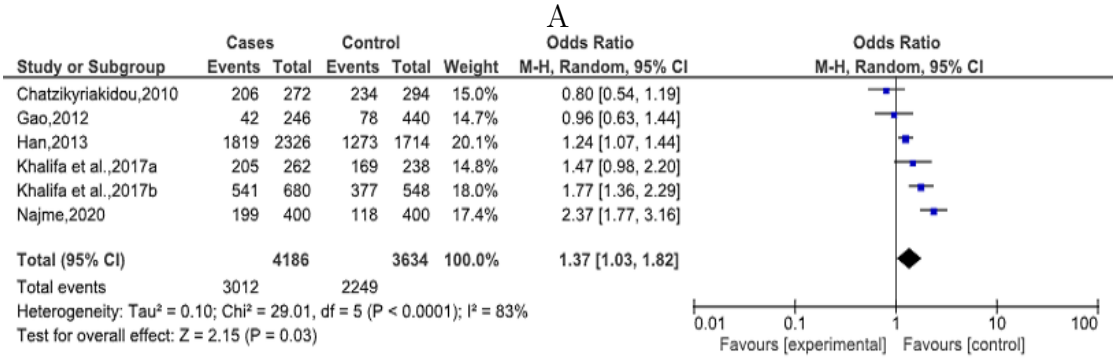
FIGURE 4.14: Meta-Analysis Showed Association of IRAK1 rs1059703 T>C Polymorphisms with Susceptibility to ADs; A) Homozygote Model (CC vs TT), B) Allele Model (C vs T), C)Dominant Model (TC+CC vs TT), D) Recessive Model (CC vs TT+TC), E) Heterozygote Model (TC vs TT).

was significantly high among controls than ADs as supported by significant p-value, OR as well as position of diamond. Random models was selected in four models due to high heterogeneity except recessive model where fixed model was applied (Figure 4.14, A-E).

In sub-group analysis on the basis of the diseases, six studies were selected for analysis of rs1059703 T>C polymorphisms with RA. Significant increased risk was depicted in allele model (C versus T; OR=1.37, 95% CI=1.03-1.82, P=0.03, I_2=83%) based on significant p-value, OR as well as position of diamond. Dominant model (TC+CC vs TT; OR=1.26, 95% CI=0.69-2.31, P=0.44, I_2=92%), heterozygote model (TC vs TT; OR=1.49, 95% CI=0.81-2.73, P=0.20, I_2=91%) indicated slight association with ADs risk based on OR as well as position of diamond but not statistical significant due to greater p-value.

Recessive model (CC vs TT+TC; OR=0.78, 95% CI=0.45-1.35, P=0.37, I_2=83%) and homozygote model (CC vs TT; OR=0.91, 95% CI=0.45-1.83, P=0.79, I_2=84%) were slightly high among control based on OR as well as position of diamond but not statistical significant due to greater p-value. Random effect model was used in all models due to high heterogeneity (Figure 4.15, A-E).

A



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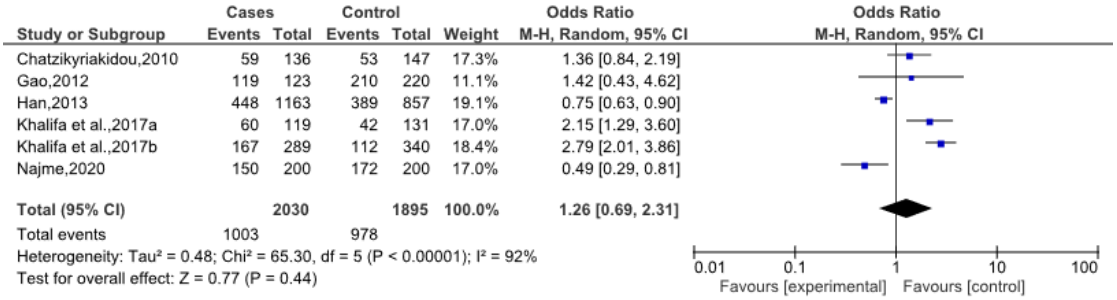


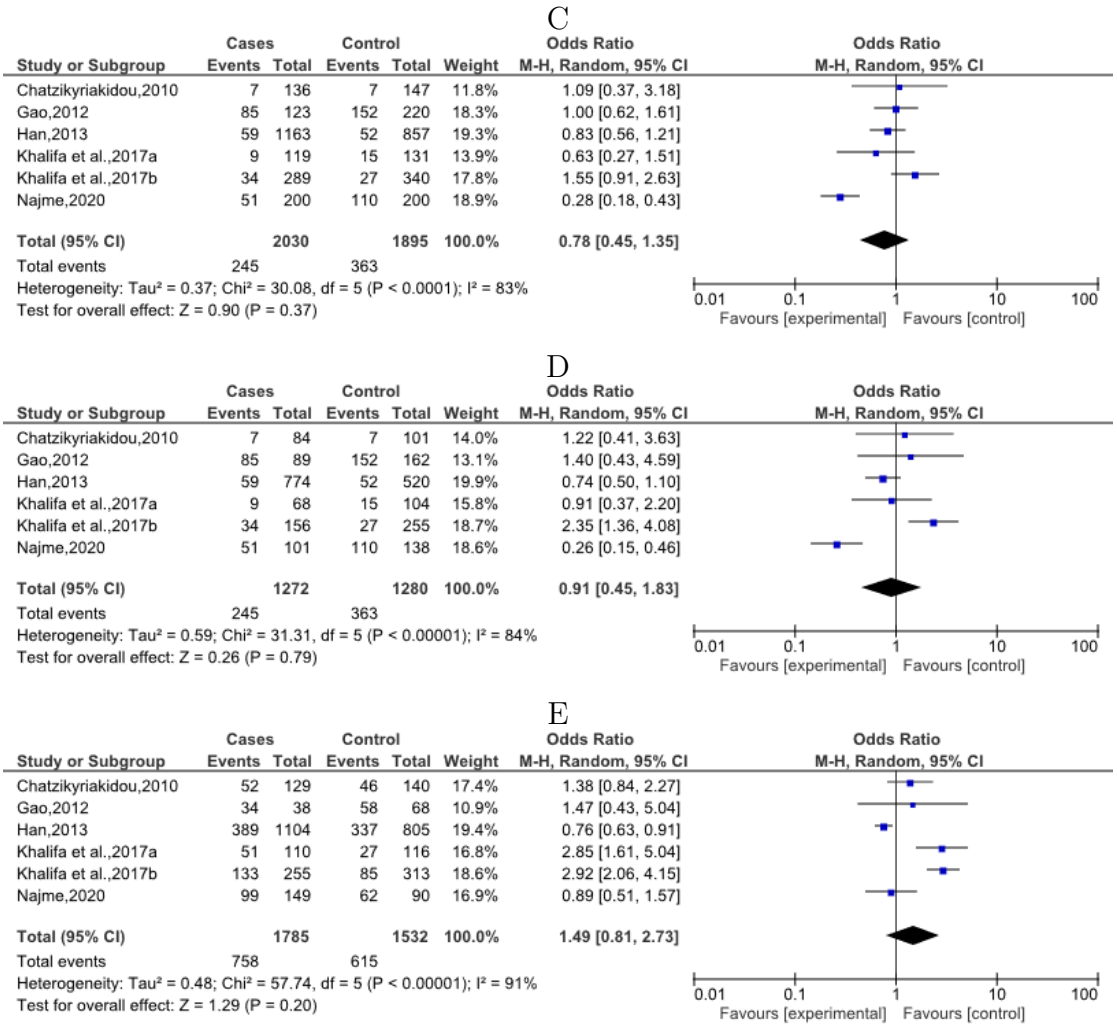


FIGURE 4.15: Meta-Analysis Showed Association IRAK1 rs1059703T>C with RA Susceptibility. A) Allele Model (C vs T), B) Dominant Model (TC+CC vs TT), C)Recessive Model (CC vs TT+TC), D)Homozygote Model (CC vs TT), E) Heterozygote Model (TC vs TT).

For AITDs, three studies were included for meta-analysis. Results indicated significant increased risk for allele model (C vS T; OR=1.40, 95% CI=1.24-1.58, P<0.0001, I_2=0%) based on significant p-value, OR as well as position of diamond. Recessive model (OR=0.71, 95% CI=0.62-0.82, P<0.00001, I_2=8%) and homozygote model (OR=0.63, 95% CI=0.46-0.86, P=0.004, I_2=0%) were high among in control as supported by p-value, OR as well as position of diamond. No association was depicted in dominant model (OR=0.74, 95% CI=0.51-1.08, P=0.12, I_2=0%) as well as heterozygote model (OR=0.96, 95% CI=0.69-1.32, P=0.78, I_2=0%) because p-value was not significant. Fixed model was used in all models due to low heterogeneity (Figure 4.16, A-E). Stratified analysis were

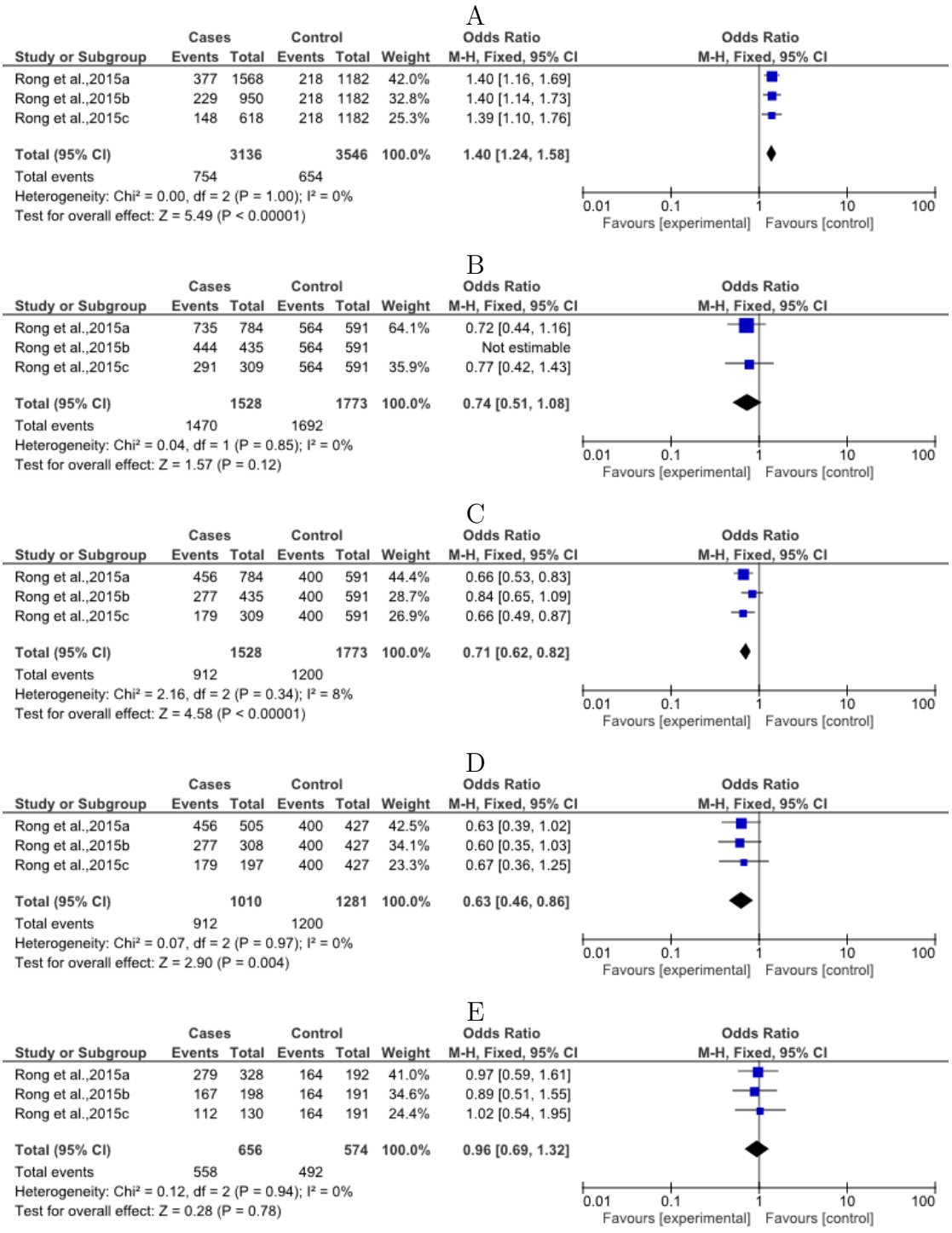


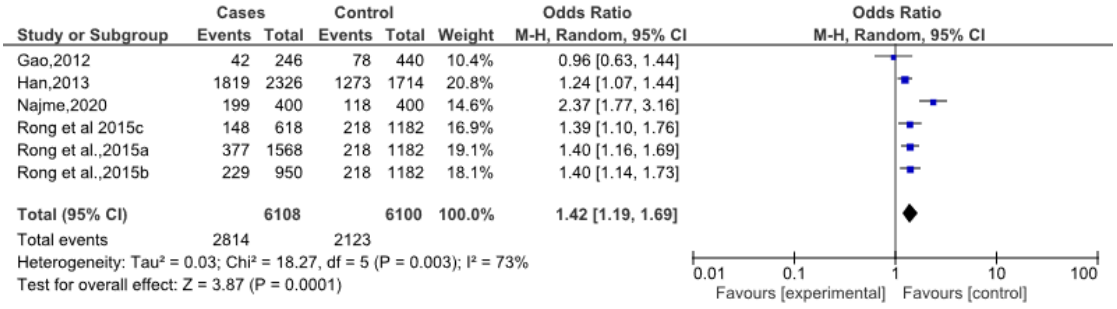
FIGURE 4.16: Meta-Analysis Showed Association of IRAK1 rs1059703 T>C with AITDs Susceptibility. A) Allele Model (C vs T), B)Dominant Model (TC+CC vs TT), C)Recessive Model (CC vs TT+TC), D)Homozygote model (CC vs TT), E) Heterozygote model (TC vs TT).

also performed on the basis of populations to find association of rs1059703 T>C polymorphisms with susceptibility to ADs. In Asian, six studies were found for computational analysis. Significant association was observed with ADs in allele

model (C vs T; OR=1.42, 95% CI=1.19-1.69, P=0.0001, I_2=73%) evidenced by significant p-value, OR as well as position of diamond. P-value, OR as well as diamond position favoring high amount in control for dominant models ((TC+CC vs TT; OR=0.73, 95% CI=0.63-0.84, P<0.0001, I_2 =0%) , recessive model (CC vs TT+TC; OR=0.64, 95% CI=0.49-0.83, p=0.008, I_2 =75%), homozygote model (CC vs TT; OR=0.59, 95% CI=0.42-0.85, p=0.004,I_2 =57%) and heterozygote model (TC vs TT; OR=0.81, 95% CI=0.70-0.95, P=0.009, I_2=0%) than ADs.

The heterogeneity was low in dominant model and heterozygote model suggesting use of fixed effect model. There was moderate heterogeneity in homozygote model, high in allele model and recessive model suggesting application of random effect model (Figure 4.17, A-E, Table 4.3).

A



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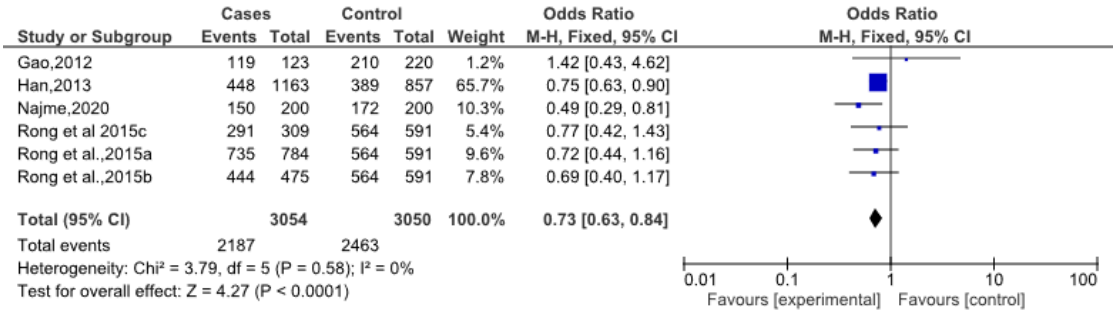

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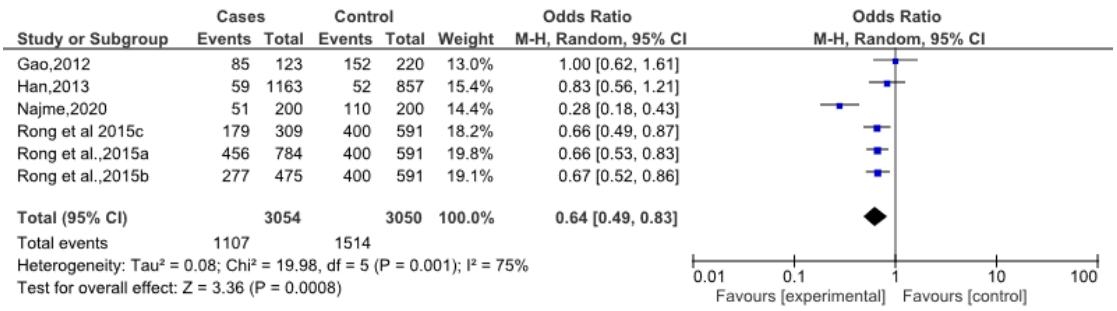

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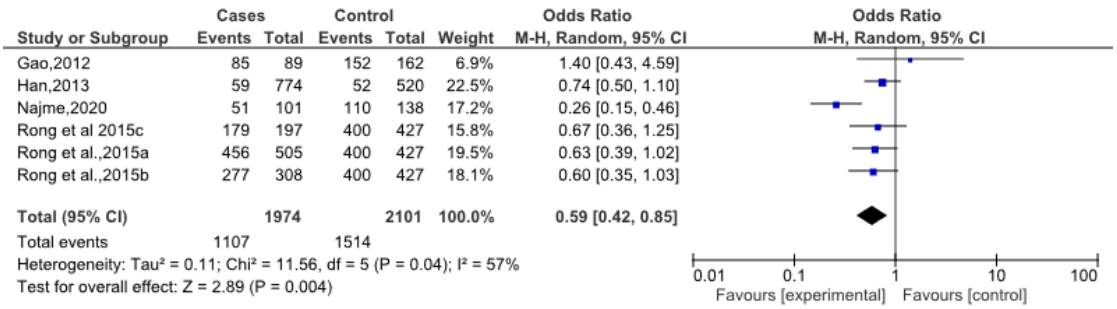

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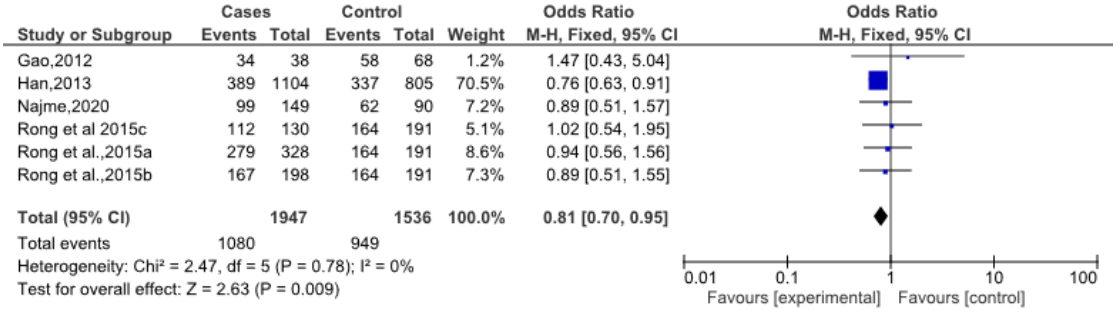

FIGURE 4.17: Meta-Analysis Showed Association of IRAK1 rs1059703 T>C with AITDs Susceptibility. A) Allele Model (C vs T), B)Dominant Model (TC+CC vs TT), C)Recessive Model (CC vs TT+TC), D)Homozygote Model (CC vs TT), E) Heterozygote Model (TC vs TT).

For meta-analysis of rs1059703 T>C polymorphisms in Europe, three studies were included. There was high risk was observed in homozygote model (CC vs TT; OR=2.09, 95% CI=1.32-3.33, P=0.002, I^2=0%) for ADs as indicated by the p-value, OR as well as position of diamond.

Slight association was found in allele model (C vs T; OR=1.13, OR=0.62-2.05, 95% CI=0.62-2.05, P=0.69, I_2 =83%), dominant model (TC+CC vs TT;OR=1.52, 95% CI=0.74-3.14, P=0.26%, I_2=83%), recessive model (CC vs TT+TC; OR=1.55, 95% CI=0.99-2.43, P=0.06, I_2 =0%) and heterozygote model (TC vs TT; OR=1.34, 95% CI=0.55-3.30, P=0.52, I_2=86%) based on OR as well as position of diamond but not statistical significant due to greater p-value. Fixed model was applied in homozygote model as well as recessive model due to low heterogeneity. While high heterogeneity in three models dominant model, allele model and heterozygote model suggesting use of random effect model (Figure 4.18, A-E, Table 4.3).

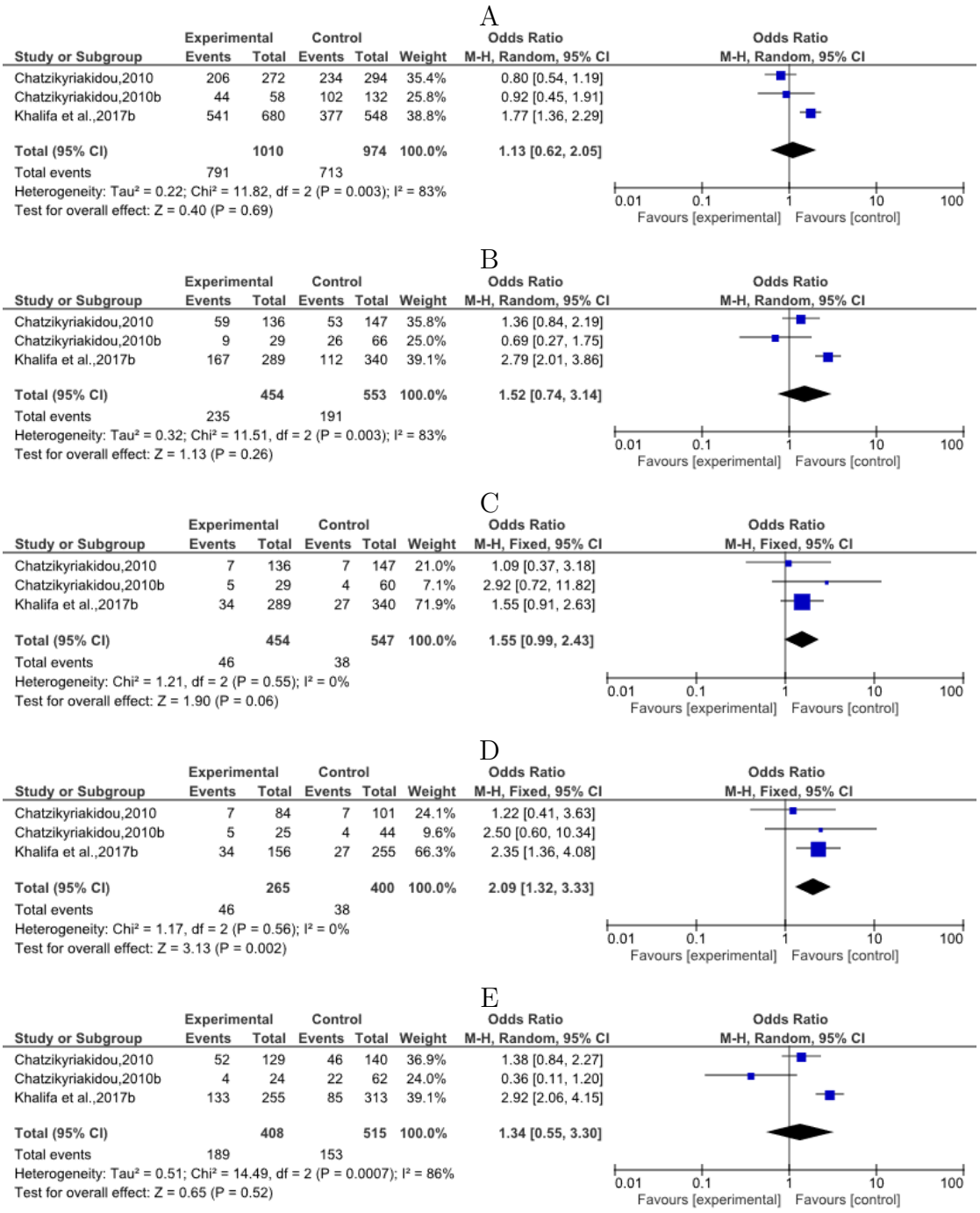

FIGURE 4.18: Meta-Analysis of Association of IRAK1 rs1059703 in Asian. A) Allele Model (C vs T), B)Dominant Model (TC+CC vs TT), C)Recessive Model (CC vs TT+TC), D)Homozygote Model (CC vs TT); E) Heterozygote Model (TC vs TT).

In African only one study was found and therefore heterogeneity was not applicable. Allele model (C vs T; OR=1.47, 95% CI=0.98-2.20, P=0.06), dominant model (TC+CC vs TT;OR=2.15, 95% CI=1.29-3.60, P=0.003), recessive model (CC vs TT+TC;OR=0.63, 95% CI=0.27-1.51, P=0.30), heterozygote model (TC

vs TT; OR=2.85, 95% CI=1.61-5.04, P= 0.0003), homozygote model (CC vs TT; OR=0.91, 95% CI=0.37-2.20, P=0.83) (Table 4.3).

TABLE 4.1: Results Summary of Meta-Analysis of IRAK1 rs3027898 C>A Gene Polymorphisms Association with ADs Susceptibility in Different Populations.

Polymorphisms	**Population**	**No. of Studies**
rs3027898		
Allele Model A vs C	Overall	17
	Europe	3
	Asian	10
	African	4
Dominant Model CA+AA vs CC	Overall	17
	Europe	3
	Asian	10
	African	4
Recessive Model AA vs CC+CA	Overall	17
	Europe	3
	Asian	10
	African	4
Homozygote Model AA vs CC	Overall	17
	Europe	3
	Asian	10
	African	4
Heterozygote Model CA vs CC	Overall	17
	Europe	3
	Asian	10
	African	4

Association Test			Heterogeneity Test		
OR	CI 95%	P-Value	Model	P-Value	I_2%
1.07	0.83-1.39	0.6	R	0.00001	93
1.86	0.65-5.30	0.24	R	0.0001	89
0.82	0.63-1.07	0.15	R	0.00001	92
1.52	0.75-3.06	0.24	R	0.00001	92
0.9	0.64-1.27	0.55	R	0.00001	92
0.3	0.16-0.55	0.0001	F	0.59	0
1.05	0.69-1.59	0.82	R	0.00001	94
1.1	0.49-2.49	0.82	R	0.0002	85
0.94	0.68-1.29	0.69	R	0.00001	84
1.24	0.50-3.09	0.64	R	0.01	78
1.21	0.90-1.63	0.2	R	0.005	70
0.43	0.20-0.95	0.04	R	0.0001	88
0.82	0.58-1.18	0.29	R	0.00001	80
0.26	0.15-0.48	0.0001	F	0.18	42
1.19	0.81-1.74	0.38	R	0.0001	76
2.7	0.96-7.62	0.06	R	0.0003	84
1.11	0.77-1.59	0.58	R	0.00001	91
0.11	0.02-0.66	0.02	R	0.02	75
1.13	0.77-1.66	0.53	R	0.0001	93
0.62	0.31-1.26	0.19	R	0.006	76

4.5 Sensitivity Analysis

Sensitivity analysis were accomplished by eliminating single study one after another in order to check the effect of single study on overall estimates. In all models no prominent change was observed indicating results were reliable for all models.

TABLE 4.2: Result Summary of Meta-Analysis of Association of IRAK1 rs1059702T>C Polymorphisms with ADs Susceptibility in Different Populations.

Polymorphisms	**Population**		**No. of Studies**		
rs1059702	Overall		7		
	Europe		4		
	Asian		2		
Allele Model C vs T	African		1		
Dominant Model	Overall		7		
TC+CC vs TT	Europe		4		
	Asian		2		
	African		1		
Recessive Model	Overall		7		
CC+TT vs TC	Europe		4		
	Asian		2		
	African		1		
Heterozygote Model	Overall		7		
TC vs TT	Europe		4		
	Asian		2		
	African		1		
Homozygote Model	Overall		7		
CC vs TT	Europe		4		
	Asian		2		
	African		1		
Association Test			**Heterogeneity Test**		
OR	CI 95%	P-Value	Model	P-Value	I_2%
1.23	1.09-1.37	0.0005	R	0.006	67
1.19	1.0-1.41	0.04	R	0.003	79
1.3	1.16-1.46	0.00001	F	0.42	0
1.26	8.85-1.0	0.25	NA	NA	NA
1	0.62-1.61	0.99	R	0.00001	93

0.73	0.60-0.89	0.002	F	0.51	0
0.68	0.53-0.86	0.001	R	0.09	65
16	8.23-31.2	0.00001	NA	NA	NA
0.99	0.73-1.34	0.93	R	0.00001	91
0.85	0.69-1.04	0.12	R	0.001	81
0.69	0.51-0.93	0.02	F	0.36	0
8.55	4.48-16.3	0.00001	NA	NA	NA
1	0.69-1.45	0.99	R	0.00001	87
0.8	0.65-0.99	0.04	F	0.72	0
0.76	0.66-0.88	0.0002	F	0.84	0
10.1	4.83-21.24	0.0001	NA	NA	NA
1.02	0.54-1.92	0.96	R	0.00001	93
0.7	0.57-0.86	0.0005	F	0.27	24
0.62	0.46-0.85	0.002	F	0.38	0
26.7	12.04-59.2	0.00001	NA	NA	NA

TABLE 4.3: Results Summary of Meta-Analysis Indicated Association of IRAK1 rs1059703T>C Polymorphisms with ADs Susceptibility in Different Populations.

Polymorphisms	**Population**	**No. of Studies**
rs1059703		
Allele Mode C vs T	Overall	10
	Europe	3
	Asian	6
	African	1
Dominant Model TC+CC vs TT	Overall	10
	Europe	3
	Asian	6
	African	1
	Overall	10

Recessive Model CC vs TT+TC	Europe	3
	Asian	6
	African	1
	Overall	10
Heterozygote Model TC vs TT	Europe	3
	Asian	6
	African	1
	Overall	10
Homozygote Model CC vs TT	Europe	3
	Asian	6
	African	1

Association Test			**Heterogeneity Test**		
OR	CI 95%	P-Value	Model	P-Value	I_2 %
1.37	1.17-1.60	0.0001	R	0.0004	70
1.13	0.62-2.05	0.69	R	0.003	83
1.42	1.19-1.69	0.0001	R	0.003	73
1.47	0.98-2.20	0.06	NA	NA	NA
1.02	0.68-1.52	0.94	R	0.00001	87
1.52	0.74-3.14	0.26	R	0.003	83
0.73	0.63-0.84	0.0001	F	0.58	0
2.15	1.29-3.60	0.003	NA	NA	NA
0.43	0.640.82	0.00001	R	0.08	43
1.55	0.99-2.43	0.06	F	0.55	0
0.64	0.49-0.83	0.0008	R	0.001	75
0.63	0.27-1.51	0.3	NA	NA	NA
1.18	0.79-1.78	0.42	R	0.00001	85
1.34	0.55-3.30	0.52	R	0.0007	86
0.81	0.70-0.95	0.009	F	0.78	0
2.85	1.61-5.04	0.0003	NA	NA	NA
0.84	0.55-1.27	0.41	R	0.0001	75
2.09	1.32-3.33	0.002	F	0.56	0

0.59	0.42-0.85	0.004	R	0.04	57
0.91	0.37-2.20	0.83	NA	NA	NA

4.6 Discussion

IRAK1 is a protein kinase that is responsible for regulation of Toll/interleukin-1 receptor family signaling pathways [95]. The IRAK1 activates NF-kB by mediating interaction between TLR and TNF receptor-associated factor (TRAF) 6, or by forming complex with MyD88 which in turn assist transcription of different inflammatory cytokines genes including TNF-α and IL-8 [33,34,35]. Various experimental studies on animal models reported that deficiency of IRAK1 leads to disturbance in inflammatory responses and reduced level of IL-17 [36]. However IRAK1 reported to be in association with ADs susceptibility in patients of different ethnicities.

IRAK1 has three SNPs that were reported to be associated with ADs susceptibility; rs3027898 with RA [147,148,149,150,151], SLE [51,127], AS [52,133], AITDs [152] rs1059702 with RA [120,153], SSc [138,154] and rs1059703 with RA [52, 147, 155], SLE [51] but findings were conflicting. Hence, we executed this meta-analysis in order to well understanding of whether these SNPs showed susceptibility to ADs.

For current meta-analysis, we identified 18 studies for analysis, with 17 studies for rs3027898 involving 4375 cases and 5836 healthy subjects, 7 studies for rs1059702 involving 7237 patients and 7551 healthy subjects and 10 studies for rs1059703 involving 3256 patients and 3017 healthy subjects. we found all three SNPs showed association with ADs susceptibility. rs3027898 showed no risk for allele C or CC genotype with overall ADs in all genetic models. At the same time, subgroup analysis were executed on the basis of disease category and population. For RA, slight risk was observed for C allele in allele model. Little data was available for SLE, C allele showed increased susceptibility to SLE in allelic model. A slight increased risk was noticed for CC genotype with disease in heterozygote model.

Similarly, little data was available for AS for analysis. Increased susceptibility was observed CC genotype only in recessive model with AS. Four models except allele model showed highly associated risk of T allele and TT genotype for AITDs. Stratified analysis showed increased susceptibility for ADs in different population. In Europe, slight increased risk was observed in case of allele C with ADs. Allele model and heterozygote model revealed significantly increased disease susceptibility for allele C and genotype CC to ADs in African. However, in Asian increased susceptibility of C allele as well as CC genotype was found in homozygote model, heterozygote model and recessive with ADs.

In case of rs1059702, T allele was found be increased risk with overall ADs in only allele model. Sub-group analysis on the basis of disease category indicated T allele was seem to be related with increased risk to RA in allelic model. Allele model indicated increased risk of T allele for SSc. Stratified indicated increased risk of T allele with AD in Europe and Asian as shown by allele model comparison. For rs1059703, significantly increased association of T allele was observed with ADs in allele model comparison. In sub-group analysis, allele T in allelic model model was found with increased RA susceptibility. Stratified analysis denoted allele T was associated significantly in Asian to risks of ADs as shown by allele comparison and also same findings noticed in AITDs. Significant association was noticed for ADs in TT genotype and TT+TC genotype among homozygote model and recessive model. However, our findings of meta-analysis were not according to meta-analysis performed by Changzheng et al. However, their meta-analysis contained ten articles that showed association of rs3027898, rs1059702 and rs1059703 with susceptibility to ADs in different genetic models.

Since IRAK1 is important element of myddosome and responsible for activation of NLRP3 inflammasome. Immunity and inflammation are regulated by IL-1 and TLR- signaling cascades that are mediated by the IRAK1 [156]. IL-1 is a cytokines essential for regulation of inflammation. IRAK1 is a intracellular kinase that is responsible for regulation of non-specific immune response. These responses are important mediators of inflammation, antiviral responses and ultimately stimulation of adaptive immune response as well as control of autoimmune diseases.

Pattern recognition receptors (PRRs) are responsible for recognition of pathogens in non-specific immune signaling cascade. One of the pattern recognition receptor is Toll-like receptors (TLRs) [157]. TLR signaling is regulated by IRAK1. Disturbances in these processes result in implication in autoimmune inflammatory diseases and development of different types of cancer [156]. IL-1/TLR signaling cascades and the IRAK members are crucial for anti-pathogenic responses, inflammation as well as autoimmunity [6]. Researches reported that inhibitors of IRAK1 inhibit NF-kB results in suppression of inflammatory conditions. Previous studies reported mice deficient of IRAK1 showed impairment in Th1 T cell formation with low secretion of IFN-γ during disease induction by immunization. This finding concluded that defective adjuvant effect on antigen presenting cells due to sub-optimal activation of TLR involve in mechanism of IRAK-1 deficient ability in modulation of immune responses [158]. Due to involvement of IRAK1 in immune responses both innate and adaptive, it might be a better therapeutic potential target for cure of autoimmune diseases. The recognition of genetic variants that are involve in development of autoimmune diseases is essential for understanding of autoimmune diseases pathogenesis. The polymorphisms in IRAK1 has influence on its gene expression and in turn its function, which might be responsible for development of ADs. Several studies reported association of IRAK1 polymorphisms with susceptibility to ADs like systemic lupus erythematosus [126], systemic sclerosis [154], RA [147], AITDS [152] and MS. However, on the whole, these studies indicated that Xq28 was involve in complex diseases, particularly risk for the inflammatory diseases. Study of Han as well as Khlifa et al reported strong association of rs1059703 with RA in allelic model in Korean population, French and Tunisian population [120,153] same results were also found in our meta-analysis suggesting IRAK1 play important role in common inflammatory mechanism involve in autoimmune diseases While results of Najme et al reported association of rs1059703 with RA in recessive and dominant model in IRAN. However no association of rs1059703 was reported by Chatzikyriakidou in Greek population [147]. Zhang et al [48] reported genotype AA of IRAK1 rs3027898 C/A was significantly associated with increased susceptibility to RA in a Chinese population (OR=1.91, 95%

CI=1.12-3.26; P=0.017). The meta-analysis results of song et al [159] showed that genotype CC was associated with high RA risk (OR=2.602, 95% CI=1.387-4.879; P=0.003). The investigations of Chatzikyriakidou in Greece population showed significant increased risk for rs3027898 with RA. The study of Atabaki et al [148] on IRAN reported no association of rs3027898 with RA. In Egyptian population increased risk of CA and AA was observed by Shaker et al. The results of our meta-analysis were contradict to these studies. Our results observed increased risk of IRAK1 rs3027898 C allele with RA similar to findings of Yang et al. while results of meta-analysis by Changzheng et al [146] indicated increased susceptibility of genotype CC within heterozygote model (CA versus CC: OR = 0.83, 95% CI = 0.71–0.97, p= 0.021). Han [120] also reported association of rs1059702 with susceptibility to RA and results of our meta-analysis also according to results of Han. Labib et al [127] reported association of IRAK1 rs3027898 with susceptibility to SLE and noticed statistically significant variability between cases and healthy subjects. Our results of meta-analysis also found association of rs3027898 with SLE in allele model similar findings of Zhai et al [51] (C versusA: OR=1.438, 95 % CI=1.180–1.753, p<0.001) among Chinese population and Changzheng et al's meta-anaysis [146]. Study of wang reported association of rs3027898 with AS and our results also showed increased susceptibility for AS in recessive model. Dieude showed increased susceptibility of allele T and genotype TT (OR=1.20 and 95%, 95% CI=1.06–1.35 for the T allele P=0.003) and OR =1.49, 95% CI = 1.06–2.10 for the genotype TT, P=0.023) for SSc and our results also indicated increased susceptibility of T allele in allele model.

The study has some limitations. First, since 18 articles were selected for analysis, the studies for stratified analysis on the basis of disease category as well as population were limited. For example, only one study was available for analysis of rs1059702 and rs1059703 in African population, two studies of AS and SLE for rs3027898, two studies of SSc and Asian population for rs1059702. Second, considerable heterogeneity was found among different genetic models that may distorted the analysis, although sensitivity analysis were carried out and no potential source of heterogeneity was observed. Thirdly, our stratified analysis were only based on

European, Asian and African populations and our findings are only useful for these ethnicities. More work is demanded among others ethnic groups. Fourthly, analysis were carried out by including three SNPs, however, there are some other SNPs related to IRAK1 gens that are involved in TLR signaling cascade. The influence of these SNPs and their interaction in immune responses should be searched in the future.

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

The current meta-analysis of published studies on three SNPs of IRAK1 including rs3027898, rs1059702, rs1059703 indicated their association with susceptibility to ADs. However, the association was only observed within peculiar genetic model, peculiar population or particular disease category not in all sorts of subjects or ADs involved in study. IRAK1 is important regulator of innate and adaptive immune responses and its used as drug target will be helpful in prevention of ADs in coming years.

5.2 Recommendations

- Further new studies are encouraged to clarify the association of IRAK1 in other ethnicities as well as diseases since IRAK1 may have a difference in performance in different ethnicities and different diseases.
- we did not carried out analysis by stratifying analysis on the basis of gender or clinical and environmental variables and their role in clinical manifestation

of the disease due to insufficient data. Future studies must be conducted on the bases of gender differences.

- ADs are a comprehensive group of diseases, more work should be carried out in other ADs such as inflammatory bowl syndrome, MS, PsA, AITDs, seronegative spondyloarthropathies and sepsis to find the association of IRAK1 genetic polymorphisms.

- Future functional studies must be conducted on other populations to find association of IRAK1 with ADs risk.

- Their are some other SNPs of IRAK1 in addition to rs3027898, rs1059702 and rs1059703. The influence of these SNPs and their interaction in immune responses should be searched in the future.

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