IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISMS PREDICTING SUSCEPTIBILITY OF ANKYLOSING SPONDYLITIS AND THE RESPONSE TO ANTI-TNFα THERAPY

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A thesis submitted to the University of Huddersfield in partial fulfilment of the requirements for the degree of Doctor of Philosophy

August 2018

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ABSTRACT

INTRODUCTION: Different genetic features may result in different incidences of diseases, treatment response and adverse events following medical therapy. The studies included in this thesis collectively aim to identify polymorphisms associated with ankylosing spondylitis (AS) and those can predict therapeutic efficacy and adverse events of anti-tumor necrosis factor-α (TNFα) treatment in Chinese Han patients.

METHODS: We performed a case-control study including 149 Chinese Han ankylosing spondylitis patients ethnicity- and gender-matched to 106 healthy controls, and genotyped 14genes encompassing 28 short nucleotide polymorphisms (SNPs) by using the Matrix-assisted Laser Desorption/ionization-time of Flight Mass Spectrometry technique in order to evaluate which genes and SNPs are associated with AS. Furthermore, we performed genotyping on 106 Chinese Han patients with AS, who received infliximab or a recombinant human TNFα receptor II–IgG Fc fusion protein (rhTNFR–Fc) therapy, for evaluating associations between drug response and polymorphisms of TNFα gene -238, -308, -857, and -1031. We also conducted a meta-analysis on the same set of SNPs in 211 spondyloarthritis (SpA) patients and 392 inflammatory bowel disease (IBD) to validate their capability in predicting response toward anti-TNFα therapy. Finally, 402 Chinese AS patients with anti-TNFα monotherapy were monitored for short-term adverse events within two hours. Additionally, long-term adverse effects of anti-TNF therapy were profiled for 172 patients at 8, 12, 52, and 104 weeks, and their incidences were analyzed for the frequency of the aforementioned SNPs of TNFα gene.

RESULTS: We demonstrated that SNPs in TNFα-857 (rs1799724, p=0.0002), TNFα-308 (rs1800629, p=0.0484), tumor necrosis factor receptor super-family 1A (TNFRSF1A) (rs4149577, p=0.0087), human leukocyte antigen B27tagged (HLA-B27tagged) (rs4349859, p=0.0004), and interleukin 23 receptor (IL-23R) (rs1004819, p=0.0131), have close associations with AS. Regarding the pharmacogenomics of infliximab and rhTNFR–Fc therapy, we revealed that TNFα -857 C/C and -1031 T/T genotypes were significant predictors of treatment response. The results from meta-analysis showed that TNFα genotypes -308 G/G (p = 0.0020) and -857 C/C (p = 0.0100) responded better to anti-TNFα therapy. By analyzing the side effects of TNF inhibitors (TNFi), we found elevated C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) as well as the duration of disease are highly associated with increased risk for short-term and long-term

adverse events ($p < 0.05$). Additionally, rhTNFR-Fc therapy was associated with less adverse events than Infliximab during long-term treatment ($p < 0.01$). However, when we correlated the SNP frequencies in TNFα genes to the occurrence of adverse events, none of them was found to be an effective predictor of side effects in AS patients treated with TNF blockers.

CONCLUSION: Collectively, these studies outline AS-related SNPs in Chinese Han patients. In particular, SNPs at promoter regions of TNFαare closely associated with the predisposition of SpA and IBD, and responsiveness to anti-TNFα therapy. The prevalence of short- and long-term adverse events of TNFi monotherapy were profiled and were not associated with SNPs at TNFα promoter. Future studies are expected to confirm these findings in larger cohorts and perhaps establish these SNPs as reliable biomarkers at clinics.

Key words: Ankylosing spondylitis, TNFα, single nucleotide polymorphism, adverse events, prediction

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List of Abbreviations

AS, Ankylosing spondylitis

ASAS, Assessment of SpondyloArthritis International Society

BASDAI, Bath Ankylosing Spondylitis Disease Activity Index

BASFI, Bath AS Functional Index

CRP, C-reactive protein

CTC, Common Terminology Criteria

DMARDs, Disease modifying anti-rheumatic drugs

ERAP1, Endoplasmic reticulum aminopeptidase 1

ESR, Erythrocyte sedimentation rate

FcTR, Fcgamma transduction receptors

HCC, Hepatic cell carcinoma

HLA-B27, Human leukocyte antigen B27

IBD, Inflammatory bowel disease

ICH, Intracranial haemorrhage

IL-23R, Interleukin 23 receptor

IQR, Interquartile range

LTA, Lymphotoxin alpha

MCL, Mucocutaneous leishmaniasis

MHC, Major histocompatibility complex

MS, Multiple sclerosis

NSAIDs, Non-steroidal anti-inflammatory drugs

OLP,Oral lichen planus

OR, Odds ratio

PASI, Psoriasis Area and Severity Index

PS, Psoriasis

PsA, Psoriatic arthritis

rAOM, Recurrent acute otitis media

RCC, Renal cell carcinoma

rhTNFR–Fc, Recombinant human TNF-α receptor II–IgG Fc fusion protein

SAD, Sporadic Alzheimer's disease

SD, Standard deviation

SNP, Single nucleotide polymorphism

SpA, Spondyloarthritis

STAT3, Signal transducer and activator of transcription 3

TNFRSF1A, Tumor necrosis factor receptor super-family 1A

TNFα, Tumor necrosis factor-α

Acknowledgments

First and foremost I would love to express my gratitude and appreciation to my supervisor, **Dr. Patrick McHugh**, director of the Centre for Biomarker Research (CeBioR), for your mentorship of this PhD thesis. You provided an incredible research environment, alongside with support, encouragement and resources without which this work could not have been accomplished. Beyond this, you have helped me sharpen my research acumen and establish myself as an independent scientist.

To Dr. Christopher Cooper, D.O., your extensive knowledge in basic science has been invaluable in the completion of this work. Thank you. Getting to know and work with you has been the experience of a lifetime.

To Dr. Tristan de Mooij., one of my best friends, I would like to thank you for your sincere help and supports. I could not have done this without your inspiration and decent backup.

To Professor Qing Cai from the Department of Rheumatology and Immunology at Changhai hospital affiliated to the Second Military Medical University in China, thank you for all your guidance and insights in genotyping and detecting SNPs, without your guidance and fully support, I could not have been completed my research and finished my PhD project.

To Professor Sheng-Ming Dai, Director of the Department of Rheumatology and Immunology, Shanghai Jiao Tong University Affiliated Sixth People's Hospital in China, I'm so grateful for being working with you since 2008 that I have been acquiring tremendous help and fantastic support from him, he always encourages me to pursue academic training and also teach me how to become a professional Rheumatologist.

To Jia He from the Department of Statistics at the Second Military Medical University in China, for your support with the statistical analyses of these studies.

To Punam Bajracharya from Shree Birendra Military hospital in Nepal for your help with the editing and revising of the manuscripts that are the basis of this thesis.

To the Shanghai Scientific Committee Basic Research Fund and the National Natural Science Foundation of China, thank you for supporting this work.

To my colleagues Dan Zhou, Jiaqi Wang, Yiwen Xu, at Mayo Clinic, the Second Military Medical University, and Shanghai Jiao Tong University, thank you for friendship and encouraging me becoming a stronger physician-scientist.

To my patients, thank you for your support and cooperation. Without your trust in me as your physician, these scientific discoveries would not have been possible. I sincerely hope your efforts will prevent or lessen the suffering of future ankylosing spondylitis patients.

Also, finally, to my family, my parents, my wife Ying Zhu and our son Zeyan Tong (Leo), you shaped me into the man I am today, and I could not be more grateful for your love and support.

1.INTRODUCTION

Working as a rheumatologist in China, I have many patients with ankylosing spondylitis (AS)visiting the clinic every day. At present, the first-line therapy for AS is the nonsteroidal anti-inflammatory drugs (NSAIDs).However, some patients are still in need of tumor necrosis factor (TNF) antagonists (Taurog, Chhabra, & Colbert, 2016), a novel biological agent which has been proved effective in inflammatory arthropathy. However, not all patients respond to TNF antagonists (Maneiro, Souto, Salgado, Mera, & Gomez-Reino, 2015), not to mention that the cost of long-term use of this drug is not affordable to most of the Chinese patients. Additionally, TNF inhibitor (TNFi) can result in a variety of adverse events; the side effects of this approach are not yet very clear among Chinese patients in the real world. Therefore, biomarkers that can predict the outcomes, including both efficacy and safety, in patients are urgently demanded.

Recently, pharmacogenomics combining approaches of pharmacology and genomic analysis has been widely applied to predict individual response toward specific medication. Several single nucleotide polymorphisms (SNPs) in association with clinical outcomes of TNFi therapy in patients with AS have been identified through Pharmacogenomics studies. Our study herein performed the first systemic investigation on these SNPs in Chinese AS patients, focusing on their association with AS susceptibility, and with the efficacy and adverse outcomes of TNFi therapy.

1.1 The clinical features of SpA and the role of TNFα in AS

1.1.1 Specific features of SpA

Spondyloarthritis (SpA) refers to a spectrum of inflammatory diseases including AS, Reactive arthritis (RA), Psoriatic arthritis (PsA), Inflammatory bowel diseases (IBD)-related arthritis, Juvenile SpA, and undifferentiated SpA. AS is one of the most prevalent chronic inflammatory rheumatic disease, affecting the axial skeleton which may additionally involve peripheral joints and non-articular structures, causing chronic progressive back pain, spinal motion restriction and disability (Corona-Sanchez et al., 2012). IBD is also defined as an autoimmune disease, which is characterized by chronic inflammation of the gastrointestinal tract. The manifestation includes vomiting, abdominal pain, rectal bleeding, and diarrhea. Gastrointestinal inflammation usually occurs in 60% of SpA patients. Among these patients, nearly 10% will develop IBD eventually (Rizzo, Ferrante, Guggino, & Ciccia, 2017). More importantly, AS patients exhibited a similar profile of immunological changes to IBD patients. For instance, E-cadherin, a cell surface

protein dominating the cell type between mesenchymal and epithelial, is enriched in the gastrointestinal tissues in AS and IBD patients. This holds true to some other gene/protein, including TNFα, a dominant cytokine, and Toll-like receptors-2 and -4 (Tong et al., 2013).

1.1.2 TNFα as a therapeutic target of SpA

TNFα is the cytokine mainly produced by monocytes, macrophages and other cell types, such as T cells and B cells, natural killer cells, which can generate TNFα in a smaller amount (Grivennikov et al., 2005). TNF is a 26kD transmembrane protein (tmTNF), with its extracellular domain being cleaved by TNFα-converting enzyme (TACE) to generate soluble TNF (sTNF) (Kalliolias & Ivashkiv, 2016). Two receptors interact with both tmTNF and sTNF, among which TNF receptor I can activate cell death and NF-κB signaling, which is an anti-apoptotic response. In contrast, TNF receptor II stimulates pro-survival and pro-inflammatory pathways (Kalliolias & Ivashkiv, 2016).

TNFα was considered as "a fire alarm of the body", in that it initiates defense process against injury(Feldmann & Steinman, 2005). It conveys benefits to the body against infections when present at low concentrations by facilitating host defense system. However, when the concentration of TNFα becomes very high, it can result in over activation of the inflammatory response and tissue injury. It coordinates with other proinflammatory cytokines, such as IL-1 and IL-17, to mediate a spectrum of pathogenic processes, leading to inflammation and tissue destruction(Feldmann & Steinman, 2005). TNFα is hence considered at the top of a proinflammatory signaling cascade.

The increased presence of TNFα at an inflammatory site could aggravate disease pathology, therefore approaches to eliminate or reduce the amount of TNFα accumulating became a therapeutic strategy(Brennan, Chantry, Jackson, Maini, & Feldmann, 1989). Animal studies demonstrated that elevated level of TNFα could lead to arthritis spontaneously, a phenomenon could be effectively attenuated by inhibition of TNFα (Keffer et al., 1991; Thorbecke et al., 1992). Hallmarks of chronic inflammation were decreased after receiving TNFα blocker, including activation and proliferation of leukocytes, and production of other inflammatory factors. Inspired by these pre-clinical results, people performed a pilot study in patients with RA, who were given a monoclonal anti-TNFα antibody, known as infliximab today. This study and a subsequent multicenter clinical trial both proved that inhibiting TNFα resulted in significant

attenuation of disease activity, signs and symptoms of RA (Elliott et al., 1994; Elliott et al., 1993). As a pro-inflammatory cytokine, TNFα also plays an essential role in SpA and IBD and has changed the treatment regimen profoundly. This will be discussed in section 1.3.2.

1.2 The genetic variants that affect AS

Ankylosing spondylitis has a strong genetic component to its pathophysiology. For example, in Caucasians, 63% of monozygotic twins are both affected, compared to only 12.5% in dizygotic twins (M. A. Brown, Kennedy, et al., 1997). 8.2% of first-degree relatives suffer from ankylosing spondylitis, whereas second and third-degree relatives are affected by 1% and 0.7% respectively. While ankylosing spondylitis disproportionally affects men, disease prevalence and severity differs between ethnicities (Braun & Sieper, 2007). For example, 0.1 to 1% of Caucasians suffer from ankylosing spondylitis (Van der Linden, Valkenburg, de Jongh, & Cats, 1984), while the prevalence in China is 0.2 to 0.5% (Ng et al., 2007). However, even in at-risk populations the incidence of AS is seemingly lower in Chinese Han patients, with first-degree relatives being diagnosed in 3.8% and second and third-degree in 0.9% and 0.3% respectively, suggesting protective environmental or genetic factors. Understanding ethnicity-specific underlying predisposing and protective genetic factors may therefore be used to identify at-risk people.

SNPs are the most basic genetic variation in the genome (Hinds et al., 2005), and specific polymorphisms are indeed associated with ankylosing spondylitis or predict disease severity (M. A. Brown, 2008; Seo et al., 2005). Furthermore, conjoined carriage of risk-associated polymorphisms compound into higher disease prevalence. Subtle differences in signal processing in inflammatory pathways between different ethnicities may lead to marked differences in outcomes (Hebert et al., 2012) and complications (Eudy, Vines, Dooley, Cooper, & Parks, 2014). Moreover, these differences are highly attributed to the genetic characters in a different population. However, coming up with a set of SNPs that accurately predicts the future onset of disease has proven difficult. This may in part be due to variation in polymorphism prevalence across populations (Gofton, Robinson, & Trueman, 1966) or environmental factors playing a role in disease development (Gu et al., 2009).

1.2.1 AS-related SNPs in TNFα gene

SNPs in TNFα have been previously reported in many disease settings, including infectious diseases, cancer, and others (**Table 1**). The advent of genetic screening for polymorphisms has also opened the door for pharmacogenomics, a novel branch of medicine that provides therapy personalization based on genetic factors. AS is a poorly controlled chronic progressive inflammatory process, which will continuously inch a patient closer to the development of bamboo spine. Thus, providing patients with the best personalized recommendations on the treatment of their disease is perhaps nowhere a paramount task. Several polymorphisms susceptible to AS, notably a G to A substitution at position -308(rs1800629) and a C to T substitution at position -857 (rs1799724), in the TNFα gene are known to increase the risk for ankylosing spondylitis, and correlate with response to anti-TNFα treatment (Chung et al., 2011; Sousa et al., 2009).

1.2.2 Status of HLA-B27 affect AS

Multiple regions and genes have been implicated throughout various studies and populations. The major histocompatibility complex (MHC) region, located on chromosome 6, encompasses 3.6 megabases including 224 known genes HLA-B27 is an allele of one such gene, and consists of over 105 different subtypes(Khan, 2013). Differences in HLA-B27 prevalence drive most of the variation in prevalence seen worldwide(M. A. Brown, 2009; M. A. Brown, Kennedy, et al., 1997).HLA-B27 is generally present in >90% of AS patients, but in <10% of the general population (3.6 – 5.7% in Chinese Han) (Zeng et al., 2008), making HLA-B27 almost synonymous with autoimmune disease(Brewerton et al., 1973; Khan, 1996). However, of the HLA-B27-positive individuals in the general population, only 5% develop AS, underlining oligogenic pathophysiology(M. A. Brown, 2009). To be sure, HLA-B27 may explain only16% of the overall genetic susceptibility of AS, and the entire HLA region only 40-50%(Braun & Sieper, 2007; Lee, Rho, Choi, Ji, & Song, 2005).

Specifically for Chinese Han patients with AS, HLA-B27 positive patients were 3x more likely to have had acute anterior uveitis (p=0.04), higher C-reactive protein levels (p=0.04) but again no differences in BASDAI or BASFI (Qian et al., 2017). *HLA-B27*-positive patients usually have an earlier disease onset, and are observed more frequently in male patients in our and previous study of Chinese patients, but are not different between genders in other research, suggesting potential racial and/or geographical predilection of the Chinese population(Yang et al., 2013).

1.2.3 Variants of non-HMC genes that affect AS

Several genome-wide association studies (GWAS) have reported SNPs related to AS by genotyping of large cohorts and have identified a number of non-major histocompatibility complex (non-MHC) immune-related risk loci for AS (Australo-Anglo-American Spondyloarthritis et al., 2010). Interleukin (IL)-23R, -12β, -6R, -27 and Tyk2 are components of IL-23-related pathway.IL-23 was known to drive the differentiation of CD4-positive Th17 cells, activation of which generates IL-17. IL-17 can further activate expression and secretion of IL-6, -8, TNF and matrix metalloproteinases(Smith & Colbert, 2014). In SpA, differentiation of Th17/Th1 cells is mediated by the genetic variants at loci in the IL-23 pathway(Coffre et al., 2013).The variant in IL-23R is also related to radiographic sacroiliitis in AS in French population(Kadi et al., 2013). Two aminopeptidases, including endoplasmic reticulum aminopeptidase 1/2 (ERAP1) and (ERAP2) are found to be associated with AS (Robinson & Brown, 2014). ERAP1 contains variants associated with either protect against or susceptible to AS, and these associations are restricted to HLA-B27-positive AS patients (International Genetics of Ankylosing Spondylitis et al., 2013). On the contrary, variants of ERAP2 are more linked with AS in HLA-B27-negative patients (International Genetics of Ankylosing Spondylitis et al., 2013). ERAP1 variants can alter the antigen presentation and stability of HLA-B27, trimming and composition of HLA-B27 ligands (L. Chen et al., 2014). ERAP1, together with HLA-B27, are the most powerful predictors of AS. Haplotype of ERAP1 ERAP2 is related to familial AS, in that the rs2549782 changes specificity and activity of ERAP2, while rs27044 and rs30187 mediate peptide-trimming capacity of ERAP1(Tsui et al., 2010). Other non-MHC loci contributable to AS risk include variations in T-cell lineages (EOMES, RUNX3, ZM1Z1, BACH2 etc.) and those in G-protein-coupled receptors (GPR35, GPR37, GPR65) (Tsui, Tsui, Akram, Haroon, & Inman, 2014). These non-MHC loci were estimated to account for 4.3% of heritable AS.

1.3 Current management of AS

1.3.1 Conventional therapy of AS

Nonsteroidal Anti-Inflammatory Drugs (NSAIDS) such as diclofenac, ibuprofen, naproxen, celecoxib and etoricoxib are recommended as the first line treatment in patients with ankylosing spondylitis which is characterized by alleviating the pain and inflammation response(Vonkeman & van de Laar, 2010). NSAIDs reduce back pain and stiffness in AS patients rapidly which

through inhibiting the production of prostaglandins by blocking the COX enzyme(Sieper & Poddubnyy, 2016). However, NSAIDs have several adverse events in association with morbidity and mortality. It may cause gastrointestinal ulcer bleeding, perforation, and obstruction. Cardiovascular events have close associations with COX-2-selective NSAIDs and the development of hypertension, renal failure, and heart failure need to be considered when short-term and long-term NSAIDs treatment initiate(Vonkeman & van de Laar, 2010). It is therefore unsurprising that the recent availability of anti-TNFα blockers has been widely applied in AS patients, as we are now able to directly target a key driver of pathological inflammation (Braun et al., 2011; Callhoff, Sieper, Weiss, Zink, & Listing, 2015).

1.3.2 TNF inhibitors as medical therapy to treat AS patients

TNF blockers bind to cognate ligands and block their binding to the two TNF receptors. They can act as antagonists by blocking tmTNF interactions with TNF receptors, or as an agonist by initiating reverse signaling, which induces apoptosis, cell activation or cytokine inhibition (Tracey, Klareskog, Sasso, Salfeld, & Tak, 2008). In particular, TNF blockers have been shown to be able to reduce manifestations in effectively. TNF inhibitors, including Etanercept, Infliximab, Adalimumab, Golimumab, and Certolizumab have delivered on that promise; they have shown to attenuate disease progression and improve both function and quality of life (Schett, Coates, Ash, Finzel, & Conaghan, 2011).Etanercept is a TNF-receptor Fc-fusion protein, binding to both TNF and lymphotoxin family proteins (Tracey et al., 2008). It is a fusion protein transcribed by a recombinant DNA fragment. It fuses the soluble TNF receptor 2, which binds to TNFα, to the constant end of the IgG1 antibody (Peppel, Crawford, & Beutler, 1991). Etanercept shares similar efficacy in treating RA with Infliximab and adalimumab but has less power on IBD. Infliximab, adalimumab and golimumab are all full-length bivalent IgG monoclonal antibodies, while certolizumab is a monovalent Fab antibody modified by polyethylene glycol (Tracey et al., 2008).Infliximab and adalimumab are monoclonal antibodies, binding specifically to TNF(Tracey et al., 2008).They share highly similar efficacy in treating RA, PsA, AS and Crohn's disease (Tracey et al., 2008).Infliximab consists of 75% human constant (human IgG1) and 25% murine variable domain (binding site of TNFα). The human tract was designed to decrease immunogenicity while preserving the functional immune capacity(Sandborn & Hanauer, 1999). This calls for pharmacogenomic testing regarding anti-TNFα therapy and drug

metabolism when Infliximab is administered to patients. rhTNFR-Fc is a soluble variant of etanercept and works as a TNFα-decoy (Tong et al., 2012).

However, an unresolved issue remains regarding the variety and uncertainty of individual response toward this type of medicine. This hurdle is required to be overcome as one has to decide whether or not to proceed with the expensive treatment. As such, it is imperative to develop an efficient and reliable method to predict the needs and outcome of this therapy. Previous reports have identified several SNPs with the predictive capacity of susceptibility and severity of SpA and potential response toward TNF blockers. Differences in outcomes and adverse events may be magnified by the characteristics lying between individual anti-TNFα treatment(Radstake et al., 2009).

1.3.2 Pharmacogenomic analysis on the association between SNPs and TNFi therapy

Given the TNF-α plays a central role in the pathogenesis of inflammatory arthritis, TNF inhibitors are effective and widely applied for treatment of Rheumatoid arthritis (RA) and SpA. However, not all patients benefit from anti-TNF-α therapy, and its side effects are still unclear. Currently, patients with inflammatory arthritis have no reliable predictors for TNFi response and -associated toxicity. As advances in pharmacogenomics developing, identification of biomarkers that can predict TNFi response and adverse events contributing to the development of the better clinical decision. Pharmacogenomics is a new study focusing on how genes influence individual response to drugs and related toxicity. The distinctive genetic features may predict the outcome of medications, which provide a helpful way to increase the efficacy and avoid the severe side effects. The pharmacogenomics of TNFi is advancing in the last decade. Single nucleotide polymorphisms (SNPs) is the variant of the gene that can be employed to predict drug responses as well as toxicity. A variety of SNPs especially in TNF-α promoter have been identified to be susceptible for RA and SpA and associated with the response to TNFi. The SNP at position -308,-238 and -857 of the TNF-α gene have been demonstrated to be associated with responsiveness of TNFi in the previous study(Song et al., 2015). However, the results of these studies are often varied due to different racial and ethnic populations. Guis et al. reported that the RA patients carrying the genotype of TNF-α-308 G/G showed a better response to TNFi than those with the genotype of TNF-α-308 A/G (Guis et al., 2007). Seitz et al. have investigated polymorphism at locus -308 of TNFα gene influence upon the response to TNFi in patients with RA and SpA (PsA and AS) (Seitz, Wirthmuller, Moller, & Villiger, 2007). The study revealed

that patients with the genotype of TNFα-308 G/G have better responsiveness to TNFi than those with either A/A or A/G genotypes. Padyukov et al. demonstrated that RA patients with TNF-α-308 G/G and IL-10-1087G/G increase the response to anti-TNFα therapy (Padyukov et al., 2003). Maxweel et al. have demonstrated that RA patients with a genotype of TNFα-308A/A were associated with the poorer response comparing to those with TNFα-308G/G in a soluble receptor (etanercept), but not a chimeric monoclonal antibody(infliximab) treated patients (Maxwell et al., 2008). In contrast, they found that genotype of TNFα-238 G/A was associated with the poorer responsiveness to infliximab rather than etanercept.

Anti- TNFα therapy has been proved to be useful in the management of inflammatory arthritis. However, 30% of patients do not respond to TNFi (Maxwell et al., 2008). Several SNPs seems to influence the response to TNFi. However, a large-scale study is demanded to unveil the associations with SNPs and the responsiveness of biologics. Due to different ethnicity may respond differently to TNFi, the investigations of pharmacogenomics in our Chinese Han patients with TNFi are required.

1.3.3 Current adverse events of anti-TNFα therapy

Long-term use of TNFi in AS remain necessary for patients to sustain remission. However, the safety of prolonged use of TNFi has not been assessed thoroughly in AS(Jani, Dixon, & Chinoy, 2018). The first generation of TNFi is a fully murine immunoglobulin, which induced intensive human anti-murine antibody responses but reduced efficacy. From there, scientist started to engineer chimeric proteins, containing murine variable but human constant regions, such as Infliximab. Further reduction of the murine component resulted in even less immunogenic inhibitors, such as Certolizumab pegol. Later, people can manage to produce fully humanized antibodies, such as Adalimumab and Golimumab. Although more humanized antibodies are expected to trigger the less immune response, Adalimumab and Golimumab are still doing so. Besides, the complicated manufacturing process may introduce subtle differences beyond the detection limit, and thus alterations in the biological function and immunogenicity of the produce (Dorner et al., 2013).

The major adverse event of TNFi is infections. TNF recruits leukocytes to infected tissues, resulting in activation of macrophage and dendritic cells and hence induces innate immunity or regulating acute-phase response(Waters, Pober, & Bradley, 2013).TNFi leads to growing risks of

infections (including serious infections) in patients with RA (Michaud, Rho, Shamliyan, Kuntz, & Choi, 2014). Regarding AS, there have not been any large longitudinal studies to investigate the side effects of TNFi. One clinical trial reported no difference lying between patients on TNFi and those not treated with TNFi (Wallis, Thavaneswaran, Haroon, Ayearst, & Inman, 2015). However, this result may be due to a lack of statistical power. Tuberculosis (TB) is one of the infectious diseases to which AS patients on TNFi therapy are susceptible. This is because TNF, by interacting with interferon (IFN)-γ, plays a vital role in the host defense system against TB(Mootoo, Stylianou, Arias, & Reljic, 2009). RA patients on TNF blockers exhibited an elevated risk of TB compared to those on classical Disease-modifying antirheumatic drug (DMARDs)(Ai et al., 2015). The incidence of TB also differs from the kind of TNF blockers in use. For example, a much higher chance of TB is found in patients on monoclonal antibodies Infliximab and Adalimumab than those on Etanercept(Ai et al., 2015). Unfortunately, fewer publications are available regarding AS on this term. Surveys in the country with an increased burden of TB did show an increased prevalence of TB in AS patients treated with TNFi (Gomes et al., 2015). In addition, six randomized controlled trials, involving 3,886 patients with RA, reported opportunistic infections(Minozzi et al., 2016). As TNF is essential to the clearance of HBV-infected hepatocytes and HBV virus, TNF blockers may in turn enhance the chance of reaction in resolved HBV/occult HBV infection, or ameliorate the active disease (Valaydon et al., 2016).

Other medical concerns following TNFi administration are summarized as follows. Because TNF regulates cell growth and death, tumor malignancies are another major concern in patients on long-term use of TNF blockers. Several publications reported an increased risk of malignancy induced by TNF inhibitors in patients with RA (Bongartz et al., 2006), but none in those with AS (Hellgren et al., 2014).TNFi induces cell apoptosis, which inevitably leads to autoantigens release and less efficient clearance of nuclear debris, resulting in the formation of autoantibodies (Gonnet-Gracia et al., 2008). Induction of antinuclear antibody was found in 52% ~ 87% AS patients treated with Infliximab, comparing to 3.3% ~ 27.1% in those at baseline, whereas anti-dsDNA antibodies were found in 2% ~ 40% (Wronski & Fiedor, 2019).Thus, autoimmune diseases, such as lupus-like syndrome and systemic lupus erythematosus, become a class of adverse events of TNFi (Perez-De-Lis et al., 2017). Discontinuing TNFi medical therapy can effectively reverse the disease (Jani et al., 2017). Infliximab was also shown to increase the risk

of heart failure in elderly RA patients (Setoguchi et al., 2008). Paradoxical adverse events, such as uveitis, psoriasis, or IBD, have been reported in AS patients on TNF blockers. Usually, these extra-articular events were treatment targets of TNFi in SpA. However, their situation could become worse by this group of inhibitors (Van der Heijde et al., 2017). One cohort study suggested that etanercept may increase the occurrence of uveitis flares in AS patients (Lie et al., 2017). Etanercept has also been related to the incidence of sarcoidosis when treating RA patients(Perez-De-Lis et al., 2017).On the other hand, more than two hundred cases of TNFi-triggered psoriasis occurred in Crohn disease, RA, and AS(G. Brown et al., 2017), probably due to the disequilibrium in cytokine balance(Seneschal et al., 2009). This mechanism also explains for the observation that etanercept triggered IBD, a rare paradoxical side effect, in patients with AS(Braun et al., 2007).

1.4 Rationale and design of the study

1.4.1 Rationale

The AS disease is characterized by a delay in diagnosis, as initial symptoms are usually dismissed as transient ailments by young patients maintaining an active lifestyle (Tam, Gu, & Yu, 2010). However, most of the radiographic progression occurs within the first decade of disease onset. Thus, significant disease progression can occur insidiously, with radiographs initially revealing irreversible erosive changes at the corners of the vertebral bodies and later the development of syndesmophytes. Ultimately, these syndesmophytes fuse with adjacent vertebral bodies to form a rigid, calcified spine, called bamboo spine (Tam, Gu, & Yu, 2010).

Considering the insidious onset of AS and the irreversible damage it causes, screening and early detection are of paramount importance to prevent disease progression and pain,as such, identifying at-risk patients before the onset of disease would mitigate the delay in both diagnosis and start of treatment. This in turn will improve treatment outcomes as currently the best predictor for disease progression is disease severity at the time of presentation (Gran & Skomsvoll, 1997).

Previous studies identified a group of genomic features that are associated with the TNFi response and adverse events of AS in the Caucasian population. However, several issues regarding the pharmacogenomics of TNFi in Chinese AS patients remained to be resolved: 1) The validation of previously identified SNP loci has not been conducted in Chinese Han patients

with AS. The question is whether they are associated with AS in Chinese population; 2) The genetic feature may result in different responses toward TNFi approach. Nevertheless, there has not been a comprehensive study to profile the potential response in Chinese Han patients with AS. Besides, it is not clear whether polymorphisms of TNFα gene are of predictive value for the TNFi treatment response; 3) The adverse events of TNFi monotherapy have not yet been reported in a large cohort of Chinese Han patients with AS., And we are not able to predict adverse events based on current genetic features. Identifying the predictors of the occurrence of adverse events in AS patients treated with TNF blockers could provide potential clues for clinical decision. Driven by these unresolved issues, we hypothesized the polymorphisms in the TNFα gene have a close association with the efficacy and safety of anti-TNF therapy. Hence, our aim is to explore the genetic indicators that can pre-determine TNFi efficacy and the side effects in AS patients. To achieve this goal, we conducted the current pharmacogenomic study.

1.4.2 Study design

Our first study recruited 149 AS patients and 106 healthy control. We collected peripheral blood and isolated genomic DNA, which is subjected to genotyping. Total 28 SNPs in 14 genes that were previously reported to be related to ankylosing spondylitis in non-Chinese Han populations were analyzed for their associations with AS in Chinese Han patients. In our second study, we examined whether polymorphisms in promoter of TNFαat -238, -308, -857, -1031(**Figure 1**) are associated with the outcomes in Chinese Han AS patients (99 patients) when treated with infliximab and rhTNFR–Fc, by stratifying patients into good, poor, and no response groups. We furtherly performed a meta-analysis to confirm the predictive value of identified SNPs indifferent ethnicities and cohorts. Based on the information from our cohort, we also profiled the detailed side effects of anti-TNF monotherapy in Chinese Han AS patients and followed-up short- (402 patients) and long-term (172 patients) adverse events. Simultaneously, because we are curious to investigate whether AS-associated TNFα SNPs are related to the adverse effects, each of the SNP was analyzed for its relationship to the occurrence of side effects of anti-TNF therapy. The detailed study design and results are described in Section 2-5 as follows. We also evaluated whether biomarkers like erythrocyte sedimentation rate and C-reactive protein which have demonstrated prognostic value in non-Chinese populations (Ingegnoli, Favalli, & Meroni, 2011; Lee, Ji, Bae, & Song, 2010; Pavy et al., 2010; Seitz et al., 2007) are predictive in Chinese Han patients too.

2. IDENTIFY ANKYLOSING SPONDYLITIS-ASSOCIATED SNPs IN CHINESE HAN PATIENTS WITH AS (unpublished)

2.1 Methods

2.1.1 Patient recruitment for AS-associated SNPs in Chinese Han patients with AS

The clinical criteria according to 1984 modified New York Criteria for AS includes 1) limitation of motion of the lumbar spine in all 3 planes; 2) a history of pain or the presence of pain at the (dorso)lumbar junction or in the lumbar spine; 3) limitation of chest expansion (relative to normal values corrected for age and sex (Van der Linden, Valkenburg, & Cats, 1984). To diagnose AS, one has to meet the following criteria: 1) grade 3-4 bilateral sacroiliitis associated with at least 1 clinical criterion; or 2) grade 3-4 unilateral or grade 2 bilateral sacroiliitis associated with clinical criterion 1 or with bother clinical criteria 2 and 3 (Van der Linden, Valkenburg, & Cats, 1984). One hundred forty-nine Chinese Han patients diagnosed with ankylosing spondylitis using the American College of Rheumatology and Modified New York criteria, as well as 106 healthy age-, gender- and ethnicity-matched controls were enrolled into this study at Chang Hai Hospital, Shanghai. The institutional review board of Changhai Hospital (Shanghai, China) approved this study. After written informed consent was obtained, 10 mL of blood was collected via venipuncture.

2.1.2 Genotyping of polymorphisms

Blood was collected in ethylenediaminetetraacetic acid coated tubes and stored at -80°C. Samples were digested overnight using 0.5 mg/mL Proteinase K on a 10% SDS gel at 37 °C. DNA was extracted by using a salting out procedure and isopropanol precipitation. Regions of interest were amplified by polymerase chain reaction. Genotyping was performed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry and MALDI-TOF mass spectrometry technique. **Table 2** lists investigated SNPs in the ankylosing spondylitis-associated SNP experiment.

2.1.3 Statistical analysis

Genotypes frequencies were estimated by direct counting and are reported in percent. A χ^2test was applied to test whether genotypes were in Hardy–Weinberg equilibrium. A p-value equal to or smaller than 0.05 was considered significant. The interactions between the protein products of

significant SNPs were investigated with STRING (http://string-db.org), with medium level confidence (0.400).

2.2 Results

Multiple genomic regions and genes have been implicated throughout various studies and populations, including European, African, and Asian. **Table 3** details the demographics of patients and controls. Twenty-eight SNPs in 14 genes that are previously known to be associated with non-Chinese Han AS patients (M. A. Brown, Kenna, & Wordsworth, 2016; International Genetics of Ankylosing Spondylitis et al., 2013; Reveille, 2012; Sims et al., 2007; Wellcome Trust Case Control et al., 2007)were genotyped. **Table 4** listed SNPs significantly associated with AS, while **Table 5** showed those are not. While FOXP3- and IL23R-associated SNPs were reported in non-Chinese Han patients, there were no associations found in our cohort for rs2232367, rs28935477 andrs11465804. However, in our cohort, HLA-associated SNPs were found to be correlated, mainly HLA-B27tagged (rs4349859).

Table 6 shows an overview of currently known AS-associated SNPs and **Figure 2** on how their genes and protein products might interact. There are a few common genes contributing to the pathogenesis of AS in Chinese Han and Europeans, such as IL-12B, IL-23R, ERAP1, and TNFRSF1A, although the variant loci are different between these two populations. Analyzing through STRING database, we found that genes containing those AS-associated genetic variants indeed have close interactions with each other, suggesting they collaborate to regulate immune functions.

2.3 Discussion

AS is an autoimmune disease with a strong genetic component and a variable penetration of causative genes in various populations. SNPs associated with AS are found in genes that have a complex interaction involving antigen mal-presentation, pro-inflammatory cytokines and auto-destructive T-cells. We demonstrated the association of SNPs in the *TNFRSF1A, IL-23R, HLA-B27* and *TNFα* genes in Chinese Han patients, in an attempt to further elucidate AS pathophysiology, improve diagnostic tools, provide treatment-guiding insights and aid in genetic counseling.

The increased serum *TNFα*levels in AS patients are of great clinical significance as they parallel pain, fatigue, joint swelling, morning stiffness and disease progression (McLeod et al., 2007;

Xueyi et al., 2013). The advent of monoclonal antibody anti-*TNFα* treatment represents a paradigm shift in rheumatology. The *TNFα*-308 SNP could even be a possible predictor of Etanercept treatment efficacy (Chen, 2017; Tong et al., 2012). The SNP rs4149577 in the *TNFRSF1A* gene was associated with AS in both Caucasian and Chinese Han patients (Davidson et al., 2011; Karaderi et al., 2012), and was confirmed in this study. SNPs rs1799724 and rs1800629 in the *TNFα*gene were reconfirmed in Chinese Han AS patients (**Table 7&8**)(Sun, Huang, Zhang, & Liu, 2013; Zhu et al., 2007).

Considering the central role that the *TNFα* pathway plays in inflammation, it is not surprising that several SNPs in the ligand and receptor have been associated with AS (Davidson et al., 2011). However, as important *TNFα* may be, the stimulus for *TNFα* induction remains unknown, and the downstream inflammatory pathways are incompletely understood (Feldmann & Maini, 2008; Tracey et al., 2008). One putative mechanism through which *TNFα*, *IFN-α*, and *IFN-γ* may contribute to AS pathophysiology is their capacity to activate the *HLA-B27* promoter (Feng, Ding, Fan, & Zhu, 2012). Another possible mechanism is through the induction of auto-destructive *CD4*+T cells and *CD8*+T cells (Duftner et al., 2003; Rudwaleit et al., 2001; Schirmer et al., 2002). Additionally, white blood cells in the sacroiliac joints of AS patients express high levels of *TNFα*(Braun et al., 1995), but anti-*TNFα*treatment does not reduce bone erosion allowing syndesmophyte formation to continue. Therefore, the interplay with other cytokines needs to be considered (Van der Heijde, Landewe, Baraliakos, et al., 2008; Van der Heijde, Landewe, Einstein, et al., 2008; Van der Heijde et al., 2009).

Here we have confirmed the association with the rs4349859 SNP in the HLA-B27 gene in Chinese Han patients, as was previously demonstrated in other populations (Evans et al., 2011). Subsequently, non-HLA SNPs have been mapped. These non-HLA SNPs are of pivotal importance to disease pathophysiology as is illustrated by Gambian Fula populations, where HLA-B27 carriage is 6% but AS does not occur (M. A. Brown, Jepson, et al., 1997).

The *IL-23R* is a hemopoietin type receptor with affinity for its proinflammatory namesake. The SNPs rs11209032, rs11209026 and rs11465817 are associated with AS in Caucasian populations (Australo-Anglo-American Spondyloarthritis et al., 2010; Burton et al., 2007; Karaderi et al., 2009), but not Asian populations (Chen, Zhang, Li, & Wang, 2012; Davidson et al., 2009; Lee, Choi, Ji, & Song, 2012; Qian et al., 2013). However, conflicting results have been reported for

SNPs rs11209032 and rs6677188 in Chinese Han patients in other reports (Wang et al., 2010). We reported that an SNP on rs1004819 is significantly associated with Chinese Han AS patients. Overall, the population-attributable risk associated with *IL-23R* is approximately 1% (Australo-Anglo-American Spondyloarthritis et al., 2010; Burton et al., 2007).

Activation of the *IL-23* receptor induces Signal transducer and activator of transcription 3 (*STAT3*) phosphorylation and thereby the production of *IL-17*, *IL-6*, *IL-8*, and *TNFα*, thus amplifying and stabilizing γδT cells and *CD4*+Th-17 autoimmune lymphocytes (Kenna et al., 2012; McGeachy et al., 2009; McKenzie, Kastelein, & Cua, 2006; Trinchieri, Pflanz, & Kastelein, 2003). The association of multiple SNPs in the genes involved in the pathogenic Th-17 cell pathway, including *IL-23R*, *STAT3*, and *IL-12B* (an *IL-23* subunit), suggests this is an essential pathway in AS. Indeed, serum levels of *IL-23* (Xueyi et al., 2013), *IL-17* (Mei et al., 2011), and *IL-1B* are elevated in AS patients. Furthermore, *IL-23* and *IL-17* serum levels increase upon binding of prostaglandin E2 to the prostaglandin E-4 receptor. SNPs in the prostaglandin E-4 receptor gene, *PTGER4*, have been associated with AS (Evans et al., 2011). Non-steroidal anti-inflammatory drugs slow heterotopic ossification and radiographic progression in AS (Baird & Kang, 2009; Poddubnyy et al., 2012), thereby underlining the importance of cytokines and pro-inflammatory autoimmune T-cells in AS pathophysiology.

SNPs in the *ERAP1* and *ERAP2* genes have been robustly associated with *HLA-B27*-positive AS in both Caucasian and Chinese Han patients. *ERAP1*trims peptides to optimal length in order that they can be presented by MHC class I molecules within the endoplasmic reticulum (Saveanu et al., 2005). Because of this function, it is presumed that SNPs in these genes lead to aberrant processing and presentation of antigenic peptides. The population-attributable risk associated with *ERAP1* is 26% (Australo-Anglo-American Spondyloarthritis et al., 2010; Burton et al., 2007). Combined *HLA-B27* and *ERAP* mutations are predictive of increased disease severity (Szczypiorska et al., 2011). Additionally, the SNP rs27037 is predictive of syndesmophyte formation in AS, suggesting subtype of AS may exhibit unique SNP characters that may be omitted when the population was mixed up (Wang et al., 2012).*ERAP1* also sheds the *TNFα*, *IL-1* and *IL-6* receptors off of the cell surface (Cui, Rouhani, Hawari, & Levine, 2003b). These receptors subsequently become cell-free, act as decoys and by less availability of respective receptors on the cell surface increase cytokine activity (Cui, Rouhani, Hawari, & Levine, 2003a;

Thomas & Brown, 2010). The clinical importance of mechanism is still under investigation. However, our results did not find associations between SNPs of ERAP1 and incidence of AS. This controversy might attribute to the limited sample size and insufficient statistical power.

In particular, the results showed that these genes are associated with defense response regulation/immune system process (*e.g.CARD9(Ruland, 2008),ERAP1(Alvarez-Navarro & Lopez de Castro, 2014), HRAS(Castellano, De Las Rivas, Guerrero, & Santos, 2007),PTPN22(Bottini & Peterson, 2014),RAF1(Odabaei et al., 2004)*) and osteoclast differentiation (*e.g.TNF(Martin, Romas, & Gillespie, 1998), IL-12B(Martin et al., 1998), PPARGC1B(Ishii et al., 2009)*), suggesting AS-related SNPs are extensive distributed in immunoregulatory and developmental related pathways. Subsequently, non-HLA SNPs have been mapped. These non-HLA SNPs are of pivotal importance to disease pathophysiology as is illustrated by Gambian Fula populations, where *HLA-B27* carriage is 6% but AS does not occur (M. A. Brown, Jepson, et al., 1997).

As illustrated by the gene polymorphism and associated gene-protein networks among susceptible genes we found and summarized from previous studies, the protein products of *TNFα* and *IL-23R* genes play a dominant role in a predictive network. Interestingly, evidence of anti-*TNFα* therapy presents a solid treatment for the patients with AS, and also new compounds that target the *IL-23* receptor may provide a potential opportunity for advances in the treatment of ankylosing spondylitis.

Overall, the etiology of AS is complex and only partly understood. SNP findings provide limited clues on the pathogenesis of AS, and the analysis of associated protein products advances the development of biologics and small molecules that specify on these predominant cytokines. The relevant lines of further investigation are to integrate with the detailed biology of the risk factors, especially with respect to how these risk factors may affect the pathogenic process and clinical outcome.

3. THE STUDY OF TREATMENT RESPONSE-PREDICTIVE SNPs IN CHINESE HAN PATIENTS WITH AS

3.1 Methods

3.1.1 Patient recruitment

One hundred six Chinese Han patients identified from the Changhai Hospital Ankylosing Spondylitis database who were diagnosed with ankylosing spondylitis using the Modified New York criteria (Van der Linden, Valkenburg, & Cats, 1984), as well as 106 healthy ethnicity-matched controls were enrolled into this study. Seven patients were lost during follow-up. The institutional review board of Changhai Hospital (Shanghai, China) approved this study. Exclusion criteria for this study were the use of disease-modifying anti-rheumatic drugs in the last three months, as well as a diagnosis of any chronic infectious diseases, cancer, hepatic or renal dysfunction, hematological and cardiac conditions or multiple sclerosis. After written informed consent was obtained, patient demographics and disease pertaining information was collected using the Assessment of SpondyloArthritis International Society (ASAS) criteria and the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)(Anderson, Baron, van der Heijde, Felson, & Dougados, 2001; Garrett et al., 1994). Four components were included in the "ASAS 20 improvement criteria", namely patient global, pain, function (assessed by BASFI), and inflammation. When three of these four essences are improved by $\geq$ 20% and a minimum of one unit on a scale of 0 to 10, a patient meets an ASAS 20 response. In the remaining component, no worsening of 20% and a minimum of 1 unit, on a 0 to 10 scale, should be met(Anderson et al., 2001). Similarly, ASAS 40, 50, and 70 response respectively require the improvement to be no less than 40%, 50% and 70%, for three out of four components, and no worsening than 40%, 50% and 70% in the remaining one(Brandt et al., 2004). BASDAI covers six questions on fatigue, spinal pain, peripheral joints, entheses, intensity of morning stiffness, and duration of morning stiffness(Garrett et al., 1994). A numerical rating scales (NRS) or a 10 cm visual analog scale (VAS) is used for scoring each question. The final BASDAI score is derived from the summation of the first four questions and the mean value of the last two questions, after dividing by five. The scale of the final score is 0 (no disease activity) to 10 (very active disease). To define active disease, the frequently used cutoff is 4.

3.1.2 Genotyping of polymorphisms

Blood was collected in ethylenediaminetetraacetic acid coated tubes and stored at -80°C. Samples were digested overnight using 0.5 mg/mL Proteinase K on a 10% SDS gel at 37 °C. DNA was extracted using a salting out procedure and isopropanol precipitation. Regions of interest were amplified by polymerase chain reaction. Genotyping was performed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry and MALDI-TOF mass spectrometry technique. **Table 9** shows the primer sequences for TNFα genotypes -238 (rs361525), -308 (rs1800629), -857 (rs1799724) and -1031 (rs1799964) used in the experiment investigating TNFα SNPs to treatment outcome.

3.1.3 Clinical Evaluations

Patients were evaluated in the end of 12 weeks of treatment by using the ASAS-score. This evaluates patients based on global, pain, function and inflammation. Patients were deemed non-responders if no improvement was shown based on their baseline ASAS-score. Patients were deemed good responders if they achieved a global improvement of ⩾40, 50and 70%, along with a ⩾2-point improvement in pain, function and inflammation and a maximum of 1-point deterioration in other the final category. All other patients were deemed poor responders. Positive cut-off points of biochemical parameters include an erythrocyte sedimentation rate of 20 mm/h in men and 15 mm/h in women and a C-reactive protein of 8 mg/L. The HLA-B27 titers were quantified. Disease activity and functionality were assessed using the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (Garrett et al., 1994) and Bath Ankylosing Spondylitis Functional Index (BASFI) (Calin et al., 1994).

3.1.4 Statistical analysis

Clinical comparisons were performed in SPSS v18 using the student's T-test for continuous normal distributed variables, the Wilcoxon's Rank Sum test for continuous non-normal distributed variables and the $\chi 2$ test was used for categorical variables. Genotype-response association analysis was also performed using the $\chi 2$. A p-value of 0.05 or smaller was considered significant.

3.2 Results

3.2.1 Demographics

Table 10 details the demographics of the patients in this clinical cohort. The average age was 41 ± 16 years with a male predominance (78%) and an average disease duration of 9.5 ± 9 years.

Symptoms started with inflammatory backpain or alternating buttock pain in 55% and peripheral involvement (e.g., enthesitis, dactylitis, arthritis and acute anterior uveitis) in 45%. HLA-B27 carrier status was found in 92%. The average C-reactive protein was 35 ± 19 mg/L. Among all subjects, 91.92% were*HLA-B27*-positive. The mean value of erythrocyte sedimentation and CRP were 36 ± 17 and 35 ± 18mg/dl. On the other hand, patients who were administered with infliximab and rhTNFR–Fc took 28% and 71% respectively. Baseline patient characteristics did not differ regarding predictive SNPs per type of therapy.

3.2.2 AS-related TNFα SNPs in patients versus controls

Table 7 details the demographics of patients and controls regarding the 4 TNFα SNPs investigated with this cohort. For each allele, these four SNPs were compared between AS and healthy controls. All genotype distributions were in Hardy-Weinberg equilibrium. In elevated frequency of G/G phenotype was observed for TNFα -308 polymorphism in AS group (p = 0.0093). However, *TNFα*-238 and *TNFα*-1031 polymorphisms were not significantly different from each other. AS patients showed a significantly distinct frequency of *TNFα*-857 (C/T genotype) than the control group (p = 0.00001). Specifically, T allele is the predominant genotype in Chinese Han AS population ($p = 2.455 \times 10^{-7}$). Additionally, the patient cohort was enriched for the G/G genotype at the -308 position as well as the C/T and the T genotype at the -857 position.

3.2.3 Predictive TNFα SNPs in TNFi non-responders versus responders

Table 8 details a comparison between non-responders and responders (ASAS $\leqslant$ 20 versus ASAS $\geqslant$ 40) for all four SNPs of interest. Different genotypes present a different level of response toward anti-TNF treatment. Specifically, at locus TNFα -857 rs1799724, patient with genotype C/C (n=1), genotype T/T (n=3), and genotype C/T (n=4) exhibited no response at all. Similarly, at locus TNFα -1031 rs1799964, patient with genotype C/C (n=3), genotype T/T (n=3), and genotype C/T (n=2) responded at ground level. Moreover, at locus TNFα -308 rs1800629 (p=0.602) and TNFα -238 rs361525 (p=0.426), G/G genotype (n=8), A/G genotype (n=1), and G/G genotype (n=7), respectively, were not responding to the therapy. In summary, TNFα SNPs investigated in this cohort did not differentiate between non-responders and responders.

3.2.4 Predictive TNFα SNPs in good responders versus not-good responders

Table 11 details a comparison between responders and the remaining patients (ASAS ⩾ 40 versus < 40) for all four SNPs of interest. The response level and quality cannot be predicted solidly from the allelic polymorphism at *TNFα*-308 rs1800629 and *TNFα*-238 rs361525. At *TNFα*-857 rs1799724, however, a good response can be foreseen (χ^2 = 12.285; *p*-value equals to 0.0021). In addition, at *TNFα*-857 rs1799724, a genotype of C/C in Chinese Han AS patients exhibited a better response toward *TNFα*blocker, comparing to those with genotypes of C/T or T/T. Besides, a good response (χ^2 = 15.645; *p*-value equals to 0.0004) and treatment outcome could also be predicted with the polymorphism at *TNFα*-1031 rs1799964 locus by bearing a T/T genotype rather than a C/T or C/C genotypes. Furthermore, another locus at -875 of TNFα can facilitate the prediction of good response (χ^2 = 10.488; *p*-value equals to 0.0012). Lastly, T allele at -1031 position exhibited effective response as well in Chinese AS patients (χ^2 = 7.473; *p*-value equals to 0.0063). Overall, polymorphisms at -857 with a C/C genotype responded well to treatment, while C/T or T/T genotypes did not. Polymorphisms at -1031 with a T/T genotype responded well to treatment, while C/T and C/C did not. The C-allele at -857 and the T-allele at -1031 predicted good treatment response.

3.3 Discussion

Remarkable variations occur in response to treatment in Chinese Han AS population. Emerging evidence, although not conclusive, suggest that certain biomarkers can predict the response to anti-TNFα therapy.

The TNFα protein is a potent pro-inflammatory cytokine and immune modulator of joint destruction, and *TNFα*is possibly considered as a risk factor for developing SpA (Romero-Sanchez et al., 2012). *TNFα*stimulates the expression of adhesion molecules and increases neutrophil activation. Polymorphisms at *TNFα*gene was shown to be the predictor of the response toward *TNFα*blocker treatment in Caucasian AS population (Seitz et al., 2007). A number of studies have reported that *TNFα*gene transcription is regulated by its polymorphisms, and in most cases the polymorphisms reduced the gene expression and lead to a more significant of *TNFα* suppression (Kooloos, Wessels, van der Straaten, Huizinga, & Guchelaar, 2009; Lv, Chen, Cai, Fang, & Sun, 2006; Marotte, Arnaud, Diasparra, Zrioual, & Miossec, 2008). Subsequently, several studies reported contradictory results: Six showed that *TNFα*-308 A/G

polymorphism are associated with *TNFα*blocker treatment in RA for both Western and Asian people(Aguillon et al., 2006; Guis et al., 2007; Maxwell et al., 2008; O'Rielly, Roslin, Beyene, Pope, & Rahman, 2009; Plant et al., 2011), whereas three studies found that *TNFα*-308 G/G polymorphism and variants spanning type 2 TNF receptor (TNFR2) and TNF cleavage enzyme (TACE) genes are not related to TNFi response(Potter et al., 2010; Soto et al., 2011; Suarez-Gestal, Perez-Pampin, Calaza, Gomez-Reino, & Gonzalez, 2010). This inconsistency might be a consequence drove us to initiate this study in Chinese AS patients.

The availability of effective anti-*TNFα*treatment has been challenged with enormous economic and social value. To identify the predictors for *TNFα* blocker responsiveness is highly valuable, due to the high medical cost and the possibility of unknown side effects. Two distinct characters lies in our study: 1) we are the first study to identify TNFi response-associated SNPs in Chinese Han AS patients; 2) the inhibitors provided to our patients include infliximab and rhTNFR-Fc (product name: YiSaiPu), a biosimilar of etanercept produced by Shanghai CP Goujian Pharmaceutical Co. in China and a most widely prescribed TNFi due to insurance coverage by Chinese government. However, the pharmacogenomics of rhTNFR-Fc has never been investigated in AS patients.

Two polymorphisms, namely TNF -308 G/A and -857 C/T, are the most commonly studied ones for assessing response toward anti-TNFα therapy. Our research failed to recapitulate the predictive value of -308 SNP but confirmed that -857 C/C genotype could predict good response. This is in contrary to a previous study, which showed -308 G/G phenotype exhibiting a better response to anti-TNF therapy(Seitz et al., 2007). Such inconsistency might be a result of inadequate sample sizes and statistical power. To overcome these issues, we performed a meta-analysis to increase the population size while reducing the random errors that may yield false-positive or -negative results. Although a few meta-analyses have examined the relationship between effects of TNF blockers and TNF polymorphism in rheumatoid arthritis (Lee et al., 2010; Pavy et al., 2010; Seitz et al., 2007; Zeng et al., 2013), less is known in SpA. Hence, our present research provided the first evidence regarding this issue.

4. META-ANALYSIS OF TNFα SNPs PREDICTING ANTI-TNFα TREATMENT RESPONSE IN PATIENTS WITH SPA AND IBD

4.1 Methods

4.1.1 Meta-analysis of TNFi-associated SNPs in SpA and IBD

We performed a meta-analysis for original studies reporting associations between single nucleotide polymorphisms at both -308 and -857 of the TNFα-gene and outcome of anti-TNFα treatment. A search using PubMed and Elsevier's Science Direct was performed for publications as recent as February 2013 using the following entry: "TNF-α OR TNFα OR Tumor necrosis factor OR TNF blocker ORTNF therapy OR infliximab OR etanercept OR adalimumab OR polymorphism 857 OR polymorphism308 OR spondyloarthritis OR SpA OR ankylosing spondylitis OR psoriatic arthritis OR Crohn's disease OR enteropathic arthritis OR PsA OR IBD". No filters were applied. References to suitable studies within the citations of publications matching to our query were included in the meta-analysis. When necessary investigators were contacted for missing information or additional data.

4.1.2 Statistical analysis

RevMan 5.0 was used in the meta-analysis. Dichotomous data were reported as the odds ratio and a 95% confidence interval. A p-value equal to or smaller than 0.05 was considered significant. A Z-test determined the significance of the pooled odds ratios. We evaluated within- and between-study heterogeneity using the Cochran's Q-statistic (Zeggini & Ioannidis, 2009) with a p-value equal to or smaller than 0.10 indicating heterogeneity across studies. Furthermore, we quantified the effect of the heterogeneity using a I^2. 25% was considered low, 25% was considered moderate and 75% high. A fixed effects model was applied using the Mantel–Haenszel method for non-heterogeneous results, otherwise a random effects model was applied using the Der Simonian and Laird method (Zeggini & Ioannidis, 2009). Publication bias was assessed using a funnel plot.

4.2 Results

4.2.1 Detailed description for studies included for meta-analysis

Because AS and IBD share similar immunologic alteration and same epitome, and previous studies have found that there are many common susceptible genes to both diseases, we investigated these two diseases together in our meta-analysis. **Table 12**and **Figure 3** detail the 6 studies that met the inclusion criteria for the meta-analysis (Duricova et al., 2009; Louis et al.,

2002; Seitz et al., 2007; Tong et al., 2012; Vasilopoulos et al., 2012; Vermeire et al., 2000), which include a total of 211 spondyloarthropathy patients (SpA) (121 ankylosing spondylitis and 90 psoriasis / psoriatic arthropathy) and 392 inflammatory bowel disease (IBD) patients. Depending on the study, patients were evaluated after 30 days to 6 years. Infliximab monotherapy was applied in three studies, whereas the remaining three studies treated with either etanercept or adalimumab. Our meta-analyses demonstrated no heterogeneity among studies ($p>0.1$). Therefore the fixed effects model was applied. Publication bias was not detected for either polymorphisms meta-analyses. Five independent research groups examined the association of -308 A/G polymorphism at TNFα promoter in one hundred thirty-one SpA patients and three hundred nighty two IBD patients. Among these reports, merely three of them successfully recapitulated the association between -857 C/T genotype and response toward *TNFα*blockers in one hundred seventy-nine SpA patients and one hundred and one IBD patients.

4.2.2 SNPs of TNFα -308 and -857 predict the TNFi response

The TNFα-308 A/G polymorphism was investigated by five studies, including 131 spondyloarthropathy and 392 inflammatory bowel disease patients. The TNFα-857 C/T polymorphism was investigated in three studies, including 179 spondyloarthropathy and 101 inflammatory bowel disease patients.

Other than A/G variants at TNFα-308 position, we also compare the genotypes between G/G and (A/A+A/G), (A/G+G/G) and A/A. The comparison illustrated both the dominant and recessive aspects of the model in the G allele. **Table 13** lists the results of the -308 polymorphisms meta-analysis. G allele was associated with better treatment response over the A allele, as did the G/G genotype over the G/A or A/A genotypes. Because inadequate data was included, a funnel plot was not able to be depicted for assessing publication bias. With a p-value greater than 0.1, the studies included seem to have little heterogeneity. Therefore, the fixed model was valid for utilization. G allele, according to the OR (2.14) and 95% CI range (1.38-3.33) as well as p-value (0.0007), suggested a better response than that of A allele. On the other hand, in patients treated with TNFα blocker, G/G genotype seems to receive a better outcome than that of G/A+A/A genotype, evidenced by OR equals 2.31, 95% CI ranges from 1.36 to 3.91, and p-value equals 0.002. There is no difference between A/A genotype and A/G+G/G. See **Figures 4-6** for details.

In addition to TNFα-308 A/G, our study also compared the allelic effect between several pairs, including C and T, C/C and C/ T+T/T, C/C+C/T and T/T. Because inadequate data was included, a funnel plot was not able to be depicted for assessing publication bias. With a p-value greater than 0.1, again, the studies related to TNFα -857 C/T included seem to have little heterogeneity. With an OR equals 2.17, 95% CI ranges from 1.17 to 4.03, and a p-value of 0.01, C allele exhibited a significant between response toward TNFα blocker than T allele. Moreover, T allele bears a significantly lower OR value (3.66) in the responder group. C/C+C/T is not significantly different than T/T genotypes. **Table 14** lists results for the -857 polymorphism meta-analysis. Here, the C allele was associated with a better treatment response over the T allele, but the C/C, C/T and T/T genotypes were not predictive. See **Figures 7-9** for details.

4.3 Discussion

The present meta-analysis suggests that *TNFα* gene polymorphisms at the -308 and -857 positions could predict therapeutic response with *TNFα* blocker, several clinical aspects remain to be clarified. Only six studies met the selection criteria which were included in our research, many more studies of different ethnic populations are needed to detect the association between *TNFα* polymorphisms and responsiveness to *TNFα* blocker. Meanwhile, sophisticated studies dissecting the co-function of *TNFα*-308, -857 and other genes with response to anti-*TNFα* therapy are required in large details. In the future, pharmacogenomics will be widely utilized in SpA and IBD with *TNFα* blocker for personalized medication. The -308 G/G genotype and/or -857 C/C genotype could guide us to select appropriate patients for anti-*TNFα* therapy. Surprisingly, an independent cohort study performed by us in the Chinese Han population showed that genetic polymorphisms at -308 and -238 of *TNFα* gene are not able to predict therapeutic responses toward anti-*TNFα* therapy. In contrast, either *TNFα* -587 C/C or -1031 T/T alone turned out to be useful predictors of good response. Combining both did improve the prediction efficacy. Our study emphasizes the fact that population-specific predictors shall be developed to account for precise medical treatment.

Our meta-analysis revealed that for both *TNFα*-308 or -857 polymorphisms, the common allele (G and C, respectively) showed a better response to the *TNFα* blocker than the minor allele (A and T, respectively). In addition, it also showed a significant difference between the minor allele carrier (GA+AA and CT+TT) and non-carrier states (GG and CC). Among the five studies

detecting the association of *TNFα*promoter -308 A/G polymorphism with responsiveness to *TNFα* blocker, only Seitz *et al.* demonstrated a positive result as to G allele and GG genotype (Seitz et al., 2007), and Vermeire found a significantly positive association between carriers of allele G and treatment response (Louis et al., 2002). The other three studies showed no relevant association of *TNFα*promoter-308 A/G polymorphism and therapeutic response (Duricova et al., 2009; Louis et al., 2002; Tong et al., 2012). Among the three studies detecting the association of *TNFα* promoter-857 C/T polymorphism with responsiveness to *TNFα* blocker, Tong showed the -857 C/C genotype responded better to therapy (**Table 15**)(Tong et al., 2012)and Vasilopoulos revealed carriage of *TNFα*-857C was related with positive response to anti-*TNFα* treatment (Vasilopoulos et al., 2012). Our meta-analysis showed entirely different results from the studies included. The explanation for this phenomenon may be because we used meta-analysis to combine inconsistent results from several studies to explore a result for SpA. However, despite belonging to SpA, different diseases such as AS and PsA may have a different mechanism for the response to *TNFα*blocker.

As the first meta-analysis study concerning *TNFα* polymorphism and therapeutic response in SpA patients, we compared our results with other meta-analyses examining RA patients. In 2010, Lee *et al.* showed that there was no significant difference in the proportions of *TNFα* promoter -308A allele carriers in a group that responded to treatment and a group that did not (Lee et al., 2010), while Pavy *et al.* came to the same conclusion (Jahan et al., 2017). In2012, Zeng concluded that there was no significant difference between GG and (GA+AA)with responsiveness to the *TNFα* blocker (infliximab, etanercept and adalimumab) (Zeng et al., 2013), which conflicted with our results. Several reasons could be considered: SpA and RA are different diseases and may have different mechanisms for the response to *TNFα* blocker; Zeng *et al.* included 15 studies with a total of 2127 patients in their meta-analysis, which is of a larger sample size than that outlined here. At the same time, subgroup analysis was performed based on different response criteria (disease activity score 28 [DAS28] and American College of Rheumatology 20% improvement criteria [ACR20]) in his study. Subgroup analysis could explore the source of heterogeneity and reduce the possible influence factor, thus providing more accurate results. There are still several limitations in our present study. Firstly, owing to the fact that only six studies met the criteria and were included in this meta-analysis, we could not rule out the existence of publication bias, which may affect the results. More studies are needed to

detect the association of *TNFα* polymorphism and responsiveness to *TNFα*blocker. Secondly, albeit differences exist among different ethnic populations, TNFα antagonists are widely used in the world and show excellent efficacy. Our meta-analysis aims to demonstrate the role of *TNFα* genes in predicting responsiveness to different *TNFα* antagonists. That is why we included a variety of *TNFα* blocker (infliximab, etanercept and adalimumab) rather than only one of them. In this meta-analysis, only six studies were included owing to our rigid inclusion and exclusion criteria. Among the six included studies, only one Asian cohort was incorporated (Tong et al., 2012); the other cohorts comprised European SpA and IBD patients. Therefore, more studies on Asians should be included in future meta-analyses.

5. ADVERSE EVENTS AND ASSOCIATED SNPS OF ANTI-TNFα THERAPY

5.1 Methods

5.1.1 Patient recruitment

Total 402 Chinese Han patients presenting at the department of rheumatology at Changhai Hospital who was diagnosed with ankylosing spondylitis using the Modified New York criteria (Van der Linden, Valkenburg, & Cats, 1984) were enrolled into this study. Signed informed consent was obtained from patients who were randomized to rhTNFR-Fc or infliximab monotherapy and outcome data regarding short-term and long-term side effects were collected during the study period June 2008 to February 2013. The institutional review board of Changhai Hospital (Shanghai, China) approved this prospective clinical study. Exclusion criteria listed under 2.1.3 were applied. There are a total of 113 patients with non-adverse event, among which 31 patients do not have SNP information. Meanwhile, 20 patients out of 51, who have adverse events, have no SNP information. Hence, 82 patients with the non-adverse event and 39 with adverse events were included to analyze the side effects-related polymorphisms of TNFα.

5.1.2 Loss to follow-up

Enrollment commenced in 2008 and concluded in 2013, and of the 402 patients in this study, 230 were lost to follow-up for the study of long-term adverse events (87 receiving infliximab and 85 receiving rhTNFR-Fc). In 162 out of 230 cases of loss to follow-up, the main reason was the financial burden associated with a not (fully) reimbursed treatment. Other causes of loss to follow-up were treatment failure (20 cases) or complications (19 cases). These patients transitioned into NSAIDs plus DMARDs co-therapy under our care. A further 23 patients were lost due to relocation, five dues to unknown reasons, and one due to non-small cell lung cancer.

5.1.3 Study design

Participants received either 200 mg infliximab (n=32) intravenously at week 0, 2 and 6, or 25 mg rhTNFR-Fc (n=74) twice weekly for 12 weeks subcutaneously. Upon administration, participants were put under observation for 2 hours to monitor for side-effects, which were categorized as mild when symptoms spontaneously resolved within the hour, moderate when medical intervention was required, and severe when hospitalization was required or in case of a fatality.

5.1.4 Statistical analysis

Clinical comparisons were performed in SPSS v17using the student's T-test or Pearson's chi-square test. Results were considered significant when the p-value is lower than 0.05.

5.2 Results

5.2.1 Demographics

Table 16 details patient demographics in this study(Tong et al., 2015). The cohort consisted of 375 men and 27 women, a total of 402 patients with an average age of 39.5 ± 16 years. Therefore, men took up to 93.3 percent of the entire cohort with an average age at 39 ± 15. Two hundred ten patients received rhTNFR-Fc monotherapy, and 192 received infliximab monotherapy. See **Table 16** for a list of side effects occurring in this study. Side effects of treatment typically comprise a spectrum of infectious diseases, such as pneumonia (thirteen cases), urinary infection (seventeen cases), otitis media, and oral candidiasis. The first two types take up to fifty percent of all events. Overall, pneumonia(22.0%) and urinary tract infections (28.9%) combined caused the majority of the toxicities. Urinary tract infections occurred at a significantly higher frequency in the infliximab-group (p equals to 0.0325).

5.2.2 Short-term side effects

Table 18 details the prevalence of short-term side effects. Twenty percent of the cohort suffered from adverse events within 2 hours since the beginning of treatment, affecting forty-six patients treated with rhTNFR-Fc and thirty-five patients that are treated with infliximab. Adverse events rate was not different between treatment groups. Patients experiencing early onset toxicities were younger, had short disease duration and primarily peripheral involvement of disease but had lower C-reactive protein levels and erythrocyte sedimentation rates($p=10^{-5}$) paradoxically.

5.2.3 Long-term side effects

Table 19 details the prevalence of long-term side effects. Total one hundred and seventy-two AS patients have follow-up data. These include eighty-seven and eighty-five patients receiving two hundred milligram Infliximab per 6 weeks and twenty-five milligram rhTNFR-Fc biweekly, respectively. The treatment lasted for one hundred and four weeks, that is from 2008 (study began) to 2013 (study concluded). Fifty-nine patients reported long-term side. These comprised nineteen patients (out of 172 patients, 11.0%) on rhTNFR-Fc treatment and forty (out of 172 patients, 23.3%) on Infliximab treatment. The percentage suffering from side effect was significantly different in these two populations (p-value equals to 0.0013). Overall, thirty-four percent of the cohort suffered from late-onset adverse events, affecting 19 patients treated with rhTNFR-Fc and 40 patients who were treated with infliximab. Patients experiencing late-onset adverse events were free from early-onset complications. Again, patients experiencing late onset side effects were slightly but not significantly younger (37 ± 20 vs 42 ± 19, p equals to 0.1461), had extended disease duration (p equals to 0.0011) and higher baseline C-reactive protein (p equals to 0.0039) and erythrocyte sedimentation rates (p equals to 0.0345).

5.2.4 SNPs in TNFα cannot predict the occurrence of adverse events

Table 20 details the events count for each genotype in both non-adverse and adverse events patients regarding the 4 TNFα SNPs investigated in this cohort. For each genotype, these four SNPs were compared between non-adverse and adverse cases. All genotype distributions were in Hardy-Weinberg equilibrium. Frequency of A/G and G/G genotype at TNFα -308 polymorphism is not significantly different between two groups (p = 0.3 and p = 0.839respectively). Similarly, adverse events incidence is not associated with these genotypes at *TNFα*-238 rs361525. At *TNFα*-1031 and *TNFα*-857 loci, three polymorphism genotypes, namely C/C, T/T, and C/T, were not significantly related to the occurrence of adverse events following anti-TNF therapy (p = 0.207 and 0.116 respectively). In summary, the SNPs investigated in this study cannot effectively predict the occurrence of adverse effects.

5.3 Discussion

Given the clinical efficacy, anti-*TNFα* therapy is chosen as an effective treatment for a broad spectrum of rheumatic diseases in China (Callhoff et al., 2015; Tong et al., 2013). However, the adverse events have been constitutively reported in clinical applications, firmly against the original drug-safety profiles (Nagy et al., 2011). In China, a lower dosage of *TNFα* blocker was prescribed to patients that reported in the literature. This might explain the milder adverse effect and better tolerability. As this therapy is becoming increasingly utilized clinically, both patients and clinicians are eager to understand in great details of the adverse effect and to make better and safer medical care to a broader patient population. To achieve so, we ought to first profile the adverse effects in Chinese Han populations to record the details of information related to clinical features, treatment, and responses.

Our study undertook this aim and provided the first report on adverse events in Chinese Han patients, separated by their onset stage. The AS patients were under long-term monotherapy of rhTNFR-Fc and Infliximab. Regarding short-term treatment, our analysis showed that about 10% adverse effect is moderate, in agreement with literature report in which 13.2% were reported with infliximab infusion (De Moraes et al., 2010; Fouache et al., 2009; Gerloni, Pontikaki, Gattinara, & Fantini, 2008). There were less than 1% of severe adverse events occurred in our study. These severe events, however, are generally fatal, suggesting monitoring treatment and making an appropriate decision is critical to patients' safety. At an early stage, patients may exhibit high tolerance, and they should be aware of adverse effects emerging later or long-term wise. Long-term treatment usually leads to infectious disease, a consistent observation with previous reports (Dixon et al., 2006; Suwannalai, Auethavekiat, Udomsubpayakul, & Janvitayanujit, 2009). This is because the function of TNF is to activate macrophage and dendritic cells, leading to an induction of innate immunity or regulating acute-phase response (Waters et al., 2013). However, TNFi blocks these roles played by TNF, resulting in increased risks of infections (Michaud et al., 2014).

To reduce the chance of side effects, we need to be able to screen patients at high risk. Our result suggests that shorter disease duration is a risk factor for both short- and long-term side effects. Additionally, if the adverse effects show within two hours of therapy initiation, these patients have a higher chance to suffer from peripheral joint symptoms. Lastly, the incidence of adverse

effect could be predicted by serum CRP and ESR. These biomarkers are elevated in patients comparing to healthy people. However, they are lower in adverse than non-adverse events patients for short-term treatment, and higher than non-adverse events patients for long-term treatment. This phenomenon might be attributed to that CRP is able to react with nuclear antigen and to indorse the phagocytosis (Peisajovich, Marnell, Mold, & Du Clos, 2008).

Also, because SNPs in TNFα have been widely investigated in the field of immune disease, and specifically AS, we set out to examine whether these SNPs can be used to determine the adverse effects. Unfortunately, with limited AS cases recruited to our study, we could not prove their efficiencies in predicting the incidences of side effects following therapy administration. This result indicated that several approaches could be taken to explore side-effects-related genotypes in future study: 1) enlarge the clinical trial by recruiting more patients, which may improve the statistical power; 2) investigate other polymorphisms on TNFα and other infection-associated genes; 3) stratify patients by their clinical characteristics and refine the association analysis.

We appreciate the fact that this study comprises a broad range of patients with varied age and disease duration, alluding to a heterogeneous population and thus an averaged result. More critically, a significant loss of follow-up rendered the study with limited information and statistical power, mainly as a result of the unsolvable financial burden of these expensive drugs. Last but not least, we were not able to evaluate the correlation between disease activity and the incidence of adverse effects since the BASDAI was not used when the study was designed initially.

6. RESEARCH SUMMARY AND FUTURE DIRECTIONS

The current research thesis, for the first time, comprehended the genomic and pharmaceutical investigations in Chinese Han AS patients. It also for the first time revealed the adverse outcomes in the real world by following up the biological agent therapy, providing the first-hand predictive data and relevant experiences regarding TNFα blocker usage. Nowadays, there are not too many therapeutic options for AS patients clinically. The evidence-based clinical evaluations are only available for NSAIDs and TNFα blocker. Unfortunately, as the first-line treatment for AS patients, NSAIDs are not efficiently benefiting the entire population (Taurog et al., 2016). As a consequence, biological agents, namely TNF blocker, inevitably become a preferred choice for Chinese AS population. However, it is well known that the cost of TNF blocker is relatively high, bringing economic burden globally, especially in developing countries like China.

Our cohort study recruited the greatest number of Chinese AS patients who were treated with TNF blocker. The advantage comes from several reasons. First, the hospital where samples were collected from locates in Shanghai, a metropolitan city which has the largest population in China. Second, the medical insurance system of Shanghai covers the cost of rhTNFR-Fc, making it possible for us to continue following these patients. However, several hurdles remain to be resolved. Specifically, when patients need to make a choice between different medical treatments, clinicians and patients both face the uncertainty of treatment effects, the high cost of medication, and unknown side effects. Due to these limitations, we took a different perspective to investigate whether any genetic variant is related to AS and whether any genetic information can predict the treatment efficacy and levels of adverse effects. Our association study revealed that AS patients carrying genotypes of TNFα -857 C/C and/or -1031 T/T exhibited a better response to TNF blockers. Meta-analysis suggested that TNFα -308 G/G and -857 C/C predicted better response toward the TNFi therapy. We also profiled multiple major adverse events for AS patients treated with TNFi monotherapy, namely infliximab and rhTNFR-Fc, with the latter being a domestic biosimilar of etanercept. Our results suggested short-term side effects occurred at a frequency of ~20% whereas long-term side effects occurred in 59% of patients. Importantly, more adverse events were found in the group treated with infliximab than those treated with rhTNFR-Fc.

Moreover, CRP, ESR and disease duration have a significant relationship to both short- and long-term side effects. However, when we tried to establish a link between AS-associated genetic variants in TNFα with the frequencies of adverse events, we could not find a significant relationship in-between. Recruiting more patients and a greater number of genetic loci for analysis might shed light on this perspective. In light of such discoveries, clinicians will have data specific to Chinese Han AS patients in the real world. When it is time to decide between TNF blocker and other treatments, the patients can first go through a test designed for TNF SNPs. If mutations occurred at such locus (loci), clinicians could make an evidence-based decision that which treatment strategy is suitable for the patient.

There are several limitations that need to be addressed regarding this study, which certainly should be taken into considerations when one needs to interpret these results for designing future studies. First, our investigation is based on a single-centered study. A multi-centered research is expected to be conducted in the future to have more and diversified patients involved. Secondly, only two TNF inhibitors were included in our study, namely Infliximab and rhTNFR-Fc, hence we are not able to provide a complete profile of adverse events for all commonly used TNFi and cannot rule out the possibility that the SNPs investigated in this study could be associated with the incidence of side effects. Thirdly, many other genetic variants, such as those on TNF receptor and IL-10, were not investigated in our study. They might convey significant associations to AS development, responses and adverse events of TNFi.

As a summary, our study presented several novel findings in the field: 1) For the first time, it was revealed and validated AS-related SNP positions in a Chinese Han population using a large cohort study; 2) these newly revealed SNP positions exhibited strong predictive values for the efficiency of AS treatment, therefore is expected to reduce the medical cost for patients significantly; 3) side effects of current AS treatment were investigated, which will profoundly improve the effectiveness in surveilling incidences of tuberculosis and infectious diseases; 4) pharmacogenomic analysis showed little relationship between TNFα variants and adverse events, suggesting a bridge between genetics and side effects of TNFi are not yet able to be established by these known and well-defined SNPs; other polymorphisms at different loci and genes might serve as better predictors. In future studies, to identify more AS-associated SNPs with prognostic and diagnostic values in the Chinese Han population, it is imperative to enlarge the cohort size in

order to acquire a shred of stronger and substantial evidence. Meanwhile, it is currently less clear how SNPs are associated with gene transcription regulation and protein function. It is hence worthy of more devotion to investigating the impact of SNPs and the exact mechanisms by which SNPs affect genes, proteins, and ultimately disease.

Table 1. SNPs in *TNFα* and associated disease.

SNP	Disease	P values and OR	Populations	References
rs1799724	MS	$p<0.001$	Turkish	(Akcali et al., 2010)
rs1800629	Asthma	OR = 1.37, $p = 0.04$	China	(Gao, Shan, Sun, Thompson, & Gao, 2006)
rs1799964	Bowel disease	$p= 0.00004$	UK white Caucasoid	(Ahamad et al., 2003)
rs1800629	Irritation		USA	(Davis, Visscher, Wickett, & Hoath, 2010)
rs1800629, *rs361525*	Ischemic strokes and silent brain infarctions	$p<0.05$	Koreans	(Kim et al., 2010)
rs1800629	SAD	OR = 2.635, $p < 0.01$	Southern China	(L. Yang, Lu, Jiang, Liu, & Peng, 2009)
rs1800629	Worsened labile anger	$p<0.05$	USA	(Lotrich, Ferrell, Rabinovitz, & Pollock, 2010)
rs361525	New ICH	$p= 0.003$	Northern California	(Achrol et al., 2006)
rs1799964	Endometriosis.	$p = 0.04$; OR = 1.75, 1.019–3.01	Japanese	(Asghar et al., 2004)
rs2229094 of LTA	Proliferative Vitreoretinopathy	$p= 0.0283$	Spain	(Rojas et al., 2010)
rs361525	Invasive breast cancer risk	OR 1.00 (0.95–1.06)	Europe, USA and Australia	(Gaudet et al., 2009)
rs1799724, *rs1800610*	Prostate cancer risk	Significant	Nutrition Cohort	(Danforth et al., 2008)
rs1800630, *rs361525*	Lung cancer	$p= 0.0001$	Taiwan	(Shih et al., 2006)
rs1800629	RCC	Significant	Turkey	(Basturk et al., 2005)
rs1800629	HCC	$p<0.001$, odds ratio [OR] = 4.75	Turkish	(Akkiz et al., 2009)

rs1800629, *rs1143627*, *rs1800896*, *rs361525*	HCC	OR = 1.74	20 studies Caucasian and Asian	(Y. Yang, Luo, Feng, & Bi, 2011)
rs1800629	rAOM	$p<0.02$	USA	(McCormick et al., 2011)
rs1800750, *rs361525*	rAOM	Crude OR: 3.10; $p = 0.05$; adjusted OR: 3.06; $p = 0.07$	Netherlands	(Emonts et al., 2007)
rs361525	Secondary dengue hemorrhagic fever	$p= 0.0009$	Thais	(Vejbaesya, Chierakul, Luangtrakool, & Sermduangprateep, 2007)
rs1799964, *rs1799724*, *rs1800750*, *rs1800629* and *rs361525*	Malaria	rs1799964 $p = 0.0178$, rs1800630, $p = 0.0481$	Indian	(Sinha et al., 2008)
rs1800629	MCL	$p<0.05$	Venezuelan	(Cabrera et al., 1995)
rs1800629, *rs361525*	OLP	OR = 10.93	Thais	(Kimkong, Hirankarn, Nakkuntod, & Kitkumthorn, 2011)

Unpublished data.

MS, multiple sclerosis; SAD, sporadic Alzheimer's disease; ICH, intracranial haemorrhage; LTA, lymphotoxin alpha; RCC, renal cell carcinoma; HCC, hepatic cell carcinoma; rAOM, recurrent acute otitis media; MCL, mucocutaneous leishmaniasis; OLP, oral lichen planus; OR, odds ratio.

Table 2. SNPs under investigation in this study.

MHC genes		**Non-MHC genes**	
*HLA-B27*tagged	*rs4349859*	*ERAP1*	*rs27044*
TNFα-238	*rs361525*		*rs30187*
TNFα-308	*rs1800629*		*rs17482078*
TNFα-857	*rs1799724*		*rs10050860*
TNFα-1031	*rs1799964*	*ERAP2*	*rs2549782*
		EXOC3L	*rs868213*
		FOXP3	*rs2232367*
			rs28935477
		IL-1	*rs1143627*
		IL-10-819	*rs1800871*
		IL-10-1087	*rs1800896*
		IL-23R	*rs10889677*
			rs11465804
			rs1495965
			rs1343151
			rs1004819
		LNPEP	*rs2303138*
		MAP3K1	*rs96844*
		MAP3K14	*rs4792847*
		TNFRSF1A	*rs4149577*
		TNFRSF11B	*rs2073618*
			rs4355801
			rs3102735

Unpublished data.

Table 3. Baseline demographics for identifying AS-related SNPs (Section 2).

	Ankylosing spondylitis patients	Healthy controls
Total number	149	106
Gender	86 male 63 female	62 male 44 female
Age (years)	35.8±7.8	37.4±8.2

Unpublished data.

Table 4. SNPs significantly associated with AS.

Gene	SNP	Genotype	Patient	Control	*p* value
TNFα-857	*rs1799724*	CC	64 (43.0%)	73 (68.9%)	
		TT	16 (10.7%)	5 (4.7%)	
		CT	69 (46.3%)	28 (26.4%)	0.0002
		T	101 (33.9%)	38 (17.9%)	
		C	197 (66.1%)	174 (82.1%)	<0.0001
*HLA-B27*tagged	*rs4349859*	AA	5 (3.4%)	0	
		AG	26 (17.4%)	4 (3.8%)	
		GG	118 (79.2%)	102 (96.2%)	0.0004
		A	36 (12.1%)	4 (1.9%)	
		G	262 (87.9%)	208 (98.1%)	<0.0001
TNFα-308	*rs1800629*	AG	8 (5.4%)	13 (12.3%)	
		GG	141 (94.6%)	93 (87.7%)	0.04838
		A	8 (2.7%)	13 (6.1%)	
		G	290 (97.3%)	199 (93.9%)	0.0535
TNFRSF1A	*rs4149577*	CC	69 (46.3%)	32 (30.2%)	
		TT	23 (15.4%)	13 (12.3%)	
		CT	57 (38.3%)	61 (57.5%)	0.008710
		T	103 (34.6%)	87 (41.0%)	
		C	195 (65.4%)	125 (59.0%)	0.136139
IL-23R	*rs1004819*	CC	32 (21.5%)	29 (27.4)	
		TT	55 (36.9%)	21 (19.8%)	
		CT	62 (41.6%)	56 (52.8%)	0.01317
		T	172 (57.7%)	98 (46.2%)	
		C	126 (42.3%)	114 (53.8%)	0.01039

Unpublished data.

Table 5. SNPs not significantly associated with AS.

Gene	SNP	Genotype	Patient	Control	*p*
TNFα-238	*rs361525*	AG	9 (6.0%)	7 (6.7%)	
		GG	140 (94.0%)	98 (93.3%)	0.8396
TNFα-1031	*rs1799964*	CC	2 (1.3%)	6 (5.7%)	
		TT	110 (73.8%)	68 (64.2%)	
		CT	37 (24.9%)	32 (30.1%)	0.0755
FOXP3	*rs2232367*	CC	149 (100%)	106 (100%)	
FOXP3	*rs28935477*	CC	149 (100%)	106 (100%)	
IL-1	*rs1143627*	CC	43 (28.9%)	23 (21.7%)	
		TT	32 (21.5%)	17 (16.0%)	
		CT	74 (49.6%)	66 (62.3%)	0.1373
IL-10-1087	*rs1800896*	AG	15 (10.1%)	15 (14.2%)	
		AA	132 (88.6%)	89 (84.0%)	
		GG	2 (1.3%)	2 (1.8%)	0.5632
IL-10-819	*rs1800871*	CC	15 (10.1%)	13 (12.3%)	
		TT	65 (43.6%)	44 (41.5%)	
		CT	69 (46.3%)	49 (46.2%)	0.8449
IL-23R	*rs10889677*	AC	60 (40.3%)	41 (38.7%)	
		CC	12 (8.0%)	14 (13.2%)	
		AA	77 (51.7%)	51 (48.1%)	0.4046
	rs11465804	TT	149 (100%)	106 (100%)	
	rs1495965	AA	31 (20.8%)	24 (23.5%)	
		AG	68 (45.6%)	58 (56.9%)	
		GG	50 (33.6%)	24 (19.6%)	0.1594
	rs1343151	CC	140 (94.0%)	101 (95.3%)	
		CT	9 (6.0%)	5 (4.7%)	0.6475
ERAP1	*rs27044*	CC	37 (24.8%)	30 (28.3%)	
		GG	41 (27.5%)	21 (19.8%)	
		CG	71 (47.7%)	55 (51.9%)	0.3640
	rs30187	CC	34 (0.228)	23 (21.7%)	
		TT	46 (30.9%)	27 (25.5%)	

		CT	69 (46.3%)	56 (52.8%)	0.5479
	rs17482078	CC	132 (88.6%)	98 (92.5%)	
		CT	17 (11.4%)	8 (7.5%)	0.3067
	rs10050860	CC	131 (87.9%)	98 (92.5%)	
		CT	18 (12.1%)	8 (7.5%)	0.2384
ERAP2	*rs2549782*	TT	46 (30.9%)	29 (27.4%)	
		GG	25 (16.8%)	24 (22.6%)	
		GT	78 (52.3%)	53 (50.0%)	0.4881
TNFRSF11B	*rs2073618*	CC	11 (7.4%)	3 (2.8%)	
		CG	62 (41.6%)	48 (45.3%)	
		GG	76 (51.0%)	55 (51.9%)	0.2807
	rs4355801	AG	62 (41.6%)	42 (39.6%)	
		AA	74 (49.7%)	58 (54.7%)	
		GG	13 (8.7%)	6 (5.7%)	0.5638
	rs3102735	CC	3 (2.0%)	0	
		TT	108 (72.5%)	66 (62.3%)	
		CT	38 (25.5%)	40 (37.7%)	0.0471
MAP3K1	*rs96844*	CC	93 (62.4%)	54 (50.9%)	
		TT	8 (5.4%)	10 (9.4%)	
		CT	48 (32.2%)	42 (39.7%)	0.1476
MAP3K14	*rs4792847*	AG	27 (18.1%)	24 (22.6%)	
		AA	118 (79.2%)	80 (75.5%)	
		GG	4 (2.7%)	2 (1.9%)	0.6341
LNPEP	*rs2303138*	AA	30 (20.1%)	17 (16.1%)	
		AG	80 (53.7%)	61 (57.5%)	
		GG	39 (26.2%)	28 (26.4%)	0.6936
EXOC3L	*rs868213*	TT	145 (97.3%)	104 (98.1%)	
		CT	4 (2.7%)	2 (1.9%)	0.6787

Unpublished data.

Table 6. Currently known AS-associated SNPs in Chinese Han and other populations.

Gene	SNP	Population	Citation
STAT4	*rs7574865*	Chinese Han	(Liu, Zhang, & Dong, 2014)
FCGR2B	*rs10917661*	Chinese Han	(Duan et al., 2012)
FCRL4b	*rs2777963*	Chinese Han	(Zeng et al., 2012)
IL-12B	*rs6871626*	Chinese Han	(Zhang et al., 2015)
IL-1R2	*rs2302589*	Chinese Han	(Xia et al., 2015)
RUNX3	*rs11249215*	Chinese Han	(Liu et al., 2015)
PPARGC1B	*rs7379457*	Chinese Han	(Liu et al., 2015)
	rs32579		(Liu et al., 2015)
PTPN22	*rs1217414*	Chinese Han	(Tang, Wang, & Chen, 2014)
ETS4	*rs112833*	Chinese Han	(Shan et al., 2014)
IL-23R	*rs17375018*	Chinese Han	(Dong, Li, Zhang, Tan, & Jiang, 2013)
ERAP1	*rs27434*	Chinese Han	(Australo-Anglo-American Spondyloarthritis et al., 2010)
FCRL4	*rs2777963*	Chinese Han	(Zeng et al., 2012)
2p15	*rs10865331*	Chinese Han	(Zhang et al., 2015)
5q14.3	*rs4552569*	Chinese Han	(Lin et al., 2011)
5q14.3	*rs17095830*	Chinese Han	(Wei et al., 2013)
12q12 (ANO6)	*rs17095830*	Chinese Han	(Lin et al., 2011)
	rs4552569		(Wei et al., 2013)
	rs1265112		(Lin et al., 2011)
	rs2516509		(Lin et al., 2011)
	rs3915917		(Lin et al., 2011)
	rs2596501		(Lin et al., 2011)
	rs13202464		(Lin et al., 2011)
TNFRSF1A	*rs11616188*	Chinese Han	(Davidson et al., 2011)
6q21	*rs13210693*	Chinese Han	(Lin et al., 2011)
HAPLN1-EDIL3	*rs4552569*	Chinese Han	(Lin et al., 2011)
2p15	*rs10865331*	Taiwanese	(Wen et al., 2014)

ORAI1	*rs12313273*	Taiwanese	(Wei et al., 2011)
	rs7135617	Taiwanese	(Wei et al., 2011)
STIM1	*rs3750996*	Taiwanese	(Wei et al., 2012)
IL12B	*rs3212227*	Taiwanese	(Wong et al., 2012)
	rs6556416	European	(Evans et al., 2011)
ETS1	*rs1128334*	Ningxia	(Shan et al., 2014)
IL-23R	*rs11209026*	European	(Australo-Anglo-American Spondyloarthritis et al., 2010)
	rs2310173	European	(Australo-Anglo-American Spondyloarthritis et al., 2010)
	rs11209026	European	(Burton et al., 2007)
2p15	*rs10865331*	European	(Australo-Anglo-American Spondyloarthritis et al., 2010)
21q22	*rs2242944*	European	(Australo-Anglo-American Spondyloarthritis et al., 2010)
	rs27037	European	(Burton et al., 2007)
	rs27434	European	(Burton et al., 2007)
ERAP1	*rs30187*	European	(Evans et al., 2011)
	rs27037	Taiwanese	(Wang et al., 2012)
RUNX3	*rs11249215*	European	(Evans et al., 2011)
ANTXR2	*rs4333130*	European	(Australo-Anglo-American Spondyloarthritis et al., 2010)
	rs4389526	European	(Evans et al., 2011)
TNFRSF1A	*rs11616188*	European	(Evans et al., 2011)
PTGER4	*rs10440635*	European	(Evans et al., 2011)
TBKBP1	*rs10781500*	European	(Evans et al., 2011)
CARD9	*rs10781500*	European	(Evans et al., 2011)

Unpublished data.

Table 7. TNFα genes associated with AS in Chinese Han population.

Gene	rs number	Genotype and allele	Case n(%)	Control n(%)	χ^2	p-value
TNFα-308	*rs1800629*	A/G	3(2.8)	13(12.3)	6.7602	0.0093
		G/G	103(97.2)	93(87.7)		
		A	3(1.4)	13(6.1)	6.4951	0.0108
		G	209(98.6)	199(93.9)		
TNFα-238	*rs361525*	A/G	7(6.6)	8(7.5)	0.0717	0.7888
		G/G	99(93.4)	98(92.5)		
		A	7(3.3)	8(3.8)	0.0691	0.7926
		G	205(96.7)	204(96.2)		
TNFα-1031	*rs1799964*	C/C	12(11.3)	6(5.7)	2.2118	0.3309
		T/T	61(57.5)	66(62.3)		
		C/T	33(31.2)	34(32)		
		T	94(62.3)	100(56.2)	1.2448	0.2645
		C	57(37.7)	78(43.8)		
TNFα-857	*rs1799724*	C/C	39(36.8)	72(67.9)	23.074	0.00001
		T/T	20(18.9)	5(4.7)		
		C/T	47(44.3)	29(27.4)		
		T	87(41)	38(18.1)	26.6368	2.455×10^-7
		C	125(59)	172(81.9)		

The cohort was comprised of 106 ankylosing spondylitis patients (cases) and 106 healthy patients (controls). p-values were determined by the χ2 test.Cited from (Tong et al., 2012).

Table 8. TNFα gene polymorphism associated with nonresponders and responders.

Gene	number	Genotype	Nonresponders	Responders	χ2	*p*-value
TNFα-308	*rs1800629*	A/G	0(0.0)	3(3.3)	0.272	0.602
		G/G	8(100)	88(96.7)		
TNFα-238	*rs361525*	A/G	1(12.5)	5(5.5)	0.634	0.4259
		G/G	7(87.5)	86(94.5)		
TNFα-1031	*rs1799964*	C/C	3(37.5)	9(9.9)	5.408	0.06694
		T/T	3(37.5	57(62.6)		
		T/C	2(30)	25(27.5)		
TNFα-857	*rs1799724*	C/C	1(12.5)	35(38.5)	3.189	0.203
		T/T	3(37.5)	15(16.5)		
		C/T	4(50)	41(45)		

The cohort comprised of 99 ankylosing spondylitis patients. Nonresponders were patients who did not fulfill the Assessment of SpondyloArthritis International Society (ASAS) improvement criteria 20, 40, 50 and 70 following anti-TNF therapy; responders were patients who fulfilled any one of the ASAS 20, 40, 50 and 70 criteria following anti-TNF therapy. p-values were determined by the χ2 test. Cited from (Tong et al., 2012).

Table 9. Primer sequences for genotyping of TNFα polymorphisms.

Amplification Target	Primer Sequence
TNFα -857 rs1799724	F-ACGTTGGATGGAGGCTCTTTCACTCCCTG
	R-ACGTTGGATGATGGGTAGGAGAATGTCCAG
TNFα -1031 rs1799964	F-ACGTTGGATGCCCTCCAGACCCTGACTTT
	R-ACGTTGGATGGGATATGTGATGGACTCACC
TNFα -308 rs1800629	F-ACGTTGGATGGATTTGTGTGTAGGACCCTG
	R-ACGTTGGATGGGTCCCCAAAAGAAATGGAG
TNFα -238 rs361525	F-ACGTTGGATGATCAAGGATACCCCTCACAC
	R-ACGTTGGATGCACACAAATCAGTCAGTGGC

Cited from (Tong et al., 2012).

Table 10. Clinical characteristics of patients with TNFα -857 before treating with TNFα blocker and response assessment after anti-TNFα therapy.

	All patients (n=99)	C/C genotype (n=36)	T/C genotype (n=45)	T/T genotype (n=18)
Age (years, mean±SD)	41.63±15.82	41.00±14.85	42.98±15.52	39.07±18.27
Men (%)	77.78	75	75.56	92.86
Duration (years, mean±SD)	9.45±9.00	10.69±10.86	8.99±7.67	7.36±5.99
Clinical features				
Axis onset† (%)	54.55	62.5	48.89	50
Biologic assessment				
HLA-B27 (%)	91.92	90	91.11	100
Serum CRP (mg/dl, mean±SD)	35.22±18.82	34.68±12.59	35.96±23.14	34.49±18.09
ESR (mg/dl, mean±SD)	36.42±17.95	37.45±16.21	36.89±18.46	32±20.27
Drug treatment				
Infliximab, n (%)	28(28.28)	7(19.44)	15(33.33)	6(33.33)
rhTNFR–Fc, n (%)	71(71.72)	29(80.56)	30(66.67)	12(66.67)
BASDAI scores				
Week0 (mean±SD)	5.92±1.87	6.18±2.02	5.79±1.83	5.64±1.46
Week12 (mean±SD)	2.93±1.39	2.86±1.22	2.96±1.56	3.03±1.24
ASAS				
Nonresponder, n (%)	8(8.08)	1(2.78)	4(8.89)	3(16.67)
ASAS20, n (%)	20(20.20)	5(13.89)	7(15.56)	8(44.44)
ASAS40, n (%)	27(27.27)	13(36.11)	12(26.67)	2(11.11)
ASAS50, n (%)	36(36.36)	12(33.33)	20(44.44)	4(22.22)
ASAS70, n (%)	8(8.09)	5(13.89)	2(4.44)	1(5.56)

Total 106 patients were recruited initially. Seven of them were lost during follow-up. †Axis onset means ankylosing spondylitis patients that presented with inflammatory back pain and alternating buttock pain symptoms at the beginning of the disease. ASAS, Assessment of SpondyloArthritis International Society; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index, ESR, Erythrocyte sedimentation rate; rhTNFR–Fc, Recombinant human TNFα receptor II–IgG Fc fusion protein; SD, Standard deviation. Cited from (Tong et al., 2012)

Table 11. TNFα gene polymorphisms associated with poor and nonresponders versus good responders.

Gene	rs number	Genotype and allele	Poor and nonresponders, n(%)	Good responders, n(%)	$\chi 2$	*p*-value
TNFα-308	*rs1800629*	A/G	1(3.6)	2(2.8)	0.039	0.8436
		G/G	27(96.4)	69(97.2)		
TNFα-238	*rs361525*	A/G	3(10.7)	3(4.2)	1.485	0.2229
		G/G	25(89.3)	68(95.8)		
TNFα-1031	*rs1799964*	C/C	9(32.1)	3(4.2)	15.645	0.0004
		T/T	15(53.6)	45(63.4)		
		T/C	4(14.3)	23(32.4)		
		T	34(60.7)	113(79.6)	7.473	0.0063
		C	22(39.3)	29(20.4)		
TNFα-857	*rs1799724*	C/C	6(21.4)	30(42.3)	12.285	0.0021
		T/T	11(39.3)	7(9.9)		
		C/T	11(39.3)	34(47.8)		
		T	33(58.9)	48(33.8)	10.488	0.0012
		C	23(41.1)	94(66.2)		

The cohort comprised of 99 ankylosing spondylitis patients. Nonresponders were patients who did not fulfill the Assessment of SpondyloArthritis International Society (ASAS) 20 improvement criteria; good responders met the ASAS 40, 50 and 70 criteria. *p*-values were determined by $\chi 2$ test. Cited from (Tong et al., 2012).

Table 12. Summary of studies included in the meta-analysis.

year	country	disease	patient number	TNF inhibitor	Follow-up period	Response criteria	TNFa-308	Responder (n)	Nonresponder (n)	TNF-a-857	Responder (n)	Nonresponder (n)	Ref
2000	Belgium	CD	77	Infliximab	-	CDAI	G/G	47	12	-	-	-	(Vermeire et al., 2000)
							G/A	7	9	-	-	-	
							A/A	1	1	-	-	-	
2002	Belgium	CD	214	Infliximab	18 weeks	CDAI	G/G	116	35	-	-	-	(Louis et al., 2002)
							G/A	39	18	-	-	-	
							A/A	3	3	-	-	-	
2007	Switzerland	AS	22	Infliximab, adalimumab and etanercept	24 weeks	BASDAI	G/G	16	0	-	-	-	(Seitz et al., 2007)
							G/A	4	2	-	-	-	
							A/A	0	0	-	-	-	
		PsA	10	Infliximab, adalimumab and etanercept	24 weeks	DAS28	G/G	10	0	-	-	-	
							G/A	0	0	-	-	-	
							A/A	0	0	-	-	-	
2009	Denmarkd	CD	16	Infliximab	30 days	Clincal Ourcome	G	26	1	C	30	2	(Seitz et al., 2007)
							A	4	1	T	0	0	
	Denmarkd	CD	15	Infliximab	6 years	Clincal Ourcome	G	23	2	C	28	2	
							A	5	0	T	0	0	
	Czech	CD	36	Infliximab	30 days	Clincal Ourcome	G	61	1	C	62	2	
							A	9	1	T	8	0	

	Czech	CD	34	Infliximab	4 years	Clincal Ourcome	G	59	0	C	8	0	
							A	9	0	T	60	0	
2012	China	AS	99	Infliximab and rhTNFR-Fc	12 weeks	BSDAI	G/G	88	8	C/C	35	1	(Tong et al., 2012)
							G/A	3	0	C/T	41	4	
							A/A	0	0	T/T	15	3	
2012	Greece	PS	80	Infliximab, adalimumab and etanercept	6 months	PASI	-	-	-	C/C	41	6	(Vasilopoulos et al., 2012)
							-	-	-	C/T	20	9	
							-	-	-	T/T	2	2	

rhTNFR-Fc (Product name: YiSaiPu), recombinant human TNF-α receptor II–IgG Fc fusion protein, is a *TNFα* blocker manufactured in China and the effect is similar to that of etanercept (Huang et al., 2011). –, Unavailable data; AS, Ankylosing spondylitis; BASDAI, Bath Ankylosing Spondylitis Activity Index; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; DAS28, Disease Activity Score 28; PASI, Psoriasis Area and Severity Index; PS, Psoriasis; PsA, Psoriatic arthritis.Cited from (Tong et al., 2013).Nonresponders were patients who did not fulfill the Assessment of SpondyloArthritis International Society (ASAS) improvement criteria 20, 40, 50 and 70 following anti-TNF therapy; responders were patients who fulfilled any one of the ASAS 20, 40, 50 and 70 criteria following anti-TNF therapy.

Table 13.Association of -308 A/G polymorphism at TNFα promoter and response to TNF blockers.

Polymorphism	Pooled studies	Test of heterogeneity			Effects model	Test of association			
		Q	*p*-value	I2(%)		OR	95% CI	Z	*p*-value
G versus A	(Duricova et al., 2009; Louis et al., 2002; Seitz et al., 2007)	4.48	0.48	0	Fixed	2.14	1.38–3.33	3.38	0.0007
GG versus GA+AA	(Louis et al., 2002; Seitz et al., 2007; Tong et al., 2012)	4.41	0.22	32	Fixed	2.31	1.36–3.91	3.11	0.002
GG+GA versus AA	(Louis et al., 2002; Seitz et al., 2007; Tong et al., 2012)	0.01	0.94	0	Fixed	1.44	0.69–11.60	1.44	0.15

Publication bias was not detected owing to not enough data included to draw a funnel plot. OR: Odds ratio. I^2 was used to quantify the effect of heterogeneity. I^2 ranges between 0 and100% and this represents the proportion of the between-study variability that can be attributed to heterogeneity rather than chance. I^2 values of 25, 50 and 75% were assigned as low, moderate and high estimates, respectively; CI, confidence interval. Cited from (Tong et al., 2013).

Table 14. Association of -857 C/T polymorphism at TNFα promoter and response to TNF blockers.

Polymorphism	Pooled studies	Test of heterogeneity			Effects model	Test of association			
		Q	*p*-value	I2(%)		OR	95% CI	Z	*p*-value
C versus T	(Duricova et al., 2009; Tong et al., 2012)	2.61	0.27	23	Fixed	2.17	1.17–4.03	2.47	0.01
CC versus CT+TT	(Tong et al., 2012; Vasilopoulos et al., 2012)	0.04	0.84	0	Fixed	3.66	1.35–9.92	2.55	0.01
CC+CT versus TT	(Tong et al., 2012; Vasilopoulos et al., 2012)	0.05	0.82	0	Fixed	3.38	1.00–11.48	1.95	0.05

Publication bias was not detected owing to not enough data included to draw a funnel plot. I^2-values of 25, 50 and 75% were assigned as low, moderate and high estimates, respectively; CI, confidence interval. CI, confidence interval. Cited from (Tong et al., 2013).

Table 15. Clinical features of patients with TNFα -1031 before treating with TNFα blockers and response assessment after anti-TNFα therapy.

	C/C genotype (n=12)	T/C genotype (n=27)	T/T genotype (n=60)
Age (years,mean±SD)	43.58±15.78	43.07±13.25	40.37±16.50
Men (%)	83.33	81.48	76.67
Duration (years,mean±SD)	10.00±7.98	9.13±8.27	9.55±9.52
Clinical features			
Axisonset†(%)	66.67	62.96	46.67
Biologic assessment			
HLA-B27(%)	91.67	96.29	91.67
Serum CRP (mg/dl,mean±SD)	38.25±17.62	36.33±24.74	34.28±15.51
ESR (mg/dl,mean±SD)	29.83±19.02	34.56±18.50	38.85±17.16
Drug treatment			
Infliximab, n (%)	3(25.00)	7(25.93)	18(30.00)
rhTNFR–Fc, n (%)	9(75.00)	20(74.07)	42(70.00)
BASDAI scores			
Week0 (mean±SD)	5.55±1.59	6.10±2.49	5.91±1.57
Week12 (mean±SD)	3.44±1.72	2.79±1.36	2.88±1.30
ASAS			
Nonresponder,n(%)	3(25.00)	2(7.41)	3(5.00)
ASAS20, n(%)	6(50.00)	2(7.41)	12(20.00)
ASAS40, n(%)	2(16.67)	7(25.92)	18(30.00)
ASAS50, n(%)	1(8.33)	13(48.15)	22(36.67)
ASAS70, n(%)	0(0.00)	3(11.11)	5(8.33)

†Axis onset indicates the symptoms presented at the beginning of the disease.ASAS, Assessment of SpondyloArthritis International Society; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index;ESR, Erythrocyte sedimentation rate; rhTNFR–Fc, Recombinant human TNF-α receptor II–IgG Fc fusion protein;Nonresponders were patients who did not fulfill the Assessment of SpondyloArthritis International Society (ASAS) improvement criteria 20, 40, 50 and 70 following anti-TNF therapy; SD, Standard deviation. Cited from (Tong et al., 2012).

Table 16. Clinical features of patients with short-term adverse events.

	All patients	non-adverse event patients	adverse event patients	*p* value
Demographics	(n=402)	(n=321)	(n=81)	
Age±SD (years)	39.6±15.8	42.1±19.0	35.9±17.2	0.075
Male (%)	93.30%	92.20%	97.5%	0.087
duration±SD (years)	7.6±8.2	10.1±9.8	6.3±7.6	0.001
Type				
Axial phenotype (%)	69.20%	75.10%	45.7%	1.02E-07
Biologic evaluation				
HLA-B27, no (%)	95.5%	98.8%	82.7%	1.76E-09
Serum CRP±SD (mg/dl)	36.4±18.5	41.5±17.4	31.3±22.4	1.11E-05
ESR±SD (mg/dl)	32.2±15.3	38.0±16.5	29.9±15.3	1.95E-05
Drug treatment				
Infliximab (%)	47.8%(192)	48.9%(157)	43.2%(35)	0.3852
rhTNFR-Fc (%)	52.2%(210)	51.1%(164)	56.8%(46)	

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Infliximab is an antibody biologic drug that works against tumor necrosis factor alpha (TNF-α); rhTNFR-Fc (Product name: YiSaiPu), recombinant human TNF-α receptor II–IgG Fc fusion protein, is a *TNFα* blocker manufactured in China and the effect is similar to that of etanercept (Huang et al., 2011); SD, Standard deviation. Axial phenotype initially mainly affects the spine and pelvic joints. Cited from (Tong et al., 2015).

Table 17. Long-term adverse events in two TNFi treatments.

	Patients with adverse events	rhRNFR-Fc	Infliximab	*p*-value
Infectious disease (%)	(n=59)	(n=19)	(n=40)	
Pneumonia	13(22%)	5(26.3%)	8(20%)	0.5844
Urinary tract infection	17(28.80%)	2(10.5%)	15(37.5%)	0.0325
Otitis media	8(13.6%)	1(5.3%)	7(17.5%)	0.1995
Tuberculosis	6(10.2%)	4(21.1%)	2(5%)	0.0566
Abscess	2(3.4%)	0(0%)	2(5%)	0.3213
Oral candidates	1(1.7%)	0(0%)	1(2.5%)	0.4869
The other AEs (%)				
Elevation of transaminase	3(5.1%)	2(10.5%)	1(2.5%)	0.1897
Anemia	2(3.4%)	1(5.3%)	1(2.5%)	0.5836
Hematuresis	1(1.7%)	0(0%)	1(2.5%)	0.4869
Constipation	4(6.8%)	4(21.1%)	0(0%)	0.0026
Weight loss	1(1.7%)	0(0%)	1(2.5%)	0.4869
Exfoliative dermatitis	1(1.7%)	0(0%)	1(2.5%)	0.4869

AE, adverse events;rhTNFR-Fc (Product name: YiSaiPu), recombinant human TNF-α receptor II–IgG Fc fusion protein, is a *TNF*α blocker manufactured in China and the effect is similar to that of etanercept (Huang et al., 2011);Infliximab is an antibody biologic drug that works against tumor necrosis factor alpha (TNF-α).Cited from (Tong et al., 2015).

Table 18. Short-term adverse events in two TNFi treatments.

	Patients with adverse events	rhRNFR-Fc	Infliximab	*p*-value
Mild (%)	(n=81)	(n=46)	(n=35)	
rash	14(17.3%)	11(23.9%)	3(8.6%)	0.0704
pruritus	5(6.2%)	2(4.4%)	3(8.6%)	0.4339
nausea	9(11.1%)	4(8.7%)	5(14.3%)	0.4277
headache	3(3.7%)	1(2.2%)	2(5.7%)	0.4032
Moderate (%)				
skin allergies	16(19.8%)	12(26.1%)	4(11.4%)	0.1007
fever	2(2.5%)	2(4.6%)	0(0%)	0.2116
palpitations	12(14.8%)	8(17.4%)	4(11.4%)	0.4542
dyspnea	2(2.5%)	1(2.2%)	1(2.9%)	0.8443
chest pain	1(1.2%)	0(0%)	1(2.9%)	0.2486
abdominal pain	4(4.9%)	1(2.2%)	3(8.6%)	0.188
hypertension	9(11.1%)	2(4.4%)	7(20%)	0.0263
Severe (%)				
papilledema	2(2.5%)	2(4.4%)	0(0%)	0.2116
laryngeal edema	1(1.2%)	0(0%)	1(2.9%)	0.2486
premature ventricular contraction	1(1.2%)	0(0%)	1(2.9%)	0.2486

Mild adverse outcomes refer to any complications that can be solved in 1 hour (include 14 cases of rushes, 5 cases of pruritus, 9 cases of nausea, 3 cases of headache). Total eleven cases were recorded in rhTNFR-Fc group.Moderate adverse outcomes refer to complications that could be resolved immediately by medical treatment (include 16 cases of akin allergies, 2 cases of fever, 12 cases of palpitations, 2 cases of dyspnea, 1 case of chest pain, 4 cases of abdominal pain, 9 cases of hypertension). Severe adverse outcomes refer to fatal events that require inpatient hospitalization (include 2 cases of papilledema, 1 case of laryngeal edema, 1 case of premature ventricular contraction). rhTNFR-Fc (Product name: YiSaiPu), recombinant human TNF-α receptor II–IgG Fc fusion protein, is a *TNFα* blocker manufactured in China and the effect is similar to that of etanercept (Huang et al., 2011);Infliximab is an antibody biologic drug that works against tumor necrosis factor alpha (TNF-α).Cited from (Tong et al., 2015).

Table 19. Clinical features of AS patients with long-term adverse events.

	All patients	non-adverse event patients	adverse event patients	*p* value
Demographics	(n=172)	(n=113)	(n=59)	
Age±SD (years)	40.6±18.5	42.3±19.6	37.7±20.2	0.1461
Male (%)	98.80%	98.20%	100%	9.39E-17
duration±SD (years)	9.8±8.5	10.9±9.5	6.3±6.5	0.0011
Type				
Axial phenotype (%)	98.30%	99.10%	98.30%	0.6381
Biologic evaluation				
HLA-B27, no (%)	100%	100%	100%	
Serum CRP±SD (mg/dl)	39.6±17.3	35.7±19.6	44.8±18.7	0.0039
ESR±SD (mg/dl)	38.5±18.7	36.9±18	42.9±16.5	0.0345
Drug treatment				
Infliximab (%)	50.6%(87)	41.6%(47)	67.8%(40)	0.0013
rhTNFR-Fc (%)	49.4%(85)	58.4%(66)	32.2%(19)	

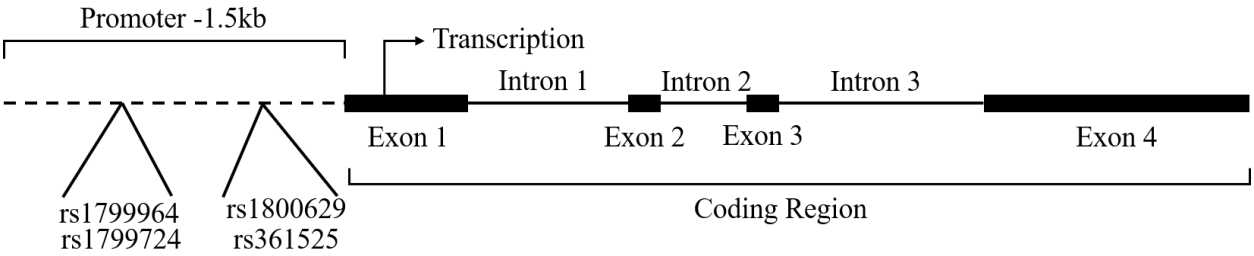
CRP: C-reactive protein. ESR: erythrocyte sedimentation rate; Infliximab is an antibody biologic drug that works against tumor necrosis factor alpha (TNF-α); rhTNFR-Fc (Product name: YiSaiPu), recombinant human TNF-α receptor II–IgG Fc fusion protein, is a *TNFα* blocker manufactured in China and the effect is similar to that of etanercept (Huang et al., 2011); SD, Standard deviation. Axial phenotype initially mainly affects the spine and pelvic joints. Cited from (Tong et al., 2015).

Table 20. TNFα gene polymorphisms associated patients with long-term non-adverse events and adverse events.

Gene	rs	Genotype	Non-adverse events patients (n=82)	Adverse event patients (n=39)	$\chi 2$	p-value
TNFα-308	rs1800629	A/G	3	4	1.074	0.3
		G/G	79	35		
TNFα-238	rs361525	A/G	4	3	0.041	0.839
		G/G	78	36		
TNFα-1031	rs1799964	C/C	5	5	3.153	0.207
		T/T	52	27		
		T/C	25	7		
TNFα-857	rs1799724	C/C	39	18	4.315	0.116
		T/T	8	9		
		C/T	35	12		

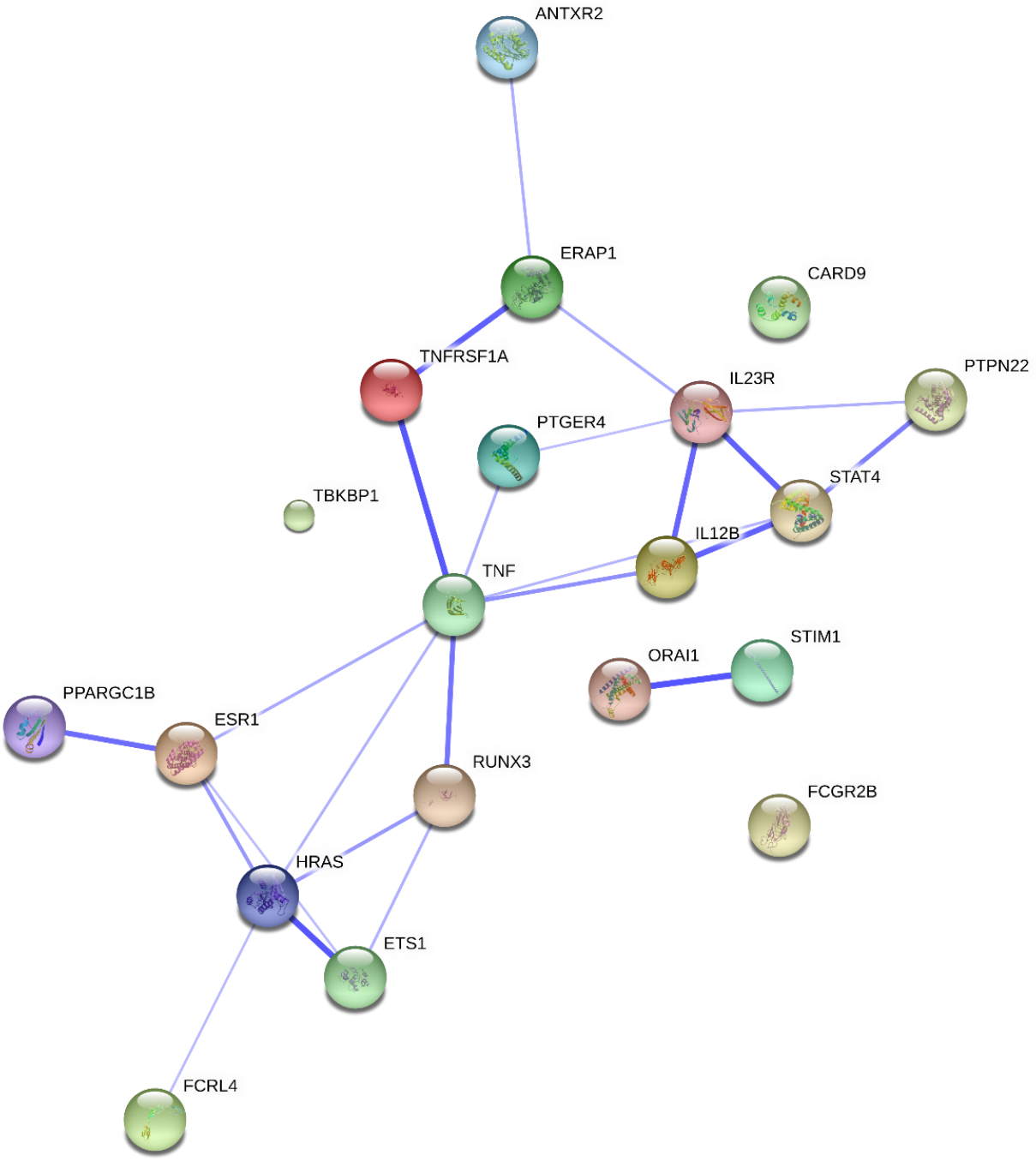
Unpublished data.

Figure 1. Genomic location of investigated SNPs on *TNF* gene.



The gene structure of *TNFα*is shown above. Each solid box indicates an exon, each line in between two boxes indicates an intronic region. Promoter (-1.5kb) of *TNFα* is drawn as dashed line, with four investigated SNPs marked at relative position.

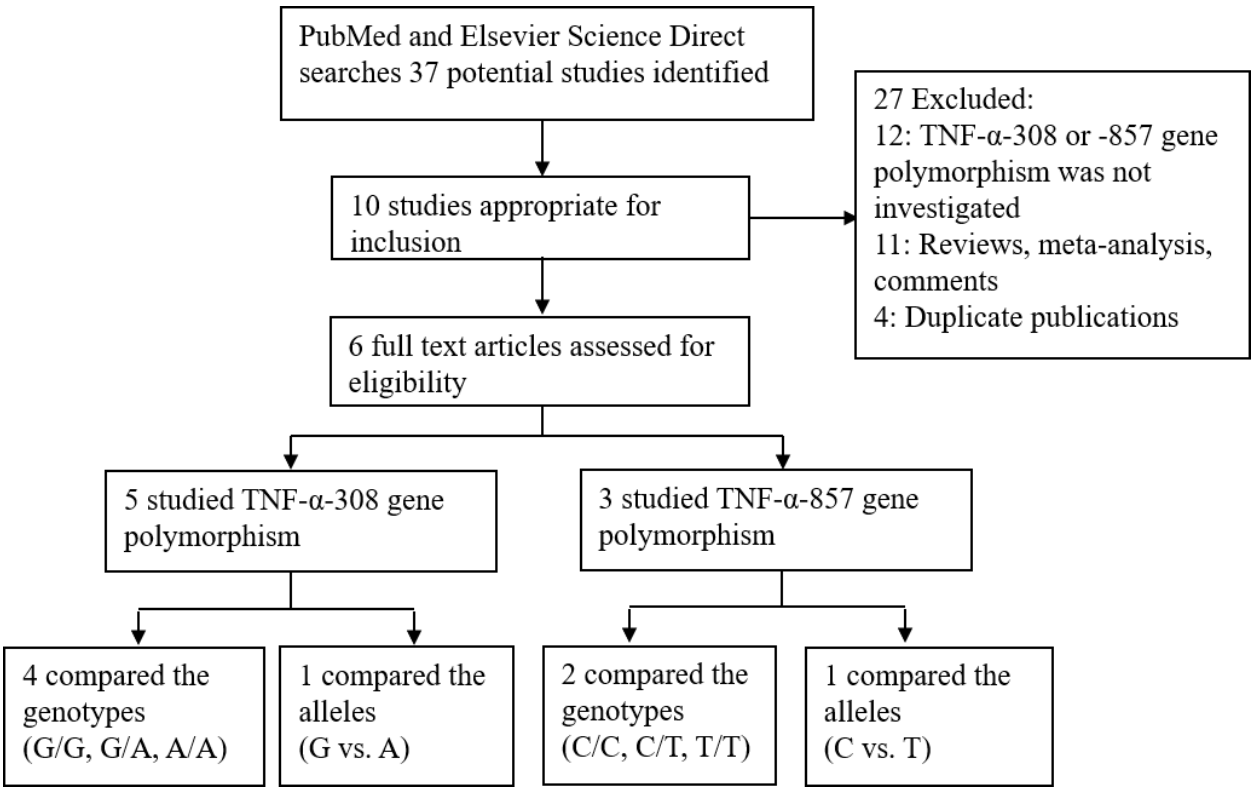
Figure 2. The networks formed by susceptible genes and protein products in AS.



Unpublished data. The network view summarizes the network constructed by the transcribed protein products of SNP-associated genes. Each node represents a protein. Blue lines connecting each node suggests that their relationship is predicted based on experimental evidences. The thickness of the lines represents the degree of confidence prediction of the interaction. Medium

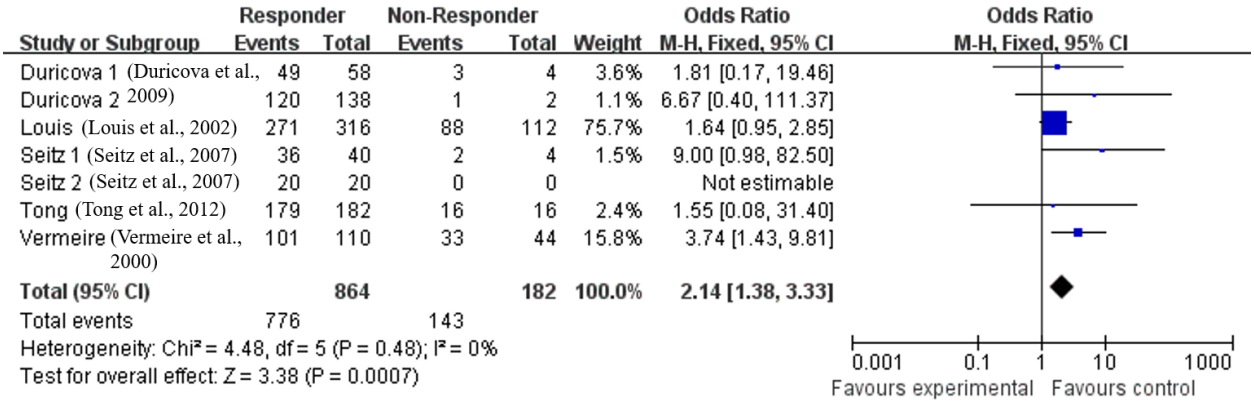
level confidence (0.400) was used to build the network. *ANTXR2*:Anthrax toxin receptor 2; *ERAP1*:Endoplasmic reticulum aminopeptidase 1;*CARD9*:Caspase recruitment domain family, member 9; *PTPN22*:Protein tyrosine phosphatase, non-receptor type 22 (lymphoid); *IL-23R*: Interleukin 23 receptor; *TNFRSF1A*: Tumor necrosis factor receptor superfamily, member 1A; *PTGER4*:Prostaglandin E receptor 4 (subtype EP4); *STAT4*:Signal transducer and activator of transcription 4; *TBKBP1*:TBK1 binding protein 1; *TNF*: Tumor necrosis factor; *IL12B*: Interleukin 12B; *PPARGC1B*:Peroxisome proliferator-activated receptor gamma, coactivator 1 beta; *ESR1*: Estrogen receptor 1; *RUNX3*:Runt-related transcription factor 3; *ORAI1*:ORAI calcium release-activated calcium modulator 1; *STIM1*:Stromal interaction molecule 1; *FCGR2B*:Fc fragment of IgG, low affinity IIb, receptor (CD32); *HRAS*:v-Ha-ras Harvey rat sarcoma viral oncogene homolog; *ETS1*:V-ets erythroblastosis virus E26 oncogene homolog 1 (avian); *FCRL4*:Fc receptor-like 4.

Figure 3. Study selection for the meta-analysis (Section 4).



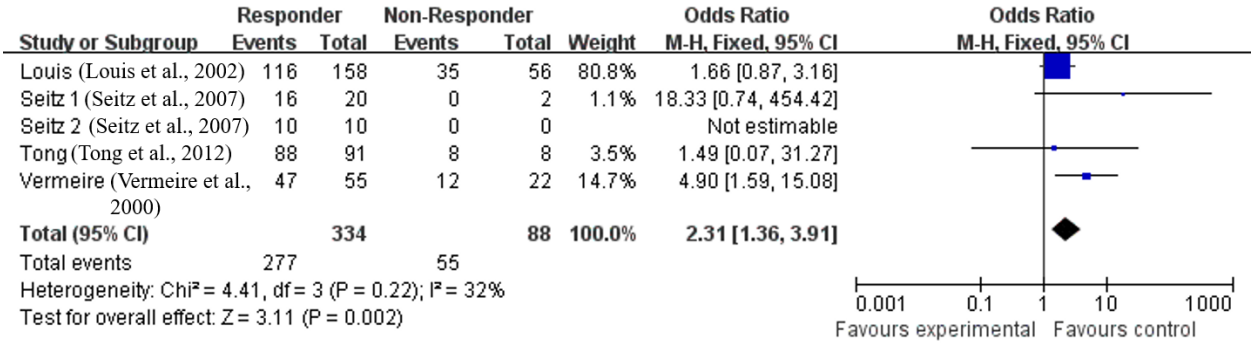
A total of six relevant studies met the inclusion criteria for the meta-analysis. The flow chart showed the process of the meta-analysis, including the inclusion and exclusion criteria for initial study selection, assessment for eligibility, separation based on SNP targets, and finally the comparison results.

Figure 4. Association of -308 A/G polymorphism at *TNFα* promoter and response to *TNF* blockers (G allele vs A allele).



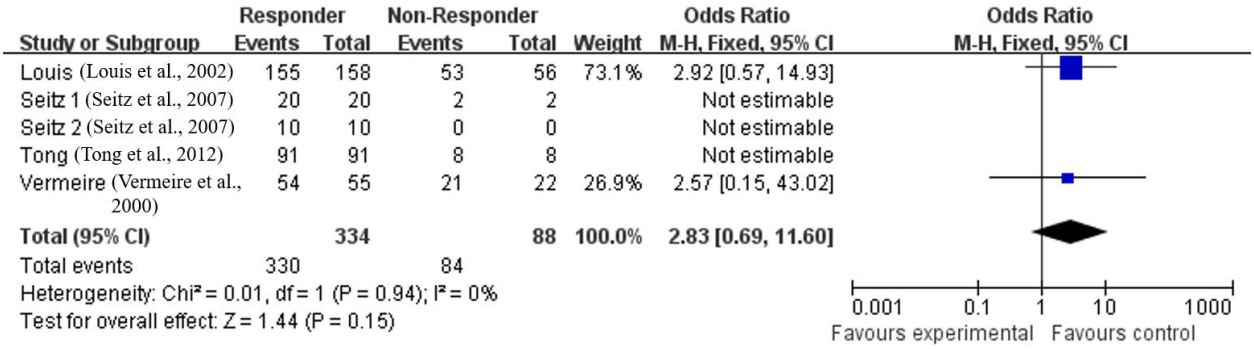
Duricova 1 and Duricova 2 refer to the results of patients from two different countries. Seitz 1 and Seitz 2 refer to the results of two different subsets. Cited from(Tong et al., 2013).

Figure 5. Association of -308 A/G polymorphism at *TNFα* promoter and response to *TNF* blockers (G/G vs [G/A+A/A] genotypes).



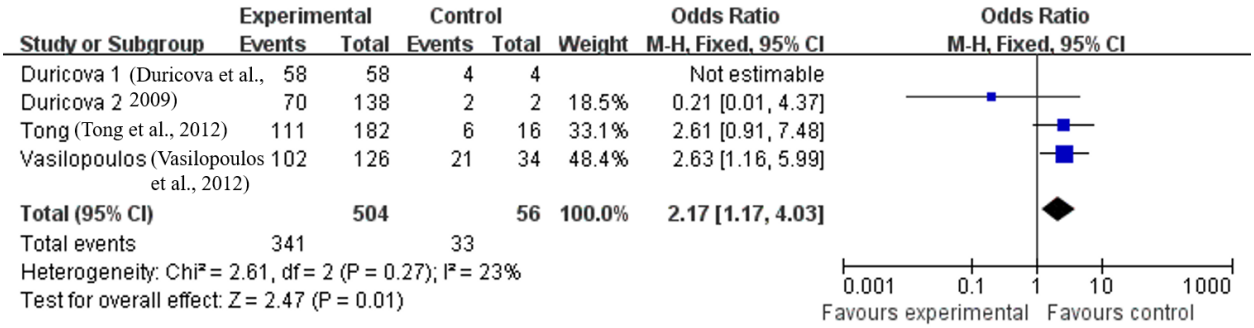
Seitz 1 and Seitz 2 refer to the results of two different subsets. Cited from (Tong et al., 2013).

Figure 6. Association of -308 A/G polymorphism at *TNFα* promoter and response to *TNF* blockers ([A/G+G/G] vs A/A genotypes).



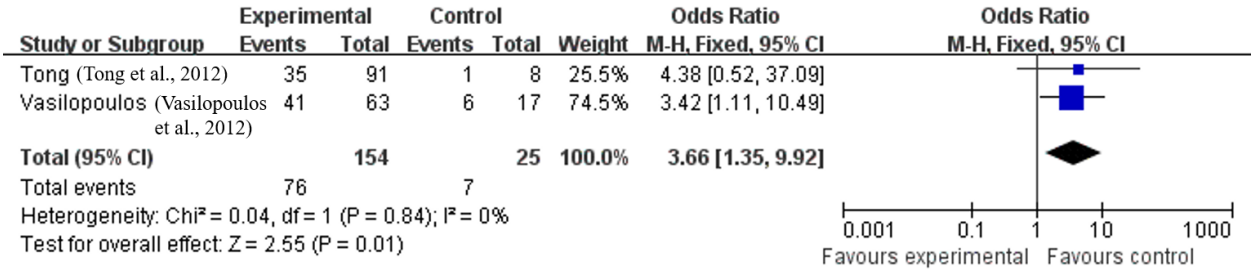
Seitz 1 and Seitz 2 refer to the results of two different subsets. Cited from (Tong et al., 2013).

Figure 7. Association of -857 C/T polymorphism at *TNFα* promoter and response to *TNF* blockers (C allele vs T allele).



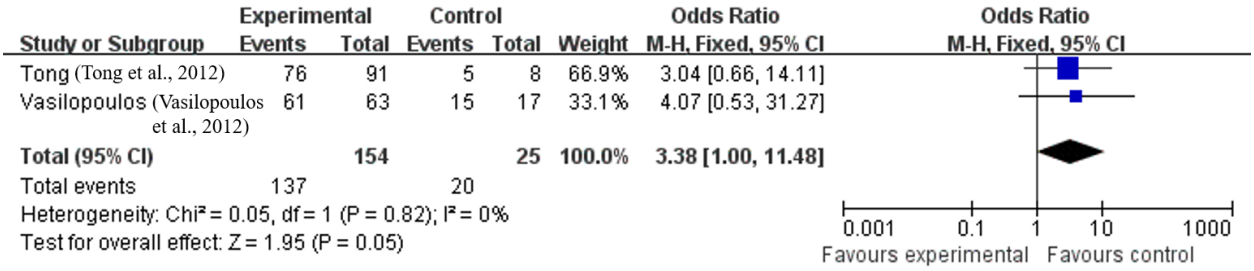
Duricova 1 and Duricova 2 refer to the results of patients from two different countries. Cited from (Tong et al., 2013).

Figure 8. Association of -857 C/T polymorphism at *TNFα* promoter and response to *TNF* blockers (C/C vs [C/T+T/T] genotypes).



Cited from (Tong et al., 2013).

Figure 9. Association of -857 C/T polymorphism at *TNFα* promoter and response to *TNF* blockers ([C/T+C/C] vs T/T genotypes).



Cited from (Tong et al., 2013).

APPENDIX: LIST OF PUBLICATIONS

1. Tong, Q., Cai, Q., de Mooij, T., Xu, X., Dai, S., Qu, W., Zhao, D. B.(2015). Adverse events of anti-tumor necrosis factor alpha therapy in ankylosing spondylitis. PLoS One, 10(3), e0119897. doi:10.1371/journal.pone.0119897
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