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Association of TYK2 polymorphisms with susceptibility to microscopic polyangiitis in a Guangxi population

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Background. Heredity and epigenetics affect the pathogenesis of microscopic polyangiitis (MPA). Tyrosine kinase 2 (TYK2) is a potential protective factor againstnovel anti-neutrophil cytoplasmic antibody (ANCA)--associated vasculitis (AAV).) potential protective factor. Current research suggests that TYK2 is associated with many autoimmune diseases, but there is currently no study on the relationship between TYK2 and AAV. The purpose of this This study aimed was to assess the effect of TYK2 polymorphisms (rs2304256A>C, rs280519G>A, and rs12720270A>G) on susceptibility to MPA. Methods. A total of 562 Chinese participants (265 MPA patients and 297 healthy volunteers) were recruited. Polymerase chain reactions were combined with high-throughput sequencing to sequence polymorphic loci and perform correlation analysis. Results. In males, individuals with the CA genotype (rs2304256) in the dominant model showed a significantly reduced risk of MPA (odds ratio [OR], 0.52; 95% confidence interval [CI], 0.29-0.91; P=0.02). In the dominant model, the rs280519 heterozygous mutation AG showed a lower risk of MPA (OR, 0.48; 95% CI, 0.27-0.84; P=0.01). The dominant model AG-AA (rs280519) genotype may be a protective factor for MPA risk compared with the homozygous mutation GG (OR, 0.56; 95% CI, 0.31-0.98; P=0.04). The risk of MPA was lower in the GA genotype (rs12720270) than in the AA-GG genotype (OR, 0.55; 95% CI, 0.31-0.97; P=0.04). Discussion. TYK2 variants (rs2304256, rs280519, rs12720270) may be associated with MPA susceptibility as demonstrated in a male Chinese population.

Association of TYK2 Polymorphisms with Susceptibility to Microscopic Polyangiitis in a Guangxi Population

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Abstract

Background. Heredity and epigenetics affect the pathogenesis of microscopic polyangiitis (MPA). Tyrosine kinase 2 (TYK2) is a potential protective factor against anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). Current research suggests that TYK2 is associated with many autoimmune diseases, but there is currently no study on the relationship between TYK2 and AAV. This study aimed to assess the effect of TYK2 polymorphisms (rs2304256A>C, rs280519G>A, and rs12720270A>G) on susceptibility to MPA.
Methods. A total of 562 Chinese participants (265 MPA patients and 297 healthy volunteers) were recruited. Polymerase chain reactions were combined with high-throughput sequencing to sequence polymorphic loci and perform correlation analysis.
Results. In males, individuals with the CA genotype (rs2304256) in the dominant model showed a significantly reduced risk of MPA (odds ratio [OR], 0.52; 95% confidence interval [CI], 0.29-0.91; *P*=0.02). In the dominant model, the rs280519 heterozygous mutation AG showed a lower risk of MPA (OR, 0.48; 95% CI, 0.27-0.84; *P*=0.01). The dominant model AG-AA (rs280519) genotype may be a protective factor for MPA risk compared with the homozygous mutation GG (OR, 0.56; 95% CI, 0.31-0.98; *P*=0.04). The risk of MPA was lower in the GA genotype (rs12720270) than in the AA-GG genotype (OR, 0.55; 95% CI, 0.31-0.97; *P*=0.04).
Discussion. TYK2 variants (rs2304256, rs280519, rs12720270) may be associated with MPA susceptibility as demonstrated in a male Chinese population.

Introduction

Anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a heterogeneous group of small vasculitis of unknown etiology, characterized by the presence of proteinase 3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA) in the serum. It is often accompanied by inflammation and necrosis of the vessel wall. There are several types of this systemic vasculitis, including granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (Li, Huang et al. 2021). The distribution of AAV subtypes varies by region. GPA is common in Northern Europe (Berti, Cornec et al. 2017), while MPA is more common in China and Japan (Fujimoto, Watts et al. 2011, Watts and Scott 2012). The incidence of MPA ranges from 0.5 to 24.0 cases per million person-years, and the prevalence ranges from 9.0 to 94.0 cases per million person-years, with onset ages ranging from 55 to 75 years (Kitching, Anders et al. 2020). The prevalence of AAV has been reported to be 46–421 per million population in Asia(Kawasaki and Tsuchiya 2021). Kidney damage occurs in nearly all patients with MPA, which is characterized by necrotizing and crescentic pauci-immune glomerulonephritis. Deterioration of renal function has the potential to lead to end-stage renal disease and mortality (Binda, Moroni et al. 2018).

The HLA region is considered the most important risk factor for AAV (Trivioli, Marquez et al. 2022). Genome-wide studies have revealed a unique pattern of MPA, namely its association with HLA-DQ (Li, Huang et al. 2021). In a Chinese population, HLA DQA1*03:02, DQB1*03:03 was associated with MPO-AAV susceptibility (Wang, Cui et al. 2019). In a Japanese population, HLA DQA1*03:02, DQB1*03:03 was found to be associated not only with MPO-AAV susceptibility, but also with the risk of recurrence (Kawasaki, Sada et al. 2023). The genetic studies of AAV enhance our understanding of the genetic susceptibility factors contributing to this disease, thereby improving patient management and treatment strategies.

Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway is a signaling pathway that affects biological processes such as cell proliferation, differentiation, apoptosis, and immune regulation (Xin, Xu et al. 2020). This pathway plays a direct role in the communication between transmembrane receptors and the nucleus (O'Shea and Plenge 2012, O'Shea, Schwartz et al. 2015). The basic process can be summarized as follows: cytokines bind to the corresponding receptors, receptors aggregate with JAKs, and JAKs are phosphorylated and activated by each other; STAT proteins are recruited to a docking site formed by the phosphorylation of tyrosine residues; STAT is phosphorylated to form a dimer; STATs enter the nucleus and interact with other transcription factors to regulate gene transcription (Xin, Xu et al. 2020). Tyrosine kinase 2 (TYK2) is a vital signal transduction kinase in the JAK/STAT signaling pathway (Gonciarz, Pawlak-Bus et al. 2021), which participates in the differentiation of Th1 and Th17 cells. Dysfunction of TYK2 can contribute to the development of autoimmune and inflammatory diseases (Muromoto, Shimoda et al. 2021). The necessity of TYK2 activity for interleukin (IL)-12, IL-23 and type I interferon (IFN) signaling has been demonstrated experimentally in mice with TYK2 deficiency (Gorman, Hundhausen et al. 2019). Moreover, it has been shown that blocking TYK2 activity inhibits the downstream signal transduction of IL-12 and other cytokines (Elyoussfi, Rane et al. 2023). Novel TYK2 inhibitors are expected to

have significant clinical impacts (Muromoto, Shimoda et al. 2021). TYK2 gene mutations are genetically linked to ankylosing spondylitis, psoriasis, Crohn's disease, ulcerative colitis, type 1 diabetes (T1DM), multiple sclerosis (MS), lupus erythematosus (SLE), and rheumatoid arthritis (RA) (Parkes, Cortes et al. 2013). To our knowledge, there are no reports of an association between TYK2 mutations and AAV. Therefore, we investigated the association between TYK2 single nucleotide polymorphisms (SNPs) (rs2304256C/A, rs280519G/A, rs12720270A/G) and susceptibility to MPA in the Chinese population.

Materials & Methods

Ethics approval

This study was approved by the Medical Ethics Committee of The Second Affiliated Hospital of Guangxi Medical University (2018 KY-0100) and was carried out in compliance with the Declaration of Helsinki. Informed consent was given by all subjects.

Study subjects

The MPA cohort comprised 265 patients with microscopic polyangiitis admitted to the Second Affiliated Hospital of Guangxi Medical University, whose diagnosis criteria met the International Chapel Hill Consensus Conference Nomenclature of Vasculitides criteria (18). Patients with secondary vasculitis caused by other factors (external infections, tumors, drugs) and with other autoimmune diseases(rheumatoid arthritis, systemic lupus erythematosus, Henoch-Schonlein purpura) were excluded. In addition, 297 healthy subjects from the same hospital were included in the normal control group who were confirmed to be free of MPA, other autoimmune diseases and malignancies (Table 1).

Genotyping and data quality control

Genotyping was performed using multiplex PCR and high-throughput sequencing (Sangon Biotech, Shanghai, China). Data quality control: First, any part of the sequence containing the sequenced splitter sequence was excised using cutadapt (v 1.2.1) software, then the remaining sequence was quality-controlled using PRINSEQ-lite (v 0.20.3) software, and bases with a quality threshold <20 were removed from the 3' end to the 5' end of the sequence. The remaining sequences were considered to be qualified for quality control. Genotyping: BWA (v 0.7.13-r1126) software was used to align the qualified sequences to the reference genome (the parameters were default). According to the comparison results, the genotypic results of the target locus were calculated by samtools software (version 0.1.18). Finally, Annovar software was used to annotate the mutation sites. Genomic DNA was extracted using the corresponding kit (Tiangen Biotech, Beijing, China). DNA concentration and purity were determined by ultraviolet spectrophotometer (Thermo Scientific, USA).

Statistical analysis

The SPSS 26.0 software was used for statistical analysis. Allele frequency and genotype frequency , Hardy-Weinberg equilibrium (HWE) , and haplotype frequency analysis were performed by SHEsis online software (http://analysis.bio-x.cn). The odds ratio (OR) and 95% confidence interval (CI) were calculated by SNPstats software to evaluate the relationship

between the genetic model and the incidence of MPA in the subgroup analysis. P<0.05 indicated that the difference was statistically significant.

Results

The genotyping success rate was 100%. For rs2304255, the minor allele frequency (MAF) did not reach the threshold value (MAF ≤5%), lacking statistical significance, and was therefore excluded from subsequent correlation analysis. The genotype frequency distribution of the other three SNPs did not deviate from Hardy–Weinberg equilibrium (HWE, *P*>0.05). Preliminary analysis of the distribution differences of SNP genotypes, alleles, haplotypes, and genetic models in the two groups showed that each SNP was not correlated with MPA (*P*>0.05) (Tables 2-4). The association between SNPs and MPA risk was influenced by sex, particularly in males (*P*<0.05). Rs2304256 reduced the risk of MPA with the CA genotype in the overdominant model (OR, 0.52; 95% CI, 0.29-0.91; *P*=0.02). In rs280519, carriers of the AG genotype in the overdominant model and the AG-AA genotype in the dominant model showed a significantly lower risk of MPA (overdominant: OR, 0.48; 95% CI, 0.27-0.84; *P*=0.01; dominant: OR, 0.56; 95% CI, 0.31-0.98; *P*=0.04). The GA genotype in the codominant model (rs12720270) had a lower incidence of MPA (OR, 0.55; 95% CI, 0.31-0.97; *P*=0.04) (Table 5). However, negative results were found in the female subgroup (Supplementary Table 1). Similarly, we conducted a stratified analysis of MPA patients by age and ethnicities and no statistically significant differences were found (all *P*>0.05) (Supplementary Tables 2).

Discussion

Research on the relationship between MPA risk and mutations in autophagy genes has demonstrated that autophagy gene polymorphisms are significantly correlated with MPA risk (Zhu, Rao et al. 2021, Lan, Zhu et al. 2023, Li, Rao et al. 2023, Li, Li et al. 2023). TYK2 may affect the pathophysiological processes of AAV (Ortiz-Fernandez, Lopez-Mejias et al. 2020). Our study provides the first evidence that its polymorphisms may be associated with MPA risk. TYK2 is a 27.9-kb gene with 25 exons located on chromosome 19p13.2 (Lindqvist, Steinsson et al. 2000). TYK2 is a member of the JAK family that moderates immune responses to IL-12, IL-23, and IFNα (Gonzalez Lopez de Turiso and Guckian 2022).

A published European cohort study of patients with AAV further suggested that the IL-12/IL-23 cytokine signaling pathway, along with Th1 and Th17 cells, plays a significant role in the pathophysiology of AAV (Ortiz-Fernandez, Lopez-Mejias et al. 2020). In an ANCA-mediated mouse model of experimental vasculitis, Hoshino et al. found that activated neutrophils produced IL-17A and IL-23 via the classical complement pathway in response to MPO-ANCA. This pathway provides a local environment that promotes IL-17 production and Th17-mediated autoimmunity, becoming the first step in the initiation of chronic autoimmune inflammation (Hoshino, Nagao et al. 2008). The pathogenesis of AAV involves a vicious cycle of neutrophil

extracellular trap (NET) formation and ANCA production (Nakazawa, Masuda et al. 2019). NET formation is directly induced by IL-17 (Tohme, Yazdani et al. 2019). Wilson et al. proposed that neutrophils drive a feed-forward loop through the production of NETs, which directly promotes the development of Th17 cell subsets and further enhances NET formation, thus playing a significant role in the pathogenesis of autoimmunity (Wilson, Randall et al. 2022). We speculate that a mutation in TYK2 may result in a reduction in the function or activity of its own gene, thus affecting downstream signal transduction. Dysregulation of signal transduction may lead to the dysregulation of NET release and degradation, This process is closely related to the pathogenesis of AAV. Rs2304256 is one of the most common SNPs of TYK2, and its genetic association has been widely investigated(Gonciarz, Pawlak-Bus et al. 2021, Morand, Merola et al. 2024). The genetic association between rs2304256 and SLE is contradictory across different populations. This variant is significantly associated with in Finnish, Swedish, British (Sigurdsson, Nordmark et al. 2005, Cunninghame Graham, Manku et al. 2007, Hellquist, Jarvinen et al. 2009, Suarez-Gestal, Calaza et al. 2009), and Chinese Han (Tang, Wan et al. 2015) populations, but shows a reversed association in Hong Kong-based (Li, Chang et al. 2011) and Japanese (Kyogoku, Morinobu et al. 2009) populations. TYK2 rs2304256 was verified to be closely related to systemic sclerosis susceptibility (SSc) in the European population (Lopez-Isac, Campillo-Davo et al. 2016), while the results were negative in the Chinese Han SSc population (Liu, Yan et al. 2021). These inconsistencies appear to arise from differences in geography and ethnicity. TYK2 gene mutations are genetically linked to SLE, ankylosing spondylitis, psoriasis, Crohn's disease, ulcerative colitis, type 1 diabetes, MS, and RA. To our knowledge, there are currently no reports of an association between TYK2 mutations and AAV. We decided to investigate the association between TYK2 SNPs in the Chinese Han population (Pellenz, Dieter et al. 2021). Pellenz et al. (2021b) pooled data from 34 articles for a meta-analysis assessing the impact of multiple TYK2 variants on susceptibility to autoimmune diseases. Their data suggest that rs2304256 is a susceptibility factor for several autoimmune diseases, including SLE, MS, and rheumatoid RA, with the A allele acting as a protective factor (Pellenz, Dieter et al. 2021). Rs2304256 was found to be a non-synonymous SNP located in the exon 8 region of the TYK2 gene. The A allele contributes to the substitution of valine 362 to phenylalanine in the JAK homologous 4 (JH4) region, which is a key domain in the interaction of TYK2 with IFNAR1 and its function, which is crucial for maintaining IFNAR1 expression on the cell membrane. Amino acid substitution affects the processing of precursor mRNA (Li, Rotival et al. 2020). In addition, both the PolyPhen-2 and Sorting Intolerant From Tolerant (SIFT) tools indicate that rs2304256 is a benign variant (Kumar, Henikoff et al. 2009, Adzhubei, Schmidt et al. 2010). Our study illustrates that the rs2304256(A>C) variant, in the dominant model, potentially decreases susceptibility to MPA in males. The authors hypothesized that its protective effect operates as described above.

As for rs280519, the AG-AA genotype reduced MPA risk compared with the GG genotype in the dominant model, and the AG genotype may reduce MPA risk in the overdominant model. In the overdominant model of rs12720270, the GA genotype protected against MPA. The above

conclusions also followed from the analysis of the male subgroup. The polymorphisms reported in this paper, rs280519 and rs12720270, are both located in the intron region. The backbone of a gene is the exon, which contains the coding sequences that are transcribed into mRNA and subsequently translated into a protein. Splicing abnormalities caused by intron variation can disrupt the encoded protein, resulting in frameshifts of multiple residues and intra-frame deletions or insertions (Bryen, Joshi et al. 2019), with devastating effects. The impact of single nucleotide abnormalities in introns on human disease should not be ignored. An earlier meta-analysis, including 16,335 SLE patients and 30,065 controls, revealed that the rs280519 polymorphism was significantly associated with SLE risk in Caucasians and Asians (Lee and Bae 2016). A Turkish study involving 60 patients with Crohn's disease and 151 patients with ulcerative colitis found that the rs280519 AA genotype was a risk factor for ulcerative colitis, while the AG genotype was a protective factor for ulcerative colitis and Crohn's disease (Can, Tezel et al. 2015). Affected by the interferon (IFN) signaling pathway, the rs280519G allele may influence the severe National Institute on Aging classification in patients with chronic hepatitis C (Lopez-Rodriguez, Hernandez-Bartolome et al. 2017). Rizi et al. studied patients with mild and severe coronavirus disease 2019 (COVID-19) and found that the rs12720270 polymorphism may down-regulate the risk of COVID-19 severity, speculating that the G allele might affect COVID-19 severity by decreasing TYK2 expression (Zabihi Rizi, Ghorbani et al. 2023), which is a process similar to the previously mentioned protective effect of rs2304256. Besides, both the rs280519 and rs12720270 variants reduced the risk of juvenile idiopathic arthritis in a Chinese Han population in both the recessive and dominant models (Qian, Chen et al. 2022). TYK2 induces intracellular signal transduction. We speculated that the rs280519 and rs12720270 variants might result in the abnormal expression of TYK2, thereby affecting cytokine signaling pathways and ultimately reducing susceptibility to MPA. However, Molecular mechanisms underlying the increased risk of MPA in the Chinese population caused by the SNP rs280519 and rs12720270 mutation are still unknown. ATG4 SNPrs280519 and rs12720270 are associated with MPA; future research should explore the molecular mechanisms involved in this association.

Conclusions

This study found that some mutations in the TYK2 gene were associated with a reduced risk of MPA in a male, Chinese Han population. This study was a single-center retrospective study with a small number of cases and the conclusions need to be further confirmed in a larger sample as part of future multi-center, prospective, large-scale randomized controlled clinical studies.

Acknowledgements

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