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Association between *MEFV* polymorphisms and the susceptibility to ankylosing spondylitis in a Chinese Han population

A case-control study

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Abstract

The aim of this study was to explore the genetic association of Mediterranean fever (*MEFV*) gene polymorphisms rs3743930 and rs11466023 with ankylosing spondylitis (AS) susceptibility in a cohort of Chinese Han population.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for genotyping *MEFV* polymorphisms in 131 AS patients and 127 healthy controls. Chi-square test was employed to compare the genotype and allele distributions between the case and control groups. Odds ratio (OR) with 95% confidence interval (CI) was calculated to assess the association between *MEFV* gene polymorphisms and AS incidence.

The frequency of the G allele of *MEFV* polymorphism rs3743930 in the AS group was significantly higher than that in the healthy control group (36.64% vs 28.35%, $P < .05$). And individuals carrying the GG genotype showed 2.896 folds higher risk of developing AS when compared with CC genotype carriers (OR = 2.896, 95% CI = 1.115–7.519). But no significant differences were detected in either genotype or allele distributions between case and control groups for the polymorphism rs11466023 ($P > .05$).

MEFV gene polymorphism rs3743930 might be significantly associated with AS susceptibility in Chinese Han population, and its G allele might predict high risk of AS.

Abbreviations: AS = ankylosing spondylitis, CI = confidence interval, ERAP1 = endoplasmic reticulum aminopeptidase 1, FMF = familial Mediterranean fever, HRH4 = histamine H4 receptor, MEFV = Mediterranean fever, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, proIL-1β = pro-interleukin-1β, VEGF = vascular endothelial growth factor.

Keywords: ankylosing spondylitis, Mediterranean fever, polymerase chain reaction, polymorphism

1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease that mainly affects the axial skeleton and limb joints, and accompanied by various degrees of inflammations in the eyes, kidneys, and gastrointestinal tract.[1] AS patients always suffer from back pain and stiffness, which sharply decreases the life quality of the patients.[2] Various risk factors have been confirmed for AS, including biological, genetic, and environmental factors.[3] Among others, genetic factors play important roles in the pathogenesis of AS. Previously, twin and family studies have demonstrated that genetic factors contribute to 90% of overall AS susceptibility.[4–7] Up to now, genetic variants in multiple genes have been proved to be associated with AS, such as vascular endothelial growth factor (*VEGF*),[8] endoplasmic reticulum aminopeptidase 1 (ERAP1),[9] and histamine H4 receptor (*HRH4*).

Mediterranean fever (*MEFV*), encoding for pyrin, has been firstly identified as a candidate gene for familial Mediterranean fever (FMF).[10] Pyrin is reported to be expressed only in neutrophils and monocytes, and to play key roles in innate immune response. Previous evidences have shown that pyrin and several other proteins containing the pyrin domain have close association with autoinflammatory disorders, which are considered as intracellular modules of inflammatory signaling.[11] Besides, the crucial role of pyrin in the regulation of inflammasome activity and pro-interleukin-1β (proIL-1β) processing has also been reported.[12,13]

In human, the *MEFV* gene is located on the short arm of chromosome 16p13.3, containing 10 exons.[14] More than 100 mutations have been identified in the *MEFV* gene, and are associated with FMF susceptibility. It is noted that *MEFV* gene mutations are significantly correlated with enhanced inflammatory response, which may further lead to inflammatory diseases.[15] Furthermore, earlier study has reported the association of *MEFV* mutant with AS susceptibility already.[16]

In the present study, we adopted a case-control design to compare the frequencies of *MEFV* polymorphisms rs3743930 (E148Q) and rs11466023 (P369S) between patients with AS and healthy controls, so as to assess potential association between these polymorphisms and AS risk in a Chinese Han population.

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The authors declare no conflicts of interest.

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2. Materials and methods

2.1. Study population

This case-control study was carried out in the Harrison International Peace Hospital. All the subjects included in our study must meet the following criteria: the patients with AS were diagnosed according to the modified New York criteria[17]; the individuals in control group were healthy people with normal physical examination results; being adults; being Han people living in China; and without blood relationship. In addition, the participants presented any of the following conditions would be excluded: receiving hormone and/or immune-suppressing drugs within 3 month; with the histories of tumors, atherosclerosis and autoimmune diseases, such as rheumatoid disorders; accompanied by endocrine disorders, such as thyroid diseases and adrenal gland diseases; presenting FMF; and with serious liver or kidney diseases. In addition, the case and control groups were matched in age and gender. Finally, 131 AS patients including 104 males and 27 females, and 127 healthy individuals (101 males and 26 females) were enrolled in our study.

The whole study was approved by the Ethics committee of Harrison International Peace Hospital. Sample collection was in accordance with the ethnic criteria of national human genome research. Before sample collection, all participants signed informed consents, and agreed to provide blood samples for clinical investigation.

2.2. DNA extraction

Here 5 mL venous blood was obtained from each subject and stored into anticoagulative tube with EDTA-disodium salt. Genomic DNA was extracted from peripheral blood using TaKaRa Genome DNA Extraction Kit (Dalian Biological Engineering CO., LTD, China), and then stored at –20°C for later application.

2.3. Determination of the polymorphisms

To analyze genotype distributions of *MEFV* polymorphisms, the mutant region of rs3743930 and rs11466023 was partially amplified using polymerase chain reaction (PCR) and genotyped through restriction fragment length polymorphism (RFLP) method. The primer sequences for rs3743930 and rs11466023 were designed using Primer Premier 5.0 (Table 1), and synthesized by Shanghai Sangon biotech Co., Ltd. PCR procedures were performed in a total volume of 25 μL, including 2 μL genomic DNA, 2 μL primers (1 μL of upstream and downstream primers), 1.5 μL Mg^{2+}, 2 μL dNTP, 0.3 μL Taq DNA polymerase, 2.5 μL 10 × buffer, and 14.7 μL ddH_2O.

Table 1

Primer sequences of *MEFV* gene rs3743930 and rs11466023 polymorphisms.

Variations		Primer sequences
rs3743930	Forward	5'-CCTGAAGACTCCAGACCACCCCG-3'
	Reverse	5'-GGCCCTCCGAGGCCTTCTCTCTG-3'
rs11466023	Forward	5'-TCCCCGAGGCAGTTTCTGGGCACC-3'
	Reverse	5'-TGGACCTGCTTCAGGTGGCGCTTAC-3'

MEFV = Mediterranean fever.

Then, the restriction enzymes *BstNI* and *AluI* were used for RFLP. Next, the digested products were separated using 2% agarose gel with ehidium bromide staining. Additionally, the PCR products of the polymorphisms rs3743930 and rs11466023 were also randomly selected for confirmation via direct sequencing, and the results were perfectly concordant with PCR-RFLP results.

2.4. Statistical analysis

All statistical analysis in this study was performed using the PASW statistics 18.0 statistical software. The genotype and allele frequencies of the polymorphisms rs3743930 and rs11466023 were calculated through direct counting. The conformity to Hardy–Weinberg equilibrium (HWE) among our study population was estimated via chi-square test, to assess the quality of the study sample. Chi-square test was performed to compare the genotype and allele distributions of the rs3743930 and rs11466023 polymorphisms between groups. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to evaluate the association between *MEFV* gene polymorphisms and AS susceptibility. All the tests were 2-tailed, and *P* values less than .05 were considered as significant.

3. Results

3.1. HWE test

Table 2 presented the genotype and allele distributions of *MEFV* gene polymorphisms rs3743930 and rs11466023 in both case and control groups. In chi-square test, we noted that the genotype

Table 2

Genotype and allele distributions of *MEFV* gene rs3743930 and rs11466023 polymorphisms in case and control groups.

Genotype/Allele	Case $n=131$ (%)	Control $n=127$ (%)	χ^2	*P*	OR (95% CI)
rs3743930					
CC	52 (39.69)	62 (48.82)	–	–	1
CG	62 (47.33)	58 (45.67)	0.857	.355	1.275 (0.762–2.131)
GG	17 (12.98)	7 (5.51)	5.044	.025	2.896 (1.115–7.519)
C	166 (63.36)	182 (71.65)	–	–	1
G	96 (36.64)	72 (28.35)	4.041	.044	1.462 (1.009–2.118)
rs11466023					
GG	112 (85.50)	111 (87.40)	–	–	1
GA	19 (14.50)	16 (12.60)	0.200	.655	1.177 (0.576–2.406)
AA	0 (0)	0 (0)	–	–	–
G	243 (92.75)	238 (93.70)	–	–	1
A	19 (7.25)	16 (6.30)	0.185	.667	1.163 (0.584–2.316)

CI = confidence interval, MEFV = Mediterranean fever, OR = odds ratio.

distributions of the detected SNPs did not deviate from HWE in control group ($P > .05$), demonstrating their good representativeness.

3.2. Genetic association of MEFV polymorphisms with AS

Significant association was found between *MEFV* rs3743930 polymorphism and AS susceptibility. A remarkable increase in the frequency of the GG genotype was detected in AS patients group when compared with healthy controls (12.98% vs 5.51%) while the CC genotype frequency decreased in case group (39.69% vs 48.82%), and these differences were statistically significant ($P < .05$). In healthy control group, we noted that the proportion of the subjects carrying the heterozygote CG was 45.67%, similar with that in case group (47.33%). Besides, the C and G allele frequencies were 63.36% and 36.64% in case group and 71.65% and 28.35% in control group, showing statistically significant difference between the groups ($P < .05$). These data suggested that *MEFV* gene polymorphism rs3743930 was associated with AS susceptibility in Chinese Han population, and individuals carrying G allele showed 1.462 folds higher risk of developing AS when compared with C allele carriers (OR = 1.462, 95% CI = 1.009–2.118).

For rs11466023, only 2 genotypes of GG and GA were detected in our study population. As shown in Table 2, the GG and GA genotype frequencies were 85.50% and 14.50% in AS patients group and 87.40% and 12.60% in control group. Besides, the G and A allele frequencies were 92.75% and 7.25% in cases and 93.70% and 6.30% in controls. But all differences did not reach significant level ($P > .05$). According to the χ^2 test results, *MEFV* gene polymorphism rs11466023 might have no obvious association with AS susceptibility in Chinese Han population.

4. Discussion

AS is a common inflammatory rheumatic disease with back pain and stiffness as its main clinical characteristics.[18] AS more likely occurs in young and middle-aged adults, and patients with AS frequently suffer complicated lesions in eyes, lung, cardiovascular, and renal in different degrees. In China, the prevalence of this disease has reached approximately 0.2%, with a ratio of female to male about 4:10.1 in terms of its incidence rate.[19] As a polygenic disease, AS is caused by the combined influences from environmental and genetic factors. Recently, exploring polymorphisms in candidate genes for the development of AS has become a promising method in molecular genetics research. As reported in previous literature, a number of genes have been identified to be related to AS risk.[20,21]

In the present study, we used PCR-RFLP method for genotyping 2 polymorphisms rs3743930 and rs11466023 in *MEFV* gene. Up to now, more than 90 mutant sites have been identified in human *MEFV* gene, and half of them have been proved to be associated with human diseases. The most common mutations in *MEFV* gene mainly distribute in exon 10, and 4 missense mutations M694V, M6801, V726A, and M694 have been widely explored for their associations with FMF risk.[22,23] The human *MEFV* gene is located on chromosome 16p and encodes the protein pyrin. Pyrin has been reported to be involved in the regulation of inflammation through leukocyte apoptosis and interleukin-1 and NF-κB activation, suggesting a potential role of *MEFV* gene in the development of certain inflammatory diseases. Previous evidences have shown that *MEFV* gene variants are associated with a number of inflammatory rheumatic disorders, such as Behçet's disease and palindromic rheumatism.[24] Furthermore, a major study has demonstrated a significant association between AS susceptibility and *MEFV* gene mutations which mainly concentrate on the B30.2 domain.[25]

The results of our present study indicated a significant association between *MEFV* gene polymorphism rs3743930 and AS susceptibility. We noted that individuals carrying the GG genotype showed 2.896 folds higher risk of developing AS when compared with CC genotype carriers. And the frequency of the G allele of this polymorphism in the AS group was 36.64%, significantly higher than that in healthy control group (28.35%). Meanwhile, the polymorphism rs3743930 (E148Q) is located on the second exon of *MEFV* gene, and shows higher mutation rate. In previous study, *MEFV* gene polymorphism rs3743930 has been reported to be a contributor to Henoch–Schönlein purpura (HSP) and HSP-related joint syndromes in Chinese children,[26] which was in accordance with our present results. Nonetheless, *MEFV* gene polymorphism rs11466023 showed no significant association with AS risk in our study population, and only 2 genotypes of GG and GA were detected in our study sample.

In conclusion, *MEFV* gene polymorphism rs3743930 might be associated with AS risk in Chinese Han population. However, the relatively small sample size and low frequency of *MEFV* gene mutations in this study led to a low statistical power (less than 0.9). Additionally, the molecular mechanisms underlying the association between *MEFV* and AS risk remained unclear. Further functional studies will be required to address this issue. So studies with larger sample sizes and various populations should be performed to verify our findings.

Author contributions

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References

[1] Erkol Inal E, Gorukmez O, Eroglu S, et al. Associations between polymorphisms of IL-17F and IL-17A genes with disease activity and clinical outcome of Ankylosing Spondylitis. Acta Reumatol Port 2016;41:232–9.
[2] Tyrrell JS, Redshaw CH. Physical activity in ankylosing spondylitis: evaluation and analysis of an eHealth tool. Inform Prim Care 2016;23:169.
[3] Sparks JA, Costenbader KH. Genetics, environment, and gene-environment interactions in the development of systemic rheumatic diseases. Rheum Dis Clin North Am 2014;40:637–57.
[4] Vidal-Castineira JR, Lopez-Vazquez A, Diaz-Pena R, et al. A single nucleotide polymorphism in the Il17ra promoter is associated with functional severity of ankylosing spondylitis. PloS One 2016;11: e0158905.

[5] O'Rielly DD, Uddin M, Rahman P. Ankylosing spondylitis: beyond genome-wide association studies. Curr Opin Rheumatol 2016;28:337–45.
[6] Brown MA, Kennedy LG, MacGregor AJ, et al. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. Arthritis Rheum 1997;40:1823–8.
[7] Brown MA, Laval SH, Brophy S, et al. Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. Ann Rheum Dis 2000;59:883–6.
[8] Wang M, Zhou X, Zhang H, et al. Associations of the VEGF level, VEGF rs2010963 G/C gene polymorphism and ankylosing spondylitis risk in a Chinese Han population. Immunol Lett 2016;179:56–60.
[9] Lee YH, Song GG. Associations between ERAP1 polymorphisms and susceptibility to ankylosing spondylitis: a meta-analysis. Clin Rheumatol 2016;35:2009–15.
[10] Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean, fever. The International FMF Consortium. Cell 1997;90:797–807.
[11] Gumucio DL, Diaz A, Schaner P, et al. Fire and ICE: the role of pyrin domain-containing proteins in inflammation and apoptosis. Clin Exp Rheumatol 2002;20:S45–53.
[12] Ting JP, Kastner DL, Hoffman HM. CATERPILLERs, pyrin and hereditary immunological disorders. Nat Rev Immunol 2006;6:183–95.
[13] Papin S, Cuenin S, Agostini L, et al. The SPRY domain of Pyrin, mutated in familial Mediterranean fever patients, interacts with inflammasome components and inhibits proIL-1beta processing. Cell Death Differ 2007;14:1457–66.
[14] Pras E, Aksentijevich I, Gruberg L, et al. Mapping of a gene causing familial Mediterranean fever to the short arm of chromosome 16. N Engl J Med 1992;326:1509–13.
[15] Booth DR, Gillmore JD, Lachmann HJ, et al. The genetic basis of autosomal dominant familial Mediterranean fever. QJM 2000;93: 217–21.
[16] Akar S, Birlik M, Sari I, et al. M694 V mutation may have a role in susceptibility to ankylosing spondylitis. Rheumatol Int 2009;29: 1259–60.
[17] van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. Arthritis Rheum 1984;27:361–8.
[18] Zhang S, Li Y, Xu X, et al. Effect of cigarette smoking and alcohol consumption on disease activity and physical functioning in ankylosing spondylitis: a cross-sectional study. Int J Clin Exp Med 2015;8: 13919–27.
[19] Wang J, Li H, Gao X. Association between ERAP1 gene polymorphisms and ankylosing spondylitis susceptibility in Han population. Int J Clin Exp Pathol 2015;8:11641–6.
[20] Zhang X, Ding W. Association of genetic variants in pentraxin 3 gene with ankylosing spondylitis. Med Sci Monit 2016;22:2911–6.
[21] Laza IM, Hervella M, DE-LA-Rúa C. Genetic markers in a medieval case of ankylosing spondylitis. J Rheumatol 2016;43:679–81.
[22] Atoyan S, Hayrapetyan H, Sarkisian T, et al. MEFV and SAA1 genotype associations with clinical features of familial Mediterranean fever and amyloidosis in Armenia. Clin Exp Rheumatol 2016;34:72–6.
[23] Koca SS, Etem EO, Isik B, et al. Prevalence and significance of MEFV gene mutations in a cohort of patients with rheumatoid arthritis. Joint Bone Spine 2010;77:32–5.
[24] Wu Z, Zhang S, Li J, et al. Association between MEFV mutations M694 V and M680I and Behcet's disease: a meta-analysis. PloS One 2015;10: e0132704.
[25] He C, Li J, Xu W. Mutations in the B30.2 domain of pyrin and the risk of ankylosing spondylitis in the Chinese Han population: a case-control study. Immunol Lett 2014;162:49–52.
[26] He X, Lu H, Kang S, et al. MEFV E148Q polymorphism is associated with Henoch-Schonlein purpura in Chinese children. Pediatr Nephrol 2010;25:2077–82.