

## ***TNFR1* -383 A>C polymorphism and ankylosing spondylitis in a Russian Caucasian population: a preliminary study**

V. Mordovskii<sup>1\*</sup>, A. Semenchukov<sup>2\*</sup>, S.Y. Nikulina<sup>1</sup>, A.B. Salmina<sup>3</sup>,  
A. Chernova<sup>1</sup>, E. Kapustina<sup>1</sup>, A. Kents<sup>1</sup>, A. Ohapkina<sup>1</sup>, E. Moskaleva<sup>1</sup>,  
P.E. Maltese<sup>4</sup>, P. Convertini<sup>4</sup> and M. Bertelli<sup>5</sup>

<sup>1</sup>Department of Internal Diseases N.1,  
Krasnoyarsk State Medical University, Krasnoyarsk, Russia

<sup>2</sup>Russia-Italy Laboratory of Medical Genetics MAGI-Russia,  
Research Institute of Molecular Medicine & Pathobiochemistry,  
Krasnoyarsk State Medical University named after Prof. V.F. Voino-Yasenetsky,  
Krasnoyarsk, Russia

<sup>3</sup>Department of Biochemistry, Research Institute of Medical,  
Pharmaceutical and Toxicological Chemistry,  
Krasnoyarsk State Medical University named after Prof. V.F. Voino-Yasenetsky,  
Krasnoyarsk, Russia

<sup>4</sup>MAGI Non-Profit Human Medical Genetics Institute, Rovereto, Italy

<sup>5</sup>MAGI Non-Profit Human Medical Genetics Institute, Bolzano, Italy

\*These authors contributed equally to this study.

Corresponding author: P. Convertini

E-mail: paolo.convertini@assomagi.org

Genet. Mol. Res. 16 (1): gmr16019581

Received December 13, 2016

Accepted February 20, 2017

Published March 30, 2017

DOI <http://dx.doi.org/10.4238/gmr16019581>

Copyright © 2017 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution ShareAlike (CC BY-SA) 4.0 License.

**ABSTRACT.** The aim of this study was to assess the association between the *TNFR1* rs2234649 polymorphism and ankylosing spondylitis susceptibility in a Russian Caucasian population. A total of 41 ankylosing spondylitis patients and 43 healthy controls, matched

according to age and sex, were enrolled, and polymerase chain reaction-restriction fragment length polymorphism analysis was used to genotype the rs2234649 variant. We evaluated genotype distributions in the patient and control groups with the chi-square test, and assessed the relationship between genotypes and ankylosing spondylitis using the odds ratio. Our analysis showed that the rs2234649 polymorphism does not increase ankylosing spondylitis risk. In conclusion, the *TNFR1* gene polymorphism tested does not appear to be useful for assessing predisposition to this disease or for its diagnosis or prognosis.

**Key words:** Ankylosing spondylitis; Tumor necrosis factor receptor 1; Inflammation; Genetic polymorphism; Rheumatic disease; Case-control studies

## INTRODUCTION

Ankylosing spondylitis (AS) is a common inflammatory rheumatic disease affecting the axial skeleton, leading to inflammatory back pain and structural and functional impairments that decrease quality of life (Braun and Sieper, 2007). Over 80% of AS patients are young people (from 20 to 35 years old), and the associated disability rate reaches 40-60% by 5 years after the onset of symptoms. Disability related to this disease is caused by structural changes involving syndesmophyte formation and ankylosis of the vertebrae (Feldtkeller et al., 2003; Wang et al., 2012). According to the Assessment of SpondyloArthritis international Society (ASAS) criteria, 2009, AS is characterized by insidious onset at <40 years of age and pain and stiffness after periods of inactivity that improve with exercise and worsen with rest (Sieper et al., 2009). Physicians do not yet completely understand the exact pathogenesis and etiology of AS, but it is accepted that genetic and environmental factors are implicated (Daryabor et al., 2014). To date, there exist no methods for its early diagnosis. After the appearance of symptoms, diagnosis takes 8 years on average, causing the worsening of the disease (van der Linden and van der Heijde, 1998). AS is more frequent in Caucasian populations (Braun and Sieper, 2007; Brown, 2008; Daryabor et al., 2014) and has a heritable component, as demonstrated by studies of patients' families and twins, who demonstrate a much higher risk of developing AS (Brown, 2008; Dong et al., 2013; Daryabor et al., 2014). More than 80% of AS patients carry the human leukocyte antigen-B (*HLA-B*)27 allele, but only 1-5% of *HLA-B*27 carriers develop AS, suggesting that additional variants are likely to influence susceptibility (Brown et al., 2000). Besides *HLA-B*27, 12 loci have been associated with AS in Europeans (*ANTXR2*, *CARD9*, *ERAP1*, *IL12B*, *IL23R*, *KIF21B*, *PTGER4*, *RUNX3*, *TBKBP1*, *TNFR1*, and chromosomes 2p15 and 21q22) (Burton et al., 2007; Reveille et al., 2010; Evans et al., 2011).

Tumor necrosis factor (TNF) is a proinflammatory cytokine that plays a key role in chronic inflammation (Wajant et al., 2003). Given its involvement in the pathology of many autoimmune diseases, such as rheumatoid arthritis, Crohn's disease, and psoriasis, researchers have developed anti-TNF therapies to treat these conditions (Kontermann et al., 2009). TNF exerts specific and often opposing effects via two distinct receptors, TNF receptor superfamily member 1a (TNFR1) and TNF receptor 2 (TNFR2). TNFR1 has proinflammatory effects (Akassoglou et al., 1998; McCoy and Tansey, 2008), whereas TNFR2 (Grell et al., 1995)

promotes both neuroprotection and remyelination (Arnett et al., 2001; Fontaine et al., 2002). Accordingly, full inhibition of TNF leads to side effects such as increased inflammation; thus, there is a clear need to avoid the risks associated with total TNF inactivation. One solution is the development TNFR1-specific antagonists that leave TNFR2 signaling unaffected.

The cytogenetic location of *TNFR1* is 12p13.31. Mutations in this gene have been associated with multiple sclerosis. Ferrante (1989) previously identified several single nucleotide polymorphisms in *TNFR1*. Of the various *TNFR1* sequence variants, the 5' untranslated region (UTR) rs2234649 polymorphism, resulting in an adenine to cytosine transversion at position -383 relative to the translation start site, has been associated with AS in the Mexican population (Corona-Sanchez et al., 2012) and type 1 diabetes (Nishimura et al., 2003). Indeed, Nishimura et al. (2003) showed a significantly increased frequency of the -383C allele among patients with type 1, likely causing higher expression of the *TNFR1* gene, and resulting in the increased inflammation-observed in these patients. Recently, Fletes-Rayas et al. (2016) established an association between the -383C allele and primary Sjögren's syndrome, an autoimmune disease.

Therefore, our aim was to study the association between the rs2234649 *TNFR1* polymorphism and AS in a Russian Caucasian population.

## MATERIAL AND METHODS

### Patients and healthy subjects

This study was performed in a regional clinical hospital in Krasnoyarsk, Russia, from June, 2014 to August, 2016, and included 41 AS patients and 43 control subjects. To be included, patients had to: i) be diagnosed with AS based on the modified New York criteria (Sieper et al., 2009); ii) have been born in the Krasnoyarsk region and be long-term residents; iii) be more than 18 years old; iv) be Caucasian; v) be able to perform the necessary procedures; and vi) sign an informed consent form for this research. In addition, we excluded patients with an overlapping syndrome or inflammatory bowel disease.

We selected a healthy control group from the general population of the Krasnoyarsk region, in which they had been born and of which they were residents. Control subjects of unknown ancestry or suspected of having a chronic disease were excluded from our study. Each control group member was chosen from a different family, and there were no blood relationships between controls and cases.

For clinical assessment, we used a protocol based on the ASAS handbook guidelines for evaluation of spondyloarthritis. The protocol took into account clinical and demographic characteristics. Disease activity was assessed by the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), and functioning by the Bath Ankylosing Spondylitis Functional Index and Health Assessment Questionnaire for the Spondyloarthropathies (Sieper et al., 2009).

### Ethical considerations

Our protocol conformed to ethical standards and was approved by the Local Ethical Committee of Krasnoyarsk State Medical University named after Prof. V. F. Voyno-Yasenetsky (protocol No. 16 of 23.06.2014).

## **Polymerase chain reaction (PCR)-restriction fragment length polymorphism screening of the *TNFR1* -383 A>C polymorphism**

On the day of the clinical evaluation, blood samples were collected in tubes containing ethylenediaminetetraacetic acid. DNA was extracted from 0.5 mL whole blood using an E.N.Z.A. Blood DNA kit (Omega Bio-tek; Norcross, GA, USA) following the manufacturer protocol. Genotyping of the rs2234649 polymorphism was performed using the primers 5'-TTATTGCCCTTGGTGTGGTTG-3' (forward) and 5'-GGAGGGGAAGAGTGAGGCAGTGT-3' (reverse), in order to obtain a PCR product incorporating a BglIII enzyme restriction site. Each 25- $\mu$ L PCR contained 30-50 ng DNA, 10  $\mu$ M each primer, 2.5  $\mu$ L 10X PCR buffer, 0.5 mM each deoxynucleotide, 1.5 mM MgCl<sub>2</sub>, and 0.75 U *Taq* polymerase (Fermentas, Vilnius, Lithuania). The reactions were conducted in a thermocycler (Life ECO, model TC-96/G/H(b)C BIOER, Binjiang, China). After amplification, 3  $\mu$ L PCR product was analyzed by GelRed staining after electrophoresis on a 2% agarose gel (Biotium, Hayward, CA, USA) and observation under ultraviolet light. PCR fragment sizes were evaluated by comparison to a 100-bp DNA ladder (Fermentas). The following cycling profile was used for PCR: 95°C for 5 min (initial denaturation), then 40 cycles of 95°C for 15 s (denaturation), 58°C for 30 s (annealing), and 72°C for 40 s (extension), followed by a final extension at 72°C for 5 min.

### **Statistical analysis**

We used the chi-square test to evaluate the differences between patients and controls in the distribution of rs2234649 alleles and genotypes, and the odds ratio (OR) to measure the association between AS and the observed genotype frequencies, as a measure of AS risk. Statistical significance was considered to be represented by P values < 0.05 for the chi-square test and OR (MedCalc Software, Mariakerke, Belgium).

Deviation from Hardy-Weinberg equilibrium (HWE) was tested in the control group using the online tool "Hardy-Weinberg 2-Allele Calculator" (<http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html>).

We used Fisher's exact test to calculate the difference between patients and controls in the distribution of rs2234649 alleles and genotypes. P values < 0.05 were considered statistically significant (MedCalc Software).

Deviation from Hardy-Weinberg equilibrium (HWE) was tested in the control group using the on-line tool "Hardy-Weinberg 2-Allele Calculator" (<http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html>).

We used Fisher's exact test to calculate the difference between patients and controls in the distribution of rs2234649 alleles and genotypes; P values < 0.05 were considered statistically significant (MedCalc Software, Mariakerke, Belgium).

## **RESULTS**

### **Demographic and clinical characteristics**

In this study, we compared 41 patients with AS and 43 controls in terms of allele and genotype frequencies of the rs2234649 *TNFR1* 5'-UTR polymorphism. Table 1 shows the

general characteristics of the AS patients, all of whom were men. The mean age in this group was 41.5 years. Mean disease duration was 12.4 years, and mean age at onset was 29.7 years. The average BASDAI score was greater than 4.0.

**Table 1.** Clinical characteristics of the ankylosing spondylitis patients (N = 41) recruited in this study.

Characteristic	
Age (years)	41.5 ± 6.9
Disease duration (years)	12.4 ± 6.7
Age at onset (years)	29.7 ± 5.4
BASDAI score	4.87 ± 0.8
BASFI score	4.99 ± 0.75
HAQ-S score	1.2 ± 0.6
Morning stiffness (VAS)	52.24 ± 19.71
Schober (cm)	3.6 ± 0.46
Occiput to wall (cm)	7.68 ± 9.59

Quantitative variables are reported as means ± standard deviations. The visual analogue scale (VAS) was evaluated from 0 to 100 mm. BASDAI = Bath Ankylosing Spondylitis Disease Activity Index; BASFI = Bath Ankylosing Spondylitis Functional Index; HAQ-S = Health Assessment Questionnaire for the Spondyloarthropathies.

### Analysis of the *TNFRI* -383 A>C polymorphism

The results of restriction enzyme digestion for analysis of the rs2234649 polymorphism showed that the homozygous A/A genotype yielded a 199-bp fragment, the heterozygous A/C genotype resulted in three products of 199, 135, and 64 bp, and the homozygous C/C genotype generated two fragments of 135 and 64 bp. Table 2 shows the *TNFRI* rs2234649 allele and genotype frequencies recorded in each group.

The control group was found to be in HWE with respect to this polymorphism (P = 0.8759). Genotype and allele frequencies of this *TNFRI* variant did not differ between patients and controls (P = 1.0).

**Table 2.** Distribution of *TNFRI* rs2234649 alleles and genotypes in Russian Caucasian patients with ankylosing spondylitis and control subjects.

Allele or genotype	Patients (N = 41)	Controls (N = 43)	P*
A	81 (98.78%)	84 (97.67%)	1.0
C	1 (1.22%)	2 (2.33%)	
A/A	40 (97.56%)	41 (95.35%)	1.0
A/C	1 (2.44%)	2 (4.65%)	
C/C	0	0	

\*P value from the Fisher exact test.

## DISCUSSION

The result of this preliminary study performed on a relatively small cohort showed that the *TNFRI* polymorphism examined is not associated with AS in the Russian Caucasian population. This finding, if confirmed in a larger cohort, suggests that differences exist in genetic susceptibility to AS between ethnic groups, and is consistent with some previous

investigations. For example, Bridges et al. (2002) found no difference in rs2234649 allele frequencies between African-Americans with rheumatoid arthritis and control subjects. Moreover, AS is a multifactorial pathology and genetic factors are only one element of its pathogenesis. Thus, even if an association had been established between rs2234649 and AS in the present work, we would not have considered this variant to be the unique cause of this disease.

In conclusion, our results suggest that genetic testing of the rs2234649 polymorphism cannot be used to estimate AS risk and routine screening of this variant should not be recommended to determine prognosis or anticipate treatment response.

### Conflicts of interest

The authors declare no conflict of interest.

### REFERENCES

- Akassoglou K, Bauer J, Kassiotis G, Pasparakis M, et al. (1998). Oligodendrocyte apoptosis and primary demyelination induced by local TNF/p55TNF receptor signaling in the central nervous system of transgenic mice: models for multiple sclerosis with primary oligodendroglialopathy. *Am. J. Pathol.* 153: 801-813. [http://dx.doi.org/10.1016/S0002-9440\(10\)65622-2](http://dx.doi.org/10.1016/S0002-9440(10)65622-2)
- Arnett HA, Mason J, Marino M, Suzuki K, et al. (2001). TNF alpha promotes proliferation of oligodendrocyte progenitors and remyelination. *Nat. Neurosci.* 4: 1116-1122. <http://dx.doi.org/10.1038/nn738>
- Braun J and Sieper J (2007). Ankylosing spondylitis. *Lancet* 369: 1379-1390. [http://dx.doi.org/10.1016/S0140-6736\(07\)60635-7](http://dx.doi.org/10.1016/S0140-6736(07)60635-7)
- Bridges SL, Jr., Jenq G, Moran M, Kuffner T, et al. (2002). Single-nucleotide polymorphisms in tumor necrosis factor receptor genes: definition of novel haplotypes and racial/ethnic differences. *Arthritis Rheum.* 46: 2045-2050. <http://dx.doi.org/10.1002/art.10463>
- Brown MA (2008). Breakthroughs in genetic studies of ankylosing spondylitis. *Rheumatology (Oxford)* 47: 132-137. <http://dx.doi.org/10.1093/rheumatology/kem269>
- Brown MA, Laval SH, Brophy S and Calin A (2000). Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. *Ann. Rheum. Dis.* 59: 883-886. <http://dx.doi.org/10.1136/ard.59.11.883>
- Burton PR, Clayton DG, Cardon LR, Craddock N, et al.; Wellcome Trust Case Control Consortium; Australo-Anglo-American Spondylitis Consortium (TASC); Biologics in RA Genetics and Genomics Study Syndicate (BRAGGS) Steering Committee; Breast Cancer Susceptibility Collaboration (UK) (2007). Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat. Genet.* 39: 1329-1337. <http://dx.doi.org/10.1038/ng.2007.17>
- Corona-Sanchez EG, Muñoz-Valle JF, Gonzalez-Lopez L, Sanchez-Hernandez JD, et al. (2012). -383 A/C tumor necrosis factor receptor 1 polymorphism and ankylosing spondylitis in Mexicans: a preliminary study. *Rheumatol. Int.* 32: 2565-2568. <http://dx.doi.org/10.1007/s00296-011-1997-5>
- Daryabor G, Mahmoudi M, Jamshidi A, Nourijelyani K, et al. (2014). Determination of IL-23 receptor gene polymorphism in Iranian patients with ankylosing spondylitis. *Eur. Cytokine Netw.* 25: 24-29.
- Dong H, Li Q, Zhang Y, Tan W, et al. (2013). IL23R gene confers susceptibility to ankylosing spondylitis concomitant with uveitis in a Han Chinese population. *PLoS One* 8: e67505. <http://dx.doi.org/10.1371/journal.pone.0067505>
- Evans DM, Spencer CC, Pointon JJ, Su Z, et al.; Spondyloarthritis Research Consortium of Canada (SPARCC); Australo-Anglo-American Spondyloarthritis Consortium (TASC); Wellcome Trust Case Control Consortium 2 (WTCCC2) (2011). Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat. Genet.* 43: 761-767. <http://dx.doi.org/10.1038/ng.873>
- Feldtkeller E, Khan MA, van der Heijde D, van der Linden S, et al. (2003). Age at disease onset and diagnosis delay in HLA-B27 negative vs. positive patients with ankylosing spondylitis. *Rheumatol. Int.* 23: 61-66.
- Ferrante A (1989). Tumor necrosis factor alpha potentiates neutrophil antimicrobial activity: increased fungicidal activity against *Torulopsis glabrata* and *Candida albicans* and associated increases in oxygen radical production and lysosomal enzyme release. *Infect. Immun.* 57: 2115-2122.
- Fletes-Rayas AL, Palafox-Sánchez CA, Muñoz-Valle JF, Orozco-Barocio G, et al. (2016). TNFR1-383 A>C polymorphism

- association with clinical manifestations in primary Sjögren's syndrome patients. *Genet. Mol. Res.* 15: gmr4177. <http://dx.doi.org/10.4238/gmr.15024177>
- Fontaine V, Mohand-Said S, Hanoteau N, Fuchs C, et al. (2002). Neurodegenerative and neuroprotective effects of tumor Necrosis factor (TNF) in retinal ischemia: opposite roles of TNF receptor 1 and TNF receptor 2. *J. Neurosci.* 22: RC216.
- Grell M, Douni E, Wajant H, Löhden M, et al. (1995). The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell* 83: 793-802. [http://dx.doi.org/10.1016/0092-8674\(95\)90192-2](http://dx.doi.org/10.1016/0092-8674(95)90192-2)
- Kontermann RE, Scheurich P and Pfizenmaier K (2009). Antagonists of TNF action: clinical experience and new developments. *Expert Opin. Drug Discov.* 4: 279-292. <http://dx.doi.org/10.1517/17460440902785167>
- McCoy MK and Tansey MG (2008). TNF signaling inhibition in the CNS: implications for normal brain function and neurodegenerative disease. *J. Neuroinflammation* 5: 45. <http://dx.doi.org/10.1186/1742-2094-5-45>
- Nishimura M, Obayashi H, Mizuta I, Hara H, et al. (2003). TNF, TNF receptor type 1, and allograft inflammatory factor-1 gene polymorphisms in Japanese patients with type 1 diabetes. *Hum. Immunol.* 64: 302-309. [http://dx.doi.org/10.1016/S0198-8859\(02\)00799-1](http://dx.doi.org/10.1016/S0198-8859(02)00799-1)
- Reveille JD, Sims AM, Danoy P, Evans DM, et al.; Australo-Anglo-American Spondyloarthritis Consortium (TASC) (2010). Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat. Genet.* 42: 123-127. <http://dx.doi.org/10.1038/ng.513>
- Sieper J, Rudwaleit M, Baraliakos X, Brandt J, et al. (2009). The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. *Ann. Rheum. Dis.* 68 (Suppl 2): ii1-ii44. <http://dx.doi.org/10.1136/ard.2008.104018>
- van der Linden S and van der Heijde D (1998). Ankylosing spondylitis. Clinical features. *Rheum. Dis. Clin. North Am.* 24: 663-676, vii. [http://dx.doi.org/10.1016/S0889-857X\(05\)70036-3](http://dx.doi.org/10.1016/S0889-857X(05)70036-3)
- Wajant H, Pfizenmaier K and Scheurich P (2003). Tumor necrosis factor signaling. *Cell Death Differ.* 10: 45-65. <http://dx.doi.org/10.1038/sj.cdd.4401189>
- Wang CM, Ho HH, Chang SW, Wu YJ, et al. (2012). ERAP1 genetic variations associated with HLA-B27 interaction and disease severity of syndesmophytes formation in Taiwanese ankylosing spondylitis. *Arthritis Res. Ther.* 14: R125. <http://dx.doi.org/10.1186/ar3855>