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Lack of association between the CC genotype of the rs7903146 polymorphism in the TCF7L2 gene and rheumatoid arthritis

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ABSTRACT

Introduction: TCF7L2 is a transcription factor involved in Wnt/beta-catenin signaling and which has a variant known to be consistently associated with type 2 diabetes risk and some studies have also indicated its association with risk of certain types of cancer. **Objective:** Since this pathway may be involved in the pathophysiology of other chronic inflammatory diseases such as rheumatoid arthritis, we aimed to investigate the effect of TCF7L2 polymorphism rs7903146 on rheumatoid arthritis severity in a Brazilian population. **Patients and methods:** This polymorphism was genotyped in 208 patients with rheumatoid arthritis and in 104 healthy controls. We also analyzed the association of this polymorphism to smoking history, functional status classification and radiological indicators of disease severity. **Results:** The distribution of CC, CT and TT genotypes of SNP rs7903146 of the TCF7L2 gene was not different between patients and controls, and no association between the genotype and indicators of disease severity or smoking history was found. When data were evaluated using the dominant model, in which carriers of the CT and TT genotypes were grouped, an increase in the T allele was observed in patients positive for rheumatoid factor and erosions, although this was not significant. The frequency of T allele was also increased in patients with functional class II compared to class I (P = 0.032). **Conclusion:** It is possible that the small number of patients included in this study may have restrained additional findings. Further studies are therefore needed to investigate the role of TCF7L2 gene variants in the risk of rheumatoid arthritis and its severity.

Keywords: Wnt proteins, genetics, polymorphism, genetic.

INTRODUCTION

Rheumatoid Arthritis (RA) is a progressive and chronic systemic inflammatory disease of unknown etiology. It involves primarily the synovial membrane, and can lead to the destruction of bone and cartilage.[1] RA affects about 0.5%–1% of the world population; this figure can reach up to 5% depending on the age and ethnic group studied.[2]

Despite significant advances in the treatment of RA in the last few decades due to the development of appropriate laboratory and imaging methods, such methods still have a limited value for the early diagnosis of RA and also for the definition of an individual prognosis, and these aspects may limit the therapeutic effect of available medications.[3] A better understanding of the physiopathological factors related to this disease would be of great value in establishing early and effective treatment.

A number of studies have emphasized the critical role of activated fibroblast-like synovial cells (FLS) in the pathogenesis of RA, since a hyperplastic FLS population could potentially promote leukocyte infiltration and retention.[4] The rheumatoid

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synovium eventually turns into a pannus that destroys the articular cartilage and bone.[4] The underlying mechanisms involved in FLS activation are still unknown. It has been suggested that the Wingless MMTV-integration site (Wnt) – Frizzled (Fz) signaling pathways could be important in autonomous FLS activation.[4]

In fact, genes encoding proteins in the Wnt-Fz signaling pathway are highly expressed in synovial tissues in RA.[4] Wnt is a family of secreted glycoproteins that bind to cell surface G-protein coupled receptors from Fz family to induce intracellular cascades involved in cell growth and differentiation.[5] It has recently been suggested that these cascades could also contribute to initiate an activated FLS phenotype in a process of cell maintenance after joint injury,[6] and that untimely activation of FLS cells could lead to accumulation of activated kinases and transcription and growth factors that could drive RA pathogenesis.[5]

Two different Wnt-Fz pathways are recognized, that are known as the canonical (or β-catenin-dependent) and non-canonical (or β-catenin-independent) signaling pathways.[5] One of the proteins in the intracellular cascades activated by β-catenin-dependent signaling is transcription factor-7-like 2 (TCF7L2), which has recently been the focus of various studies on human diseases, since some common variants of TCF7L2 gene have been associated with the risk of developing type 2 diabetes (T2D)[6] and certain types of cancer.[7–9]

TCF7L2 encodes TCF4, a transcription factor involved in the Wnt/β-catenin signaling pathway; it has a critical role in embryogenesis and in control of cell proliferation and differentiation, and it is also involved in diverse physiological processes in adult life.[10] Recently, the rs7903146 polymorphism in the TCF7L2 gene has been associated with the risk of developing T2D, possibly through impairment of pancreatic beta cells function.[11–14] Recently, a strong correlation between the T allele of the rs7903146 polymorphism in the TCF7L2 gene and T2D was described in Denmark, the United States and Iceland.[7,15] This same correlation has been verified in other populations, including those of East Asia, Europe, West Africa and Scandinavia.[16–19] This genetic variant has also been associated with the risk of malignant neoplasms of prostate,[20] colon,[8] and breast.[9]

There are still data that suggest involvement of the Wnt pathway in the inflammatory response and in the physiopathology of chronic inflammatory disease, which is the case for RA.[4] The Wnt/Fz complex is responsible for controlling the tissue formation in embryogenesis and during limb development and joint formation.[4] Sen et al.[21] have studied the role of one of the receptor-ligand pairs of Wnt-Fz signaling pathway, namely Wnt-5A and Fz5, in FLS activation, which results in the production of inflammatory and chemotactic cytokines in the joints of patients with RA. The blocking of Wnt-5A/Fz5 signaling diminishes IL6 and IL15 induced cytokine expression, such as RANKL, and even diminishes synovial activation.[21] Another study demonstrated that the activation of Wnt signaling in chondrocytes induces degradation of the cartilage matrix, which is similar to what occurs in osteoarthritis and rheumatoid arthritis.[22] Various studies have suggested that Wnt signaling increases bone formation by regulating the proliferation and differentiation of osteoblasts and osteoclasts.[23] Moreover, Wnt signaling can promote upregulation of osteoprotegerin expression in the osteoblasts. As osteoprotegerin inhibits osteoclast differentiation, the signaling can partially increase bone mass by blocking bone reabsorption through the osteoclasts.[23]

The activation of the immune system before the beginning of the clinical manifestations of the disease suggests that clinical RA already represents a chronic illness. Studies show that the earliest possible treatment of auto-immune diseases, before the development of disease manifestations, can delay disease progression and improve the patient's prognosis.[24,25] In this way, genes that codify proteins of the Wnt signaling pathway represent plausible candidates whose variants could be related to the risk of development of the disease or to its severity.

The mechanisms that underlie the association of TCF7L2 polymorphisms with T2D and malignant neoplasms risk are still unclear. Considering that these diseases have in common with RA an inflammatory response component, and considering the role of the Wnt-Fz signaling pathway in this response, the investigation of the frequency of TCF7L2 common variants in systemic inflammatory diseases like RA could contribute to improve the understanding of the mechanisms involved in increased disease risk in situations of abnormal TCF7L2 gene expression.[26] Given these possible benefits, a case-control study was proposed to assess the association between the single nucleotide polymorphism rs7903146 of TCF7L2 gene and RA activity.

PATIENTS AND METHODS

Patients

After approval from the Ethics Research Committee of the Universidade de Brasília (CEP/UnB), the selected patients were informed about the content and objectives of the study, expected benefits, freedom of refusal, and the guarantee of confidentiality and privacy. Those who agreed to participate signed a free and informed consent form.

A total of 208 patients, which represents the number of patients with RA that attend the Rheumatoid Arthritis Clinic at Hospital Universitário de Brasília, were selected by convenience for the study; they met the classificatory criteria for the disease according to the American College of Rheumatology (ACR 1987).

The following patient information was obtained from a questionnaire or retrospectively by chart review: age, gender, personal history of T2D, smoking history, age at RA diagnosis, time since RA diagnosis and functional status classification. The results of rheumatoid factor measurement in serum were also obtained by review of medical records; in all cases rheumatoid factor was measured by nephelometry and values above 15 UI/mL were considered positive.

Radiographic information was also obtained, and included the presence of erosion in the x-rays of patients' hands, wrists, feet and ankles. Members of the Radiology Team of Hospital Universitário de Brasília, who routinely assess these exams, evaluated the images.

Collection, purification and DNA genotyping

A 5-mL sample of venous blood was obtained from a peripheral vein puncture using disposable material, and the blood was stored in bottles that contained EDTA. The sample was collected during a visit to the clinic or laboratory.

DNA was extracted from the samples through the Chelex-100 method[27] and genotyping of the rs7903146 polymorphism was conducted through an allele-specific polymerase chain reaction (AS-PCR); the primers and conditions have been previously described.[28]

Statistical analysis

The data obtained were submitted to appropriate statistical testing. The measures of smoking, rheumatoid factor, erosions, and functional class were described in the patients from a polymorphism analysis with the use of absolute and relative frequencies. The testing verified the presence of polymorphism association with measures that utilized chi-squared tests or likelihood ratio tests.[29] The chi-squared test was used to assess if the observed genotype frequency was consistent with Hardy-Weinberg equilibrium. Logistic regression models were implemented for each one of the following data, with age and gender controls: smoking history, functional class status and radiographic erosions. The models also estimated the odds ratio with the respective 95% confidence intervals.[30] Statistically significant values were considered at $P < 0.05$.

RESULTS

A total of 208 patients with the diagnosis of RA were included. Their mean age was 51.55 ± 13.19 years, and most were female (87.5%). The mean age at diagnosis was 37.3 years, and the mean disease duration, assessed by time since diagnosis, was 8.1 years.

The distribution of TCF7L2 rs7903146 genotype was 47.6% CC, 45.2% CT and 7.2% TT, and was in agreement with Hardy-Weinberg equilibrium. This resulted in the following allele frequencies: 70.2% C allele and 29.8% T allele (Table 1).

Statistical differences were not observed between the CC, CT, and TT genotypes and the following indicators of rheumatoid disease severity: smoking (P = 0.691), rheumatoid factor (P = 0.418), erosions (P = 0.261), and functional classes I, II, III, and IV (P = 0.328) (Table 2).

Table 1
Genotype distribution according control and patient groups

Variable	Group						P
	Control		RA		Total		
	N	%	N	%	N	%	
Gender							< 0.001
Male	62	59.6	26	12.5	88	28.2	
Female	42	40.4	182	87.5	224	71.8	
TCF7L2 genotype							0.07
CC	43	41.3	99	47.6	142	45.5	
CT	45	43.3	94	45.2	139	44.6	
TT	16	15.4	15	7.2	31	9.9	
TCF7L2 genotype							0.296
CC	43	41.3	99	47.6	142	45.5	
CT or TT	61	58.7	109	52.4	170	54.5	
Total	**104**	**100**	**208**	**100**	**312**	**100**	
Allele							0.069
C	131	63.0	292	70.2	423	67.8	
T	77	37.0	124	29.8	201	32.2	
Total	**208**	**100**	**416**	**100**	**624**	**100**	

RA: rheumatoid arthritis.

Table 2
Genotype distribution and correlation with smoking and indicators of disease severity in patients

Variable	TCF7L2 genotype								P
	CC		CT		TT		Total		
	N	%	N	%	N	%	N	%	
Smoking									0.691
No	62	62.6	62	66.0	11	73.3	135	64.9	
Yes	37	37.4	32	34.0	4	26.7	73	35.1	
RF									0.418*
No	29	31.2	21	22.8	3	23.1	53	26.8	
Yes	64	68.8	71	77.2	10	76.9	145	73.2	
Erosions									0.261
No	54	56.3	48	51.1	11	73.3	113	55.1	
Yes	42	43.8	46	48.9	4	26.7	92	44.9	
Functional class									0.328*
I	44	44.4	30	31.9	6	40.0	80	38.5	
II	25	25.3	36	38.3	6	40.0	67	32.2	
III	18	18.2	21	22.3	2	13.3	41	19.7	
IV	12	12.1	7	7.4	1	6.7	20	9.6	

RF: rheumatoid factor. *Result of the Likelihood Ratio test.

Table 3
Genotype distribution and correlation with smoking and indicators of disease severity in patients, adjusted by gender and age (logistic regression)

Variable	TCF7L2 genotype				OR*	CI (95%)	OR**	CI (95%)	P
	CC		CT or TT						
	N	%	N	%					
Smoking									
No	62	62.6	73	67	1				
Yes	37	37.4	36	33	0.83	0.47-1.46	0.81	0.44-1.48	0.485
RF									
No	29	31.2	24	22.9	1				
Yes	64	68.8	81	77.1	1.53	0.81-2.88	1.5	0.79-2.84	0.214
Erosions									
No	54	56.3	59	54.1	1				
Yes	42	43.8	50	45.9	1.09	0.63-1.89	1.13	0.65-1.98	0.668
Functional class									
I	44	44.4	36	33	1				
II	25	25.3	42	38.5	2.05	1.06-3.98	2.11	1.07-4.19	0.032
III	18	18.2	23	21.1	1.56	0.73-3.33	1.59	0.73-3.46	0.247
IV	12	12.1	8	7.3	0.81	0.3-2.21	0.8	0.27-2.31	0.675

RF: rheumatoid factor. *Not adjusted; **Adjusted by gender and age.

The data were also evaluated using the dominant model, in which carriers of the CT and TT genotype were grouped for logistic regression statistical analysis with gender and age controls (Table 3). A non-significant increase was observed for the likely appearance of the T allele in patients positive for rheumatoid factor and erosions. However, in the analysis of the functional class status, there was an increase in the frequency of T allele in functional class II patients as compared to functional class I patients (P = 0.032). For other functional classes, a statistically significant increase in the likely appearance of the polymorphism was not observed (P = 0.247 and P = 0.675 for class III and IV, respectively).

DISCUSSION

This was a pioneering study in the assessment of the association between the CC genotype of the rs790146 polymorphism in the TCF7L2 gene and some features of RA.

Although an association between the rs7903146 polymorphism of the TCF7L2 gene and RA was not found, our results suggest a tendency, although not statistically significant, that the T allele is less represented in the patient group than in the control group.

In this study, a non-significant increase in the frequency of the C allele of the rs7903146 polymorphism in the TCF7L2 gene was found in RA patients. A statistically relevant correlation was observed between the T allele and functional class II of RA. The significance of these findings is uncertain, and it is possible that the small number of patients included in this study was a factor, as well as the fact that the control group was selected by convenience and was hence not matched to the patient group.

To our knowledge, the allele frequencies of TCF7L2 rs7903146 polymorphism have not been shown to vary according to gender or ethnic groups, except in the Chinese population, in which the allele T of the rs7903146 polymorphism was shown to be low.[31] The allele frequencies found in the control group of present study are similar to those described in healthy individuals included in control groups of other studies involving this polymorphism. However, the gender and also the average age differences between patient and control groups in this study are recognized as important limitations, since RA is more common in women after the age of 40 years. This may have precluded finding possible genotypic differences between them.

This is the first study, to the best of our knowledge, that has assessed the correlation between a TCF7L2 variant and a rheumatologic disease. Furthermore, it is the first to demonstrate a polymorphism of this gene that is associated with a functional class status of RA. Although this study has many limitations, which prevent definite conclusions about the significance of these findings, it opens new avenues for the investigation of the physiopathology of RA. Additional studies are necessary, therefore, to confirm these results and

clarify the role of the TCF7L2 gene in the risk and determination of RA severity.

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