BRAZILIAN JOURNAL
OF MEDICAL AND BIOLOGICAL RESEARCH
www.bjournal.com.br

ISSN 0100-879X
Volume 43 (5) 381-496 May 2011

BIOMEDICAL SCIENCES
AND
CLINICAL INVESTIGATION

Braz J Med Biol Res, May 2011, Volume 44(5) 418-420

doi: 10.1590/S0100-879X2011007500036

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Brazilian Journal of Medical and Biological Research (2011) 44: 418-420
ISSN 0100-879X Short Communication

Novel regulatory SNPs in the promoter region of the *TNFRSF*18 gene in a Gabonese population

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Abstract

Parasites are accountable for driving diversity within immune gene families. We identified and investigated regulatory single nucleotide polymorphisms (SNPs) in the promoter regions of the tumor necrosis factor receptor superfamily member 18 (*TNFRSF*18) gene by direct sequencing in a group of male Gabonese individuals exposed to a wide array of parasitic diseases such as malaria, filariasis and schistosomiasis. Two new promoter variants were identified in 40 individuals. Both novel variants were heterozygous and were linked to SNP #rs3753344 (C/T), which has been described. One of the SNP variants (ss2080581728) was close to the general transcription factor site, the TATA box. We further validated these new promoter variants for their allelic gene expression using transient transfection assays. One new promoter variant with two base changes (C/T - ss2080581728/rs3753344) displayed an altered expression of the marker gene. Both novel variants remained less active at the non-induced state in comparison to the major allele. The allele frequencies observed in this study were consistent with data for other African populations. The detection and analysis of these human immune gene polymorphisms contribute to a better understanding of the interaction between host-parasite and expression of Treg activity.

Key words: Tumor necrosis factor receptor superfamily; Polymorphism; Transfection; Regulatory T cells

Introduction

Failure of the immune system to clear parasites may lead to prolonged disease or exacerbated pathology. One reason for this failure may lie in the immunomodulatory effects induced by parasites, which then favor the expansion of regulatory T cells (Tregs). Tregs are actively involved in the control of pathological and physiological immune responses, thereby contributing to the maintenance of immunological self-tolerance and immune homeostasis (1-3). Regulatory single nucleotide polymorphism (SNPs) can cause significant changes in gene expression in functional immune genes. Promoter regions are potential candidates for the presence of such functional SNPs, as they are involved in transcription initiation and many of the *cis*-acting elements that regulate gene expression possibly harbor functional polymorphisms (4). Investigation of these human polymorphisms in the promoter region of immune genes possibly reflects the level of susceptibility to parasitic infection and of Treg expression. We investigated the tumor necrosis factor receptor superfamily member 18 (*TNFRSF*18) locus, a 228-amino acid type I transmembrane protein known to play a key role in the regulation of T cell receptor-mediated cell death (5). In the current study, the occurrence of different promoter variants inducing differential expression of *TNFRSF*18 was validated using transient transfection assays.

Material and Methods

Forty DNA samples obtained from unrelated Gabonese individuals were screened for SNPs in the promoter region of the *TNFRSF*18 by the polymerase chain reaction (PCR) and subsequent sequencing. The study was approved by the local Ethics Committee of the International Foundation of the Albert Schweitzer Hospital. Fragments of the promoter of the *TNFRSF*18 genes were amplified using the primer pairs - *TNFRSF*18-forward: 5'-GCGCCTCTTTCT ATCCAGG-3', *TNFRSF*18-Intern01: 5'-GGCTCCTCC TCAACTCCCTCC-3', and with *TNFRSF*18-reverse:

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Received July 9, 2010. Accepted March 10, 2011. Available online March 25, 2011. Published May 16, 2011.

5'-AACGCCGGTCTGAGCAC-3' (MWG Operon, Germany). PCR amplifications were carried out in 20-µL reaction volumes with 5 ng genomic DNA, 1X PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, and 1.5 mM $MgCl_2$; Qiagen, Germany), 0.125 mM dNTPs, 0.5 mM of each primer, and 1 U Taq DNA polymerase (Qiagen) in a PTC-200 Thermal cycler (MJ Research, USA). Thermal cycling parameters were: initial denaturation at 94°C for 2 min, followed by 34 cycles of 30 s at 94°C denaturation, 30 s at 61°C annealing temperature, 1 min and 30 s at 72°C extension, followed by a final extension of 2 min at 72°C. The PCR products (1 µL) were used directly as templates for sequencing, using the BigDye terminator v. 2.0 cycle sequencing kit (Applied Biosystems, USA) on an ABI 3100 DNA sequencer. Using the program BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html), polymorphisms were identified when assembled with the reference sequence of the *TNFRSF*18 gene obtained from the SNPper database (http://snpper.chip.org/). The respective genomic DNA identified to have SNPs was amplified with infusion primers and were cloned to the pGL3 basic vector using the infusion advantage PCR cloning kit (Clontech, USA). Two independent colonies were picked from these transformations and maxi prep was performed using the Endofree plasmid maxi kit (Qiagen). We tested the activities of the observed polymorphic promoters in unstimulated Jurkat T cell lines. In principle, five independent transfection experiments for each construct were performed in duplicate with Jurkat T cells using the TransIT-Jurkat Transfection Reagent (Mirus Bio Corporation, USA) as recommended by the manufacturer. Data were analyzed by the StatView software (http://www.statview.com). The activity of the *TNFRSF*18 promoter variants was compared to the activity of the major allele based on their luciferase activities. P values were calculated by ANOVA corrected by the Bonferroni/Dunn test. The differences were considered to be significant when $P \leq 0.05$.

Table 1. Genetic variants identified in the promoter regions of the *TNFRSF*18 locus.

SNP (rs#)	Polymorphism	Flanking sequences	Genotype	Analyzed individuals	Allele	Frequency
ss2080581724	C>T	GGAAC[C/T]GCCTT	CC	39	C	0.975
			CT	1	T	0.025
ss2080581728	C>T	ATAAA[C/T]GCCGC	CC	39	C	0.975
			CT	1	T	0.025
rs3753344	C>T	ATCCC[C/T]GCCAG	CC	34	C	0.850
			CT	6	T	0.150

SNP = single nucleotide polymorphism.

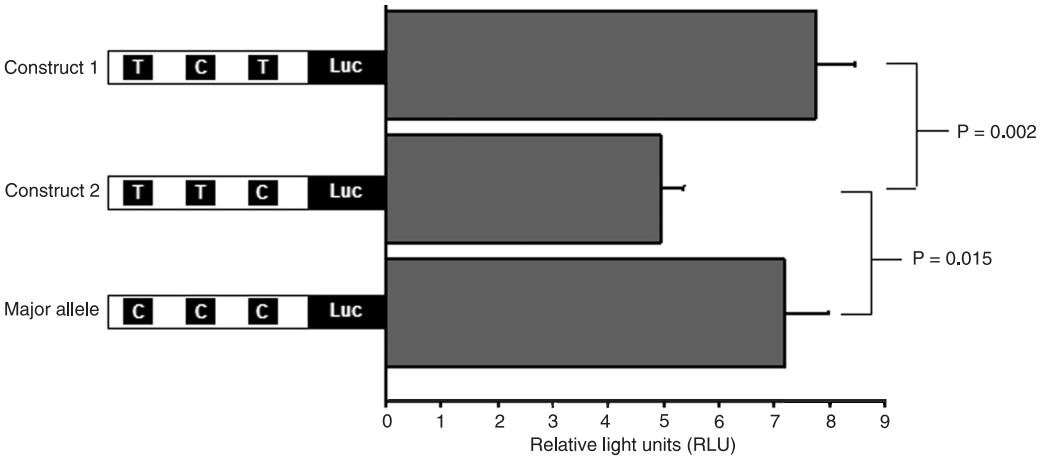

Figure 1. Comparison of the activity of the three different *TNFRSF*18 promoter variants analyzed based on luciferase (Luc) activity. The ratio of the relative light units (firefly/renilla) is presented. P values were calculated by ANOVA corrected by the Bonferroni/Dunn test. The letters indicate the presence of C/T mutations.

Results

Three SNPs were identified in 40 subjects. Two novel SNPs were identified at position #ss2080581724 (C/T) and #ss2080581728 (C/T) and an SNP that has been described earlier in the SNPper database was identified at position #rs3753344 (C/T). Their respective genotype and allelic frequencies are summarized in Table 1. All three SNPs observed were heterozygous. Both the novel SNPs were linked to the described SNP #rs3753344 (C/T). Comparison of the activity of all three *TNFRSF*18 promoter variants based on luciferase activity indicated decreased activity for one variant compared to the Major allele (Figure 1). The P values were significant for Construct 2 (#ss2080581728/#rs3753344) compared to the major allele (P = 0.015). The P values indicated statistical significance when Construct 1 (#ss2080581724/#rs3753344) was compared to construct 2 (#ss2080581728/#rs3753344; P = 0.002).

Discussion

In the current study, we identified two novel promoter variants in the *TNFRSF*18 gene locus. A study in the context of regulatory polymorphisms in the TNF receptor superfamily had reported a similar polymorphism (#rs3753344 C/T) with an allele frequency of 10% among 24 Korean individuals (6) and an allele frequency of 20% was observed in Japanese individuals as obtained from the JSNP database (http://www.snp.ims.u-tokyo.ac.jp). The current study revealed that the SNPs (#rs3753344 C/T) contribute to 15% of allelic distribution among 40 Gabonese individuals, in agreement with the allele frequencies of individuals from an Afro-American ethnic background (http://snpper.chip.org). In the Nigerian Yorubas, both alleles occur at a frequency of 0.5 compared to the NCBI HapMap database. It appears that a significant difference in the distribution of SNP allele frequencies occurs among diverse ethnic groups, possibly reflecting the role of these regulatory SNPs in confined infectious challenges. In order to associate any disease severity with any of these newly identified SNPs, a very large cohort of samples is obligatory. The regulatory SNPs identified in the current study will provide useful information for understanding the relevance of sequence polymorphisms in populations of different ethnic background and may serve as a basis to study parasite susceptibility in association studies.

Acknowledgments

We thank A. Weierich, V. Galinat and V. Grummes from Institute for Tropical Medicine, Tübingen, Germany, for technical assistance during the study period. The project received funding from a grant of the European Commission (TRANCHI; INCO-CT-2006-032436).

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