

Different Allele Frequency between Males and Females of a SNP of the Human Beta T Cell Receptor

P. Tripputi ¹ and M. Bignotto ²

1 Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche
Facoltà di Medicina e Chirurgia, Ospedale San Paolo
Università degli Studi, Milano, Italy

2 Dipartimento di Scienze della Salute, Facoltà di Medicina e Chirurgia
Ospedale San Paolo, Università degli Studi, Milano, Italy

Corresponding author:

Pasquale Tripputi M.D.

Mailing address: Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche
Laboratorio di Medicina Molecolare, V piano Blocco C, Ospedale S. Paolo,
via di Rudinì 8, 20142 Milano, Italy
e-mail: pasquale.tripputi@unimi.it

Keywords: DNA- Gender- Linkage disequilibrium – Polymorphism-T cell receptor

Abstract

We studied a single nucleotide polymorphism (SNP) located in the human beta T-cell receptor, in association studies and different allele frequency in the two sexes. Here we report a new series of 200 subjects (100 males and 100 females) in a restricted age range, 20-30 years.

We found the following results: in males 57/100 (57%) were heterozygous, 26/100 (26%) were homozygous for the two digestion fragments and 17/100 (17%) were homozygous for the 603bp fragment. Females were 44/100 (44%) heterozygous, 24/100 (24%) homozygous for the two digestion fragments and 32/100 (32%) homozygous for the 603bp fragment. The allele frequency was significantly different according to chi square analysis (X square statistic (df = 2) = 7.412; p = 0.025). Our study shows that in

females, but not in males, there is a significant increase of CC homozygous status and a proportionate decrease of heterozygous status compared with Hardy-Weinberg expectations.

This study could explain the controversial results obtained by association studies between this SNP (rs1800907) and autoimmune diseases made in the ninety and unconfirmed in more recent papers. Moreover it could be a starting point to search for other autosomal DNAs differences between the two sexes.

INTRODUCTION

Gene polymorphisms, in particular single nucleotide polymorphism (SNP), are being evaluated for their role in multi-factorial diseases as cancer, inflammation and autoimmunity [8].

A very common SNP (rs1800907) in the promoter region of the beta T cell receptor (TRCB) has been extensively studied in the nineties and associated to autoimmune disease as insulin-dependent diabetes [6,7], autoimmune hepatitis [5], IgA nephropathy [10], membranous nephropathy [6], Graves' disease and Hashimotos thyroiditis [4].

All these studies were made with Southern blot analysis of restriction fragment length polymorphisms (RFLP), obtained by cutting genomic DNA with restriction enzyme BglIII and DNA sequencing as described in [14].

These studies suggested that particular alleles can be found at different frequencies in complex diseases as compared to healthy population, in our study for example, we found an higher frequency of CC homozygous genotype in females and a proportionate decrease of heterozygous status.[11,12]

We tried to make a metanalysis study with other authors data, and in no paper but one [5], controls subjects were selected by sex. In this paper were described 60 patients, 45 female e 15 males, with genotypes: TT 12 - TC 23 - CC 25 .

All these data lead us to make another allele frequency study between sexes in a new series. and in this study with a more restricted range of age, only subjects between 20 and 30 years old were admitted.

MATERIALS AND METHODS

The study included 200 individuals. All subjects were caucasian of European origin and were recruited from the single metropolitan area of Milan (Italy). The age range was 20 to 30 years. These subjects were healthy, unrelated individuals: 100 males and 100 females, consecutively admitted in this study after informed consent, as blood donors and staff personal. Males were 80 blood donors and 20 staff, females were 60 blood donors and 40 staff.

DNA was extracted from 5 ml of peripheral blood by proteinase K digestion, phenol extraction and ethanol precipitation as described [13].

PCR was performed with 40 cycles of 93° for 1 min, 55° for 1 min and 72° for 1 min and was carried out with primers designed around the polymorphic Bgl II site, according to sequences of the NCBI program

(GenBank accession number U66061, OMIN number 186930).

Forward primer: 5' -TAATTTTGAAATAAGGGAAGATGAC- 3'

Reverse primer: 5' -TTTTGTATCCACCCTATGGGTTGGC- 3'

Restriction digestion was made at 37° for 6h and samples were run on an ethidium bromide stained 2% agarose gel. PCR amplification product is a 603 bp fragment and restriction with Bgl II gives rise to two fragments of 203 and 400 bp.

The presence of three bands showed heterozygosity, the 603 band alone homozygosity for the C nucleotide and the two bands of 203 and 400 homozygosity for the T nucleotide (Fig. 1).

Direct nucleotide sequence analysis of the TCR polymorphism was performed following PCR amplification on both strands (ABI PRISM 310 sequencer) using Big Dye kit as described [1].

Chi square analysis was done to investigate difference in the distribution of genotypes between sexes. The results have been considered significant at the 0.05 level, correcting for the degree of freedom (df). T test and one-way analysis of variance have been used as appropriate.

RESULTS

The two groups, males and females, were comparable for age and selection criteria. Mean age (SD) was 24.7 ± 5 years in the males and 25.2 ± 4 years in the females ($p = \text{NS}$).

Eighty males were blood donors and twenty were health professionals; sixty females were blood donors and forty were health professionals.

Genotype analysis gave the following results: in males 57/100 (57%) were heterozygous, 26/100 (26%) were homozygous for the two digestion fragments and 17/100 (17%) were homozygous for the 603bp fragment (Fig 1). Females were 44/100 (44%) heterozygous, 24/100 (24%) homozygous for the two digestion fragments and 32/100 (32%) homozygous for the 603bp fragment. All these results were confirmed by direct nucleotide sequence analysis as shown in material and methods and in Table 1.

Table 1 summarizes the genotype analysis: a significant difference ($\chi^2 = 7.412$; $p = 0.025$) was found between males and females in the allele frequency according to χ^2 analysis. Age was not different among genotypes by one way analysis of variance.

DISCUSSION

Our study shows that in females, but not in males, there is a significant increase of CC homozygous status and a proportionate decrease of heterozygous status compared with Hardy-Weinberg expectations.

As suggested by Payami et al. [9], different association studies results could be avoided by a better selection of subjects.

In particular some ranking of age could be useful to uniform these studies, and so, to limit any selection bias associated to increased morbidity and mortality with age, only subjects between 20 and 30 years were included in this study.

We are working to have an explanation for the different allele frequencies found in our study: the higher frequency of CC homozygosity in females, and the higher frequency of heterozygosity in males. Actually, selective pressures [2] and different recombination rates between males and females seems to be the possible mechanism of our finding and so will be intensively studied in our lab.

ACKNOWLEDGEMENT This work was supported by grants of University of Milan to P. Tripputi, and PUR to M. Bignotto. The authors declare to have no conflict of interest.

REFERENCES

- [1] D. Cigognini, G. Corneo, E. Fermo, A. Zanella and P. Tripputi. HIC gene, a candidate suppressor gene within a minimal region of loss at 7q31.1 in myeloid neoplasm's, *Leukemia Res*, 31 (2007), 477-482.
- [2] J.V. Clark, E.S. Ptak, I. Tiemann, Y. Qian, G. Coop. Combining Sperm Typing and Linkage Disequilibrium Analyses Reveals Differences in Selective Pressures or Recombinant Rates Across Human Populations, *Genetics*, 175 (2007), 795-804.
- [3] A.G. Demaine, R.W. Vaughan, D.H. Taube, K.I. Welsh. Association of membranous nephropathy with T-cell receptor constant beta chain and immunoglobulin heavy chain switch region polymorphisms. *Immunogenetics*, 27 (1988), 19-23.
- [4] M. Ito, M. Tanimoto, H. Kamura, M. Yoneda, Y. Morishima, K. Yamauchi, T. Itatsu, K. Takatsuki, H. Saito. Association of HLA antigen and restriction fragment length polymorphism of T cell receptor beta-chain gene with Graves disease and Hashimotos thyroiditis, *J Clin Endocrinol Metab*, 69 (1989), 100-104.
- [5] K. Manabe, M.L. Hibberd, P.T. Donaldson, J.A. Underhill, D.G. Doherty, A.G. Demaine, G. Mieli-Vergani, A.L. Eddleston, R.T. Williams. T-cell receptor constant beta germline gene polymorphisms and susceptibility to autoimmune hepatitis. *Gastroenterology*, 106 (1994), 1321-1325.
- [6] S.A. McMillan, C.A. Graham, P.J. Hart, D.R. Hadden, T.A. McNeill. A T cell receptor beta chain polymorphism is associated with patients developing insulin-dependent diabetes after the age of 20 years, *Clin Exp Immunol*, 82 (1990), 538-541.
- [7] B.A. Millward, K.I. Welsh, R.D. Leslie, D.A. Pyke, A.G. Demaine. T cell receptor beta chain polymorphisms are associated with insulin-dependent diabetes, *Clin Exp Immunol*, 70 (1987), 152-157.
- [8] M.J. Niven, C. Caffrey, R.H. Moore, J.A. Sachs, V. Mohan, H. Festenstein, M.L. Hoover, G.A. Hitman. T-cell receptor beta-subunit gene polymorphism and autoimmune disease. *Hum Immunol* 27(1990), 360-367.

- [9] H. Payami, M. Zhu, J. Montimuro, R. Keefe, C.C. McCulloch, L. Moses. One step closer to fixing association studies: evidence for age- and genderspecific allele frequency variations and deviations from Hardy-Weinberg expectations in controls, *Hum Genet*, 118 (2005), 322-330.
- [10] N. Tatsuma, H. Tsugu, M. Murakami. HLA-DQ region and TCR gene polymorphism associated with primary IgA nephropathy in Japanese children, *Nippon Jinzo Gakkai Shi*, 3 (1997), 734-739.
- [11] P. Tripputi, S. Bianchi, L. Fedele. A possible mechanism for non replication of allelic association between a single nucleotide polymorphism of the human T-cell receptor and autoimmune diseases, *Int J Immunogenet*, 35 (2008), 141-144.
- [12] P. Tripputi, D. Cigognini, S. Bianchi, L. Fedele. Different allelic distribution of a single SNP between sexes in humans, *Biochem Genet*, 46 (2008), 733-736.
- [13] P. Tripputi, S. Guerin, D.D. Moore. Two mechanisms for the extinction of gene expression in hybrid cells, *Science*, 241 (1988), 1205-1207.
- [14] P. Tripputi, D. Graziani, R.M. Alfano, B. Cassani, G. Coggi. A common T/C polymorphism in the promoter region of the beta T-cell receptor gene, *Mol Cell Probes*, 14 (2000), 195-197.

TABLE 1

GENOTYPE	MALES	FEMALES
TC^a	57/100 (57%)	42/100 (42%)
TT^b	26/100 (26%)	25/100 (25%)
CC^c	17/100 (17%)	33/100 (33%)

a = heterozygous; b = homozygous for the two digestion fragments; c = homozygous for the 603bp fragment. X square statistic (df = 2) = 7.412; p = 0.025.

Table 1. Difference of allele frequency between males and females of an SNP of the human beta T cell receptor.

Figure 1. Agarose gel stained with ethidium bromide showing in lane 1 the heterozygous pattern TC, in lane 2 the homozygous genotype CC (without restriction by Bgl II), and in lane 3 the homozygous genotype TT (complete restriction by Bgl II). Molecular weights of PCR products are shown on the left.



Received: July, 2012