Short Communication

RNASEL Alleles and Susceptibility to Infection by Human Retroviruses and Hepatitis Viruses

Miguel Arredondo,¹ Fermín de Bethencourt,² Ana Treviño,¹ Antonio Collado,³ Pilar Torres,⁴ Luz Barbolla,⁴ Vincent Soriano,¹ and Carmen de Mendoza¹

Abstract

RNASEL seems to function as an intracellular restriction factor blocking the establishment of infections caused by viral agents. Herein, we investigated whether allelic variants at the RNASEL gene might influence the susceptibility to viral infections or conditions potentially linked to viral agents. The allelic distribution at codon 462 was 139 (33.9%), 204 (49.8%), and 67 (16.3%) for RR, RQ, and QQ, respectively, in 410 individuals in Spain. There were no significant differences comparing 105 blood donors and 71 patients with HIV-1 infection, 27 with chronic hepatitis C, 67 with prostate cancer, and 107 with chronic fatigue syndrome. In contrast, two-thirds of 18 patients with HTLV-1 infection and 15 with chronic hepatitis B harbored RR. Thus, polymorphisms at the RNASEL gene do not seem to influence the susceptibility to common viral infections or conditions potentially of viral etiology. The role in influencing the susceptibility to HTLV-1 or HBV chronic infection warrants further examination in larger patient populations.

RESTRICTION FACTORS COMPRISE an important layer of host defenses against viral infections. RNASEL is a part of the interferon-mediated immune response to some viruses, blocking infections through cleavage of viral and cellular single-stranded RNA.^{1–4} The RNASEL gene variant R462Q has been suggested to increase the susceptibility for prostate cancer (PrCa)^{5–7} although it can be found in only 13% of PrCa patients.⁸ Hypothetically, R462Q could increase the risk of infection by some viral agents, such as the xenotropic murine leukemia virus-related gammaretrovirus (XMRV).⁹ The discovery of XMRV and its involvement in PrCa¹⁰ and chronic fatigue syndrome (CFS)¹¹ has generated enormous controversy with reports of conflicting results by different investigators.^{12–17} Information about the role of RNASEL variants in the susceptibility to other viral infections or putative viral-related conditions is scarce.

Plasma and peripheral blood mononuclear cells (PBMCs) were collected from 410 individuals, classified as follows: 71 with HIV-1 infection, 18 with HTLV-1 infection, 27 with chronic hepatitis C, 15 with chronic hepatitis B, 67 with PrCa, 107 with CFS, and 105 healthy blood donors. All individuals chronically infected with human retroviruses or hepatitis viruses were on regular follow-up at Hospital Carlos III, a reference infectious diseases clinic located in Madrid. Samples

from patients with PrCa were obtained at one urology clinic in Madrid. Specimens belonging to patients with CFS were obtained from the National DNA Repository located in Salamanca, although a subset was collected at Hospital Carlos III. Finally, samples from healthy blood donors were provided by blood banks located in Madrid.

DNA was isolated from PBMCs. All patients were genotyped for RNASEL R462Q variants (rs486907) using Taqman probes. A 7000 real-time PCR system (Applied Biosystems, Foster City, CA) was used for amplification. XMRV gag and env sequences were tested using sensitive nested-PCR protocols. Finally, XMRV antibodies were examined using a prototype ARCHITECT XMRV p15 and gp70 research assay (Abbott Diagnostics, Abbott Park, IL).

The majority of individuals with CFS were female (86.1%) with a mean age of 46 years. The most common symptoms of CFS patients were recurrent headache (82%), non-restorative sleep (98%), chronic pain (79%), muscle weakness (93%), and extreme exhaustion lasting more than 24 h after physical exercise (98%). All patients with PrCa were white with a mean age of 63.7 years. The mean prostate serum antigen titer was 12.5 (1.8–199) ng/ml at the time of diagnosis. The tumor stage was T1 in 84.6%, T2 in 9.6%, T3 in 3.8%, and T4 in 1.9%.

¹Infectious Diseases Department, Hospital Carlos III, Madrid, Spain.

²Urology Department, Hospital La Paz, Madrid, Spain.

³Fibromyalgia Unit, Hospital Clínic, Barcelona, Spain.

⁴Blood Transfusion Center, Madrid, Spain.

TABLE 1. FREQUENCY (%) OF CODON 462 ALLELIC VARIANTS AT THE RNASEL GENE

Category	No.	RR	RQ	QQ
HIV-1 infection	71	36.6	43.7	19.7
HTLV-1 infection	18	61.1	27.8	11.1
Chronic hepatitis C	27	33.3	51.9	14.8
Chronic hepatitis B	15	66.7	20	13.3
Prostate cancer	67	25.4	59.7	14.9
Chronic fatigue syndrome	107	34.6	51.4	14
Healthy blood donors	105	26.7	54.3	19
Total	410	33.9	49.8	16.3

The allelic frequency at the R462Q in the whole study population was 139 (33.7%), 204 (49.9%), and 67 (16.3%) for RR, RQ, and QQ, respectively. There were no significant differences comparing blood donors (27%, 54%, and 19%, respectively), HIV-1 infection (37%, 44%, and 20%, respectively), chronic hepatitis C (33%, 52%, and 15%, respectively), PrCa (25%, 60%, and 15%, respectively), and CFS patients (35%, 51%, and 14%, respectively). However, patients infected with HTLV-1 or hepatitis B virus (HBV) showed a significantly lower rate of RQ and QQ variants than the rest ($p=0.01$). The rate of the RR variant in HTLV-1 and HBV carriers was 67% and 61%, respectively (Table 1).

A total of 14 (3.6%) individuals were reactive for XMRV antibodies, although only one of them was positive for XMRV gag sequences. A trend toward a higher rate of XMRV seroreactivity was found in individuals with the RR genotype compared to the rest (6.1% vs. 2.3%, respectively; $p=0.083$). The single patient positive for XMRV gag sequences had PrCa and harbored RR at the RNASEL gene. It is noteworthy that Casey *et al.*¹⁶ estimated that individuals with the common missense mutation R462Q in homozygosis had a more than 2-fold increased risk of PrCa. We found an overall low rate of the QQ variant in all different study groups. These results are consistent with data reported by others^{18–20} who failed to show a significant association between codon 462 variants and the risk of PrCa in whites. Likewise, other studies have failed to show any linkage between PrCa, XMRV, and R462Q polymorphisms at the RNASEL gene.^{12,14,21}

Despite the limited size of the population examined with HTLV-1 infection or chronic hepatitis B, nearly two-thirds of these patients harbored codon 462 RR homozygosis. In contrast, it was found in only around a third of the rest of individuals tested in our study. Further studies testing a larger number of patients with these conditions will clarify whether RNASEL variants may influence the susceptibility to HTLV-1 infection and/or chronic hepatitis B.

In summary, we did not find any evidence of an association between RNASEL polymorphisms and the risk of infection with human retroviruses, hepatitis viruses, and/or conditions potentially linked to XMRV infection, such as CSF or PrCa. Thus, the evidence for any influence of R462Q variants on human viral diseases is limited if there is any at all.

Acknowledgments

This work was supported in part by grants from Fundación Investigación y Educación en SIDA (IES), Fondo de Investigación Sanitaria (FIS, PI10/0520), Red de Investigación

de SIDA (RIS, ISCIII, RD06/0006/040), Ministerio de Ciencia e Innovación (MICINN, SAF2010/22232), and the European NEAT Project. We would like to thank Dr. John Hackett (Abbott Diagnostics, North Chicago, IL) for investigational XMRV serological testing.

Author Disclosure Statement

No competing financial interests exist.

References

1. Austin B, James C, Silverman R, and Carr D: Critical role for the oligoadenylate synthetase/RNase L pathway in response to IFN-beta during acute ocular herpes simplex virus type 1 infection. *J Immunol* 2005;175:1100–1106.
2. Bisbal C and Silverman R: Diverse functions of RNase L and implications in pathology. *Biochimie* 2007;89:789–798.
3. Klein E and Silverman R: Inflammation, infection, and prostate cancer. *Curr Opin Urol* 2008;18:315–319.
4. Scherbik S, Paranjape J, Stockman B, Silverman R, and Brinton M: RNase L plays a role in the antiviral response to West Nile virus. *J Virol* 2006;80:2987–2999.
5. Rennert H, Zeigler-Johnson C, Addya K, Finley M, Walker A, Spangler E, *et al.*: Association of susceptibility alleles in ELAC2/HPC2, RNASEL/HPC1, and MSR1 with prostate cancer severity in European American and African American men. *Cancer Epidemiol Biomarkers Prev* 2005;14:949–957.
6. Breyer J, McReynolds K, Yaspan B, Bradley K, Dupont W, and Smith J: Genetic variants and prostate cancer risk: Candidate replication and exploration of viral restriction genes. *Cancer Epidemiol Biomarkers Prev* 2009;18:2137–2144.
7. Rökman A, Ikonen T, Seppälä E, Nupponen N, Autio V, Mononen N, *et al.*: Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer. *Am J Hum Genet* 2002;70:1299–1304.
8. Casey G, Neville P, Plummer S, Xiang Y, Krumroy L, Klein E, *et al.*: RNASEL Arg462Gln variant is implicated in up to 13% of prostate cancer cases. *Nat Genet* 2002;32:581–583.
9. Danielson B, Ayala G, and Kimata J: Detection of xenotropic murine leukemia virus-related virus in normal and tumor tissue of patients from the southern United States with prostate cancer is dependent on specific polymerase chain reaction conditions. *J Infect Dis* 2010;202:1470–1477.
10. Urisman A, Molinaro R, Fischer N, Plummer S, Casey G, Klein E, *et al.*: Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. *PLoS Pathog* 2006;2:e25.
11. Lombardi V, Ruscetti F, Das Gupta J, Pfost M, Hagen K, Peterson D, *et al.*: Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Science* 2009;326:585–589.
12. Fischer N, Hellwinkel O, Schulz C, Chun F, Huland H, Aepfelbacher M, *et al.*: Prevalence of human gammaretrovirus XMRV in sporadic prostate cancer. *J Clin Virol* 2008;43:277–283.
13. Hohn O, Krause H, Barbarotto P, Niederstadt L, Beimforde N, Denner J, *et al.*: Lack of evidence for xenotropic murine leukemia virus-related virus (XMRV) in German prostate cancer patients. *Retrovirology* 2009;6:92.
14. Schlager R, Choe D, Brown K, Thaker H, and Singh I: XMRV is present in malignant prostatic epithelium and is associated with prostate cancer, especially high-grade tumors. *Proc Natl Acad Sci USA* 2009;106:16351–16356.

15. Verhaegh G, de Jong A, Smit F, Jannink S, Melchers W, and Schalken J: Prevalence of human xenotropic murine leukemia virus-related gammaretrovirus (XMRV) in Dutch prostate cancer patients. *Prostate* 2011;71: 415–420.
16. Switzer W, Jia H, Zheng H, Tang S, and Heneine W: No association of xenotropic murine leukemia virus-related viruses with prostate cancer. *PLoS One* 2011;6:e19065.
17. Shin C, Bateman L, Schlager R, Bunker A, Leonard C, Huguen R, *et al.*: Absence of XMRV and other MLV-related viruses in patients with chronic fatigue syndrome. *J Virol* 2011;85:7195–7202.
18. Wiklund F, Jonsson B, Brookes A, Strömquist L, Adolfsson J, Emanuelsson M, *et al.*: Genetic analysis of the RNASEL gene in hereditary, familial, and sporadic prostate cancer. *Clin Cancer Res* 2004;10:7150–7156.
19. Meyer M, Penney K, Stark J, Schumacher F, Sesso H, Loda M, *et al.*: Genetic variation in RNASEL associated with prostate cancer risk and progression. *Carcinogenesis* 2010; 31:1597–1603.
20. Martinez-Fierro M, Leach R, Gomez-Guerra L, Garza-Guajardo R, Johnson-Pais T, Beuten J, *et al.*: Identification of viral infections in the prostate and evaluation of their association with cancer. *BMC Cancer* 2010;10:326.
21. Hong S, Klein E, Das Gupta J, Hanke K, Weight C, Nguyen C, *et al.*: Fibrils of prostatic acid phosphatase fragments boost infections with XMRV (xenotropic murine leukemia virus-related virus), a human retrovirus associated with prostate cancer. *J Virol* 2009;83:6995–7003.

Address correspondence to:

Vincent Soriano

Laboratory of Molecular Biology

Infectious Diseases Department

Hospital Carlos III

Calle Sinesio Delgado 10

Madrid 28029

Spain

E-mail: vsoriano@dragonet.es