ANTICANCER RESEARCH *24*: 2547-2550 (2004)

Lack of Association Between *RNASEL* Arg462Gln Variant and the Risk of Breast Cancer

AKIN SEVINÇ[1], DRAKOULIS YANNOUKAKOS[2], IRENE KONSTANTOPOULOU[2], ESRA MANGUOGLU[3], GÜVEN LÜLECI[3], TANER ÇOLAK[3], CEMALIYE AKYERLI[1], GÜLSEN ÇOLAKOGLU[1], MESUT TEZ[4], ISKENDER SAYEK[5], GERASSIMOS VOUTSINAS[6], GEORGE NASIOULAS[6], EIRENE PAPADOPOULOU[7], LINA FLORENTIN[8], ELENA KONTOGIANNI[9], BETÜL BOZKURT[10], NESLIHAN AYGÜN KOCABAS[11], ALI ESAT KARAKAYA[11], ISIK G. YULUG[1] and TAYFUN ÖZÇELIK[1,12]

[1]*Department of Molecular Biology and Genetics, Bilkent University, Ankara, Turkey;*
[2]*Molecular Diagnostics Laboratory, I/R-RP, National Center for Scientific Research Demokritos, Athens, Greece;*
[3]*Departments of Medical Biology and Genetics, and Surgery, Faculty of Medicine, Akdeniz University, Antalya;*
[4]*Atatürk Chest Disease Research Hospital, Ankara;*
[5]*Department of Surgery, Faculty of Medicine, Hacettepe University, Ankara, Turkey;*
[6]*Institute of Biology, National Center for Scientific Research Demokritos, Athens;*
[7]*Molecular Biology Research Center "HYGEIA" - "Antonis Papayiannis", Athens;*
[8]*Alfalab, Molecular Biology and Cytogenetics Center, Athens;*
[9]*IVF & Genetics, Athens, Greece;*
[10]*Department of Surgery, Ankara Numune Research and Teaching Hospital, Ankara;*
[11]*Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Gazi University, Ankara;*
[12]*Ayhan Sahenk Foundation, Istanbul, Turkey*

Abstract. *Background: The RNASEL G1385A variant was recently found to be implicated in the development of prostate cancer. Considering the function of RNase L and the pleiotropic effects of mutations associated with cancer, we sought to investigate whether the RNASEL G1385A variant is a risk factor for breast cancer. Patients and Methods: A total of 453 breast cancer patients and 382 age- and sex-matched controls from Greece and Turkey were analyzed. Genotyping for the RNASEL G1385A variant was performed using an Amplification Refractory Mutation System (ARMS). Results: Statistical evaluation of the RNASEL G1385A genotype distribution among breast cancer patients and controls revealed no significant association between the presence of the risk genotype and the occurrence of breast cancer. Conclusion: Although an increasing number of studies report an association between the RNASEL G1385A variant and prostate cancer risk, this variant does not appear to be implicated in the development of breast cancer.*

Correspondence to: Tayfun Özçelik, Department of Molecular Biology and Genetics, Bilkent University, Bilkent – Ankara 06800, Turkey. Fax: +90-312-266-5097, e-mail: tozcelik@fen.bilkent.edu.tr

Key Words: RNASEL, breast cancer.

RNASEL (MIM# 180435) encodes for the ubiquitously expressed ribonuclease L (RNase L) that mediates antiviral and pro-apoptotic activities of the 2-5A system (1). The *RNASEL* Arg462Gln (G1385A) variant, which has three times less enzymatic activity than the wild-type, was recently found to be implicated in up to 13% of prostate cancer cases (2, 3). Furthermore, germ-line *RNASEL* mutations segregating with disease within hereditary prostate cancer (HPC) families and loss of heterozygosity (LOH) involving the *RNASEL* locus in tumor tissues has been observed (4). RNase L has been proposed to suppress the development of prostate cancer through its ability to degrade RNA and initiate a cellular stress response that leads to apoptosis (1). By fluorescence *in situ* hybridization, *RNASEL* was assigned to 1q25 (5). Cytogenetic studies have shown that one of the most frequently observed karyotypic changes seen in breast cancer involve the long arm of chromosome 1. Analysis of polymorphic DNA markers to search for allelic losses at this chromosome region suggested that inactivation of a gene(s) located on 1q23-32, which encompasses the *RNASEL* locus, might contribute to the genesis of breast cancer (6). Breast cancer is a polygenic disorder and inherited mutations have been observed in *BRCA1, BRCA2, ATM, p53* and *CHEK2* genes (7). Interestingly, germ-line mutations in *CHEK2* (checkpoint kinase 2, a ubiquitously expressed protein

Table I. *Distribution of RNASEL G1385A genotypes and breast cancer risk in the age-matched controls and breast cancer patients.*

Population	Genotype	Case n=453 (%)	Control n=382 (%)	OR (95% CI) Crude	OR (95% CI) Adjusted[a,b]
Gr + Tr	G/G	206 (45.48)	168 (43.98)	1.00	1.00
	G/A	191 (42.16)	153 (40.05)	1.02 (0.76- 1.37)	0.95 (0.70- 1.29)
	A/A	56 (12.36)	61 (15.97)	0.75 (0.49- 1.14)	0.72 (0.46- 1.12)
	G/A or A/A	247 (54.52)	214 (56.02)	0.94 (0.72- 1.24)	0.89 (0.66-1.18)
Gr	G/G	60 (39.47)	59 (35.98)	1.00	1.00
	G/A	67 (44.08)	65 (39.63)	1.01 (0.62- 1.66)	0.78 (0.42- 1.46)
	A/A	25 (16.45)	40 (24.39)	0.62 (0.33- 1.14)	0.67 (0.32- 1.42)
	G/A or A/A	92 (60.53)	105 (64.02)	0.86 (0.55- 1.36)	0.74 (0.42- 1.31)
Tr	G/G	146 (48.50)	109 (50.00)	1.00	1.00
	G/A	124 (41.20)	88 (40.37)	1.05 (0.73- 1.52)	0.77 (0.46- 1.28)
	A/A	31 (10.30)	21 (9.63)	1.10 (0.60- 2.02)	1.07 (0.48- 2.39)
	G/A or A/A	155 (51.50)	109 (50.00)	1.06 (0.75- 1.50)	0.82 (0.51-1.33)

Gr: Greek, *Tr*: Turkish populations. OR: Odds Ratio, CI: Confidence Interval. ORs and 95% CIs were calculated using binary logistic regression. Adjusted for [a]age and menopausal status (*Gr, Gr+Tr*) and [b]smoking status, body mass index, age at menarche, age of 1st pregnancy, number of children, family history of breast cancer (*Tr*).

kinase) were found to be associated with prostate cancer risk as well (8). Based on the chromosomal localization and function of *RNASEL*, and pleiotropic effects of cancer-associated mutations as exemplified by *CHEK2* in both breast and prostate cancers or *BRCA1* in breast and ovarian cancers, we sought to investigate the hypothesis that the Arg462Gln variant of this gene is associated with breast cancer risk.

Patients and Methods

Peripheral blood samples were collected from 152 Greek and 301 Turkish breast cancer patients (invasive breast carcinoma, mean age: 49.65, standard deviation: 12.95, age range: 20-86). They were divided into two groups as premenopausal (n= 203; mean age: 40.29, standard deviation: 7.82, age range: 20-58), and postmenopausal (n=250; mean age: 57.40, standard deviation: 11.15, age range: 31-86). At the time of blood donation, each individual completed a standardized questionnaire that included information about age and menopausal status (Greece); and age, menopausal status, age at menarche, age at full term pregnancy, number of full term pregnancies, family history of breast cancer, smoking history and height and weight (Turkey). Histopathology of the tumor was obtained through medical records. The age-matched control group comprised 164 Greek and 218 Turkish apparently healthy women with no history of cancer. They were also divided into two groups as premenopausal (n=180; mean age: 37.91, standard deviation: 8.05, age range: 15-52) and postmenopausal (n=202; mean age: 58.55, standard deviation: 9.77, age range: 30-88). Informed consent was obtained from all subjects.

DNA was extracted from peripheral blood and the *RNASEL* G1385A mutation was detected using the Amplification Refractory Mutation System (ARMS) (2). Genotyping was performed and confirmed by two independent researchers. The association between the G1385A genotype and incidence of breast cancer was evaluated statistically using binary logistic regression (SPSS 9.0.0).

Results and Discussion

The *RNASEL* Arg462Gln variant was analyzed in 453 female breast cancer patients and 382 age- and sex-matched controls. The combined Greek and Turkish population allele frequencies of the A allele was 0.334 and 0.359 for cases and controls, respectively. Although the A allele frequency was slightly different between the two populations (cases: 0.385 and controls: 0.442 Greek; and cases: 0.309 and controls: 0.298 Turkish), the genotype distributions in the control groups were in Hardy-Weinberg equilibrium in both populations. Our study showed that there is no significant association

between *RNASEL* G1385A mutation and breast cancer risk (*t*-test, $p=0.66$) (Table I). Stratification of the data according to age and menopausal status in the Greek population; age, menopausal status, smoking status, body mass index, age at menarche, age of first pregnancy, number of children, family history of breast cancer in the Turkish population; or age and menopausal status in both populations combined, did not change the results. Inclusion of two different Eastern Mediterranean populations and a fairly large number of cases and controls makes this study relatively strong. Given the sample size and allele frequencies, the study has a power of 90% to confirm an odds ratio as low as OR = 1.6 at a significance level of $\alpha = 0.05$.

In conclusion, our study suggests no significant association between the *RNASEL* G1385A variant and breast cancer risk in the Greek and Turkish populations. These results may need to be further corroborated by other investigations and in different populations since this is the first study reporting on the association of the *RNASEL* G1385A variant and breast cancer.

Acknowledgements

We gratefully acknowledge Dr. Atilla Halil Elhan for help in statistical analyses. All experiments were performed in accordance with Greek and Turkish laws and regulations.

Grant sponsors: The Scientific and Technical Research Council of Turkey (TÜBITAK-GSRT-11), Bilkent University, Turkey, the Greek General Secretary for Research and Technology (97EKBAN2-1.2-112) and the Stavros Niarchos Foundation for Charity, Greece.

References

1 Silverman RH: Implications for RNase L in prostate cancer biology. Biochem *42*: 1805-1812, 2003.
2 Casey G, Neville PJ, Plummer SJ, Xiang Y, Krumroy LM, Klein EA, Catalona WJ, Nupponen N, Carpten JD, Trent JM, Silverman RH and Witte JS: *RNASEL* Arg462Gln variant is implicated in up to 13% of prostate cancer cases. Nature Genet *32*: 581-583, 2002.
3 Nakazato H, Suzuki K, Matsui H, Ohtake N, Nakata S and Yamanaka H: Role of genetic polymorphisms of the *RNASEL* gene on familial prostate cancer risk in a Japanese population. Br J Cancer *89*: 691-696, 2003.
4 Carpten J, Nupponen N, Isaacs S, Sood R, Robbins C, Xu J, Faruque M, Moses T, Ewing C, Gillanders E, Hu P, Bujnovszky P *et al*: Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nature Genet *30*: 181-184, 2002.
5 Squire J, Zhou A, Hassel BA, Nie H and Silverman RH: Localization of the interferon-induced, 2-5A-dependent RNase gene (RNS4) to human chromosome 1q25. Genomics *19*: 174-175, 1994.
6 Chen LC, Dollbaum C and Smith HS: Loss of heterozygosity on chromosome 1q in human breast cancer. Proc Natl Acad Sci USA *86*: 7204-7207, 1989.
7 Balmain A and Ponder B: The genetics and genomics of cancer. Nature Genet *33*: 238-244, 2003.
8 Dong X, Wang L, Taniguchi K, Wang X, Cunningham JM, McDonnel SK, Qian C, Marks AF, Slager SL, Peterson BJ, Smith DI, Cheville JC *et al*: Mutations in *CHEK2* associated with prostate cancer risk. Am J Hum Genet *72*: 270-280, 2003.

Received January 20, 2004
Accepted March 4, 2004