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SEPP1 and *SEP15* gene polymorphisms and susceptibility to breast cancer

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Breast cancer is the most commonly diagnosed type of cancer among women worldwide, (second most common cancer overall) [1]. Cancer onset and progression have been linked to oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability and cell proliferation, and therefore antioxidant agent could interfere with carcinogenesis [2]. Single nucleotide polymorphisms may have functional effects on gene expression and protein activity [3]. Selenoprotein P (Sepp1) is the most abundant selenoprotein in serum and delivers selenium to tissues, carrying ten selenocysteine (Sec) residues per polypeptide. Sepp1 has been postulated as a defence mechanism to defend against oxidative injury in the extracellular space [4]. The best-studied polymorphism of this selenoprotein includes Ala234Thr (rs3877899) that is associated with a G/A transition at position 24731 of mRNA, with the amino acid change from alanine to threonine in the codon 234. The major allele G favours the production of a Se-rich 60 kDa isoform, while allele A favours the 50 kDa low Se isoform. This polymorphism affects selenium's bioavailability for the synthesis of all other selenoproteins by influencing the body's selenium status, effectiveness of supplementation, and selenium supply to target tissues [5]. *SEP15* is localized on chromosome 1p31, a locus often deleted or mutated in human cancers. The gene product, 15 kDa selenoprotein (Sep15), exhibits redox activity and may have antioxidant properties [6]. G1125A (rs5859) is a single nucleotide polymorphism within the *SEP15* SECIS element (3'UTR), and is associated with G/A transition at position 1125. SNPs in the gene regions corresponding to 3'UTR could potentially influence expression because of the key role of the SECIS and 3'UTR–protein interactions in Sec incorporation [7]. *SEPP1* (rs3877899) and *SEP15* (rs5859) are two important polymorphisms that several studies have linked with various cancers, including risk of breast cancer, lung cancer in smokers, rectal cancer risk, and risk of prostate cancer. Data from these association studies have shown that the investigated SNPs altered the expression or function of selenoproteins and may increase the risk of developing cancer**.** We hypothesized that the *Sepp1* and *Sep15* polymorphisms are linked to breast cancer.

The study population comprised 350 women with a minimum age of 30 years recruited between August 2014 and February 2016 from patients attending Razi hospital in Rasht, Iran. Of these, 150 had histologically breast cancer (mean/SD age = 50.8 (10.8) years). Pathobiological features (histology type, lymph node involvement and hormone receptor status) were collected. Two hundred controls (aged 49.2 (11.1) years) were nonrelated women who had never been diagnosed with breast tumours, other tumours, or chronic disease, and who were selected from those seeking health care in the same hospitals during the same period as patient recruitment. All subjects filled out a short questionnaire including questions on age, breastfeeding, family history of breast cancer and hormone replacement therapy (HRT). Written informed consent for the genetic analysis was obtained from each subject participating in the study. The study was conducted in accordance with the Declaration of Helsinki regarding the use of human samples.

The total DNA was isolated from a 1 ml blood sample using the GPP Solution kit (Gene Pajoohan Pouya, Iran) according to the manufacturer's recommendations. Quality of the extracted DNA was confirmed by electrophoresis on 1% agarose gel containing ethidium bromide. The *SEPP1* polymorphism was genotyped by Polymerase Chain Reaction-Restricted Fragment Length Polymorphism (PCR-RFLP). The oligonucleotide primers used to amplify *SEPP1* rs3877899 were (F: 5'TAGGAGCCAACTCTGAATCTGT3') and (R: 5'GTTGAAACTCCATCGCCTCA3'). Each reaction mixture (25 µl) contained 30 ng DNA template, 1x PCR kit, 0.5 µM

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of each primer and nuclease free water. Samples were initially denatured at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45s, annealing at 57 °C for 45s, extension at 72 °C for 45s, and then a final extension at 72 °C for 5 min. The amplified PCR products were digested with restriction enzyme *MwoI* at 37 °C for 3 h. PCR products for *SEPP1* rs3877899 were 301 bp in size. The G allele (Ala) was cut into two fragments of 198 bp and 103 bp, while the A allele (Thr) remained uncut (301 bp) (Figure 1). The genotype of *SEP15* polymorphism was detected by Allele Specific-PCR (AS-PCR). The procedure was performed using primer pairs, specific for the two alleles. Primers F1 (5′ATCTGATCCACACAAATCCC3′) and R1 (5′GATTACTATGCCTCATGTGCT3′) were used to amplify the G allele, and primers F2 (5′ATCTGATCCACACAAATCCT3′) and R2 (5′TCCTGCATTTGTTGATACCAC3′) were used to amplify the A allele. Each reaction mixture (20 µl) contained 25 ng DNA template, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM deoxyribonucleotide triphosphate, 0.5 µM of each primer and 1.5 U Taq DNA polymerase. The reaction conditions were the same as described for *SEPP1* gene (except annealing at 53 °C for G allele and 57 °C for A allele). The size of PCR products of A and G alleles were 441 bp and 306 bp, respectively (Figure 1).

The statistical significance of differences between groups was calculated by the Chi-square test. A p-value < 0.05 was considered statistically significant. To estimate the association between individual SNPs and breast cancer, we calculated odds ratios (ORs) and 95% confidence intervals (95% CI) using logistic regression. All statistical analyses were conducted using the MedCalc (version 12.1).

There were no statistically significant differences between cases and controls in relation to breast cancer risk factors including a positive family history (4.6% vs. 4.0%, $p = 0.95$), history of breastfeeding (75.3% vs. 68.0%, $p = 0.17$), HRT use (11.33% vs. 7.5%, $p = 0.3$), and menopausal status (57.33% vs. 54.5% post-menopausal women, $p = 0.67$). Once excised, of 143 tumours, 127 were ductal and 16 were non-ductal. Of 112 tumours, 81 were positive for oestrogen/progesterone receptor status whilst 34 were negative. Of 123 women examined, lymph node metastases were present in 89 and absent in 34.

Genotype frequency of *SEPP1* polymorphism showed significant differences in two groups. Individuals carrying the AA genotype were 3.8-fold at a higher risk of BC compared with GG genotype (OR = 3.89; 95% CI, 2.02–7.49; $p < 0.0001$). Also, heterozygous carriers (AG) have a higher BC risk (OR = 2.11; 95% CI, 1.23–3.63; $p = 0.006$). There was a significant difference in the distribution of allele frequencies between the control and patient groups ($p < 0.0001$). The prevalence of A allele was higher in patients than controls. The variant A allele (Thr) of *SEPP1* increased breast cancer risk (OR = 2.69; 95% CI, 1.88–3.85; $p < 0.0001$). We observed a significant difference in genotype distribution of *SEP15* polymorphism between breast cancer patients and the controls ($p < 0.0001$). It was also observed the homozygous AA had a 2.8-fold increased risk of breast cancer compared with homozygous GG (OR = 2.82; 95% CI, 1.04–7.62; $p = 0.04$). However, there was no significant increase in the A allele frequency among patients when compared with healthy controls ($p = 0.11$) (Table 1).

Oxidative stress contributes to oncogenic development via induction of DNA damage that lead to mutation and increased cell proliferation, survival and migration [2]. Many of selenoproteins have redox function contributing to decrease of oxidative stress. Several studies of the selenoprotein promoter indicated that it interacts with cytokine and growth factor pathways [8]. It is believed that selenoproteins can affect cell-signalling molecules such as nuclear factor-κB and hence influence important cellular functions such as gene transcription and cell growth [9]. It is possible that functional polymorphisms in selenoprotein genes might influence selenoproteins expression, stability or activity.

A number of epidemiological studies have been carried out to examine the relationship between this polymorphism (rs3877899) and the risk of various cancers in

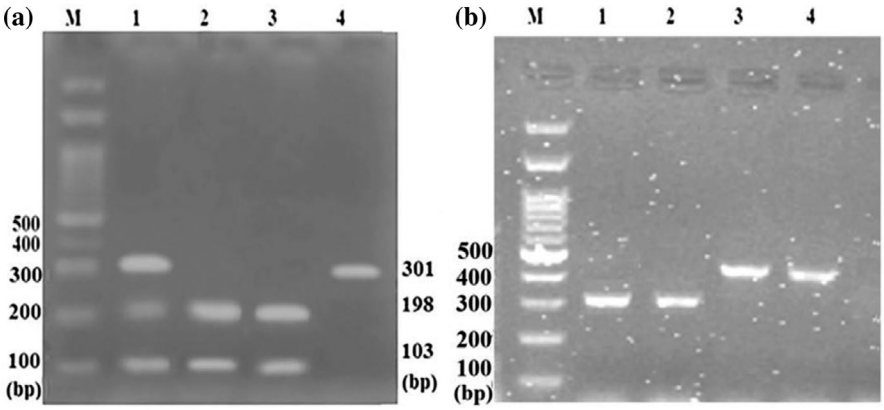

Figure 1. (a): PCR-RFLP analyses of *SEPP1* polymorphism, (1): AG with 301, 198 and 103 bp; (2, 3): GG with 198 and 103 bp; (4): AA with 301 bp. (b): AS-PCR results of *SEP15* polymorphism, (1, 2): fragments presenting the G allele; (3,4): fragments indicating the A allele. (M): 100 bp DNA marker.

Table 1. Genotype and allele frequencies of SEPP1 and SEP15 in cases and controls.

Polymorphic site	Cases (*n*)	Controls (*n*)	OR (95% CI)	*P*-value
SEPP1(rs3877899)				
GG	80 (53.3)	151 (75.5)	1.00 (Reference)	–
AG	37 (24.7)	33 (16.5)	2.11 (1.23–3.63)	0.006
AA	33 (22.0)	16 (8.0)	3.89 (2.02–7.49)	< 0.001
AA+AG	70 (46.7)	49 (24.5)	2.69 (1.71–4.24)	< 0.001
G	197 (65.7)	335 (83.7)	1.00 (Reference)	–
A	103 (34.3)	65 (16.3)	2.69 (1.88–3.85)	< 0.001
SEP15 (rs5859)				
GG	17 (11.3)	15 (7.5)	1.00 (Reference)	–
AG	101 (67.3)	175 (87.5)	0.5 (0.24–1.06)	0.07
AA	32 (21.4)	10 (5.0)	2.82 (1.04–7.62)	0.04
AA+AG	133 (88.6)	185 (92.5)	0.63 (0.3–1.31)	0.22
G	135 (45.0)	205 (51.2)	–	0.11
A	165 (55.0)	195 (48.8)	–	

Note: OR = Odd ratio, CI = confidence interval, Data are number of women (*n*) and percentage (%).

different populations. Contrary to our findings, Méplan et al. found that individuals with the AA genotype for *SEPP1* (rs3877899) have a reduced risk of breast cancer [5], whilst, Pellatt et al. found no association between breast cancer and *SEPP1* rs3877899 [10]. Geybels et al. reported that *SEPP1* rs3877899 was associated with regional/distant stage but not local stage prostate cancer [11]. Another study by Al-Taie et al. *has* shown there is no association between *SEPP1* (rs3877899) and colorectal cancer (CRC) [12].

In addition, a number of molecular epidemiological studies have been done to evaluate the association between *SEP15* polymorphism and different types of cancer risk in diverse population. Hu et al. observed an association between rs5859 and BC in African Americans but not in Caucasians [7]. In the study carried out by Sutherland et al. [13], a positive correlation was found between the minor allele (A) for rs5859 and rectal cancer risk in men. However, there was no increased risk in women or in patients with colon cancer [13]. In the Physician's Health Study, a nested case control study involving 1286 cases and 1267 controls, *SEP15* rs5859 was not significantly associated with prostate cancer risk [14]. Jablonska et al. reported that among smoking individuals, those with the AA genotype for *SEP15* (rs5858) may benefit most from a higher Se intake, whereas in those with the GG or GA genotype, a higher Se status may increase the risk for lung cancer [15]. In another study, Jablonska et al. have not found a statistical relationship between rs3877899 *(SEPP1*) and rs5859 (*SEP15*) with breast cancer risk [16]. Méplan et al. found that the interaction of rs5859 (*SEP15*) with rs3877899 (*SEPP1*) was associated with an increased risk of CRC for individuals carrying at least one rare A allele for rs3877899 [17]. These conflicting results even on the same diseases may be due to variability of sample features, disease stages, different study designs and different genetic background.

This work represents an advance in biomedical science because it suggests the possible importance of *SEPP1* rs3877899 and *SEP15* rs5859 polymorphisms in susceptibility to breast cancer. However, additional large and prospective studies with carefully collection of detailed clinical characteristics are required to achieve a definitive conclusion.

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References

[1] Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136:359–386. 10.1002/ijc.2910.
[2] Mileo AM, Miccadei S. S. Polyphenols as modulator of oxidative stress in cancer disease: new therapeutic strategies. Oxid Med Cell Longev. 2016;2016:6475624.
[3] Shastry B. SNPs: impact on gene function and phenotype. Methods Mol Biol. 2009;578:3–22.
[4] Mostert V. Selenoprotein P: properties, functions, and regulation. Arch Biochem Biophys. 2000;376:433–438.
[5] Méplan C, Dragsted LO, Ravn-Haren G, et al. Association between polymorphisms in glutathione peroxidase and selenoprotein P genes, glutathione peroxidase activity, HRT use and breast cancer risk. PLoS ONE. 2013;8:e73316.
[6] Wu H, Lin C, Zha Y, et al. Redox reactions of Sep15 and its relationship with tumor development. Ai Zheng. 2003;22:119–122.
[7] Hu Y, Korotkov K, Mehta R, et al. Distribution and functional consequences of nucleotide polymorphisms in the 3′-untranslated region of the human Sep15 gene. Cancer Res. 2001;61:2307–2310.
[8] Mostert V, Wolff S, Dreher I, et al. Identification of an element within the promoter of human selenoprotein P responsive to transforming growth factor-β. Eur J Biochem. 2001;268:6176–6181.
[9] Hayashi T, Ueno Y, Okamoto T. Oxidoreductive regulation of nuclear factor kappa B. Involvement of a cellular reducing catalyst thioredoxin. J Biol Chem. 1993;268:11380–11388.
[10] Pellatt A, Wolff R, John E, et al. SEPP1 influences breast cancer risk among women with greater native American ancestry: the breast cancer health disparities study. PLoS ONE. 2013;8:e80554.
[11] Geybels MS, Hutter CM, Kwon EM, et al. Variation in selenoenzyme genes and prostate cancer risk and survival. Prostate. 2013;73:734–742.
[12] Al-Taie OH, Uceyler N, Eubner U, et al. Expression profiling and genetic alterations of the selenoproteins GI-GPx and SePP in colorectal carcinogenesis. Nutr Cancer. 2004;48:6–14.

[13] Sutherland A, Kim D-H, Relton C, et al. Polymorphisms in the selenoprotein S and 15-kDa selenoprotein genes are associated with altered susceptibility to colorectal cancer. Genes Nutr. 2010;5:215–223.
[14] Penney KL, Schumacher FR, Li H, et al. A large prospective study of SEP15 genetic variation, interaction with plasma selenium levels, and prostate cancer risk and survival. Cancer Prev Res (Phila). 2010;3:604–610.
[15] Jablonska E, Gromadzinska J, Sobala W, et al. Lung cancer risk associated with selenium status is modified in smoking individuals by Sep15 polymorphism. Eur J Nutr. 2008;47:47–54.
[16] Jablonska E, Gromadzinska J, Peplonska B, et al. Lipid peroxidation and glutathione peroxidase activity relationship in breast cancer depends on functional polymorphism of GPX1. BMC Cancer. 2015;15:1126–657.
[17] Meplan C, Hughes DJ, Pardini B, et al. Genetic variants in selenoprotein genes increase risk of colorectal cancer. Carcinogenesis. 2010;31:1074–1079.