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ORIGINAL CONTRIBUTION

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Lung cancer risk associated with selenium status is modified in smoking individuals by *Sep15* polymorphism

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Abstract *Background* Selenium (Se) is a trace element suggested to act chemopreventive in lung cancer. The mechanism by which Se suppresses tumour development may be associated with some of the functions of selenoproteins, including 15 kDa selenoprotein (Sep15). This protein exhibits antioxidant properties and thus may be involved in the process of carcinogenesis. Recently, it has been shown that the genetic polymorphism of Sep15, resulting in different response of the protein to Se, is associated with the risk of breast and head and neck cancers. *Aim of the study* The aim of the study was to investigate the possible association between lung cancer risk and *Sep15* polymorphism in combination with Se status in the Polish population. *Methods* The study concerned 325 cases and 287 controls. All the participants were smokers. Plasma Se concentration was determined using graphite furnace atomic absorption spectrometry, and *Sep15* polymorphism (1125 G/A transition within 3′-untranslated region) was detected with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. *Results* The adjusted odds ratios (ORs) for lung cancer cases, compared to individuals with *Sep15* wild type variant (GG), were: 0.91 (95% CI: 0.64–1.32) for the heterozygous variant (GA) and 0.80 (95% CI: 0.39–1.65) for the homozygous variant (AA). Although plasma Se concentration was statistically lower in lung cancer cases (49.4 ± 17.4 ng/ml) compared to controls (53.3 ± 14.0 ng/ml, $p < 0.002$), the analysis of the joint effect of *Sep15* polymorphism and Se status for lung cancer development revealed that lung cancer risk differed between the *Se15* genotype groups. An increasing Se concentration was associated with a decreased risk in all individuals; however, at Se concentration above 80 ng/ml, the risk started to increase in individuals possessing the *Sep15* 1125 GG or GA genotype. *Conclusions* It appears that among smoking individuals, those with the *Sep15* 1125 AA genotype 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.

Key words *Sep15* – selenium – lung cancer

Introduction

Since selenium (Se) was shown to prevent liver necrosis in rats [34] and exudative diathesis in chicks [28], it was regarded as an essential trace element. In recent years, most attention was paid to Se in the context of cancer incidence reduction, which was demonstrated in animal studies (recently reviewed by Whanger) [37] and human clinical trials in relation to prostate, colon, lung and liver cancers [6, 39]. The mechanism by which Se suppresses tumour development has not as yet been elucidated. It is supposed that some of the anticancer properties of Se may be associated with specific proteins, which contain this element in the form of selenocysteine, the 21st naturally occurring amino acid [13]. Presently, 25 human selenoproteins are known, including 11 enzymes (four glutathione peroxidases, three iodothyronine deiodinases, three thioredoxin reductases and selenophosphate synthetase 2) and proteins: 15 kDa, H, I, K, M, N, S, O, P, R, S, T, V and W [19].

One of the selenoproteins of greater interest, due to its relevance to cancer risk, is 15 kDa selenoprotein (Sep15) [31]. This protein was first identified by Kacklösch et al. in the epithelial cells of rat prostate and by Gladyshev et al. in human Jurkat T-cells [10, 15]. Sep15 exhibits redox activity [21, 38] and belongs to the thiol-disulfide oxidoreductase family of selenoproteins [7]. Compared to other selenoproteins, Sep15 is unique as it is located in endoplasmic reticulum (ER) and forms a complex with another protein, UDP-glucose: glycoprotein glucosyltransferase (UGGT), which is involved in the quality control of protein folding [18, 22]. As Sep15 contains a thioredoxin-like structure and an unusual CXU motif (one residue (X) between cysteine (C) and selenocysteine (U)), which is similar to CXXC motif characteristic for thiol-disulfide oxidoreductases, it was suggested that this selenoprotein may take part in the process of correct disulfide bond formation, which has been confirmed recently by Ferguson et al. [7].

The cDNA library search shows that *Sep15* is expressed in a wide range of tissues, with the highest levels in thyroid, parathyroid and prostate [10]. With the use of northern blot technique, high levels of *Sep15* mRNA were also detected in prostate, liver, brain, kidney and testis [20]. Interestingly, the expression of this gene is dysregulated in certain human and animal tumours. Apostolou et al. [2] found that *Sep15* was downregulated in approximately 60% of human malignant mesothelioma (MM) cell lines and tumours. Another study showed a lack of expression of 15 kDa selenoprotein in murine adenocarcinoma prostate and liver tumours, compared to the high levels of this protein exhibited by normal mouse liver and prostate [20]. These observations, together with the fact that Sep15 genetic locus (1p31) is commonly mutated or deleted in different human tumours [3, 20, 25] including lung cancer [17], imply that 15 kDa protein may be involved in the process of carcinogenesis. The link between Se and lung cancer has been shown in human clinical and epidemiological studies. Clark et al. [6], in their randomized controlled trial showed that Se supplementation decreased lung cancer incidence (46% reduction) and lung cancer mortality (53% reduction) in the Se-treated group, in comparison with the placebo group. Some epidemiological studies indicate also that high nutritional levels of Se are associated with a decreased incidence of lung cancer in humans [16]. Since Sep15 expression is regulated by dietary Se [27], it can be assumed that this protein is involved in the development of lung cancer. Notably, Sep15 compared to other selenoproteins, was preferentially synthesized in lung of Se-deficient rats after Se administration, which indicates the importance of this protein in this organ [4].

It is possible that *Sep15* polymorphism is responsible for the link between Sep15 and cancer development. Two polymorphic sites located at 811 (C/T) and 1125 (G/A) within human *Sep15* 3′ untranslated region (3′-UTR) were found [10]. Further analyses revealed that allele C^{811} was exclusively associated with allele G^{1125}, whereas allele T^{811} was exclusively associated with allele A^{1125}, so there can be only two possible haplotypes existing within human *Sep15* gene: C^{811}/G^{1125} and T^{811}/A^{1152} [14, 20]; 1125 (G/A) is located in a specific stem-loop structure within 3′-UTR that is called Sec insertion sequence (SECIS), an element responsible for Sec incorporation in selenoprotein during translation [20]; 811 (C/T) was also found at a SECIS-like structure, but this element is not functional [20]. The functional meaning is attributed to site 1125. It was observed that allele containing A instead of G is responsible for a lower responsiveness of the cultured cells to Se [2, 14, 20]. The authors suggest that dietary Se may be less beneficial in chemoprevention for individuals carrying 1125A allele in *Sep15* gene [2, 14]. So far, an association between *Sep15* 1125G/A polymorphism in breast and head and neck cancers among African Americans has been found [14].

The aim of this study was to evaluate whether the genetic polymorphism of Sep15 in combination with Se status is associated with lung cancer risk.

Materials and methods

Study population

The study was a part of a case–control study conducted by Reszka et al. [33] and was approved by the local Biomedical Ethics Committee. All the subjects

Table 1 Study population characteristics

Characteristics	Cases (n = 325)	Controls (n = 287)	p
Age	58.8 ± 8.8 (30–78)	59.2 ± 8.9 (33–74)	NS[a]
Male	252	237	–
Female	73	50	–
SCC	91	–	–
NSCC	232	–	–
SI	765 ± 331	552 ± 354	<0.0002[a]

NSCC non-small cell lung cancer, *SCC* small cell lung cancer, *SI* smoking index, *NS* not statistically significant
[a]Student's t test: cases vs. controls

were Polish. A total of 325 cases of histologically confirmed lung cancer, aged 30–78 years (mean 58.8 ± 8.8), and 287 controls of similar age (33–74, mean 59.2 ± 8.9 years) were analyzed. All the cases were the patients treated in Lodz Hospitals in 1998–2002, and the controls were selected from among hospital patients, excluding those with other cancers or pulmonary diseases. After obtaining written informed consent to take part in the study, blood samples and personal data concerning the age, gender and the smoking status were collected. No data concerning the use of Se supplements were available. Non-smoking individuals were not included due to the too small sample size and the possible misclassification of the smoking status. The cases were categorized into two histological types: small cell lung cancer (SCC) and non-small cell lung cancer (NSCC). 232 Cases were classified as NSCC, accounting for the majority of cases (71.4%), 91 cases were SCC (28%) and 2 cases were classified generally as lung cancer (0.6%). The characteristics of the study population are displayed in Table 1.

Selenium status determination

The Se concentration was determined in the plasma of 325 lung cancer cases (100% of samples) and 276 controls (96% of samples), using graphite furnace atomic absorption spectrometry (GFAAS) [26]. The method was validated using reference material (lyophilized human reference serum samples of Seronorm™ from Nycomed Pharma AS, Oslo, Norway) and through participation in interlaboratory comparison trials [11].

Genotype analysis

DNA was isolated from whole blood using QIAamp DNA Blood Mini Kit (Qiagen) according to the manufacturer's instructions. Genotype analysis was performed using polymerase chain reaction-restriction fragment length polymorhism assay (PCR-RFLP) and electrophoresis techniques. Oligonucleotide sequences for PCR primers were: forward 5′-CAG ACT TGC GGT TAA TTA TG-3′ and reverse 5′-GCC AAG TAT GTA TCT GAT CC-3′ [14]. The amplicons were digested with *Dra*I (*Deinococcus radiophilus*, Fermentas) and *FspB*I (*Flavobacterium* species RFL1, Fermentas) and the digestion products were analyzed in 1.5% agarose gel.

Statistical analysis

Hardy–Weinberg equilibrium was assessed using chi square test. Odds ratios (ORs) adjusted for age, sex and smoking index were calculated as the estimates of lung cancer risk using unconditional logistic regression. Smoothing splines were used for continuous variables. For the smoking index, we used log transformation and for the age, the linear trend as a good approximation suggested by a preliminary analysis with splines regression. For plasma Se concentration, spline term was used. Nonlinearity in the trend for Se was assessed comparing the model with the linear trend to the model with the spline term with a fixed smoothness parameter. For testing significance, the likelihood ratio and confidence intervals (CI) based on likelihood profile were used. Mean plasma Se concentrations and smoking index in the cases and controls were compared using Student's t test (assuming equal variances). Standard level of significance of 0.05 was adopted for statistical inference. All the statistical tests were two sided and data analysis was performed using R statistical environment. MGCV package, version 1.3–23, was used for logistic regression with splines.

Results

The control group was in Hardy–Weinberg equilibrium for *Sep15* genotype. In all DNA samples, allele C^{811} was exclusively associated with G^{1125} and allele T^{811} was exclusively associated with allele A^{1125}. Allele frequencies and odds ratios with 95% confidence intervals for *Sep15* 1125G/A are shown in Table 2. The comparison of allele frequency in the cases and controls showed no statistically significant association between *Sep15* polymorphism and lung cancer risk. Compared to the individuals with the wild type variant (GG), the risk estimates for lung cancer were: 0.91 (95% CI: 0.64–1.32) for the heterozygous variant (GA) and 0.80 (95% CI: 0.39–1.65) for the homozygous variant (AA) (Table 2). No significant differences were also observed in SCC (OR = 1.01, 95% CI: 0.60–1.70 for variant GA, and OR = 0.50, 95% CI: 0.11–1.62 for variant AA; Table 3) and NSCC (OR = 0.91, 95%

Table 2 *Sep15* genotype frequency in lung cancer cases and cancer-free individuals

Sep15 genotype	Cases *n*, (%)	Controls *n*, (%)	OR (95% CI)[a]	*p*
Total	325 (100%)	287 (100%)		
GG	189 (58.2%)	161 (56.1%)	1.00	–
GA	117 (36.0%)	108 (37.6%)	0.91 (0.64–1.32)	0.631
AA	19 (5.8%)	18 (6.3%)	0.80 (0.39–1.64)	0.534
Males	252 (100%)	237 (100%)		
GG	151 (59.9%)	131 (55.3%)	1.00	–
GA	86 (34.1%)	90 (38.0%)	0.85 (0.57–1.28)	0.444
AA	15 (6.0%)	16 (6.7%)	0.73 (0.33–1.61)	0.433
Females	73 (100%)	50 (100%)		
GG	38 (52.0%)	30 (60.0%)	1.00	–
GA	31 (42.5%)	18 (36.0%)	1.27 (0.56–2.88)	0.566
AA	4 (5.5%)	2 (4.0%)	1.17 (0.21–9.04)	0.866

OR Odds ratio, *95% CI* 95% confidence interval
Interaction test sex and genotype $p = 0.690$
[a]Adjusted for smoking index, age and sex (where appropriate)

Table 4 *Sep15* genotype frequency in non-small cell lung cancer (NSCC) cases and cancer-free individuals

Sep15 genotype	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)[a]	*p*
Total	228 (100%)	287 (100%)		
GG	131 (57.5%)	161 (56.1%)	1.00	–
GA	81 (35.5%)	108 (37.6%)	0.91 (0.61–1.35)	0.634
AA	16 (7.0%)	18 (6.3%)	0.92 (0.43–1.96)	0.820
Males	186 (100%)	237 (100%)		
GG	111 (59.7%)	131 (55.3%)	1.00	–
GA	61 (32.8%)	90 (38.0%)	0.87 (0.55–1.36)	0.537
AA	14 (7.5%)	16 (6.7%)	0.91 (0.40–2.08)	0.820
Females	49 (100%)	50 (100%)		
GG	27 (55.1%)	30 (60.0%)	1.00	–
GA	20 (40.8%)	18 (36.0%)	1.22 (0.51–2.93)	0.658
AA	2 (4.1%)	2 (4.0%)	0.86 (0.10–7.80)	0.889

OR Odds ratio, *95% CI* 95% confidence interval
Interaction test sex and genotype $p = 0.800$
[a]Adjusted for smoking index, age and sex (where appropriate)

CI: 0.61–1.35 for variant GA, and OR = 0.92, 95% CI: 0.43–1.96 for variant AA; Table 4). The distribution of *Sep15* alleles in lung cancer differed between the males and females with SCC. In this type of cancer, allele A was less frequent in men (39.3%) than in women (54.1%), whereas in the controls, the frequency was higher for males (44.7%) than for females (40.0%). Odds ratios for genotype AA were: OR = 0.22 (95% CI: 0.12–1.14) for men and OR = 1.78 (95% CI: 0.17–18.25) for women. The difference in the risk (OR) for the males and females was not statistically significant ($p = 0.261$; Table 3).

Plasma Se concentration was significantly lower among smoking individuals with lung cancer compared to smoking cancer-free individuals (49.4 ± 17.4 vs. 53.3 ± 14.0 ng/ml; $p < 0.002$). Although these results suggest a decrease in cancer risk for higher Se levels, we observed a decreased risk only for Se concentrations of up to 70 ng/ml and an increased risk (OR = 10.32; $p = 0.025$; Table 5) for levels above 90 ng/ml. Lower plasma Se levels (not statistically significant) were found in females compared to males, both in the cases (48.0 ± 14.1 vs. 49.9 ± 18.4 ng/ml; $p = 0.407$) and controls (51.8 ± 12.6 vs. 53.5 ± 14.3 ng/ml; $p = 0.458$). In the group with *Sep15* 1125AA genotype, Se concentration was lower in females compared to males only among the cases (38.9 ± 11.8 vs. 45.5 ± 13.2; $p = 0.382$), whereas in the controls, a reverse situation was found (64.4 ± 4.0 vs. 54.8 ± 17.2; $p = 0.459$).

Table 3 *Sep15* genotype frequency in small cell lung cancer (SCC) cases and cancer-free individuals

Sep15 genotype	Cases *n*, (%)	Controls *n*, (%)	OR (95% CI)[a]	*p*
Total	91 (100%)	287 (100%)		
GG	52 (57.1%)	161 (56.1%)	1.00	–
GA	36 (39.6%)	108 (37.6%)	1.01 (0.60–1.70)	0.972
AA	3 (3.3%)	18 (6.3%)	0.50 (0.11–1.62)	0.261
Males	66 (100%)	237 (100%)		
GG	40 (60.6%)	131 (55.3%)	1.00	–
GA	25 (37.9%)	90 (38.0%)	0.88 (0.48–1.59)	0.683
AA	1 (1.5%)	16 (6.7%)	0.22 (0.12–1.14)	0.076
Females	24 (100%)	50 (100%)		
GG	11 (45.8%)	30 (60.0%)	1.00	–
GA	11 (45.8%)	18 (36.0%)	1.57 (0.51–4.82)	0.429
AA	2 (8.3%)	2 (4.0%)	1.78 (0.17–18.25)	0.608

OR Odds ratio, *95% CI* 95% confidence interval
Interaction test sex and genotype $p = 0.261$
[a]Adjusted for smoking index, age and sex (where appropriate)

Table 5 Lung cancer risk associated with plasma selenium concentration

Plasma Se concentration (ng/ml)	Cases (*n*)	Controls (*n*)	OR (95% CI)[a]	*p*
4–49	186	112	1.90 (1.30–2.77)	0.001
50–69	98	129	1.00	–
70–89	32	33	1.21(0.67–2.20)	0.531
90–125	9	2	10.32 (1.88–138.2)	0.025

OR Odds ratio, *95% CI* 95% confidence interval
Test for trend $p < 0.001$, test for non-linear trend $p < 0.001$
[a]Adjusted for smoking index, age and sex

The results for cancer risk model with interaction for *Sep15* polymorphism and plasma Se concentration are presented in Fig. 1. There was a general significant association between Se concentration and lung cancer risk for all three *Sep15* genotypes. For the GG, GA and AA genotypes, the *p* values for the trends in risk were: $p = 0.038$, 0.035, and 0.030, respectively (Fig. 1). Statistically significant differences were also found in the trends between AA and GG genotypes ($p = 0.049$) and between AA and GA genotypes ($p = 0.025$; Fig. 1). For Se concentration close to the population average (53 ng/ml), lung cancer risk was similar for all indi-

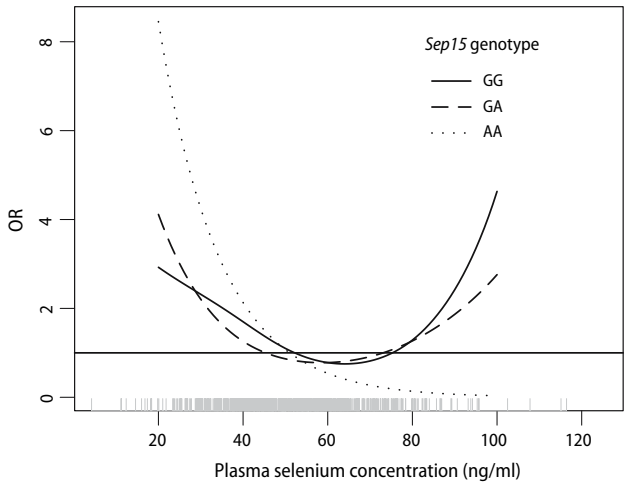

Fig. 1 Joint effect of plasma selenium concentration and *Sep15* 1125G/A polymorphism for lung cancer development. Test for trends in *Sep15* genotypes: $p = 0.038$ for GG, $p = 0.035$ for GA, $p = 0.030$ for AA. Test for trend differences: AA vs. GG: $p = 0.049$, AA vs. GA: $p = 0.025$

viduals, but for lower Se concentrations, the risk in the group with AA genotype was increasing more sharply than in those with the other two genotypes (GG and GA). Above this concentration, the risk for GG and GA genotypes stopped decreasing and then for the concentrations higher than 80 ng/ml, it started to increase again. This trend is consistent with the results shown in Table 5 presenting association between Se concentration and lung cancer risk in the whole study population (regardless the *Sep15* genotype). In the group with the AA genotype, lung cancer risk was still decreasing even at concentrations above 50 ng/ml. This trend is supported by our data up to the concentration of 80 ng/ml (the highest concentration in this group).

We estimated an average risk for two Se concentration intervals, with the cut-off point of 50 ng/ml, as this concentration was close to the population average. Among individuals with a lower Se status (below 50 ng/ml), the risk was higher for those with the AA genotype compared to those with the GG genotype (OR = 1.42, 95% CI: 0.43–4.42; Table 6). On the contrary, among individuals with a higher Se status (above 50 ng/ml), the risk was lower for those with the AA genotype compared to those with the GG genotype (OR = 0.37, 95% CI: 0.12–1.17; Table 6).

Table 6 Lung cancer risk associated with *Sep15* polymorphism in two groups of individuals: with lower and higher plasma selenium concentration

Sep15 genotype	Cases, *n* (%)	Controls, *n* (%)	OR, 95% CI[a]	*p*
Se < 50 ng/ml	187 (100%)	117 (100%)		
GG	112 (59.9%)	70 (59.8%)	1.00	–
GA	61 (32.6%)	42 (35.9)	0.98 (0.57–1.69)	0.948
AA	14 (7.5%)	5 (4.3%)	1.48 (0.52–4.95)	0.488
Se > 50 ng/ml	139 (100%)	159 (100%)		
GG	78 (56.1%)	85 (53.5%)	1.00	–
GA	56 (40.3%)	61 (38.4%)	0.92 (0.55–1.52)	0.733
AA	5 (3.6%)	13 (8.2%)	0.38 (0.11–1.12)	0.091

OR Odds ratio, *95% CI* 95% confidence interval
[a]Adjusted for smoking index, age and sex

The smoking index, expressed as cigarettes per day multiplied by the years of smoking, was significantly higher in lung cancer cases than in the controls (765 ± 331 vs. 552 ± 354, $p < 0.0002$). Unlike the Se status, smoking did not modify the risk of lung cancer due to *Sep15* polymorphism ($p = 0.210$, results not shown).

Discussion

In our study, we investigated an association between lung cancer risk and *Sep15* polymorphism combined with Se status in the smoking individuals of Polish origin.

Both the polymorphic sites in *Sep15* (811 and 1125 within 3′-UTR) were analyzed in order to confirm the presence of two exclusive haplotypes (C^{811}/G^{1125} and T^{811}/A^{1125}) in the Polish population. As expected, genotype analysis yielded only two haplotypes: CG or TA, and this exclusiveness is consistent with the results obtained by Hu et al. [14] and the analysis of the EST database. Also the distribution of alleles was similar to that found by Hu et al. in the Caucasian population [14].

In our further analysis, we are referring only to the polymorphic site at 1125, as this polymorphism is supposed to be functionally related to 15 kDa selenoprotein. It was found that the SECIS element with A at 1125 was more efficient in stimulating Sec insertion as compared to the SECIS element containing G at 1125. However, 1125A allele was also associated with a lower response to added Se in cultured cells [20] and these findings were confirmed by other studies [2, 14]. The authors suggest that *Sep15* 1125 G/A polymorphism may affect individual response to dietary Se and that Se supplementation may be less beneficial in chemoprevention for individuals carrying allele 1125A [2, 14]. This hypothesis is partially consistent with our findings. In the context of lung cancer risk, our study confirms the association between individual response to Se and *Sep15* polymorphism. Depending on plasma Se concentration, the risk of lung cancer in the study population was significantly different in the individuals with AA genotype compared to the individuals with GG and GA genotypes. As regards individuals with AA genotype, an increasing plasma Se concentration was associated with a significantly decreased risk of lung cancer, whereas GG and GA genotypes were associated with decreased risk of lung cancer only among individuals with lower Se status.

These results suggest that the individuals carrying AA genotype, compared to individuals with GG or GA genotypes, appear to benefit most from increasing dietary Se, which is not consistent with the findings of *in vitro* studies [2, 14].

Interestingly, in individuals carrying the GG or GA genotype, the increase in plasma Se concentration above 50 ng/ml was not associated with a decreased risk of lung cancer, and what is even more interesting, the highest plasma Se concentration (above 80 ng/ml) was associated with an increase in lung cancer risk. Some human epidemiological studies show an inverse correlation between nutritional levels of Se and the incidence of lung cancer [16, 35]. We also found that among smoking individuals, the Se status was lower in lung cancer cases than in controls (49.4 ± 17.4 vs. 53.3 ± 14.0 ng/ml, $p < 0.002$; Table 5) and these results were similar to those obtained in a case–control study by Reszka et al. [33], in which both smoking and non-smoking individuals were included (49.7 ± 17.1 ng/ml in the cases vs. 54.3 ± 14.3 ng/ml in the controls, $p < 0.001$). However, a large meta-analysis of epidemiological data showed that Se might have a protective function against lung cancer only in the populations with low Se levels [40]. Also the reanalysis of clinical trial conducted by Clark et al. [6] showed that Se supplementation decreased lung cancer incidence only among individuals with low baseline Se concentration [32]. As indicated by our findings, not only the low but also the high Se status may be associated with lung cancer risk, this depending on one's genetic background. To our knowledge, this is the first epidemiological study demonstrating a significant association between elevated levels of Se and an increased risk of lung cancer. A similar association, although not significant, was observed in the prospective Nurses' Health Study conducted by Garland et al. [8] who showed an increased smoking-adjusted risk for lung cancer (RR = 4.33, 95% CI: 0.54–34.60) in the highest tertile of toenail Se concentration. The explanation for the increased risk of cancer associated with a higher Se intake, may lie in the prooxidant properties of this micronutrient. Se, when not incorporated into proteins, is present in the form of different Se compounds. *In vitro* studies indicate that inorganic Se compounds exert prooxidant activity, and are able to induce apoptosis in cancer cells, which is a beneficial effect. On the other hand, some of these prooxidant compounds, when present at high concentrations, are able to cause oxidative DNA damage in normal cells and some of them also have an ability to inactivate the DNA repair processes. According to these observations, it is suggested that Se, depending on the dose and metabolic activity, may be carcinogenic [23]. Some authors postulate that an increased intake of Se may promote tumour formation in a certain genetic background. Therefore, it is very important to distinguish between individuals who may benefit from Se supplementation and those who may not, before the supplementation begins [27]. Our results fully support this hypothesis.

Among individuals with lower plasma Se concentration, those with AA genotype seem to have a higher risk for lung cancer, compared to the individuals carrying GG genotype (OR = 1.48; Table 6). The difference was not statistically significant, which may have been due to the too small number of individuals with both the polymorphic variants 1125A in this group (14 cases vs. 5 controls). Hu et al. [14] found that 1125A allele was four times as frequent among the African Americans as among the Caucasian population. Interestingly, African Americans present a higher prevalence and mortality from lung cancer than do the Whites [1] and this observation was also made among cigarette smokers of different ethnicity [12]. Notably, serum Se concentration was shown to be lower in the Blacks than in the Whites [36]. Although our results were not statistically significant and concerned only smoking individuals, an assumption that people with low Se status who carry *Sep15* 1125AA genotype are more susceptible to developing lung cancer would contribute to explaining the racial disparity. It may also partially account for the gender differences which we found in our study in relation to SCC: the women were at a higher risk of developing this type of tumour. It is notable that the risk associated with the *Sep15* 1125AA genotype was increased for females (OR = 1.78 CI: 0.20–16.20; Table 3) whereas it was decreased for males (OR = 0.22 CI: 0.03–1.71; Table 3). The difference in the risk was not statistically significant; however, it could be due to the relatively small number of female cases. Interestingly, an association between *Sep15* and cancer in females was found by Nasr et al. [25] who analyzed microsatellite markers for *Sep15* and found allelic loss at this gene locus in breast cancer. We may assume that *Sep15* polymorphism may be a stronger risk factor for women than for men. It is also possible that women who possess *Sep15* 1125AA genotype are more prone to developing lung cancer than the men with the same *Sep15* genotype, because women have a lower Se status. However, the gender difference in Se concentration was not statistically significant in our study population, possibly due to, again, the small number of female cases. Therefore, studies involving a larger female population would be necessary to investigate the above.

Generally, the association between Sep15 and lung cancer merits further investigation. It was shown that the number of different selenoproteins is expressed in human lung cancer cells [5] but Sep15 seems to be particularly important. As indicated by *in vivo* stud-

ies, this protein is one of the selenoproteins preferentially synthesized in the lung under selenium deficiency [4]. Interestingly, the study conducted by Apostolou et al. [2] showed that Sep15 was downregulated in malignant mesothelioma cells that were isolated from the patients' tumour [2]. Most of these cells displayed loss of heterozygosity (LOH) at 1p31, genetic locus for *Sep15*. However, LOH did not correlate with Sep15 downregulation. Some authors investigate Sep15 as a tumour suppressor gene referring to the fact that 1p31 is often deleted in human cancer development. LOH at 1p31 in lung cancer was detected by different authors but the frequency of regional loss varied from 53 to 28% and 27 to 14% [9, 24, 29, 30]. It should, however, be remembered that there are more genes mapped to 1p31 that have been implicated in human lung cancer as tumour suppressor genes [17].

As indicated by our study, the genetic polymorphism of Sep15 may modulate the influence of dietary Se on cancer development. Further studies are necessary in order to clarify this novel interaction between gene and nutrition in human lung cancer. The limitation of the present study is the lack of data concerning the use of Se supplements among the cases and controls. It should also be noted that our findings concern only the smoking individuals and smoking as a very strong risk factor for lung cancer may have influence on the modifying effect of *Sep15* polymorphism. Therefore, a larger population should be investigated in order to analyze the differences between smoking and non-smoking individuals as well as to elucidate the possible gender differences in susceptibility to lung cancer in relation to *Sep15* polymorphism. The controversial finding of an increased risk of lung cancer in association with the high Se status should be interpreted with great caution and needs further research.

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References

1. Abidoye O, Ferguson MK, Salgia R (2007) Lung carcinoma in African Americans. Nat Clin Pract Oncol 4:118–129
2. Apostolou S, Klein JO, Mitsuuchi Y, Shetler JN, Poulikakos PI, Jhanwar SC, Kruger WD, Testa JR (2004) Growth inhibition and induction of apoptosis in mesothelioma cells by selenium and dependence on selenoprotein SEP15 genotype. Oncogene 23:5032–5040
3. Bockmühl U, Schwendel A, Dietel M, Petersen I (1996) Distinct patterns of chromosomal alterations in high- and low-grade head and neck squamous cell carcinoma. Cancer Res 56:5325–5329
4. Bukalis K, Alber D, Bukalis G, Behne D, Kyriakopoulos A (2007) Effects of selenium diet on expression of selenoproteins in the lung of the rat. Ann N Y Acad Sci 1095:467–472
5. Bukalis K, Wolf C, Behne D, Kyriakopoulos A (2007) Studies on the selenoproteome in the cultured cells of lung and trachea by gel electrophoretic techniques. J Chromatogr A 1155:180–186
6. Clark LC, Combs GF Jr, Turnbull BW, Slate EH, Chalker DK, Chow J, Davis LS, Glover RA, Graham GF, Gross EG, Krongrad A, Lesher JL Jr, Park HK, Sanders BB Jr, Smith CL, Taylor JR (1996) Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. JAMA 276:1957–1963
7. Ferguson AD, Labunskyy VM, Fomenko DE, Araç D, Chelliah Y, Amezcua CA, Rizo J, Gladyshev VN, Deisenhofer J (2006) NMR structures of the selenoproteins Sep15 and SelM reveal redox activity of a new thioredoxin-like family. J Biol Chem 281:3536–3543
8. Garland M, Morris JS, Stampfer MJ, Colditz GA, Spate VL, Baskett CK, Rosner B, Speizer FE, Willett WC, Hunter DJ (1995) Prospective study of toenail selenium levels and cancer among women. J Natl Cancer Inst 87:497–505
9. Girard L, Zochbauer-Muller S, Virmani AK, Gazdar AF, Minna JD (2000) Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non-small cell lung cancer, and loci clustering. Cancer Res 60:4894–4906
10. Gladyshev VN, Jeang KT, Wootton JC, Hatfield DL (1998) A new human selenium-containing protein. Purification, characterization, and cDNA sequence. J Biol Chem 273:8910–8915
11. Gromadzinska J, Wasowicz W, Rydzynski K, Szeszenia-Dabrowska N (2003) Oxidative-stress markers in blood of lung cancer patients occupationally exposed to carcinogens. Biol Trace Elem Res 91:203–215
12. Haiman CA, Stram DO, Wilkens LR, Pike MC, Kolonel LN, Henderson BE, Le Marchand L (2006) Ethnic and racial differences in the smoking-related risk of lung cancer. N Engl J Med 354:333–342
13. Hatfield DL, Gladyshev VN (2002) How selenium has altered our understanding of the genetic code. Mol Cell Biol 22:3565–3576
14. Hu YJ, Korotkov KV, Mehta R, Hatfield DL, Rotimi CN, Luke A, Prewitt TE, Cooper RS, Stock W, Vokes EE, Dolan ME, Gladyshev VN, Diamond AM (2001) Distribution and functional consequences of nucleotide polymorphisms in the 3′-untranslated region of the human Sep15 gene. Cancer Res 61:2307–2310
15. Kalcklosch M, Kyriakopoulos A, Hammel C, Behne D (1995) A new selenoprotein found in the glandular epithelial cells of the rat prostate. Biochem Biophys Res Commun 217:162–170
16. Knekt P, Marniemi J, Teppo L, Heliovaara M, Aromaa A (1998) Is low selenium status a risk factor for lung cancer? Am J Epidemiol 148:975–982

17. Kohno T, Yokota J (1999) How many tumor suppressor genes are involved in human lung carcinogenesis? Carcinogenesis 20:1403–1410
18. Korotkov KV, Kumaraswamy E, Zhou Y, Hatfield DL, Gladyshev VN (2001) Association between the 15-kDa selenoprotein and UDP-glucose:glycoprotein glucosyltransferase in the endoplasmic reticulum of mammalian cells. J Biol Chem 276:15330–15336
19. Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN (2003) Characterization of mammalian selenoproteomes. Science 300:1439–1443
20. Kumaraswamy E, Malykh A, Korotkov KV, Kozyavkin S, Hu Y, Kwon SY, Moustafa ME, Carlson BA, Berry MJ, Lee BJ, Hatfield DL, Diamond AM, Gladyshev VN (2000) Structure–expression relationships of the 15-kDa selenoprotein gene. Possible role of the protein in cancer etiology. J Biol Chem 275:35540–35547
21. Kyriakopoulos A, Bukalis K, Roethlein D, Hoppe B, Graebert A, Behne D (2004) Prevention against oxidative stress of eukaryotic cell membranes by selenium compounds of the rat. Ann N Y Acad Sci 1030:458–461
22. Labunskyy VM, Ferguson AD, Fomenko DE, Chelliah Y, Hatfield DL, Gladyshev VN (2005) A novel cysteine-rich domain of Sep15 mediates the interaction with UDP-glucose:glycoprotein glucosyltransferase. J Biol Chem 280:37839–37845
23. Letavayová L, Vlcková V, Brozmanová J (2006) Selenium: from cancer prevention to DNA damage. Toxicology 227:1–14
24. Michelland S, Gazzeri S, Brambilla E, Robert-Nicoud M (1999) Comparison of chromosomal imbalances in neuroendocrine and non-small-cell lung carcinomas. Cancer Genet Cytogenet 114:22–30
25. Nasr MA, Hu YJ, Diamond AM (2004) Allelic loss at the Sep15 locus in breast cancer. Cancer Ther 1:293–298
26. Neve J, Chamart S, Molle L (1987) Optimization of a direct procedure for the determination of selenium in plasma and erythrocytes using Zeeman effect atomic absorption spectroscopy. In: Brätter P, Schramel P (eds) Trace Elem-Anal Chem Med Biol., vol 2. Walter de Gruyter, Berlin, pp 349–358
27. Novoselov SV, Calvisi DF, Labunskyy VM, Factor VM, Carlson BA, Fomenko DE, Moustafa ME, Hatfield DL, Gladyshev VN (2005) Selenoprotein deficiency and high levels of selenium compounds can effectively inhibit hepatocarcinogenesis in transgenic mice. Oncogene 24:8003–8011
28. Patterson EL, Milstrey R, Stokstad ELR (1957) Effect of selenium in preventing exudative diathesis in chicks. Proc Soc Exp Biol Med 95:617
29. Petersen I, Bujard M, Petersen S, Wolf G, Goeze A, Schwendel A, Langreck H, Gellert K, Reichel M, Just K, du Manoir S, Cremer T, Dietel M, Ried T (1997) Patterns of chromosomal imbalances in adenocarcinoma and squamous cell carcinoma of the lung. Cancer Res 57:2331–2335
30. Ragnarsson G, Eiriksdottir G, Johannsdottir JT, Jonasson JG, Egilsson V, Ingvarsson S (1999) Loss of heterozygosity at chromosome 1p in different solid human tumours: association with survival. Br J Cancer 79:1468–1474
31. Rayman MP (2005) Selenium in cancer prevention: a review of the evidence and mechanism of action. Proc Nutr Soc 64:527–542
32. Reid ME, Duffield-Lillico AJ, Garland L, Turnbull BW, Clark LC, Marshall JR (2002) Selenium supplementation and lung cancer incidence: an update of the nutritional prevention of cancer trial. Cancer Epidemiol Biomarkers Prev 11:1285–1291
33. Reszka E, Wasowicz W, Gromadzinska J, Winnicka R, Szymczak W (2005) Evaluation of selenium, zinc and copper levels related to GST genetic polymorphism in lung cancer patients. Biol Trace Elem Res 22:33–36
34. Schwarz K, Foltz CM (1957) Selenium as an integral part of factor 3 against dietary liver degeneration. J Am Chem Soc 79:3292
35. van den Brandt PA, Goldbohm RA, van 't Veer P, Bode P, Dorant E, Hermus RJ, Sturmans F (1993) A prospective cohort study on selenium status and the risk of lung cancer. Cancer Res 53:4860–4865
36. Vogt TM, Ziegler RG, Patterson BH, Graubard BI (2007) Racial differences in serum selenium concentration: analysis of US population data from the Third National Health and Nutrition Examination Survey. Am J Epidemiol 166:280–288
37. Whanger PD (2004) Selenium and its relationship to cancer: an update. Br J Nutr 91:11–28
38. Wu HJ, Lin C, Zha YY, Yang JG, Zhang MC, Zhang XY, Liang X, Fu M, Wu M (2003) Redox reactions of Sep15 and its relationship with tumor development. Ai Zheng 22:119–122
39. Yu SY, Zhu YJ, Li WG (1997) Protective role of selenium against hepatitis B virus and primary liver cancer in Qidong. Biol Trace Elem Res 56:117–124
40. Zhuo H, Smith AH, Steinmaus C (2004) Selenium and lung cancer: a quantitative analysis of heterogeneity in the current epidemiological literature. Cancer Epidemiol Biomarkers Prev 13:771–778