



The *CASPASE-8* Insertion/Deletion Polymorphism and Risk of Non-small Cell Lung Cancer

Shanbeh Zienolddiny^{1,*}, Kine Martinsen¹, Vidar Skaug¹, Per Olav Ekstrøm² and Aage Haugen¹

¹Department of Chemical and Biological Working Environment, Section of Toxicology, National Institute of Occupational Health, N-0033 Oslo, Norway.

²Department of Surgical Oncology, The Norwegian Radium Hospital, Montebello, Oslo, Norway.

***Corresponding author**

Shan Zienolddiny

Department of Chemical and Biological Working Environment

Section of Toxicology

National Institute of Occupational Health

N-0033 Oslo, Norway

Tel.: +4723195100; fax +4723195203

E-mail: shan.zienolddiny@stami.no

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Abstract

The cysteine-dependent aspartate-specific protease 8 (CASP8) is a key enzyme in the apoptosis and genetic polymorphisms in this gene have been reported to affect the gene expression and its enzymatic activity. A six-nucleotide insertion-deletion polymorphism (-652 6N ins/del, rs3834129) in the *CASP8* promoter has been shown to be associated with susceptibility to multiple cancers in the Asian populations. Here we report results of genotyping the *CASP8* ins/del polymorphism in non-small cell lung cancer (NSCLC) cases and controls from Norway, all of which are of Caucasian origin. The overall odds ratio for NSCLC was 1.40 (95% CI, 0.95-2.06). When subjects were grouped according to gender, males carrying *del/del* genotype had an increased odds ratio for developing NSCLC with an OR of 1.61 (95% CI, 1.02-2.53, $P=0.039$). Somatic mutations of the *TP53* gene in tumors were also analyzed and the results showed no interaction between alterations in *TP53* and the *CASP8* polymorphism. These results contradict a protective effect of this polymorphism on lung cancer as it has been reported in the Asian populations. The results are discussed in relation to population and exposure specific effects of this polymorphism.

Keywords: *CASP8*; lung cancer; NSCLC; polymorphism; *TP53*.

1. Introduction

Smoking, exposure to occupational and environmental factors are the major causes of lung

cancer, but genetic factors may play a role [1]. The cysteine-dependent aspartate-specific proteases (CASPs) are important in apoptotic cell signaling [2]. *CASP8* is a key enzyme in this

pathway and functional polymorphisms in the gene have been reported to affect gene expression and enzyme activity.

Another and very important apoptotic signaling pathway is controlled by the *TP53* gene. *TP53* encodes a tumor suppressor protein which is activated to prevent carcinogenic effects of DNA damage caused by various DNA damaging agents and carcinogens [3]. Upon activation, TP53 induces apoptosis, cell cycle arrest and promotes DNA repair. The importance of this protein is underlined by the fact the *TP53* gene is mutated in more than half of NSCLC tumors and mutation of the gene is considered to be an early event in lung carcinogenesis [4].

A six-nucleotide insertion-deletion polymorphism (-652 6N *ins/del*, rs3834129) in the *CASP8* gene promoter was recently described and reported to be associated with susceptibility to multiple cancers [5]. The *del* variant abolishes an Sp1 transcription factor binding site and was shown to be associated with decreased RNA levels, lower Caspase-8 enzyme activity and lower apoptotic activity in T lymphocytes. Further, this variant was found to be associated with almost 25% reduced risk (per copy) of lung cancer in a Han Chinese population. The authors also reported a protective effect for the *del* variant in breast cancer in this Chinese population [5].

Most of the studies on the effects of *CASP8* polymorphisms in Caucasian populations have not found evidence for *CASP8* variants to be genetic susceptibility markers for cancer. An analysis of 6N *ins/del* polymorphism in breast, colorectal and prostate cancer cases and controls did not show any influence of the *del* variant in these cancer types in several Caucasian populations [6-8].

The *CASP8* gene is important in regulation of the immune system through controlling the apoptosis of inflammatory T lymphocytes. We and others have previously shown that certain functional polymorphisms in the immune system genes coding for cytokines may predispose individuals to lung cancer [9,10]. To examine the hypothesis whether the *ins/del* polymorphism in *CASP8* is a susceptibility marker for lung cancer, we conducted a case-control study among nonsmall cell lung cancer (NSCLC) cases and

controls from Norway, all of which are smokers of Caucasian origin.

2. Materials and methods

The details of the ongoing lung cancer study (Table 1) and population have been recently published [11]. Briefly, lung cancer patients were admitted for surgery at the university hospitals in Oslo or Bergen between 1986 and 2001. Diagnosis of lung cancer was confirmed by two pathologists and NSCLC cases were enrolled in the study consecutively whenever practically feasible. Controls were recruited from a general health survey conducted by the National Health Surveys in the Oslo area (HUBRO) of the general population. The purpose of the surveys was to monitor the health status of the general population. A total of 440 smokers without any known history of cancer were randomly selected and frequency matched with the cases on age, smoking dose (pack-years) and sex. Cases and controls were interviewed by trained health personnel using questionnaires containing comparable information on demographic and lifestyle details. All subjects gave written consent to participate in the study which was approved by the Regional Ethical Committee.

DNA was extracted from whole blood or normal lung tissue with standard proteinase K digestion and phenol/chloroform extraction. The *CASP8* 6bp -652 *ins/del* was genotyped by capillary fragment length analysis as described elsewhere [12]. A representative picture showing fragment analysis of three subjects with *ins/del*, *del/del* and *ins/ins* genotypes, respectively, is shown in Figure 1. There was 99% genotyping success rate in both cases and controls and no deviation from Hardy-Weinberg equilibrium was observed ($P > 0.05$). A 10% blinded replicate samples were re-genotyped with a concordance rate of 100%. There were 272 lung tumor specimens available for analysis of mutations in exons 4-9 of the *TP53* gene. Mutational analysis was performed as described in a recent publication from our group [13].

Differences in demographic variables, smoking and grouped genotypic frequencies between cases and controls were evaluated by χ^2

test and reported *P* values are two-sided with *P* <0.05 considered as significant. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated from unconditional logistic regression

analyses using the SPSS (version 15.0) with age, sex and pack-years as covariates using *ins/ins* as reference.

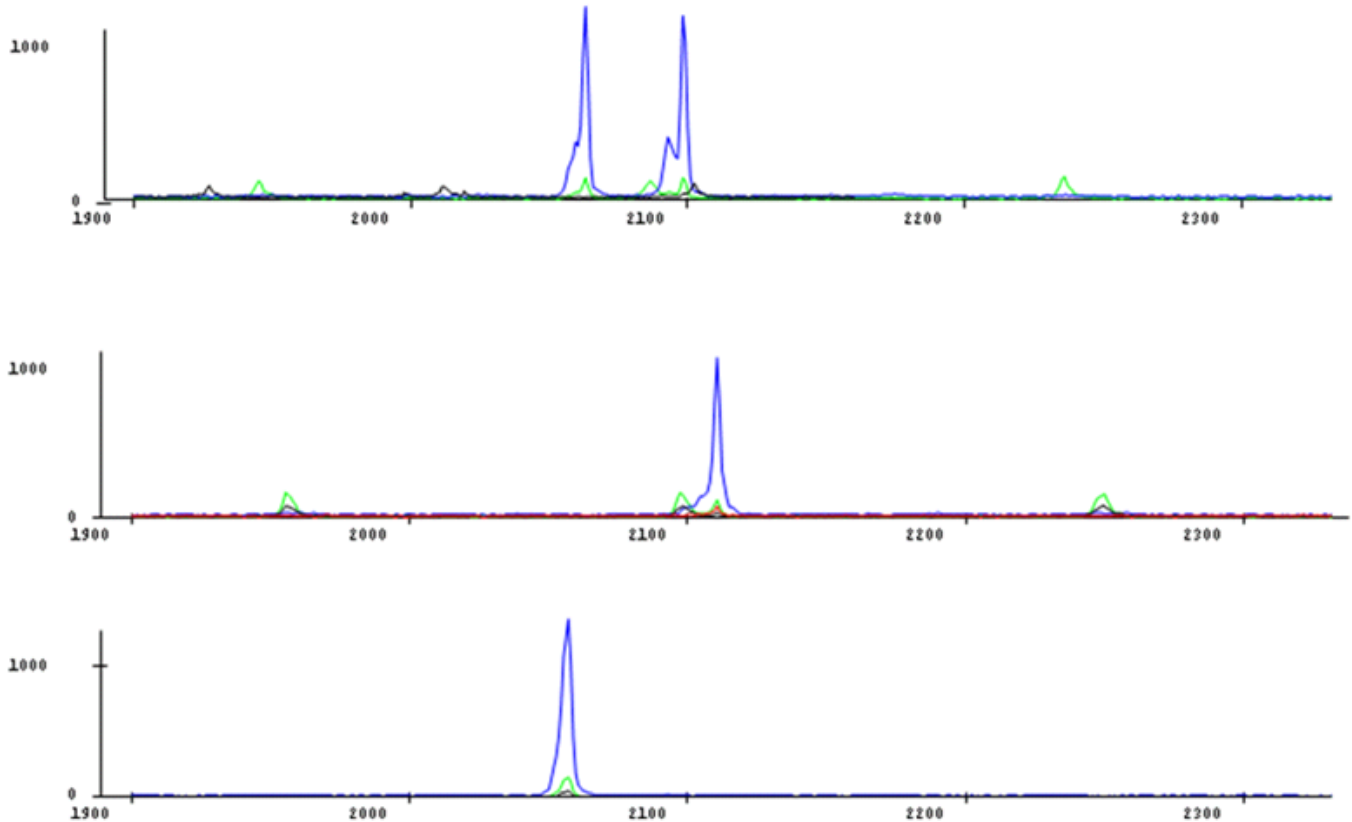


Figure 1. Representative flurogram from *CASP8* genotyping showing *ins/del* heterozygote (top); *del/del* homozygote (middle) and *ins/ins* homozygote (bottom).

3. Results

The observed frequency of *del* variant was 0.47 in controls and 0.51 in cases whereas the frequency of *ins* allele was 0.53 in controls and 0.49 in cases, respectively (Table 2) and are in agreement with frequencies in other Caucasian populations [6,7]. Male subjects with homozygote *del/del* genotype had an increased risk of NSCLC with an odds ratio of 1.61 (95% CI, 1.02-2.53, *P* = 0.039) after adjustment for age and smoking (Table 2). The OR for male subjects carrying at least one deletion allele (*ins/del* + *del/del*) was 1.43 (95% CI, 1.0-2.06, *P* = 0.05). There was no association between *CASP8* genotypes and

NSCLC histological subtypes of adenocarcinoma, squamous cell carcinoma and large cell carcinoma (Table 2). The risk was neither modified by smoking dose, light smokers (≤ 20 pack-years), moderate smokers ($>20 \leq 40$ pack-years) and heavy smokers (>40 pack-years) nor smoking status (current/former smokers, data not shown).

TP53 mutational data for 272 lung tumors were analyzed. Lung cancer cases were grouped into two groups either having a *TP53* mutation in the tumor or *TP53* wild-type and the risk associated with the *CASP8* genotypes was compared with healthy controls. The results showed no significant interaction between *TP53*,

CASP8 ins/del polymorphism and risk of NSCLC
(Table 2).

Table 1. Characteristics of lung cancer patients and healthy controls

Parameter	Cases N = 442	Controls N = 440	P
Median age (min – max)	66 (31 – 85)	60 (50 – 83)	
Sex (male/female)	325/116	335/105	0.40*
Number of cigarettes per day			
Mean ±SD	15.52 ± 8.21	14.59 ± 6.29	
Median (min.-max)	14.0 (2 – 60)	15.00 (3 – 40)	0.368†
Total smoking years			
Mean ±SD	41.07 ± 12.03	42.30 ± 8.45	
Median (min.-max.)	42.00 (2 – 69)	41.00 (15 – 65)	0.501†
Total pack-years			
Mean ± SD	31.41 ± 17.58	31.18 ± 15.07	
Median (min – max)	28.50 (1 – 113)	28.50 (5 – 84)	0.521†
TP53 status	n = 272 (100%)		
Mutated	151 (55.5%)		
Wild-type	121 (44.5%)		
Histological details	n = 442		
Adenocarcinoma	159		
Squamous cell carcinoma	192		
Large cell carcinoma	71		
NSCLC (not classified otherwise)	20		

*The two-sided P value was calculated from a 2x2 table using Pearson chi-square test.

†The two-sided P values were obtained from Wilcoxon’s non-parametric test for two independent groups.

Table 2. Frequency of *CASP8* polymorphism in NSCLC cases and controls

Gene/polymorphism	Controls/cases*	†Odds ratio (95% CI)‡
<i>CASP8</i> (-652 ins/del, rs3834129)		
<i>ins/ins</i>	125/107	1.0
<i>ins/del</i>	210/212	1.22 (0.87-1.70)
<i>del/del</i>	101/118	1.40 (0.95-2.06)
<i>ins/del+del/del</i>	311/330	1.28 (0.93-1.75)
Males		
<i>ins/ins</i>	104/80	1.0
<i>ins/del</i>	159/159	1.36 (0.92-1.99)
<i>del/del</i>	69/80	1.61 (1.02-2.53) P=0.039
<i>ins/del+del/del</i>	228/239	1.43 (1.0-2.06) P=0.050
Females		
<i>ins/ins</i>	32/27	1.0
<i>ins/del</i>	51/51	0.71 (0.34-1.48)
<i>del/del</i>	21/38	0.73 (0.33-1.62)
<i>ins/del+del/del</i>	72/89	0.72 (0.36-1.43)
<i>TP53</i> mutated		
<i>ins/ins</i>	125/35	1.0
<i>ins/del</i>	210/80	1.31 (0.82-2.09)
<i>del/del</i>	101/33	1.18 (0.68-2.06)
<i>ins/del+del/del</i>	311/113	1.27 (0.82-1.97)
<i>TP53</i> wildtype		
<i>ins/ins</i>	125/23	1.0
<i>ins/del</i>	210/49	1.17 (0.66-2.06)
<i>del/del</i>	101/40	1.89 (1.03-3.46)
<i>ins/del+del/del</i>	311/89	1.40 (0.83-2.38)
Adenocarcinoma		
<i>ins/ins</i>	125/38	1.0
<i>ins/del</i>	210/77	1.29 (0.79-2.09)
<i>del/del</i>	101/43	1.46 (0.84-2.51)
<i>ins/del+del/del</i>	311/115	1.34 (0.85-2.12)
Squamous cell		
<i>ins/ins</i>	125/45	1.0
<i>ins/del</i>	210/93	1.18 (0.75-1.86)
<i>del/del</i>	101/51	1.48 (0.88-2.50)
<i>ins/del+del/del</i>	311/144	1.28 (0.83-1.95)
Large cell		
<i>ins/ins</i>	125/21	1.0
<i>ins/del</i>	210/31	0.90 (0.49-1.65)
<i>del/del</i>	101/18	0.92 (0.45-1.90)
<i>ins/del+del/del</i>	311/49	0.91 (0.51-1.60)

*Four controls and five cases failed to yield genotype results.

†Odds ratios were calculated in an unconditional logistic regression model using the common genotype as reference (1.0). All ORs were adjusted for age, sex and smoking (pack-years). *P* values (two-sided) were obtained from the same logistic regression by comparing frequency of heterozygotes or variant homozygotes with common homozygotes. CI, ‡95% confidence interval.

4. Discussion

There was no main effect of the *CASP8 ins/del* polymorphism on NSCLC but male subjects with homozygote *del/del* genotype had an increased risk of NSCLC. This is in contrast to the proposed protective effect of the *del* variant in lung cancer patients from the Chinese population [5]. Whether these sex differences are attributed to the inheritance of the *del* allele or differences in level of exposure to tobacco carcinogens is not known since men and women have different smoking habits. In our study population the cumulative smoking dose in terms of total life time pack-years was significantly higher in male cases and controls compared to female cases and controls (data not shown). Furthermore, sex differences in occupational exposure to carcinogens and life style factors such as alcohol consumption and diet may also affect the risk. Some studies suggest gender differences in lung cancer susceptibility being higher for females [14] whereas others do not support such a hypothesis [15,16].

Another apoptotic pathway is regulated by the *TP53* tumor suppressor gene and TP53-deficient cells are resistant to apoptosis. There are variable reports on the effects of genetic variants of *TP53* gene in relation to lung cancer susceptibility [17]. Furthermore, the interaction between *CASP8* and *TP53* genetic alterations has not been investigated. While somatic mutations of *CASP* genes are rare in lung tumors [18], *TP53* mutations are common and occur early during lung tumorigenesis [19]. The lack of any effect of the *CASP8* polymorphism in a tumor genetic background where *TP53* was somatically mutated could suggest a lack of interaction between TP53 alterations and *CASP8 ins/del* polymorphism.

The differential effects of this polymorphism in conferring susceptibility to lung cancer between the Asian and the European Caucasian populations could be due to several reasons. Firstly, genetic factors may vary between the Asian and the Caucasian populations as it is evident from several studies that the frequency of the *del* variant is almost two-fold higher in Caucasians [6,7]. Secondly, it is possible that exposure to the environmental factors such as

indoor pollutions, infectious agents and life style factors may be different in the two populations [20]. Thirdly, the associations observed in the Chinese population may be unique to this study population and may not be replicated in other populations.

With the sample size of 882 individuals we had more than 80% power to detect an OR of 1.5 assuming co-dominant and recessive models, a two-sided test using $\alpha = 0.05$ and a variant allele frequency of 0.47 observed in our population. This study included only subjects of Norwegian origin and the Norwegian population has a homogeneous European ancestry. It is therefore unlikely that the lack of any significant association is attributable to bias or population stratification.

In conclusion, our results complement the results obtained from colon, breast and prostate cancer studies in various European populations indicating no role for *CASP8* variants as cancer susceptibility markers in Caucasian populations.

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Conflict of interest statement

We declare that no conflict of interest exists for any of the authors.

References

1. Schwartz, AG.; Prysak, GM.; Bock, CH.; Cote, ML. The molecular epidemiology of lung cancer. *Carcinogenesis* 2007, 28, 507-518.
2. Salvesen, GS.; Riedl, SJ. Caspase mechanisms. *Adv Exp Med Biol* 2008, 615,13-23.
3. Hussain, SP. and Harris, CC. p53 biological network: at the crossroads of the cellular-stress response pathway and molecular carcinogenesis. *J Nippon Med Sch* 2006, 73,54-64.

4. Pfeifer, GP.; Denissenko, MF.; Olivier, M.; Tretyakova, N.; Hecht, SS.; Hainaut, P. Tobacco smoke carcinogens; DNA damage and p53 mutations in smoking-associated cancers. *Oncogene* 2002, 21,7435-7451.
5. Sun, T.; Gao, Y.; Tan, W.; Ma, S.; Shi, Y.; Yao, J.; Guo, Y.; Yang, M.; Zhang, X.; Zhang, Q.; Zeng, C.; Lin, D. A six-nucleotide insertion-deletion polymorphism in the CASP8 promoter is associated with susceptibility to multiple cancers. *Nat Genet* 2007, 39,605-613.
6. Haiman, CA.; Garcia, RR.; Kolonel, LN.; Henderson, BE.; Wu, AH.; Le, ML. A promoter polymorphism in the CASP8 gene is not associated with cancer risk. *Nat Genet* 2008, 40,259-260.
7. Pittman, AM.; Broderick, P.; Sullivan, K.; Fielding, S.; Webb, E.; Penegar, S.; Tomlinson, I.; Houlston, R.S. CASP8 variants D302H and -652 6N ins/del do not influence the risk of colorectal cancer in the United Kingdom population. *Br J Cancer* 2008, 98,1434-1436.
8. Frank, B.; Rigas, SH.; Bermejo, J.L.; Wiestler, M.; Wagner, K.; Hemminki, K.; Reed, M.W.; Sutter, C.; Wappenschmidt, B.; Balasubramanian, S.P.; Meindl, A.; Kiechle, M.; Bugert, P.; Schmutzler, R.K.; Bartram, C.R.; Justenhoven, C.; Ko, Y.D.; Bruning, T.; Brauch, H.; Hamann, U.; Pharoah, P.P.; Dunning, A.M.; Pooley, K.A.; Easton, D.F.; Cox, A.; Burwinkel, B. The CASP8 -652 6N del promoter polymorphism and breast cancer risk: a multicenter study. *Breast Cancer Res Treat* 2008,,111,139-144.
9. Zienolddiny, S.; Ryberg, D.; Maggini, V.; Skaug, V.; Canzian, F.; Haugen, A. Polymorphisms of the interleukin-1 beta gene are associated with increased risk of non-small cell lung cancer. *Int J Cancer* 2004,109,353-356.
10. Engels, E.A.; Wu, X.; Gu, J.; Dong, Q.; Liu, J.; Spitz, M.R. Systematic evaluation of genetic variants in the inflammation pathway and risk of lung cancer. *Cancer Res* 2007, 67,6520-6527.
11. Zienolddiny, S.; Campa, D.; Lind, H.; Ryberg, D.; Skaug, V.; Stangeland, L.B.; Canzian, F.; Haugen, A. A comprehensive analysis of phase I and phase II metabolism gene polymorphisms and risk of non-small cell lung cancer in smokers. *Carcinogenesis* 2008, 29,1164-1169.
12. Ekstrom, P.O.; Khrapko, K.; Li-Sucholeiki, X.C.; Hunter, I.W.; Thilly, W.G. Analysis of mutational spectra by denaturing capillary electrophoresis. *Nat Protoc* 2008, 3,1153-1166.
13. Lind, H.; Ekstrom, P.O.; Ryberg, D.; Skaug, V.; Andreassen, T.; Stangeland, L.; Haugen, A.; Zienolddiny, S. Frequency of TP53 mutations in relation to Arg72Pro genotypes in non small cell lung cancer. *Cancer Epidemiol Biomarkers Prev* 2007, 16,2077-2081.
14. Mollerup, S.; Ryberg, D.; Hewer, A.; Phillips, D.H.; Haugen, A. Sex differences in lung CYP1A1 expression and DNA adduct levels among lung cancer patients. *Cancer Res* 1999,,59,3317-3320.
15. Cook, M.B.; Dawsey, S.M.; Freedman, N.D.; Inskip, P.D.; Wichner, S.M.; Quraishi, S.M.; Devesa, S.S.; McGlynn, K.A. Sex disparities in cancer incidence by period and age. *Cancer Epidemiol Biomarkers Prev* 2009, 18,1174-1182.
16. Freedman, N.D.; Leitzmann, M.F.; Hollenbeck, A.R.; Schatzkin, A.; Abnet C.C. Cigarette smoking and subsequent risk of lung cancer in men and women: analysis of a prospective cohort study. *Lancet Oncol* 2008, 9,649-656.
17. Whibley, C.; Pharoah, P.D.; Hollstein, M. p53 polymorphisms: cancer implications. *Nat Rev Cancer* 2009, 9,95-107.
18. Shivapurkar, N.; Toyooka, S.; Eby, M.T.; Huang, C.X.; Sathyanarayana, U.G.; Cunningham, H.T.; Reddy, J.L.; Brambilla, E.; Takahashi, T.; Minna, J.D.; Chaudhary, P.M.; Gazdar, A.F. Differential inactivation of caspase-8 in lung cancers. *Cancer Biol Ther* 2002, 1,65-69.
19. Shimmyo, T.; Okada, A.; Hashimoto, T.; Kobayashi, Y.; Miyagi, Y.; Ishikawa, Y.; Nakagawa, K.; Osada, H.; Tsuchiya, E. Etiologic value of p53 mutation spectra and

differences with histology in lung cancers. *Cancer Sci* 2008, 99,287-295.

20. Zhang, J.J. and Smith, K.R. Household air pollution from coal and biomass fuels in

China: measurements; health impacts; and interventions. *Environ Health Perspect* 2007, 115,848-855.