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Polymorphisms of DNA repair genes and risk of non-small cell lung cancer

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Lung cancer is a leading cause of cancer mortality with an inter-individual difference in susceptibility to the disease. The inheritance of low-efficiency genotypes involved in DNA repair and replication may contribute to the difference in susceptibility. We investigated 44 single nucleotide polymorphisms (SNPs) in 20 DNA repair genes including nucleotide excision repair (NER) genes *XPA*, *ERCC1*, *ERCC2/XPD*, *ERCC4/XPF* and *ERCC5/XPG;* base excision repair (BER) genes *APE1/APEX*, *OGG1*, *MPG*, *XRCC1*, *PCNA*, *POLB*, *POLι*, *LIG3 and EXO1;* double-strand break repair (DSB-R) genes *XRCC2*, *XRCC3*, *XRCC9*, *NBS1* and *ATR;* and direct damage reversal (DR) gene *MGMT/AGT*. The study included 343 non-small cell lung cancer (NSCLC) cases and 413 controls from Norwegian general population. Our results indicate that SNPs in the NER genes *ERCC1* (Asn118Asn, 15310G>C, 8902G>T), *XPA* (-4G>A), *ERCC2/XPD* (Lys751Gln) and *ERCC5/XPD* (His46His); the BER genes *APE1/APEX* (Ile64Val), *OGG1* (Ser326Cys), *PCNA* (1876A>G) and *XRCC1* (Arg194Trp, Arg280His, Arg399Gln); and the DSB-R genes *ATR* (Thr211Met), *NBS1* (Glu185Gln), *XRCC2* (Arg188His) and *XRCC9* (Thr297Ile) modulate NSCLC risk. The level of polycyclic aromatic hydrocarbon–DNA (PAH–DNA) adducts in normal lung tissue from 211 patients was analysed. The variant alleles of *XRCC1*(Arg280His), *XRCC1* (Arg399Gln), *ERCC1*(G8092T), *ERCC5*(His46His) and *MGMT/AGT*(Lys178Arg) were more frequent in patients with PAH–DNA adduct levels lower than the mean whereas the *XRCC1*(Arg194Trp) variant was more frequent in cases with higher adduct levels than the mean.

Introduction

Smoking, along with occupational exposure, is major cause of lung cancer. The relative risk for lung cancer in current smokers is up to 20-fold higher than never smokers (1). However, only a fraction of cigarette smokers develop lung cancer suggesting inter-individual differences in susceptibility. It has been hypothesized that these differences may be due to genetic variations in DNA repair (2).

There are ~50–60 known carcinogens in cigarette smoke (3). Many of these compounds are converted into reactive metabolites by Phase I cytochrome P450 (CYP450) metabolic enzymes (4). If not inactivated, the reactive molecules may bind to cellular DNA and form adducts. However, genomic integrity can still be restored through DNA repair mechanisms.

Smoking-related bulky DNA adducts are mainly repaired by the nucleotide excision repair (NER) pathway (5). The NER pathway consists of ~30 proteins involved in DNA damage recognition, incision, DNA ligation and resynthesis (6). The NER pathway consists of several genes termed Xeroderma Pigmentosum (XP) or excision repair cross complementing (ERCC) where *XPA*, *ERCC1*, *ERCC2/XPD*, *ERCC4/XPF* and *ERCC5/XPG* are central (7).

Oxidized bases such as 7,8-dihydro-8-oxoguanine (8-oxo-G), apurinic/apyrimidinic site (AP-site) and single-strand breaks, which are strongly pro-mutagenic, are repaired by the base excision repair (BER) pathway (8). 8-oxo-G is excised from DNA by the 8-oxo-guanine glycosylase-1 (OGG1) protein leaving an AP-site which is then acted upon by the AP-endonuclease-1 (APEX/APE1) enzyme (9). BER also involves X-ray repair cross complementation-1 (*XRCC1*), methylpurine glycosylase (*MPG*), polymerase B (*POLB*), ligase-3 (*LIG3*), exonuclease-1 (*EXO1*) and proliferating cell nuclear antigen (*PCNA*) genes (10).

O^6-Methylguanine (O^6-meG) is formed in DNA by alkylating compounds present in tobacco smoke and it may mispair with thymine during replication. O^6-meG and other alkylated bases are repaired by O^6-meG–DNA methyltransferase (MGMT/AGT) in human cells (11).

Repair of DNA double-strand breaks (DSB) involves homologous and non-homologous recombinational repair pathways. These pathways include several proteins such as RAD51, ataxia telangiectasia-mutated (ATM), ATM- and Rad3-related (ATR) and X-ray repair cross complementing (XRCC), which are important for maintenance of genomic stability (12).

Lung cancer patients have been found to have lower DNA repair capacity compared with healthy individuals (13). Molecular epidemiology studies have demonstrated that the variant DNA repair genotypes may alter susceptibility to lung

Abbreviations: AP-site, apurinic/apyrymidinic site; APE1, AP-endonuclease-1; APEX, arrayed primer extension; AT, ataxia telangiectasia; ATM, ataxia telangiectasia-mutated; ATR, ATM- and Rad3-related; BER, base excision repair; CYP450, cytochrome P450; DSB-R, double-strand break repair; DR, direct damage reversal; ERCC, excision repair cross complementing; EXO1, exonuclease-1; LIG3, ligase-3; MPG, methylpurine glycosylase; NBS, nijmegen breakage syndrome; NER, nucleotide excision repair; NSCLC, non-small cell lung cancer; O^6-meG, O^6-Methylguanine; OGG1, 8-oxo-guanine glycosylase-1; 8-ox-G, 7,8-dihydro-8-oxoguanine; PAH, polycyclic aromatic hydrocarbon; PCNA, proliferating cell nuclear antigen; POLB, polymerase B; SNP, single nucleotide polymorphism; XP, xeroderma pigmentosum; XRCC, X-ray repair cross complementing.

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cancer (14,15). Most studies have evaluated a small number of single nucleotide polymorphisms (SNPs) in a few DNA repair genes in lung cancer. We have extended these studies by genotyping 44 SNPs in 20 genes involved in NER, BER, DSB-R and DR pathways in a single moderate sized study. In addition, we have determined the PAH–DNA adduct levels in normal lung tissue of 211 lung cancer patients and have analysed the frequency of variant genotypes in relation to adduct levels.

Materials and methods

Study subjects

The cases ($n = 343$) are Caucasians born in Norway and of Norwegian origin. The cases were newly diagnosed lung cancer patients admitted for surgical treatment of their lung tumours at the surgery departments at University hospitals in Oslo and Bergen between 1986 and 2001. The diagnosis of non-small cell lung cancer (NSCLC) was confirmed by qualified pathological reviews of all the histological slides and cases with cancer history other than lung neoplasms being excluded from the study. NSCLC patients were recruited consecutively whenever practically feasible.

The controls were randomly selected from individuals of Norwegian origin who took part in the general health survey conducted by the health authorities in Oslo to evaluate the health status of the general population. Controls ($n = 413$) were recruited from 8100 individuals from the general population in the age cohorts 59/60 and 75/76 participating in the Oslo Health Survey 2000–2001 (HUBRO) arranged by the National Health Screening Service. About 4100 healthy individuals participated in this project and contributed blood samples. The controls in this study were selected from this group based on the following criteria: (i) if they were $\geq$59 years of age; (ii) had smoked >5 cigarettes per day; and (iii) were current smokers or quit smoking for <5 years. About 950 individuals met these criteria, from which 413 were randomly selected as controls in this study. Cases and controls were matched on age, smoking and male/female ratio. The controls were healthy individuals without any known history of cancer.

Cases and controls were personally interviewed by a trained health personnel using questionnaires to record demographic and lifestyle characteristics. Information on smoking, age and sex was used for the purposes of the present study. Both cases and controls gave written consent to participate in the study and to allow their biological samples to be genetically analysed. Approval for the study was given by the Regional Ethical Committee.

DNA extraction

DNA was extracted from whole blood samples or normal lung tissue with standard proteinase K digestion, phenol/chloroform extraction and ethanol precipitation.

Genotyping

A total of 44 SNPs in 20 genes related to different DNA repair pathways were chosen. The selection was based on the criteria of being related to tobacco-induced cancer types, having a frequency $\geq$5% in Caucasians, having proven or inferred biological activity, being a newly discovered SNP or being the only SNP in the gene. The relevant data on genes and SNPs are shown in Table VII available at http://www.stami.no/metabochip/DNArepair/.

Genomic DNA was amplified to enrich the fragments carrying the SNPs by using specific primer pairs shown in Table VII, as described previously (16,17). Genotyping was performed by Arrayed Primer Extension (APEX). APEX consists of a sequencing reaction primed by an oligonucleotide anchored with its 5′ end to a glass slide and terminating just one nucleotide before the polymorphic site. A DNA polymerase extends the oligonucleotide by adding one fluorescently labelled dideoxy-nucleotide (ddNTP) complementary to the variant base. Reading the incorporated fluorescence identifies the base in the target sequence. This method is suitable not only for SNPs but also for small insertion/deletion polymorphisms. Since both sense and anti-sense strands are sequenced, two oligonucleotides were designed for each polymorphism. In general, two 30mers, one for each strand, complementary to each side of the polymorphism were designed both with their 3′ ends pointing towards the polymorphism. 5′-(C-12) aminolinker oligonucleotides were synthesized by Sigma Genosys (Cambridge, UK) and spotted onto silanized slides as reported previously (18,17). PCR products were pooled, purified, concentrated using Millipore Microcon MY30 columns and fragmented as reported in detail previously (19). For single-base extension reaction, fragmented PCR products were incubated onto the slides together with the fluorescently labelled ddNTPs (4×50 pmol), 10× buffer and 4 U of Thermo Sequenase (Amersham Biosciences, Uppsala, Sweden), as reported previously (19). Slides were imaged by a Genorama-003 four-color detector equipped with the Genorama image analysis software (Asper Biotech, Tartu, Estonia). Fluorescence intensities at each position were converted automatically into base calls by the software, under the supervision of an operator. In the case of more than one signal being present on a given position, only the main signal was considered when the intensity of the weaker signal was <10% of the main signal.

Genotyping of SNPs in *ATR*, *XPA*, *NBS1*, *EXO1* and *POLI* genes were performed using TaqMan genotyping assays provided by Applied Biosystems (Foster City, USA). The Assays-On-Demand or Assays-by-Design genotyping kits were used as instructed by the manufacturer supplemented with 10 ng genomic DNA in 384-well plates. The TaqMan assay numbers, primer and probe sequences are shown in Table VII, available at http://www.stami.no/metabochip/DNArepair/. For both genotyping assays, the number of successfully genotyped cases and controls for different SNPs varies and may be affected by the sequence context surrounding each SNP. However, both TaqMan and APEX genotyping assays have thoroughly been validated and published previously (16,19). The SNPs were in Hardy–Weinberg equilibrium and the quality control of genotyping was determined by reanalysis of genotyping of the subjects independently by two persons.

Determination of PAH–DNA adducts

PAH–DNA adduct levels were determined in normal lung tissue by ^{32}P-postlabelling as described previously (20,21). Cases were divided into two groups on the basis of having greater than, or less than, the mean number of DNA adducts/10^8 nt for all cases. The frequency of genotypes were determined for each genotype.

Statistical analysis

Differences in demographic variables, smoking and grouped genotypic frequencies between the cases and control subjects were evaluated by using the χ^2-test. All reported P-values are two-sided with $P < 0.05$ considered as significant. Hardy–Weinberg equilibrium was tested by the χ^2-test as well, in controls and cases for each polymorphism. The association between the variant genotypes and risk of lung cancer was estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from unconditional logistic regression analyses using the SPSS (version 11.5) statistical package. Age, sex and smoking (pack-years) were used as the covariates. For polymorphisms, homozygosity for the more frequent allele among controls was set as the reference group. All reported odds ratios have been adjusted for age, sex and smoking (pack-years). Cumulative cigarette dose (pack-years) was calculated by the following formula: pack-years = [(pack/day) × (years smoked)]. Cases and controls were stratified on the basis of average number of the pack-years less than and equal to median of pack-years in controls ($\leq$29 pack-years) or greater than the median (>29 pack-years). The odds ratios associated with the variant genotypes were calculated in each subgroup.

Results

This study explored 44 SNPs in 20 genes related to DNA damage and repair mechanisms including NER, BER, DSB-R and DR in association with NSCLC risk in a Norwegian population-based case–control study. The relevant characteristics of the study subjects are shown in Table I. Cases and controls were similar in terms of gender distribution and smoking habits and all were smokers. Prior to genotyping, the SNPs previously reported to modify risk of tobacco-related cancers were identified in the genes from four DNA repair pathways using the published literature and available public databases. The frequencies and distribution of the genotypes and corresponding ORs for the association of each polymorphism with NSCLC risk are reported in Tables II–V.

For the NER pathway, the *ERCC1* (Asn118Asn, C > T), *ERCC1* (C15310G) and *ERCC2* (Lys751Gln) variants increased the risk of NSCLC, whereas the variant genotypes of *XPA*, *G23A* and *ERCC5/XPG* (His46His) were associated with a significant reduction of the risk of lung cancer (Table II). The other tested SNPs in the *ERCC1*, *ERCC2* and neither of the two polymorphisms in the *ERCC4/XPF* gene modified the risk of NSCLC.

For the BER pathway, the *OGG1* (Ser326Cys) and *PCNA* (A1876G) polymorphisms were associated with increased risk

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Table I. Characteristics of lung cancer patients and healthy controls

Parameter	Lung cancer patients ($n = 343$)	Healthy controls ($n = 413$)	P-values
Median age (min–max)	65 (31–85)	60 (50–85)	
Male/female	260/83	316/97	0.9[a]
Smoking habits			
Mean cigarettes per day ± SD	15.6 ± 8.3	14.8 ± 6.3	0.6[b]
Mean smoking years ± SD l	40.4 ± 12.1	42.3 ± 7.9	0.17[b]
Mean pack-years ± SD	31.1 ± 17.7	31.6 ± 15.1	0.16[b]
Current smokers (n)[c]	188	295	
Median pack-years	27	29	
PAH–DNA adducts	$n = 211$		
Mean (±SD)[d]	12.06 ± 8.51		

n, number of subjects; SD, standard deviation.
[a]χ^2-test.
[b]Non-parametric Wilcoxon's test for two independent samples.
[c]Stopped smoking ≤2 years.
[d]Number of bulky/hydrophobic DNA adducts/10^8 nucleotides.

of NSCLC (Table III). However, when heterozygous and homozygous variant genotypes of PCNA1876 were grouped together the difference was not statistically significant, indicating a recessive effect of the variant allele. The variant genotype in *APE1*/*APEX* (Ile64Val) was associated with a decreased risk. The variant T allele of PCNA2352 SNP had a marginal effect on cancer risk ($P = 0.05$). Several SNPs in the *APEX*/*APE1, XRCC1, PCNA, POLB, POLI* and *EXO1* genes were not associated with altered risk of lung cancer. The frequency of the variant alleles of *APEX*/APE1 (Gly241Arg), *MPG* (Arg59Cys) polymorphisms and the three SNPs in the *LIG3* gene were too low to calculate reliable odds ratios (Table III).

For the DSB-R pathway, the variant allele of the DSB gene *XRCC2* (Arg188His) was associated with significantly increased risk of NSCLC (Table IV). The variant allele of the *XRCC9* (Thr297Ile) and *ATR* (Thr211Met) genes may play a protective role and were associated with a significantly decreased risk of developing NSCLC (Table IV). Distribution of the *XRCC3* polymorphism Thr241Met and the *NBS1* Glu185Gln polymorphism was not statistically different in cases and controls whereas the variant alleles of the *XRCC9*/*FANCG*, Val/464Phe and Pro330Ser SNPs were rare in the Norwegian population.

From the DR pathway, four polymorphisms in the *MGMT*/*AGT* gene were genotyped (Table V). Only subjects carrying variant genotypes of *MGMT*/*AGT* (Leu84Phe) in exon 3 tended to have a marginal higher risk of developing NSCLC ($P_{\text{trend}} = 0.08$).

The mean level of bulky/hydrophobic DNA adducts in normal lung tissue of 211 patients was 12 adducts/10^8 nt of DNA. The frequency of genotypes for the two patient groups, with >12 adducts/10^8 nt and ≤12 adducts/10^8 nt, were estimated and compared (Table VI). The variant alleles of *XRCC1*(Arg280His), *XRCC1*(Arg399Gln), *ERCC1*(G8092T), *ERCC5*(His46His) and *MGMT*(Lys178Arg) were overrepresented in cases with ≤12 adducts whereas the variant *XRCC1*(Arg194Trp) was more frequent in cases with >12 adducts (Table VI). We found no significant association between PAH–DNA adduct levels and SNPs in any of the DSB-R genes (data not shown).

The subjects were dichotomized into two groups based on the median pack-years of smoking (cumulative smoking dose)

Table II. Polymorphisms in the NER genes and NSCLC risk

Gene/polymorphism	Controls[a]	Cases[a]	OR[b] (95% CI)
XPA (G23A, −4)			
G/G	114	130	1.0
G/A	125	88	0.61 (0.42–0.89)
A/A	37	30	0.67 (0.39–1.14)
G/A+A/A	162	118	0.63 (0.44–0.89)
ERCC1			
19716C>G (intron 3)			
C/C	173	130	1.0
C/G	183	146	1.06 (0.78–1.47)
G/G	45	43	1.27 (0.78–2.04)
Asn118Asn (exon 4)			
C/C	60	46	1.0
C/T	109	118	1.45 (0.90–2.33)
T/T	44	96	3.11 (1.82–5.30)
C/T+T/T	153	214	1.91 (1.23–2.98)
17677C>A (intron 5)			
C/C	291	252	1.0
A/C	95	71	0.86 (0.60–1.22)
A/A	16	11	0.81 (0.37–1.79)
15310C>G (intron 6)			
C/C	271	210	1.0
C/G	100	102	1.34 (1.0–1.88)
G/G	10	9	1.17 (0.47–2.93)
C/G+G/G	110	111	1.30 (1.0–1.79)
8092G>T (3′ UTR)			
G/G	213	169	1.0
G/T	155	131	0.74 (0.37–1.5)
T/T	17	20	0.71 (0.35–1.4)
ERCC2/*XPD*			
His201Tyr (exon 8)			
His/His	328	272	1.0
His/Tyr	77	66	1.14 (0.77–1.60)
Tyr/Tyr	-	1	ND
Asp312Asn (exon 10)			
Asp/Asp	120	119	1.0
Asp/Asn	121	102	0.83 (0.58–1.21)
Asn/Asn	49	54	1.09 (0.68–1.73)
Lys751Gln (exon 23)			
Lys/Lys	183	127	1.0
Lys/Gln	121	101	1.16 (0.82–1.65)
Gln/Gln	82	89	1.60 (1.10–2.30)
ERCC4/*XPF*			
Pro379Ser (exon 7)			
Pro/Pro	404	336	1.0
Pro/Ser	5	1	ND
Arg415Gln (exon 8)			
Arg/Arg	178	195	1.0
Arg/Gln	21	26	1.11 (0.59–2.07)
Gln/Gln	1	3	ND
ERCC5/*XPG*			
His46His (exon 2)			
C/C	138	137	1.0
C/T	126	119	0.87 (0.68–1.37)
T/T	109	60	0.56 (0.38–0.84)
C/T+T/T	235	179	0.78 (0.57–1.06)

ND, not determined.
[a]Number of subjects. Numbers may not add up to the totals of cases and controls due to genotyping failure.
[b]ORs were adjusted for age, sex and cumulative smoking dose (pack-years).

in control group, which was 29 pack-years. The frequency of genotypes for each SNP was then determined in cases and controls who smoked ≤29 pack-years or in cases and controls who smoked >29 pack-years and associated odds ratios were calculated for each SNP. The *OGG1* Ser326Cys was associated with increased risk in subjects who smoked ≤29

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Table III. Polymorphisms in the BER genes and NSCLC risk

Gene/polymorphism	Controls[a]	Cases[a]	ORs[b] (95% CI)
APE1/APEX			
Ile64Val (exon 3)			
Ile/Ile	276	263	1.0
Ile/Val	124	76	0.64 (0.45–0.89)
Val/Val	10	1	0.10 (0.01–0.81)
Ile/Val + Val/Val	134	77	0.60 (0.43–0.83)
Asp148Glu (exon 5)			
Asp/Asp	138	117	1.0
Asp/Glu	60	67	1.24 (0.80–1.91)
Glu/Glu	122	80	0.78 (0.53–1.14)
Asp/Glu + Glu/Glu	182	147	0.95 (0.68–1.32)
Gly241Arg (exon 5)			
Gly/Gly	354	274	1.0
Gly/Arg	3	1	ND
Gln51His (exon 3)			
Gln/Gln	300	279	1.0
Gln/His	24	25	1.12 (0.62–2.02)
His/His	10	8	0.86 (0.33–2.22)
OGG1			
Ser326Cys (exon 7)			
Ser/Ser	194	182	1.0
Ser/Cys	117	100	1.45 (0.90–2.33)
Cys/Cys	75	44	1.64 (1.06–2.52)
XRCC1			
Arg194Trp (exon 6)			
Arg/Arg	368	309	1.0
Arg/Trp	35	26	0.87 (0.51–1.49)
Trp/Trp	2	1	ND
Arg280His (exon 9)			
Arg/Arg	350	296	1.0
Arg/His	24	31	1.54 (0.88–2.68)
His/His	3	2	ND
Arg399Gln (exon 10)			
Arg/Arg	151	129	1.0
Arg/Gln	186	171	1.06 (0.77–1.46)
Gln/Gln	54	31	0.67 (0.40–1.10)
MPG			
Arg59Cys (exon 2)			
Arg/Arg	360	307	
Arg/Cys	-	1	ND
Cys/Cys	-	1	ND
PCNA			
1876A>G (intron 1)			
A/A	262	209	1.0
A/G	121	115	1.19 (0.87–1.64)
G/G	6	15	3.19 (1.21–8.40)
G/G + A/G	127	130	1.29 (0.95–1.75)
1661C>G (intron 1)			
C/C	138	122	1.0
C/G	40	43	1.15 (0.69–1.90)
G/G	54	31	0.64 (0.38–1.08)
1684C>A (intron 1)			
C/C	233	183	1.0
C/A	85	64	0.96 (0.66–1.40)
A/A	13	14	1.38 (0.63–3.0)
2232C>T (intron 1)			
C/C	149	137	1.0
C/T	191	131	0.73 (0.52–1.0)
T/T	55	59	1.15 (0.74–1.79)
2352C>T (intron 1)			
C/C	215	172	1.0
C/T	32	13	0.52 (0.26–1.04)
T/T	10	6	0.76 (0.27–2.16)
C/T+T/T	42	19	0.58 (0.32–1.04)
POLB			
Pro242Arg (exon 12)			
Pro/Pro	192	190	1.0
Pro/Arg	4	7	1.75 (0.50–6.09)
Arg/Arg	2	7	3.45 (0.70–16.92)
Pro/Arg+Arg/Arg	6	14	2.31 (0.87–6.18)
POLI			
−78G>T			
G/G	61	93	1.0
G/T	105	153	0.94 (0.62–1.43)
T/T	37	59	1.03 (0.61–1.75)
Thr706Ala (exon 10)			
Thr/Thr	196	157	1.0
Thr/Ala	141	119	1.04 (0.75–1.44)
Ala/Ala	29	23	1.02 (0.56–1.84)
LIG3			
Pro899Ser (exon 21)			
Pro/Pro	374	335	ND
Lys811Thr (exon 18)			
Lys/Lys	394	319	
Lys/Thr	1	2	ND
Thr/Thr	-	1	ND
Arg780His (exon 17)			
Arg/Arg	395	319	
Arg/His	2	2	ND
EXO1			
Glu589Lys (exon 10)			
Glu/Glu	116	115	1.0
Glu/Lys	145	106	0.62 (0.35–1.08)
Lys/Lys	30	35	0.85 (0.48–1.48)

ND, not determined.
[a]Number of subjects. Numbers may not add up to the totals of cases and controls due to genotyping failure.
[b]ORs were adjusted for age, sex and cumulative smoking dose (pack-years).

pack-years (OR 2.04, CI, 1.11–3.75) whereas *ERCC1* 15310 (OR, 1.65, CI, 1.04–2.64) and *ERCC2* Lys751Glu (OR, 2.0, CI, 1.15–3.41) in subjects who smoked >29 pack-years. The presence of variant alleles of the NER genes *XPA* G23A (OR, 0.53, CI, 0.32–0.89) and *NBS1* Glu185Gln (OR, 0.41, CI, 0.17–0.97) were associated with a statistically significant reduced risk in subjects who had smoked >29 pack-years while *ERCC5/XPG* His46His (OR, 0.46, CI, 0.26–0.80) was associated with decreased risk in subjects who had smoked ≤29 pack-years.

Discussion

The DNA repair pathways NER, BER, DSB-R and DR repair specific types of lesions, and individuals with a suboptimal repair capacity may have higher susceptibility to lung cancer (2,22).

We found that two polymorphisms in the *ERCC1* gene were associated with increased risk of lung cancer. These two *ERCC1* polymorphisms have not previously been evaluated in NSCLC. The silent Asn118Asn (C>T transition) has been associated with differential mRNA levels and mRNA levels of *ERCC1* may correlate with DNA repair capacity (23,24). The intron 6 (15310 C>G) polymorphism has not been described to be associated with any disease. The variant allele of G8092T SNP was more likely to be found in cases having a higher mean level of PAH–DNA adducts. This polymorphism has been associated with increased risk of brain tumours and the base change has been hypothesized to affect the mRNA stability (25). Increased lung cancer risk associated with *XPD* Lys751Gln polymorphism supports studies on other tobacco-related cancers (26,27,28). Popanda *et al*. have recently

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Table IV. Polymorphisms in DSB genes and NSCLC risk

Gene/polymorphism	Controls[a]	Cases[a]	ORs[b] (95% CI)
XRCC2			
Arg188His (exon 3)			
Arg/Arg	246	203	1.0
Arg/His	45	102	2.77 (1.85–4.13)
His/His	2	7	4.0 (0.81–19.63)
Arg/His+His/His	47	109	2.82 (1.91–4.18)
XRCC3			
Thr241Met (exon 5)			
Thr/Thr	115	114	1.0
Thr/Met	111	90	0.83 (0.56–1.23)
Met/Met	24	16	0.61 (0.30–1.24)
XRCC9/FANCG			
Val464Phe (exon 9)			
Val/Val	161	187	ND
Val/Phe	2	2	
Pro330Ser (exon 7)			
Pro/Pro	386	330	ND
Pro/Ser	2	2	
Thr297Ile (exon 7)			
Thr/Thr	394	339	1.0
Thr/Ile	10	1	0.11 (0.01–0.91)
NBS1			
Glu185Gln (exon 5)			
Glu/Glu	162	126	1.0
Glu/Gln	165	155	1.16 (0.84–1.60)
Gln/Gln	49	29	0.74 (0.44–1.25)
ATR			
Thr211Met (exon 4)			
Thr/Thr	81	79	1.0
Thr/Met	152	90	0.63 (0.41–0.95)
Met/Met	64	42	0.63 (0.41–1.14)
Thr/Met+Met/Met	216	132	0.64 (0.44–0.95)

ND, not determined.
[a]Number of subjects. Numbers may not add up to the totals of cases and controls due to genotyping failure.
[b]ORs were adjusted for age, sex and cumulative smoking dose (pack-years).

Table V. Polymorphisms in the *MGMT/AGT* gene and NSCLC risk

Gene/polymorphism	Controls[a]	Cases[a]	ORs[b](95% CI)
MGMT/AGT			
Leu84Phe (exon 3)			
Leu/Leu	247	189	1.0
Leu/Phe	106	102	1.26 (0.90–1.77)
Phe/Phe	10	13	1.66 (0.71–3.89)
Leu/Phe + Phe/Phe	116	115	1.30 (0.94–1.79)
Ile143Val (exon 4)			
Ile/Ile	290	242	1.0
Ile/Val	99	80	0.97 (0.69–1.369
Val/Val	15	9	0.72 (0.31–1.68)
Leu53Leu (exon 3)			
C/C	324	259	1.0
C/T	78	71	1.13 (0.78–1.62)
T/T	5	3	0.75 (0.17–3.18)
Lys178Arg (exon 4)			
Lys/Lys	82	74	1.0
Lys/Arg	87	99	1.08 (0.64-1.82)
Arg/Arg	43	46	0.86 (0.50-1.47)

[a]Number of subjects. Numbers may not add up to the totals of cases and controls due to genotyping failure.
[b]Odds ratios were adjusted for age, sex and cumulative smoking dose (pack-years).

reported that this polymorphism in combination with *XPD*312Asn and *XPA*(−4AA) genotypes increased the risk for lung squamous cell carcinoma (29). The variant Gln allele has been associated with an increased level of PAH–DNA adducts (30), whereas another study has found a lower DNA repair proficiency among women carrying the Lys allele (31,32). Furthermore, presence of homozygous Gln751Gln was associated with a reduced DNA repair capacity in healthy individuals (33,34). The −4G allele of the *XPA* gene was associated with a reduced risk which was consistent with some previous studies (35,29). The −4G allele has been shown to be associated with a higher DNA repair capacity (36). The XPA protein is part of damage-recognition XPC–RPA protein complexes and plays a central role in damage recognition. The homozygote *ERCC5/XPG* (His46His) variant was associated with a significantly decreased risk of lung cancer (Table II). This polymorphism has not been evaluated in relation to lung cancer previously but another polymorphism (His1104Asp) has been associated with reduced risk of squamous cell carcinoma (37,38). The association between *ERCC5/XPG* polymorphisms and the risk of lung cancer is biologically plausible since XPG protein plays an important role in NER. When the subjects were dichotomized into two groups based on the median pack-years of smoking, the protective effect of the T/T genotype against lung cancer was significant in the group with ≤29 pack-years. In relation to PAH–DNA adducts, *ERCC1*(G8092T) and *ERCC5*(His46His) variant alleles were less frequent in the cases with higher adduct levels. The functions of these polymorphisms have not been measured and it might be speculated that cases with these alleles may have suboptimal DNA repair capacity.

A total of 21 SNPs in 9 BER genes were evaluated (Table III). We found that some SNPs in *OGG1*, *APE1/APEX* and *PCNA* genes were associated with lung cancer risk. The OGG1-Cys326 protein has been shown to have a lower ability than the OGG1-Ser326 protein to repair 8-oxo-G in an *Escherichia coli* strain that is defective in 8-oxo-G repair (39). These results have also been confirmed in human lung cancer cells (40). It has also been shown that OGG1-Cys326 has a slightly lower glycosylase activity *in vitro* (41). The AP-site created by the OGG1 activity is processed by the APE1 protein. The Val 64 variant of the APE1 protein had a protective effect (Table III). This polymorphism has not been previously evaluated in lung cancer. Similarly, there are no functional data on how it may affect the biological activity of the APE1 protein. Functional studies on Asp148Glu polymorphism of the *APE1* have shown that the Glu variant may have altered endonuclease and DNA binding activity and reduced ability to communicate with other BER proteins (42). Similar to our results, another study found a lack of association of this polymorphism with lung cancer risk (15). We found interactions between *XRCC1* SNPs and PAH–DNA adduct levels. The variant allele of the *XRCC1*Arg194Trp was most likely to be found in lung cancer cases with DNA adduct levels higher than the mean ($P = 0.015$) whereas there was a significant overrepresentation of the variant alleles of Arg280His ($P = 0.037$) and Arg399Gln ($P = 0.031$), respectively, in cases with ≤12 adducts (Table VI). Mutagen sensitivity assays have shown that individuals with the wild-type Arg194Arg genotype had significantly higher values of DNA damage compared with individuals with one or two variant Trp alleles, whereas individuals homozygous for the Gln399Gln genotype had higher DNA damage (43). Although the main pathway for removal of bulky DNA adducts is NER,

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Table VI. Distribution of genotypes in relation to PAH–DNA adducts in NSCLC cases

Gene/polymorphism	Number of cases (%) with >12 adducts ≤12[b] adducts	*P*-value[a]
XRCC1		
Arg194Trp		
Arg/Arg	72 (86.7)/118 (95.9)	Reference
Arg/Trp	11 (13.3)/5 (4.1)	0.015
Arg280His		
Arg/Arg	75 (97.4)/110 (89.4)	Reference
Arg/His	2 (2.6)/13 (10.6)	0.037
Arg399Gln		
Arg/Arg	42 (51.9)/46 (37.1)	Reference
Arg/Gln	36 (44.4)/65 (52.4)	0.09
Gln/Gln	3 (3.7)/13(10.5)	0.031
ERCC1		
8092G>T		
G/G	46 (57.5)/48 (41.0)	Reference
G/T	30 (37.5)/60 (51.3)	0.031
T/T	4 (5.0)/9(7.7)	0.023
G/T+T/T	34 (42.5)/69 (59.0)	0.023
ERCC5/XPG		
His46His		
C/C	41 (53.9)/38 (33.0)	Reference
C/T	21 (27.6)/57 (49.6)	0.001
T/T	14 (18.5)/20 (17.4)	0.29
C/T+T/T	35 (46.0)/77 (67.0)	0.004
MGMT/AGT		
Lys178Arg		
Lys/Lys	26 (52.0)/26 (36.0)	Reference
Lys/Arg	18 (36.0)/28 (38.9)	0.28
Arg/Arg	6 (12.0)/18 (25.1)	0.04

[a]*P*-values were calculated using the chi-square test comparing frequency of the cases in each subgroup carrying the variant genotypes with cases carrying the common genotype.
[b]Number of PAH–DNA adducts/10^8 nucleotides.

it has been shown that BER mechanisms may also participate in adduct repair, supporting the association of *XRCC1* SNPs with PAH–DNA adduct levels (44). A recent study found a higher risk of breast cancer associated with a combination of *XRCC1* 399Gln allele and the level of PAH–DNA adducts (45). A significant association ($P = 0.03$) was found with the *PCNA* 1876G/G genotype and lung cancer risk. *In vitro* experiments have found promoter-like regulatory elements that may act as promoter for anti-sense *PCNA* mRNA transcripts that may have repressing effects on the expression of *PCNA* (46,47). PCNA is active in many biological pathways such as DNA replication, DNA repair, cell cycle control and apoptosis, and polymorphisms may affect one or several of these functions (48). The *EXO1* (Glu589Lys) polymorphism has not been previously evaluated in relation to lung cancer. In our study a marginal reduced risk of NSCLC ($P = 0.07$) was found in heterozygotes yet not significant in homozygotes. Two other polymorphisms (Thr439Met, Pro757Leu) in the *EXO1* have been shown to affect the risk of colorectal cancer (49). Furthermore, our results could not confirm an association between lung cancer risk and *POLI* polymorphisms as recently reported by Sukiyama *et al.* (38). The functional importance of these common polymorphisms in translesion bypass of bulky DNA adducts has not been investigated.

An almost 3-fold increased risk of lung cancer was found for the *XRCC2* Arg188His heterozygote carriers as well as the combined heterozygote and homozygote group compared with subjects with the Arg188Arg genotype (Table III). The role of *XRCC2* (Arg188His) polymorphism has not been studied in NSCLC and there are conflicting results on the association of this polymorphism with other cancer types (50,51). The His allele has been associated with increased risk of breast cancer and upper aerodigestive tract cancer (52,53). The XRCC2 protein has homology to Rad51 and is a component of homologous recombination involved in the repair of double-strand breaks. Mutation or deletion of the amino acid at this site of XRCC2 protein reduces cell survival following DNA damage (54). We also observed increased risk of NSCLC in heterozygotes and this is in agreement with recent findings in *Xrcc2* knock-out mice, where it has been shown that haploinsufficiency for *XRCC2* may affect chromosomal aberrations in *Xrcc2*$^{+/-}$ cells (55). The Thr297Ile polymorphism in exon 7 of the *XRCC9*/*FANCG* gene has not been analysed previously. There were few subjects with heterozygote and homozygote variant genotypes (Table III). When subjects with variant genotypes were combined, only 0.29% of lung cancer cases carried variant genotypes compared with 2.47% of controls and this difference was associated with a significant reduced risk of NSCLC ($P = 0.014$, Fisher's exact test). However, since the frequency of variant allele is low these data should be interpreted with caution. The XRCC9/FANC-G protein has been suggested to protect cells against many genotoxic agents (56). The *ATR* (Thr211Met) SNP was associated with a decreased risk. ATR protein has a central role in phosphorylation of DNA damage sensing proteins including ATM and TP53. The ATM/ATR targets are phosphorylated by ATR in response to several types of genotoxic stresses including, DSB, oxidative stress, hypoxia and inflammation (57,58). Interestingly, the *NBS1* (Glu185Glu) homozygotes who smoked >29 pack-years also had decreased risk of NSCLC. The NBS protein is involved in DSB repair and is found in complex with replication protein A (RPA) Mre11:Rad50, which is formed in response to DNA replication blockage (59). Smoking-related bulky adducts are known to block DNA replication and may induce the Mre11:Rad50-NBS1 complex. It has also been shown that ATR is recruited to the blocked replication forks (60).

The *MGMT* Lys178Arg polymorphism was associated with PAH–DNA adduct levels. A recent study has shown that carriers of the variant alleles of Lys178Arg and Ile143Val may be at an increased risk of adenocarcinoma or small cell lung carcinoma (61). Our results, consistent with most studies, indicate that *MGMT* polymorphisms at codons 53, 84, 143 and 178 may not markedly affect lung cancer risk.

In conclusion, we report associations between a set of genetic polymorphisms of DNA repair genes and lung cancer risk. Our findings also indicate that some DNA repair gene variants may play a role in determining the PAH–DNA adduct level in the normal lung. It should be noted that the healthy controls and most of the patients were recruited from two different areas in Norway, which may cause a potential selection bias. However, only patients with Norwegian origin are included in this study and the Norwegian population is very homogeneous. Small sample size for some SNPs may result in false positive associations and must be interpreted with caution and larger studies are warranted.

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