to a certain protease. In fact, we confirmed that because the sequence containing arginine at amino acid 110 generates a cleavage site for Factor Xa (-Asp-Gly-Arg-), the R110 type was cleaved by it, whereas the Q110 type was not (data not shown). Second, disruption of the intramolecular disulfide bonds of the 2 IL-13 proteins may cause the difference because it has been demonstrated that disruption of the intramolecular disulfide bonds of IL-13 causes it to lose its biologic activity.[49] The intramolecular conformational change induced by the substitution may influence susceptibility to oxidative components. Third, it may be due to the difference of absorption or excretion of the 2 IL-13 proteins in vivo.

In conclusion, we demonstrated here that the *IL13* variant may act as a functional genetic factor of bronchial asthma. This finding is useful not only for diagnosing a risk for susceptibility to bronchial asthma but also for working out a strategy for treating it on the basis of this study. We may be able to remodel the IL-13 protein to act as an antagonist for the IL-13R or remodel the IL-13R proteins to increase their affinities with IL-13 and generate a soluble form blocking IL-13 action with a high affinity.

We thank Drs Dovie R. Wylie and Sho Matsushita for critical review of this manuscript and for providing a human T_H2 clone, SM4.6, respectively. We also thank Drs Rie Sugimoto, Yoshito Abe, and Tomohiro Hamasaki for giving special technical comments.

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TABLE I. Association between the *IL13* genotype and serum IL-13 levels

IL-13 genotype	No. of patients (% frequency)		Median serum IL-13 levels (pg/mL)		
	Nonasthmatic subjects (n = 251)	Asthmatic subjects (n = 39)	Total	Nonasthmatic subjects	Asthmatic subjects
R110/R110	141 (56%)	14 (36%)	5.02 (3.38-9.12)	4.38 (4.12-9.98)	6.05 (3.20-10.8)
Q110/R110	80 (32%)	17 (44%)	6.51 (3.10-10.8)	4.29 (3.32-8.98)	8.81 (3.32-15.8)
Q110/Q110	30 (12%)	8 (20%)	10.2 (3.21-19.8)*	7.21 (3.33-16.8)	12.3 (3.24-21.3)*

The relationship between the *IL13* genotype and serum IL-13 level was shown. Serum IL-13 levels are presented as median (95% confidence interval).
*Significantly greater than in R110/R110 subjects ($P < .05$, median χ^2 test).

would at least partially influence the circulating level of IL-13, although we can not exclude the possibility of other polymorphisms in linkage disequilibrium, with the variant enhancing the transcription activity or mRNA stability of the *IL13* gene.

DISCUSSION

Many attempts have been made to elucidate genetic factors associated with bronchial asthma, with many genes being selected by using positional cloning and candidate gene approaches.[3,42] However, very few genes are reported to be functionally correlated with the pathogenesis of bronchial asthma. It is of great importance to investigate the functional differences of variants that correlated with the disease to exclude the possibility that any other polymorphisms in linkage disequilibrium evoke a genetic influence and to validate the results of genetic analyses. Previously, after we found that an extracellular variant of *IL4RA*, Ile50Val, is associated with atopy, we performed functional analyses, revealing that this variant upregulated IL-4 signals.[43,44] Following the genetic observations that the incidence of the *IL13* variant (Q110) was higher than the wild type (R110) in certain allergic phenotypes,[10-12] we performed functional analyses of the variant in this study.

We first demonstrated its slightly lower affinity with IL-13Rα2, leading to its lesser clearance by IL-13Rα2 (Figs 2 and 3). These results indicated that local IL-13 concentration may be augmented by the variant in vivo. Furthermore, we showed an enhanced stability of the variant in vitro and in vivo (Figs 4 and 5), which meant that on the contrary, the variant may affect the systemic concentration of IL-13 in vivo. As expected, the genotype of *IL13* was positively correlated with the serum level of IL-13 (Table I), although we still can not exclude the possibility that some other polymorphisms in linkage disequilibrium with the variant enhances the transcription activity or the mRNA stability of the *IL13* gene. It was reported that another polymorphism in the 5′ flanking region of the *IL13* gene (−1055 C/T) is associated with allergic asthma and that the variant type (−1055T) augmented binding to nuclear proteins and IL-13 production in T cells.[45,46] Although we could not detect a positive association between this variant and some allergic phenotypes (data not shown), such a polymorphism may also be involved in upregulating serum IL-13 levels in vivo. The findings in this study may suggest a unique mechanism of the variant as a genetic factor of bronchial asthma to upregulate IL-13 concentration in vivo. It is assumed that various triggers for bronchial asthma induce production of IL-13 in vivo and that Q110-bearing individuals may be more susceptible to bronchial asthma or to more severe bronchial asthma as a result of upregulation of IL-13 concentration.

The present finding, that the variant has lower affinity with IL-13Rα2, indicated that the variant would play its role as a genetic factor through IL-13Rα2–expressing cells. It is, however, poorly understood what cell types express IL-13Rα2 and how the expression of IL-13Rα1 and IL-13Rα2 is regulated in each cell. In B cells stimuli for CD40 and IgM receptor augment expression of IL-13Rα1, whereas no expression of IL-13Rα2 was detected in the presence or absence of the stimuli in B cells (data not shown).[29,47] In contrast, in BECs IL-13Rα1 is constitutively expressed and was not influenced on stimulation of IL-4 or IL-13, whereas expression of IL-13Rα2 was augmented by IL-4 and IL-13 (Yuyama N et al, unpublished data). Thus the *IL13* variant would exert its action distinct from the wild type on cells such as BECs but not B cells. This may explain that IgE secretion by the variant and the wild type was invariable (data not shown). Further analyses about the expression mechanism of IL-13Rα1 and IL-13Rα2 in each cell would address this point.

In a previous study computer modeling predicted that the substitution of the amino acid at 110 would affect the binding with IL-4Rα or IL-13Rα1.[10] It has also been recently shown that IL-13, which has aspartic acid instead of arginine at amino acid 110, acted as a potent agonist of IL-13. (In a previous article the same arginine residue was numbered 112[48] because of the difference of the prediction of the signal peptide length.) In this study we demonstrated that the variant has a lower affinity with IL-13Rα2, but not with IL-13Rα1, than the wild type. These results may suggest that amino acid 110 itself or the portion around amino acid 110 faces IL-13Rα2, but not IL-13Rα1. A planned cocrystal structure analysis should elucidate this point.

We further demonstrated that the variant is more stable than the wild type both in vitro and in vivo. There are several possible explanations for these differences. First, it may be due to the difference of degradation of the 2 IL-13 proteins in plasma. The amino acid substitution itself may change the specific recognition site of a certain protease, or the intramolecular conformational change induced by the substitution may influence susceptibility

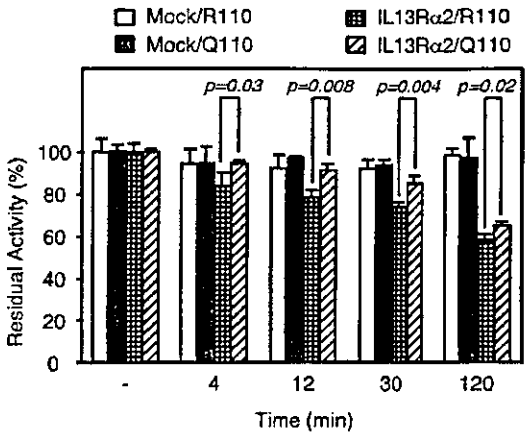

FIG 3. Clearance of R110 and Q110 by IL-13Rα2. Culture medium containing 0.5 ng/mL R110 and Q110 was preincubated with either mock-transfected (clone 1) or IL-13Rα2–transfected (clone 18) DND-39 cells for the indicated period. The removed medium was then incubated with an equal volume of IL-13Rα1–transfected DND-39 cells, and luciferase activity was measured. Fold induction was estimated, comparing the value without the preincubation. Each experiment was done with 3 samples, and the mean values are shown.

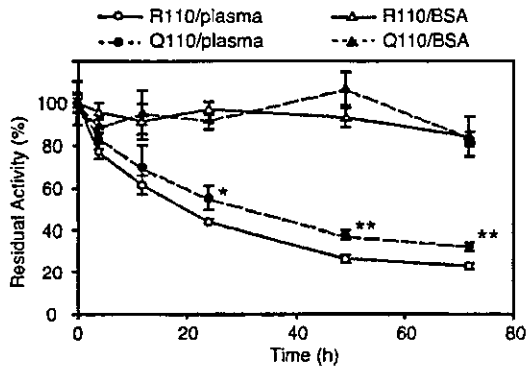

FIG 4. Stability of R110 and Q110 in human plasma. Twenty nanograms per milliliter of R110 and Q110 was incubated with either 100% human plasma or 120 mg/mL BSA for the indicated period and then incubated with IL-13Rα1–transfected DND-39 cells for the luciferase activity assay. The luciferase activities in the representative data are depicted (*solid line*, R110; *dashed line*, Q110). *Statistically significant difference at $P < .05$; **Statistically significant difference at $P < .005$.

Stability of the *IL13* variant

To test the possibility that the amino acid substitution in the variant affects stability of the protein, we first investigated it in both the presence and the absence of human plasma. When R110 and Q110 were incubated with human plasma, Iε transcription activities induced by both proteins declined in a time-dependent manner. The half-life of Q110 in plasma was estimated to be longer than that of R110 (experiment 1: 30.3 hours for Q110 and 18.9 hours for R110 [Fig 4]; experiment 2: 19.0 hours for Q110 and 13.3 hours for R110). The amount of endogenous IL-13 in human plasma was too small to be detected by the transcription activity of this system (data not shown). Substituting serum for plasma showed the same tendency (data not shown), whereas attenuation was not seen with incubation with BSA (Fig 4).

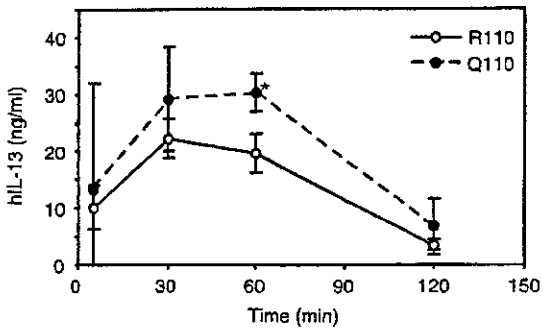

FIG 5. Stability of R110 and Q110 in mice. One microgram of R110 and Q110 was administered into BALB/c mice, and then blood samples were collected at the indicated times. The average values of IL-13 levels in plasma of 4 mice for each are depicted (*solid line*, R110; *dashed line*, Q110). *Statistically significant difference at $P < .05$.

We next analyzed the stability of IL-13 in vivo. When R110 and Q110 were administered into mice intraperitoneally, the IL-13 level in mouse plasma reached its peak 30 minutes after the injection and thereafter decreased (Fig 5). Q110 was retained to a greater degree in mouse plasma than R110 (30.3 ± 3.3 ng/mL for Q110 and 19.6 ± 3.5 ng/mL for R110 after 1 hour). We therefore hypothesize that the native structure of the IL-13 protein may be disrupted in plasma and Q110 is less susceptible to such effects of plasma or, alternatively, that Q110 may be metabolized more slowly in vivo by another mechanism.

Relationship between the genotype and serum level of IL-13

The present findings, that Q110 is less cleared by IL-13Rα2 and is more stable than R110, indicated that the variant would affect IL-13 concentrations in vivo. To explore this possibility, we investigated the relationship between the genotype and the serum level of IL-13 (Table I). The serum level of IL-13 in the Q110 homozygote tended to be higher than that in the heterozygote and the R110 homozygote in normal donors, although not statistically significant. By contrast, in asthmatic patients the serum IL-13 level was significantly higher in the Q110 homozygote than in the R110 homozygote. When the same amount of R110 and Q110 was applied to our immunoassay kit for IL-13, R110 showed a slightly higher value (1.3-fold) than Q110, and therefore the difference in serum IL-13 levels was not due to differential assay sensitivity for R110 and Q110. These results suggested that the different clearance rates, stability, or both

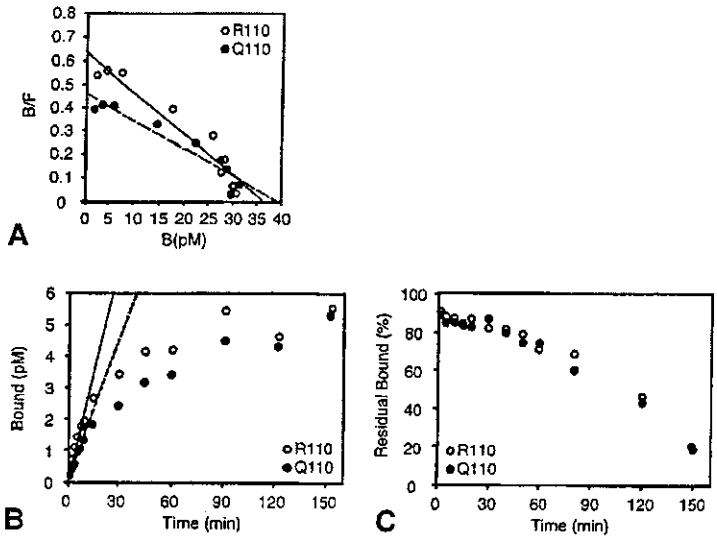

FIG 2. Binding assay of R110 and Q110 to IL-13Rα2. **A**, Scatchard plot with IL-13Rα2–expressing DND-39 cells (clone 18) incubated with 2 types of ^{125}I-labeled recombinant IL-13 (*solid line*, R110; *dashed line*, Q110) is depicted. The same experiments were performed 3 times, and the representative data are depicted. **B**, k_{on} rate of R110 and Q110. Specifically bound radioactivity of ^{125}I-labeled IL-13 with IL-13Rα2–expressing DND-39 cells (clone 15) at various times is plotted versus time. **C**, Dissociation kinetic study of R110 and Q110. Specifically bound radioactivity of preoccupied ^{125}I-labeled IL-13 with IL-13Rα2–expressing DND-39 cells (clone 15) at various times is plotted versus time.

tor with IL-13 and is capable of transducing the IL-13 signal, including STAT6 activation.[28-33] To address whether the binding activities of R110 and Q110 are different, we first used a human B-cell line, DND-39, transfected with IL-13Rα1. Scatchard analysis showed that this cell line expressed high- and low-affinity receptors, which are composed of IL-13Rα1 and IL-4Rα, and IL-13Rα1 alone, respectively (Fig 1, *A* and *B*). The equilibrium dissociation constants of R110 and Q110 with the high-affinity receptor were 21.0 ± 7 and 23.3 ± 3 pmol/L, respectively (mean ± SD, n = 3), which is consistent with a previous report,[28] and there was no difference between R110 and Q110. Furthermore, the transcription activities of the Iε promoter and STAT6 activation by R110 and Q110 were similar (Fig 1, C, and data not shown). Thus the substitution does not affect the binding activity of the IL-13R composed of IL-13Rα1 and IL-4Rα.

Binding activity of the IL-13 variant with IL-13Rα2

Another IL-13–binding molecule, IL-13Rα2, can generate a high-affinity receptor by itself.[30] To analyze the binding activity of the *IL13* variant with IL-13Rα2, we performed a binding assay using DND-39 cells on which IL-13Rα2, but not IL-13Rα1, was transfected. Scatchard analysis showed that IL-13Rα2 formed only a high-affinity receptor, as previously reported.[30] The equilibrium dissociation constants of R110 and Q110 with IL-13Rα2 were 54.7 ± 1.6 and 83.7 ± 3.1 pmol/L, respectively (mean ± SD, n = 3), which meant that Q110 had slightly lower affinity with IL-13Rα2 than did R110 (Fig 2, *A*). To find out what causes the difference in dissociation constants, we next investigated the rate constants for association (k_{on} rate) and dissociation (k_{off} rate) of each IL-13. It turned out that the k_{on} rate of Q110 was slower than that of R110 (Fig 2, *B*: 3.4 ± 0.51 vs 5.3 ± 0.89 × 10^8 × $mol^{-1} \cdot min^{-1} \cdot L$ [mean ± SD, n = 3]). In contrast, there was no apparent difference in k_{off} rates between R110 and Q110 (Fig 2, *C*). These results demonstrated that the affinity of Q110 with IL-13Rα2, which was slightly lower than that of R110, depends on the slower k_{on} rate of Q110.

Clearance of IL-13 by IL-13Rα2

IL-13Rα2 is assumed to act as a decoy receptor because it has a very short cytoplasmic domain. However, the role of IL-13Rα2 in vivo is not explicitly known. One plausible in vivo role for IL-13Rα2 is clearance of local IL-13, and the 2 IL-13 proteins may exert a different activity through this function. To explore this possibility, after either Q110 or R110 was absorbed by IL-13Rα2, we measured the residual IL-13 level by the transcription activity of the Iε promoter in IL-13Rα1–transfected cells (Fig 3). When either Q110 or R110 was preincubated with mock-transfected cells, no decrease of the transcription activity was seen; however, when IL-13 was preincubated with IL-13Rα2–transfected cells, the transcription activities were attenuated in a time-dependent manner, and Q110 caused a slightly smaller decrease than R110. These results apparently demonstrated that IL-13Rα2 is capable of its postulated role; they also indicate that Q110 may be less cleared by IL-13Rα2 than R110.

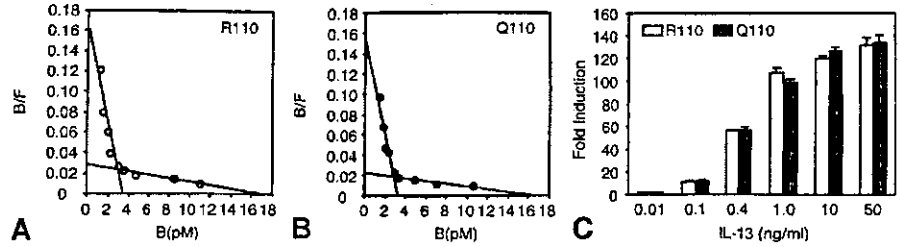

FIG 1. Binding assay of R110 and Q110 to IL-13Rα1. A and B, The Scatchard plot with IL-13Rα1–expressing DND-39 cells incubated with 2 types of ^{125}I-labeled recombinant IL-13 (A, R110; B, Q110) is depicted. The same experiments were performed 3 times, and the representative data are depicted. C, Luciferase activity of IL-13Rα1–transfected DND-39 cells incubated with either R110 or Q110 is shown. Fold induction was estimated comparing the value without the stimulus. Each experiment was done with 3 samples, and the mean values are shown.

cytometry with anti–IL-13Rα2 antibody (Diaclone, Besançon, France). The transfectants were maintained with a medium containing 1.25 mg/mL geneticin (Sigma, Saint Louis, Mo).

Harvested cells were stimulated with the indicated concentration of R110 or Q110 for the indicated period. In some experiments IL-13 was incubated with either 100% of human serum derived from healthy donors or PBS containing 120 mg/mL BSA for the indicated period.

Binding assay

Iodine 125–labeled IL-13 was generated and used in a binding assay for Scatchard plot analysis, as described before.[39] The concentration of the labeled IL-13 was determined by means of self-displacement binding to the IL-13Rα2–expressing DND-39 cells with nonradiolabeled IL-13 at a known concentration. After cells were incubated with various concentrations of ^{125}I-labeled IL-13 for 2 hours at 4°C, bound and free ligands were separated by means of centrifugation through an oil gradient. Nonspecific binding was measured by adding 100-fold or more molar excess of nonradiolabeled IL-13.

Kinetic studies to measure k_{on} rate and k_{off} rate were performed, as described earlier.[40] An association kinetic study was performed under a pseudo first-order condition with respect to both the free ligand and the unoccupied receptor. We incubated 5.0×10^6/mL cells with 15 pmol/L ^{125}I-labeled IL-13 for 2 minutes to 4 hours at 4°C. Specifically bound radioactivity at various intervals up to the equilibrium binding was plotted versus time. To yield the k_{on} rate, the initial slope of the plotted curve was divided by the initial concentrations of free ligand (15 pmol/L), and the expressed receptor was determined by means of Scatchard analysis. In the dissociation kinetic study 5.0×10^6/mL cells were first incubated with 250 pmol/L ^{125}I-labeled IL-13 for 90 minutes at 4°C to let the receptors become fully occupied by the radiolabeled IL-13. After the cells were washed once and resuspended in binding buffer at 4°C, aliquots of cells were taken at various time intervals, and the bound IL-13 was measured. Specifically bound radioactivity at various intervals was plotted versus time.

Luciferase assay

Luciferase activity assay was carried out as described previously.[29] The cells were incubated with the indicated concentrations of IL-13 for 24 hours. After the cells were washed once in PBS and lysed with reporter lysis buffer (Toyoink, Tokyo, Japan), cell lysates were mixed with luciferase assay reagent (Toyoink).

Pharmacokinetics of IL-13 in vivo

One microgram of IL-13 was intraperitoneally administered into 8- to 9-week-old female BALB/c mice, and blood samples were collected at the indicated times. Four mice were analyzed for both R110 and Q110. The procedures were conducted according to the "Guide for the Care and Use of Laboratory Animals," and the study was approved by Saga Medical School's Research Committee.

Measurement of IL-13 levels in plasma

IL-13 levels in mouse and human plasma were immunoassayed in the Mitsubishi Kagaku BCL laboratories by means of a commercial kit.[41] The minimal detectable level was 3.1 pg/mL. Because of the different sensitivities of R110 and Q110 to this assay, the values of R110 and Q110 obtained by the kit were adjusted on the basis of the known proteins as a standard. As for human serum level, blood samples were obtained between 9 and 10 AM to limit circadian variation in cytokine production, and data were presented as medians with 95% confidence intervals and were analyzed with the median χ^2 test to compare 2 groups.

Genotyping of IL-13

Genotyping of IL-13 was studied in a general population of Japanese children from Wakayama Prefecture, as described previously.[41] All the asthmatic subjects had been given diagnoses by asthma specialists of (1) recurrent breathlessness and chest tightness requiring ongoing treatment, (2) physician-documented wheeze, and (3) documented labile airflow obstruction with variability in serial peak expiratory flow rates of greater than 30%.

Genotyping of IL-13 was conducted by using the PCR method as follows.[10] DNA samples were extracted with a commercial kit (IsoQuick; Microprobe, Garden Grove, Wash). PCR primers were 5′-TGGCGTTCTACTCACGTGCT-3′ and 5′-TTTCGAAGTTTC-AGTAGTAC-3′. The underlined sequences were mutated to incorporate a restriction site for *ScaI*. The study was approved by Kyoto University's Ethics Committee.

RESULTS

Binding activity of the IL-13 variant with IL-13Rα1

It has been already demonstrated that IL-13R consisting of IL-13Rα1 and IL-4Rα forms a high-affinity recep-

arginine residue at amino acid 110 is substituted with glutamine (Arg110Gln). This variant was associated with bronchial asthma in both Japanese and British populations.[10] The same variant was thereafter reported to be positively correlated with high IgE levels and atopic dermatitis.[11,12] These results confirmed the candidacy of this variant as a novel genetic factor for allergic diseases across ethnicity, although it remains unanswered whether this variant itself or other polymorphisms that are in linkage disequilibrium affect the IL-13 signal. Such findings have led us to determine what role this variant may play in the pathogenesis of allergic diseases.

Cytokines derived from the T_H2 lymphocytes are considered to orchestrate the asthmatic phenotype.[13-15] Among T_H2 cytokines, several lines of evidence exist, implicating IL-13 in the pathogenesis of bronchial asthma. Expression of T_H2 cytokines, including IL-13, was augmented constitutively or induced by means of allergen challenge in bronchial tissues or bronchoalveolar lavage fluids derived from asthmatic patients, and the expression of IL-13 was dominant compared with that of IL-4.[16,17] Analyses of mice with disrupted IL-13 signaling molecules, such as IL-13, IL-4R α chain (IL-4Rα), and signal transducer and activator of transcription 6 (STAT6) showed that IL-13 has a pivotal role in the induction of airway hyperreactivity.[18-20] It had been assumed that IL-13's role in the pathogenesis of bronchial asthma would be an action on immune cells, such as induction of IgE switching in B cells, as well as IL-4.[21] Nevertheless, it was recently revealed that IL-13 induces asthmatic phenotype in mice independent of lymphocytes.[22-24] This result indicated that IL-13 acts directly on nonimmune cells in bronchial tissue, which may have an important role in the pathogenesis of bronchial asthma.[25,26] In fact, we have recently shown that bronchial epithelial cells (BECs) and bronchial smooth muscle cells express high levels of IL-4R and IL-13R.[10] Genetic analysis showing that the IL-13 variant was correlated with bronchial asthma rather than with atopy also supports this idea.[10]

To date, 2 IL-13–binding molecules are known to exist.[26,27] An IL-13–binding molecule, the IL-13R α1 chain (IL-13Rα1), alone shows a low affinity with IL-13; however, it forms a high-affinity receptor and transduces the IL-13 signal together with IL-4Rα.[28-33] Another IL-13–binding unit, the IL-13R α2 chain (IL-13Rα2), generates a high-affinity receptor by itself, although it is thought not to transduce the IL-13 signal,[31] acting as a decoy receptor because of its short cytoplasmic domain.[30] Although IL-13Rα2 is expressed on a variety of epithelial tumor and human fibroblast cell lines,[34-36] it is not completely clear which other cell types express IL-13Rα2 and how expression of IL-13Rα1 and IL-13Rα2 is regulated.

In this study, to address whether the IL-13 variant influences IL-13 function, thereby contributing to the pathogenesis of bronchial asthma, we generated 2 types of recombinant IL-13 proteins for this analysis: one with arginine (R110) and the other with glutamine (Q110) at position 110. It turned out that the affinity of R110 and Q110 to IL-13Rα1 was almost equal, whereas Q110 showed slightly lower affinity with IL-13Rα2, a decoy receptor, than with R110. Consequently, Q110 was slightly less cleared from the extracellular environment by IL-13Rα2 than R110. Furthermore, Q110 showed an enhanced stability in vitro and in vivo compared with R110. These results indicated that this variant could influence local and systemic concentrations of IL-13 in vivo. As expected, the serum level of IL-13 was higher in Q110-bearing individuals. Taken together, we could conclude that this variant may be a functional genetic factor in bronchial asthma.

METHODS

Generation of the IL-13 proteins

IL-13 cDNA was cloned from a human T_H2 cell clone, SM4.6.[37] Because the cloned IL-13 was the R110 type, to replace an arginine residue with a glutamine residue, we used a PCR method with the primer 5′-GCGAGGGACAGTTCAACTGAAACTTC-3′ and its complement. Two types of the *IL13* gene were incorporated into pMAL-cX vector (New England Biolabs, Beverly, Mass), with a TEV protease cleavage site (Gln-Asn-Leu-Tyr-Phe-Gln-Gly) attached to the N terminus of *IL13*. As a result, the N terminus of the cleaved product got glycine (underlined), as previously reported.[38]

Maltose-binding protein–fused IL-13 proteins were expressed in *Escherichia coli* and isolated by using an amylose affinity column (New England Biolabs), followed by cleavage by TEV protease (Life Technologies, Rockville, Md) under reducing conditions. The cleaved protein mixtures were denatured with a solution containing 5 mol/L guanidine hydrochloride, 5 mmol/L dithiothreitol, 50 mmol/L Tris/HCl (pH 8.0), 50 mmol/L NaCl, and 1 mmol/L EDTA. Then the proteins were refolded by means of sequential dialyses against the solutions containing lower concentrations (1.5, 0.9, and 0 mol/L) of guanidine hydrochloride and 2 mmol/L–0.2 mmol/L reduced-oxidized glutathione. The IL-13 proteins were purified by using a Q-Sepharose Fast Flow column, followed by a Superdex 75 HR10/30 column (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom).

Purity of the generated proteins was greater than 98% and estimated by means of silver staining of SDS-PAGE gel. Molecular weights of R110 and Q110 were 12341.2 and 12309.3, respectively, as evaluated with Voyager RP MALDI-TOF mass spectrometry (PerSeptive Biosystems, Framingham, Mass), and these weights were consistent with their theoretic values on the basis of cDNA sequences. The concentration of R110 was assessed by means of Western blotting with anti–IL-13 antibody (AF-213-NA; R&D Systems, Minneapolis, Minn), with the commercial IL-13 (PeproTech, Rocky Hill, NJ) used as a standard. The moles of the 2 IL-13 proteins were adjusted on the basis of the values calculated with an amino acid analyzer (L-8500; Hitachi, Hitachinaka, Japan).

Cells and plasmids

DND-39 cells cotransfected with IL-13Rα1 and pGL3-enhancer vector (Promega, Madison, Wis), into which the promoter region from −187 to +6 of the human Iε gene (Gε) was inserted, were constructed as described previously.[29] The cells were maintained in RPMI-1640 medium supplemented with 10% FCS, 100 μg/mL streptomycin, and 100 U/mL penicillin containing 6 μg/mL blasticidin S hydrochloride (Funakoshi, Tokyo, Japan) and 250 μg/mL hygromycin B (Wako, Osaka, Japan). A neomycin-resistant gene was inserted into the plasmid coding IL-13Rα2 in pME18S mammalian expression vector, and this plasmid was incorporated into DND-39 cells pretransfected with Gε alone by means of electroporation. Expression of IL-13Rα2 was confirmed by means of flow

Upregulation of IL-13 concentration in vivo by the *IL13* variant associated with bronchial asthma

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Background: A substantial body of evidence exists to support the pivotal role of IL-13 in the pathogenesis of bronchial asthma. We recently found that a variant of the *IL13* gene (Arg110Gln) is genetically associated with bronchial asthma, which is concordant with animal experiments using IL-13 in the development of asthma.

Objective: To address whether the Gln110 variant of *IL13* influences IL-13 function, contributing to the pathogenesis of bronchial asthma, we studied the functional properties of the variant.

Methods: We generated 2 types of recombinant IL-13 proteins, the amino acids of which at 110 were arginine or glutamine, and analyzed the binding affinities with the IL-13 receptors, as well as the stability of the proteins. We further compared the relationship between the genotype and serum levels of IL-13.

Results: The variant showed a lower affinity with the IL-13 receptor α2 chain, a decoy receptor, causing less clearance. The variant also demonstrated an enhanced stability in both human and mouse plasma. We further identified that asthmatic patients homozygous for the Gln110 variant have higher serum levels of IL-13 than those without the variant.

Conclusion: These results suggested that the variant might act as a functional genetic factor of bronchial asthma with a unique mechanism to upregulate local and systemic IL-13 concentration in vivo. (J Allergy Clin Immunol 2002;109:980-7.)

Key words: IL-13, polymorphism, IL-13 receptor α2 chain, allergy, bronchial asthma

Bronchial asthma is, like other common human diseases, a complex disease in that it is multifactorial, exhibits genetic heterogeneity, or both.[1] The increasing incidence of allergic diseases in the last few decades, including bronchial asthma, has been attributed to environmental changes, particularly in developed areas. However, genetic factors involved in the pathogenesis of bronchial asthma have been identified on the basis of analyses of inheritance patterns in families and twins.[1,2]

Several genome-wide searches for quantitative traits underlying bronchial asthma have been performed, yielding linkages to diverse chromosomal loci,[3] which is explainable by differences of ethnicity, definition for subjects, or both. However, importantly, most of the studies confirmed linkage to chromosome 5q.[4-6] T_H2 cytokine genes are clustered in 5q31-33, presenting obvious candidates. A variant of the promoter region of the *IL4* gene, −590C/T, has been shown to be related to higher IgE levels.[3] This variant associated in vitro with IL-4 expression and showed higher DNA binding affinity. However, no direct link to cellular IgE synthesis has been shown, and some other groups argued against this association.[7] Significant association of atopy with the *IL9* gene was observed,[8] although this also remains controversial.[9] We recently have found a variant of the *IL13* gene, in which

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Supported by a Research Grant for Immunology, Allergy and Organ Transplant from the Ministry of Health and Welfare of Japan; a grant-in-aid for Scientific Research from the Ministry of Education, Science, Sports, Culture, and Technology of Japan; RIKEN; Astra Zeneca Asthma Research Foundation; Japan Research Foundation for Clinical Pharmacology; grant AI43663 from the National Institute of Allergy and Infectious Diseases; and grant RSG-01-241-01-LIB from the American Cancer Society.

Received for publication December 26, 2001; revised February 27, 2002; accepted for publication March 6, 2002.

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1/84/124656

doi:10.1067/mai.2002.124656

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isms at multiple loci may help identify individuals who are at increased risk for lung cancer.

Conversely, the use of multiple comparisons in any given study raises the specter of finding an association by chance alone, if enough biomarkers and relationships are examined. Therefore, rigorous thresholds for statistical significance in such studies, far below the $P = 0.05$ level, have been proposed and warrant integration into study design and reports [121].

Although the overall risk for developing cancer in individuals with at each 'at risk' genotype may be small, lung cancer is such common malignancy that even a small increase in risk translates to a large number of excess lung cancer cases at the population level. Therefore, polymorphisms, even which were not significantly associated with lung cancer, should be an important public health issue. In addition, a susceptibility factor in one population may not be a factor in another. There are differences in the prevalence of some metabolic polymorphisms such as *CYP1A1*, *CYP2D6* and *CYP2E1*, among the various ethnic groups [6]. In a population, where the prevalence of 'at risk' genotype in given polymorphism is very low, the 'at risk' allele or 'at risk' genotype was too infrequent to assess the risk it might confer to an individual; at a population level, the attributable risk must be small simply because it is an infrequent allele.

Knowledge of the prevalence and distribution of common genetic susceptibility factors and the ability to identify susceptible individuals or subgroups will have substantial preventive implications, in particular if more data are collected to show that people with certain high-risk genotypes are more susceptible to low levels of exposure. It is conceivable that such subjects could be: (1) more easily persuaded to avoid hazardous exposure like tobacco use; (2) targeted intensive smoking cessation programs; (3) be enrolled in chemoprevention trials; and (4) be involved in cancer screening programs that are nor appropriate for the general population. However, before results of individual screening for genetic traits can be used efficiently to implement preventive measures, more cancer-predisposing genes need to be studied and gene–environment and –gene interactions elucidated.

Rapid advances in high-throughout gene analysis by DNA chip technology will spread up the identification of new mutations in predisposing cancer genes. The main task, however, will be to characterize the functional significance of these gene variants in humans. Such efforts are under way, e.g. the Human Genome Project by Human Genome Organisation, which will come to its completion in the near future, or the Environmental Genome Project pursued by the National Institute for Environmental Health Services in the United States [122].

To this purpose, future studies involving larger control and case populations, precisely and uniformly defined clinical classification of lung cancer and better exposure histories will undoubtedly lead to a more thorough understanding of the role of various genes in lung cancer development.

Acknowledgements

This study was funded in part by a Grant-in-Aid for Scientific Research (C) (12670322) from the Ministry of Education, Science, Sports and Culture, Japan and a grant from the Smoking Research Foundation, Japan. We thank Professor S. Kono (Department of Preventive Medicine, Graduate School of Medical Sciences, Kyushu University) for the collaboration.

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Table 6 (*Continued*)

Ethnicity	Cases/ controls	OR (95% CI)	Cases (histology and smoking status)	Controls (type of control and smoking status)	Quality control of genotyping	Reference
	Non-Caucasian	1.15 (0.89–1.49)				

Smoker, prevalence of both current- and ex-smokers; Current smoker, prevalence of current-smokers; Ad, adenocarcinoma; SCC, small cell carcinoma; Sq, squamous cell carcinoma; LCC, large cell carcinoma. Variant homozygous genotype vs. wild-type homozygote. Adjusted ORs were shown in table unless otherwise specified.

[a] Crude OR.

the studies of Caucasian subjects, the major ethnic group, the OR of lung cancer associated with the *p53* polymorphism was 1.08 (95% CI = 0.88–1.69). In non-Caucasian populations (African–Americans, Mexican–Americans, Japanese, Chinese and Hawaiian), the OR of this polymorphism was 1.15 (95% CI = 0.89–1.49). There was no evidence of significant heterogeneity in either subgroup. Future analyzes stratified by histology or ethnicity should be considered in future studies of the *p53* polymorphisms.

3. Conclusion

Molecular epidemiology has contributed to a growing awareness of the importance of relatively common genetic and acquired susceptibility factors in modulating risks associated with exposure to environmental carcinogens. Because lung cancer is largely preventable disease, the future challenge of molecular epidemiology is to analyze individuals who are exposed to carcinogens for a combination of genotypes associated with susceptibility to lung cancer. It is evident that use of more precisely measurable intermediary risk markers, like mutation in metabolic genes, DNA adducts and cytogenetic damage rather than lung cancer as an end point, will allow the identification of combinations of cancer-relevant genes that affect lung cancer outcome. Such associations could then be verified in epidemiological studies designed to address the association to be confirmed. Thus, further progress is expected from studies in which biomarkers for carcinogen exposure, early biological effects and susceptibility are integrated, which should allow establishment of the risk profiles of individuals and subgroups in given exposure situations.

IARC [118,119] provided state-of the art reviews of the application of biomarkers and the design and analysis of molecular epidemiological studies. The prerequisites for proper study design and conduct include: (1) clear definition of representative study populations and controls; (2) a sample size adequate to provide enough statistical power; (3) proper documentation (or measurement) of exposure; (4) avoidance of confounding because of use of study subjects of mixed ethnic background; and (5) study only genetic polymorphisms that have been shown to lead to altered phenotypic expression.

Similarly, in order to avoid some of the problems associated molecular epidemiologic studies, Todd [120] has addressed the issue of guidelines for the interpretation of results from those studies of multifactorial diseases. They expect that those studies should contain large sample size, an independent replication followed by initial study, biological plausibility and physiologically meaningful data supporting a functional role of the polymorphism in question. The initial studies showed a substantially increased/decreased risk of developing lung cancer in individuals with specific genotypes.

As discussed here, there are numerous conflicting reports on the association between different polymorphisms and lung cancer risk. The results of meta-analyzes indicate that the Arg/Arg genotype of *mEH4* His-Arg polymorphism was significantly associated with a decreased risk of lung cancer and the *MPO* polymorphism was a significant risk factor in the development of lung cancer. Those polymorphisms appear to be stronger candidates for lung cancer susceptibility genes although publication bias is always a possible limitation of combining data from various sources as in a typical meta-analysis or laboratory techniques for analysis of genotyping are different. Most of the PCR-based techniques currently used for such assignments have standardized and the quality control may not be necessarily required. The others polymorphisms, the risk estimates, although non-significant, are insufficiently precise to exclude a moderate risk, and larger studies are needed to obtain more precise risk estimates. There include polymorphisms in *NQO1*, DNA repair genes and *p53*. It is more likely, however, that the majority in variation in lung cancer susceptibility is due to genes that have not to be identified or tested. Candidate genes involved in cell cycle regulation and macro- and micro-nutrient metabolism. As stated above, the etiology of lung cancer cannot be explained by allelic variability at a single locus. Instead, the major burden of lung cancer in the population probably results from complex interaction between many genetic and environmental factors over time. An improved understanding of the interplay of environmental and genetic polymorph-

Table 6
Lung cancer and p53 Arg72 Pro polymorphism

Ethnicity	Cases/ controls	OR (95% CI)	Cases (histology and smoking status)	Controls (type of control and smoking status)	Quality control of genotyping	Reference
Mixed	78/72	0.86 (0.40–1.85)[a]	Ad: 35%, SCC: 9%, Sq: 49%, LCC: 8% Smoker: African-Americans: 47% for all subjects Caucasian: 53% for all subjects	Population	?	[106]
Mixed	31/48	0.9 (0.2–3.3)	Histology: not given Smoking: not given African-Americans: 42% Caucasian: 58%	Hospital Smoking: not given African Americans: 27% Caucasian: 74%	?	[107]
Japanese	328/347	1.57 (1.00–2.45)[a]	Ad: 46%, SCC: 15%, Sq: 33%, LCC: 6% Smoking: not given	Population Smoking: not given	?	
	Ad	0.75 (0.40–1.40)[a]				
	Sq	1.13 (0.57–2.17)[a]				
	SCC	1.27 (0.50–3.01)[a]				[108]
Caucasian	482/510	1.21 (0.80–1.83)[a]	Ad: 51%, SCC: 5%, Sq: 28% LCC: 18%, Other: 10% Current smoker: 42%	Population Current smoker: 18%	?	
	Ad	1.62 (0.99–2.64)[a]				
	Sq	0.85 (0.43–1.61)[a]				
	LCC	0.71 (0.07–3.37)[a]				
	SCC	1.30 (0.22–5.39)[a]				[109]
African-American	67/74	0.87 (0.3–2.4)	Ad: 31%, SCC: 6%, Sq: 34% LCC: 6%	Population	?	
Mexican-American	42/40	2.2 (0.2–26.6)	Histology: not given	Population		
Combined	Ad	2.71 (0.62–12.51)[a]	Smoker: 92% for all cases	Smoker: 65% for all controls		
Combined	Sq	1.11 (0.21–5.16)[a]				110
Caucasian	142/282	0.83 (0.35–1.85)[a]	Ad: 41%, SCC+LCC: 10%, Sq: 36% Smoking: not given	Population Smoking: not given	?	
	Ad	0.86 (0.24–2.52)[a]				
	Sq	1.11 (0.21–5.16)[a]				111
Caucasian	139/147	1.49 (0.56–3.97)[a]	Ad: 25%, SCC: 35%, Sq: 33% LCC: 7% Smoking: not given	Population Smoking: not given	Sequencing	
	Ad	2.19 (0.44–9.10)[a]				
	Sq	0.85 (0.08–4.64)[a]				
	SCC	1.84 (0.37–7.54)[a]				112
Chinese	194/152	1.20 (0.64–2.24)[a]	Ad: 53%, Sq: 36% LCC: 4%, Other: 8% Smoking: not given	Hospital Smoking: not given	Replicate	
	Ad	1.38 (0.66–2.86)[a]				
	Sq	0.73 (0.30–1.73)[a]				113
Japanese	111/170	1.5 (0.7–3.4)	Ad: 47.3% for all cases Sq: 22% for all cases	Population Smoking: not given	?	
Caucasian	138/173	1.4 (0.4–4.8)				
Hawaiian	85/103	1.3 (0.6–3.8)	SCC: 15% for all cases Other: 15% for all case Smoking: not given			
Combined	Ad	1.2 (0.6–2.4)				
Combined	Sq	1.0 (0.4–2.6)				
	SCC	1.4 (0.5–4.0)				114
Japanese	191/152	0.63 (0.30–1.32)[a]	Ad: 51%, Sq: 30% Other: 18%, Unknown: 7% Smoker: 94%	Hospital Smoker: 56%	?	
	Ad	0.49 (0.19–1.21)[a]				
	Sq	0.90 (0.28–2.65)[a]				115
Caucasian	109/113	0.33 (0.11–0.98)[a]	SCC: 11%, Sq: 71%, LCC: 5% Smoking: not given	Population Smoking: not given	?	116
Caucasian	1168/1256	1.29 (0.9–1.8)	Ad: 51%, SCC: 8%, Sq; 25%	Population	PCR-based pyrosequencing	
	Ad	1.33 (0.9–2.0)	LCC: 8%, Other: 6% Current smoker: 41%	Current smoker: 19%		
	Sq	0.7 (0.4–1.5)				117
Overall	3305/3639	1.16 (0.99–1.38)				
	Ad	1.22 (0.88–1.69)				
	Sq	0.98 (0.71–1.23)				
	SCC	1.32 (0.81–2.14)				
	Caucasian	1.08 (0.79–1.48)				

prevalent in Caucasians (Hungarian 0.81, English 0.76) than Asians (Japanese 0.59, Chinese 0.39) [89,92]. The differences of this polymorphism among populations will influence the interpretations and strategies for its use as potential tool for estimation of a particular individual risk. No major difference in *hOGG1* genotype distribution was observed between lung cancer cases and controls [84,93]. To date, three case-control studies were reported and none of them showed significant effects on lung cancer susceptibility. The *hOGG1* gene alone is probably not major contributor to lung cancer susceptibility (Table 5b).

A significant association between *hGPX1* genotype and lung cancer risk was observed [91]. The ORs for heterozygote and for homozygous variants were 1.8 (95% CI = 1.2–2.8) and 2.3 (95% CI = 1.3–3.8), respectively. The generalization of these results, however, may be somewhat restricted because the study was conducted among a Caucasian male smoker population. It should be attempt to examine the association between the *hGPX1* polymorphisms and lung cancer risk in other ethnic groups.

2.3. Germline polymorphism of tumor suppressor gene

The *p53* tumor suppressor gene is one of the most commonly mutated genes in all types of human cancer [94,95]. Recent studies of the function of the wild-type *p53* demonstrated that its antiproliferative effect is mediated by stimulation of a 21-kDa protein (p21cip/waf) that inhibits cyclin-dependent kinase activity and, thereby, cell division [96,97]. This negative cell cycle controller effect may explain why the wild-type *p53* gene can suppress the transformation of malignant cells in vitro [98,99]. Analysis of somatic tissue from many human cancers has shown that the wild-type *p53* allele frequently is lost and a mutant allele retained, providing a growth advantage for malignant cells [100–102]. The mutation of the *p53* gene can damage its DNA-binding properties and cell cycle control and in cell proliferation [103]. Somatic p53 mutations are found more frequently in squamous cell carcinoma than in adenocarcinoma, although this may be a function of higher exposures to tobacco in patients with squamous cell carcinoma [104].

The *p53* gene is located on chromosome 17p13. This gene is a key and potent mediator of cellular response against genotoxic insults [105]. The Pro allele of the *p53* polymorphism has been linked epidemiologically to smoking-related lung and bladder cancers in some studies. However, the exact biological mechanisms for an increased risk by the Pro allele are not fully understood.

To date, several polymorphisms in the *p53* gene has been reported. The codon 72 polymorphism on the fourth exon of the *p53* gene by *BstU* I, which produced variant proteins with an arginine (CGC) or proline (CCC), has been reported to be associated with lung cancer. *Msp* I RFLP exists in intron 6 consisting of either six or eight variable bases.

As shown in Table 6, Weston et al. [106] reported a small increased frequency of the Pro allele in adenocarcinomas among Caucasian, but a later study [107] by this same group did not confirm this finding in a different set of cancer cases and controls. They reported that the frequency of the Pro/Pro genotype was similar in lung cancer cases and controls. The Pro/Pro genotype was found to be overrepresented in a study of Japanese lung cancer, especially in Kreyberg I but not in adenocarcinoma [108]. Fan et al. [109] also found that the frequency of the Pro/Pro genotype was significantly higher in adenocarcinoma cases than controls. An enhanced risk of adenocarcinoma was reported for African–Americans with the Pro/Pro genotype [110]. A Swedish study has suggested that the codon 72 alleles may not be functionally involved in lung cancer but, rather, may be a marker in linkage disequilibrium with other cancer susceptibility sites on the *p53* gene [111]. No difference in the prevalence of the codon 72 *p53* polymorphism between lung cancer cases and controls has been found [112–115]. Murata et al. [115] reported that the genotypic frequencies of the *p53* gene in lung cancer patients were largely different between smokers and non-smokers ($P < 0.001$). Biros et al. [116] found that another polymorphism *Msp* I may modify the susceptibility to lung cancer as a single factor rather than in combination with *BstU* I polymorphism. In a Caucasian population [117], when compared with the wild-type Arg/Arg genotype the adjusted OR of lung cancer for the Pro/Pro genotype was 1.29 (95% CI = 0.9–1.8). The adjusted OR for adenocarcinoma and squamous cell carcinoma were 1.33 (95% CI = 0.9–2.0) and 0.75 (95% CI = 0.4–1.5), respectively. The 15 case-control studies of lung cancer and *p53* genotype included 6944 subjects (3305 lung cancer cases and 3639 controls). The overall OR was 1.16 (95% CI = 0.99–1.38). A small and marginally significant increased risk of lung cancer was observed in a meta-analysis. The results from 15 different studies now showed an absence of any significant correlation between lung cancer risk and the *p53* polymorphism. Adenocarcinoma and squamous cell carcinoma, which are called non-small cell lung cancer, are distinct etiological entities that should be analyzed separately. Nine studies contained information on histology in a form suitable for pooled analysis [108–115,117]. The p53 polymorphism was associated with and ORs of 1.22 (95% CI = 0.88–1.69) for adenocarcinoma and 0.98 (95% CI = 0.71–1.23) for squamous cell carcinoma. An overall OR of 1.32 (95% CI = 0.81–2.14) for small cell carcinoma was obtained from four studies [108,109,112,114]. Test for heterogeneity in tow histological subgroups analyzed showed no evidence for heterogeneity. Restricting the analyzes to

Table 5b
Lung cancer and DNA repair gene

Ethnicity	Cases/controls	OR (95% CI)	Cases (histology and smoking status)	Controls (type of control and smoking status)	Quality control of genotyping	Reference
XPD Asp312Asn polymorphism						
Caucasian	96/96	1.86 (1.02–3.40)	Ad: 26%, Sq: 68%, LCC: 6% Current smoker: 60%	Population Current smoker: 55%	Sequencing	[74]
Caucasian	195/257	1.51 (0.76–3.00)	Histology: not given Current smoker: 40%	Population Current smoker: 37%	?	[75]
XPD Lys751Gln polymorphism						
Caucasian	341/360	1.36 (0.84–2.20)	See above			[75]
Caucasian	178/453	1.34 (0.74–2.42)	Histology: not given	Population	?	
African–Americans	153/234	1.03 (0.40–2.65)	Smoking: not given	Smoking: not given		[73]
Overall	672/1047	1.50 (1.09–2.07)				
XPD Asp312Asn/Lys751Gln combined polymorphism						
Caucasian	213/268	1.84 (1.11–3.04)[b]	See above			[75]
hOGG1 Ser326Cys polymorphism						
Caucasian	37/47	1.30 (0.09–19.3)[a]	Ad: 56%, Sq: 44% Smoking: not given	Population Smoking: not given	Sequencing	[84]
Japanese	241/197	1.31 (0.65–2.62)[b]	Ad: 32%, SCC: 12%, Sq: 49%,	Population	Sequencing	
	Ad	1.34 (0.53–3.39)	Other: 7%	Current smoker: 5%		
	Sq	2.27 (0.92–5.60)	Current smoker: 13%			
	SCC	0.51 (0.09–2.87)				[92]
Caucasian	105/105	2.2 (0.41–11.79)	Ad: 48%, Sq: 48%, other: 5%	Hospital	Sequencing	
	Ad	1.84 (0.41–14.41)	Smoker: 100%	Smoker: 100%		
	Sq	1.76 (0.24–13.10)				[93]
hGPX1 Pro198Leu polymorphism						
Caucasian	315/315	2.3 (1.3–3.8)	Ad: 18%, SCC: 16%, Sq: 44%, Other: 22% Current smoker: 100%	Population Current smoker: 100%	Replication	[91]

Smoker, prevalence of both current- and ex-smokers; Current smoker, prevalence of current-smokers; Ad, adenocarcinoma; SCC, small cell carcinoma; Sq, squamous cell carcinoma; LCC, large cell carcinoma. Variant homozygote vs. wild-type homozygote unless otherwise specified. Adjusted ORs were shown in table unless otherwise specified.

[a] Crude OR.

[b] More than two variant alleles vs. other genotypes combined.

polymorphism showed no functional effect on erythrocyte GPX1 activity [83]. In addition, the presence of the ALA6 allele (the Leu allele was non-significnatly associated with reduced levels of oh^{8}Gua levels [84]. Although biochemical characterization of the human GPX1 enzymes encoded by the distinct genotype of the GPX1 polymorphism is till unclear, constitutive genotype may play a significant role in determining oh^{8}Gua levels within tissue DNA.

The formation of oh^{8}Gua in DNA causes G:C to T:A transversion, since oh^{8}Gua pairs with adenine as well as cytosine [85,86]. The human 8-oxoguanine–DNA glycosylase 1 (*hOGG1*) gene encodes base excision repair proteins for oh^{8}Gua in double-stranded DNA [87]. The OGG1 protein possesses the ability to excise oh^{8}Gua paired with cytosine [87]. The *hOGG1* Ser326Cys polymorphism was initially identified by Kohno et al. [88]. Preliminary evidence from *Escherichia coli* complementation assay suggested that the *hOGG1* Cys isoform exhibited reduced oh^{8}Gua repair activity [88]. However, Hardie et al. [89] suggested that differences in oh^{8}Gua glycosylase activity within *hOGG1* polymorphic variants were insufficient to impact on tissue oh^{8}Gua levels because levels of oh^{8}Gua did not vary with *hOGG1* genotype.

Both *GPX1* and *hOGG1* locate to regions of chromosome 3p (3p21 and 3p25/26, respectively) which are subjects to frequent and early loss of heterozygosity during lung cancer development [79,90,91].

A Japanese study indicated the Ser allele frequency was similar between controls (0.59) and lung cancer cases (0.59) but this polymorphism related to risk of squamous cell carcinoma when combined subjects with at least one Ser allele (OR = 3.01, 95% CI = 1.33–6.83) [92]. A Caucasian study also reported that the distributions of *hOGG1* Ser allele were not different between Caucasian lung cancer patient (0.74) and general population (0.76) [89]. The *hOGG1* Ser allele was more

Table 5a
Lung cancer and DNA repair gene

Ethnicity	Cases/controls	OR (95% CI)	Cases (histology and smoking status)	Controls (type of control and smoking status)	Quality control of genotyping	Reference
ERCC1[a]						
Mixed	75/95	1.76 (0.93–3.33)	Histology: not given	Population		
ERCC3[a]						
Mixed	75/95	1.56 (0.84–2.90)	Current smoker: 35%	Current smoker: 38%		
ERCC5[a]						
Mixed	75/95	2.32 (1.22–4.43)	Caucasian: 88%	Caucasian: 87%	?	
ERCC6[a]						
Mixed	75/95	2.49 (1.28–4.84)	African–Americans: 9%	African–Americans: 6%		
XPC[a]						
Mixed	75/95	2.19 (1.15–4.18)	Other: 3%	Other: 7%		[69]
XRCC1 Arg399Gln polymorphism						
Caucasian	107/208	1.3 (0.5–3.5)	Sq: > 80% Current smoker: 81%	Population Current smoker: 70%	?	[70]
Mixed	172/143	2.45 (1.1–5.8)[c]	Ad: 100% Smoker: 94% Non-Hispanic: 75% Hispanic: 25%	Hospital Smoker: 84% Non-Hispanic: 50% Hispanic: 50%	?	[71]
Caucasian	180/461	0.6 (0.3–1.3)	Histology: not given Current smoker: 61%	Population Current smoker: 18%	?	
African–American	154/243	0.6 (0.2–2.3)	Histology: not given Current smoker: 68%	Population Current smoker: 32%		[72]
Overall	613/1055	1.02 (0.54–1.93)				
XRCC1 Arg194Trp polymorphism						
Caucasian	108/210	0.7 (0.3–1.8)	See above			[70]
Caucasian	180/461	1.0 (0.5–0.8)[b]	See above			[72]
African–American	154/243	2.3 (0.2–25)	See above			[72]
XRCC1 Arg280His polymorphism						
Caucasian	106/209	1.8 (1.0–3.4)[b]	See above			[70]
XRCC3 Thr241Met polymorphism						
Caucasian	178/453	0.94 (0.51–1.77)	Histology: not given	Population	Replication	
African–Americans	153/234	1.67 (0.57–4.87)	Smoking: not given	Smoking: not given		[73]

Smoker, prevalence of both current- and ex-smokers; Current smoker, prevalence of current-smokers; Ad, adenocarcinoma; SCC, small cell carcinoma; Sq, squamous cell carcinoma; LCC, large cell carcinoma. Variant homozygous genotype vs. wild-type homozygote. Adjusted ORs were shown in table unless otherwise specified.

[a] RT-PCR. Low expression vs. high expression.

[b] Variant homozygous genotype + heterozygous genotype vs. wild-type homozygote.

[c] Variant homozygote vs. heterozygote + wild-type homozygote.

repair. The direct relationship between DNA repair capacity and expression level of the NER genes or those polymorphisms needs to be determined in future cohort studies.

2.2.2. Glutathione peroxidase 1 (GPX1) and human 8-oxoguanine–DNA glycosylase 1 (hOGG1)

In view of its abundance and mutagenicity, a number of defense mechanisms operate to minimize 8-hydroxyguanine (oh^{8}Gua) accumulation within the genome. oh^{8}Gua is a major DNA lesion produced by oxygen-radicals [76]. Primary defense mechanisms include antioxidants and enzymes such as glutathione peroxidase [77,78]. Glutathione peroxidases reduce organic peroxides and hydrogen peroxides through the coupled oxidation of reduced glutathione. Glutathione peroxidase 1 (GPX1) is the major cytosolic form of this enzyme, but other isozymes are found in the plasma and phospholipid membranes [79]. The cytosolic form of human GPX1 belongs to a family of selenium-dependent peroxidases that include another cytosolic forms, hGXP2 [80], the plasma-based hGXP3 [81] and the phospholipids hydroperoxidase hGPX4 [82]. Polymorphisms in *GPX1* are characterized by a variable polyalanine repeat and the six-alanine repeat form (ALA6, instead of ALA5 or ALA7 with the wild-type proline) also contains a proline to leucine substitution at codon 198 towards the C-terminus [79]. Recently, this

Table 4
Lung cancer and MPO (G-463A) polymorphism

Ethnicity	Cases/controls	OR (95% CI)	Cases (histology and smoking status)	Controls (type of control and smoking status)	Quality control of genotyping	Reference
Caucasian	182/459	0.30 (0.10–0.93)	Histology: not given Smoker: 96%	Population Smoker: 65%	?	
African–Americans	157/224	0.61 (0.26–1.41)	Histology: not given Smoker: 95%	Population Smoker: 69%		[54]
Caucasian Japanese Hawaiian	135/171 108/163 80/103	0.6 (0.2–2.0) 0.1 (0.0–0.5) 1.5 (0.2–1.6)	Histology: not given Smoking: not given	Population Smoking: not given	?	[55]
Caucasian	196/196	1.25 (0.34–4.52)	Histology: not given Smoking: not given	Hospital Smoking: not given	Sequencing	[56]
Caucasian	93/121	0.52 (0.30–0.90)[a]	Histology: not given Current smoker: 50%	Hospital Current smoker: 41%	?	[57]
Caucasian	315/311	0.72 (0.32–1.65)	Histology: not given Current smoker: 100%	Population Current smoker: 100%	Sequencing	[58]
Overall	1266/1748	0.63 (0.45–0.87)				

Smoker, prevalence of both current- and ex-smokers; Current smoker, prevalence of current-smokers; Ad, adenocarcinoma; SCC, small cell carcinoma; Sq, squamous cell carcinoma; LCC, large cell carcinoma. Variant homozygous genotype vs. wild-type genotype. Adjusted ORs were shown in table unless otherwise specified.

[a] Crude OR.

involved in double-strand break repair/recombination genes (X-ray cross-complementing group 3, *XRCC3*), and a gene functioning in base excision repair and the repair of radiation-induced damage (*XRCC1*)).

Polymorphisms in DNA repair genes may be associated with differences in the repair efficiency of DNA damage and may influence an individual's risk of lung cancer because the variant genotype in those polymorphisms might destroy or alter repair function. Reduced DNA repair capacity has been shown to be associated with a 5.7-fold (95% CI = 2.1–15.7) increased risk of developing lung cancer [67]. The overall 2.1-fold (95% CI = 1.5–3.0) increased risk of lung cancer was consistent with their previous small pilot study [68]. As shown in Table 5a, reduced gene expression levels of *ERCC5* (OR = 2.32, 95% CI = 1.22–4.43), *ERCC6* (OR = 2.49, 95% CI = 1.28–4.84) and *XPC* (OR = 2.19, 95% CI = 1.15–4.18) was significantly associated with an increased risk of lung cancer whereas *ERCC1* and *ERCC3* were not associated with lung cancer risk [69]. They postulated that lower DNA repair capacity in lung cancer patients may be the consequence of low expression of the NER genes. Ratnasinghe et al. [70] investigated three *XRCC1* polymorphisms (Arg194Trp, Arg280His and Arg399Gln) and found that only Arg280His allele was associated with an increased risk of lung cancer (OR = 1.8, 95% CI = 1.0–3.4). Divine et al. [71] reported that possessing the Gln/Gln genotype in *XRCC1* Arg399Gln polymorphism was significantly associated with an increased risk of adenocarcinoma of the lung among non-Hispanic (OR = 3.25, 95% CI = 1.2–10.7) and Hispanic (OR = 1.40, 95% CI = 0.3–5.9) populations. This polymorphism was non-significantly associated with decreased risk of lung cancer in both African–Americans (OR = 0.6, 95% CI = 0.2–2.3) and Caucasians (OR = 0.6, 95% CI = 0.3–1.3) while the codon 194 polymorphism was associated with increased risk (OR = 2.3, 95% CI = 0.2–25) among African–Americans [72]. In that study, neither the cases nor the controls with the genotype Trp/Trp was observed among Caucasians. *XRCC3* Thr241Met polymorphism was not associated with the risk of lung cancer among Caucasian and African–Americans [73]. For *XPD* Asp312Asn polymorphism (Table 5b), the Asp/Asp genotype has been found to have almost twice the risk of lung cancer when the Asp/Asn+Asn/Asn combined genotype served as reference [74]. The lung cancer risk was moderately associated with *XPD* Lys715Gln polymorphism among Caucasians [75,73] while an OR was 1.03 among African–Americans [73]. Spitz et al. [75] reported that OR for variant Lys751Gln genotype was 1.36 (95% CI = 0.84–2.20). For individuals with homozygous for the variant genotype at either locus, a statistically significant risk was seen (OR = 1.84, 95% CI = 1.11–3.04).

Both reduced NER gene expression [69] and low DNA repair capacity [67,68] was associated with an increased risk of lung cancer while almost polymorphisms described here were not. As DNA repair capacity might be modified by the tumor burden, a causal relationship between lung cancer and DNA repair capacity could not be determined in case-control studies. It is important to investigate precisely the biological significance of the polymorphisms involved in DNA

Table 3
Lung cancer and NQO1 (C609T) polymorphism

Ethnicity	Cases/controls	OR (95% CI)	Cases (histology and smoking status)	Controls	Quality control of genotyping	Reference
Chinese	84/84	0.74 (0.36–1.50)[a]	Ad: 58%, Sq: 42% Smoker: 54%	Hospital Smoker: 54%	?	[26]
Caucasian	82/145	1.31 (0.66–2.58)	Ad: 12%, SCC: 18%, Sq: 34%,	Hospital	?	
	Ad	1.33 (0.31–5.68)	Other: 7%	Current smoker: 26%		
	Sq	0.65 (0.21–1.98)	Current smoker: 30%			
	SCC	3.80 (1.19–12.1)				[37]
Mexican–Americans	61/161	0.53 (0.29–0.96)	Ad: 32%, SCC: 13%, Sq: 18%, LCC: 11%, other: 29% Current smoker: 46%	Population Current smoker: 28%	?	
African–Americans	116/136	0.79 (0.47–1.33)	Ad: 36%, SCC: 11%, Sq: 38%, LCC: 6%, other: 9% Current smoker: 65%	Population Current smoker: 32%		[38]
Japanese	109/167	0.6 (0.4–1.1)[a]	Histology: not given	Population		
Caucasians	135/171	0.8 (0.4–1.5)	Smoking: not given	Smoking: not given	?	[39]
Hawaiian	83/102	0.6 (0.2–1.3)				
Chinese	100/95	0.75 (0.37–1.52)	Ad: 36%, Sq: 55%, other: 29%	Hospital	?	
	Ad	0.34 (0.14–0.81)	Current smoker: 79%	Current smoker: 86%		
	Sq	1.25 (0.52–3.03)				[40]
Caucasian	814/1123	1.00 (0.82–1.21)[a]	Ad: 48%, Sq: 27%, other: 25%	Population	?	
	Ad	0.88 (0.68–1.13)[a]	Current smoker: 40%	Current smoker: 19%		
	Sq	1.20 (0.88–1.63)[a]				[41]
Overall	1584/2184	0.85 (0.69–1.05)				

Smoker, prevalence of both current- and ex-smokers; Current smoker, prevalence of current-smokers; Ad, adenocarcinoma; SCC, small cell carcinoma; Sq, squamous cell carcinoma; LCC, large cell carcinoma. Variant homozygous genotype and hetetozygote vs. wild-type genotype. Adjusted ORs were shown in table unless otherwise specified.

[a] Crude OR.

tion of carcinogens and/or production of free radicals in or near the target cells.

2.2. DNA repair genes

2.2.1. ERCC and XRCC

Physiologically, the DNA repair capacity should be correlated with the level of proteins involved in DNA repair activity, which is controlled at the transcriptional level [59]. Therefore, it is conceivable that the baseline transcriptional level of DNA repair genes reflects a cellular ability to meet repair demand once the cells are stimulated by carcinogen exposure. One major DNA repair pathway capable of removing a variety of structurally unrelated DNA lesions, including those induced by tobacco carcinogens, is nucleotide excision repair (NER). This complex DNA repair process consists of approximately 30 proteins involved in sequential damage recognition, chromatin remodeling, incision of the damaged DNA strand on both sides of the lesion, excision of the oligonuleotide containing the damage and gap-filling DNA synthesis followed by strand ligation [50]. For example, smoking-related bulky adducts induced by benzo(*a*)pyrene or other PAHs and arylamines are removed effectively by the NER pathway [61]. In xeroderma pigmentosum (XP), patients have an extraordinarily higher rate of skin cancer because of a genetically determined defect in NER [59]. Other cancer-prone patients who have deficient DNA repair also have a higher rate of internal cancer [62].

Three rare, autosomal recessive inherited human disorders are associated with impaired NER activity: XP, Cockayne Syndrome (CS) and trichothiodystrophy (TTD) [63]. XP has been studied most extensively. Seven different human NER genes, which correct seven distinct genetic XP complementation groups (XPA, XPB, XPC, XPD, XPE, XPF and XPG) have been identified [63].

A number of genes that correct defective human NER have been designated as excision repair cross-complementing (*ERCC*) genes. The human gene responsible for XP groups B, D, F and G are identified as *ERCC3*, *ERCC2*, *ERCC4* and *ERCC5*, respectively. *ERCC1* has not been found to be involved in any XP, CS or TTD [64] because defects in *ERCC1* resulting from mutations or deletions of this cause early death before the disease develop [65]. *ERCC6* is identical to CSB and mutations in this gene are involved in CS [66]. Concerning DNA repair genes, 11 genes have been reported to date (nine NER genes (*ERCC1-6*, *XPA*, *XPE*, and *XPF*), a gene

Table 2
Lung cancer and *EH4* or *EH3/EH4* combined polymorphism

Ethnicity	Cases/control	OR (95% CI)	Reference
EH4 His–Arg polymorphism			
Caucasian	50/203	1.4 (0.1–13.3)[a]	[15]
Caucasian	150/172	1.22 (0.72–2.04)[b]	[22]
Chinese	76/122	2.10 (1.03–4.27)[b]	[23]
Caucasian	175/187	0.55 (0.33–0.91)	[24]
African–Americans	155/242	1.05 (0.45–2.49)[a]	[25]
Caucasian	182/458	0.63 (0.23–1.77)[a]	[25]
Chinese	84/84	1.06 (0.07–17.34)	[26]
African–Americans	78/72	0.9 (0.39–2.24)	[27]
Mexican–Americans	60/76	3.6 (1.26–10.42)	[27]
Overall	1010/1616	1.44 (1.03–2.00)	
EH3/EH4 combined genotypes			
Caucasian	50/203	1.9 (0.6–5.9)[c]	[15]
Caucasian	150/72	0.38 (0.19–0.75)[d]	[22]
Caucasian	175/187	0.68 (0.41–1.15)[d]	[24]
African–Americans	155/242	0.69 (0.36–1.30)[d]	[25]
Caucasian	182/458	0.15 (0.87–2.86)[d]	[25]
Taiwanese	132/259	1.03 (0.66–1.61)[c]	[28]
	Ad	1.54 (0.86–2.78)	[28]
	Sq	0.51 (0.27–0.96)	[28]
Caucasian	974/1142	1.0 (0.74–1.34)[e]	[29]
	Ad	0.96 (0.67–1.35)[e]	[29]
	Sq	0.72 (0.42–1.24)[e]	[29]
Overall	1818/2563	0.96 (0.68–1.34)	

Variant homozygous genotype vs. wild-type genotype. Adjusted ORs were shown in table unless otherwise specified.
[a] Crude OR.
[b] His/Arg+Arg/Arg vs. His/His genotype.
[c] Classification based on Smith and Harrison [15].
[d] Classification based on Benhamou et al. [22].
[e] Both His/His genotypes vs. other genotypes combined.

cancer or not although a non-significant protective overall OR of 0.85 (95% CI = 069–1.05) was shown (1584 lung cancer cases and 2184 controls).

NQO2 is a polymorphic gene that encodes an enzyme with similar activity to *NQO1*. *NQO2* might be more important than *NQO1* in determining lung cancer risk. As the role of NQO1 may be different among different histology and different ethnic groups, a larger study group is warranted to evaluate the effect of smoking amount on those parameters.

2.1.6. Myeloperoxidase (MPO)

Neutrophil recruitment into lung tissue occurs after exposure to variety of insults known to increase lung cancer risk, including tobacco smoke particles, infection, asbestos and ozone [42–44]. Following immunological and/or chemical insults, neutrophils release MPO and undergo a respiratory burst, which is characterized by a massive increase in oxygen consumption and a consequent NADPH-dependent production of superoxide and other free radicals [42]. MPO is present in the primary granules of neutrophils and catalyzes the production of the potent bacteriotoxic oxidizing agent hypochlorous acid (a one- and two-electron oxidant that can attack endogenous molecules including DNA) from hydroxyl radicals and chloride ions. A significant proportion (25–40%) of the hydrogen peroxide formed by activated neutrophils may be converted to hypochlorous acids [45,46]. MPO metabolically activates a wide range of tobacco smoke mutagens and environmental pollutants to DNA-damaging metabolites, including aromatic amines [47], the promutagenic derivatives of PAHs [48–50] and heterocyclic amines [51]. The *MPO* gene is located on chromosome 17. A G to A transition at position −463 in the promoter region of the *MPO* gene, which leads to the loss of a SP1 transcription binding site in an Alu hormone-responsive element [52], has been shown to reduce *MPO* mRNA expression [53]. It is possible that possession of two copies of the A allele of the *MPO* gene reduces the risk of lung. The wild-type G allele is present in 75% of Caucasians [54–56].

Although the five studies found material associations between *MPO* genotype and lung cancer risk, estimates of the nature and extent of the association varied considerably between studies (Table 4). Possession of the A/A genotype (8–10% of the population) was associated with a decreased risk of lung cancer in Caucasians (OR = 0.30, 95% CI = 0.10–0.93) and 39% reduction (not statistically significant) in African–Americans compared with those with two G alleles [54]. A second study [55] of populations with Caucasians, Japanese or Hawaiian ethnicity reported an overall 50% reduction in risk (95% CI = 0.2–1.3) for those with the A/A genotype compared with those with the G/G genotype. In a case-control study in the Berlin area [56], possession of one or two A alleles was suggested as being a protective factor for cancer of the lung (OR = 0.58, 95% CI = 0.38–0.88) and larynx (OR = 0.63, 95% CI = 0.43–0.92) but not for cancer of the pharynx (OR = 0.82, 95% CI = 0.57–1.17). A smaller Caucasian study [57] has reported an overall reduction in risk of 48% for those possessing the A allele (95% CI = 0.30–0.90). In a case-control study nested within a Finnish clinical trial [58], no evidence of an overall association between lung cancer risk and *MPO* genotype was observed, although the A/G or A/A genotype was associated with an increased risk of lung cancer among a subset of men of >64 years old (OR = 2.92, 95% CI = 1.33–6.43). The five case-control studies of lung cancer among eight ethnic groups and *MPO* genotype included 3014 subjects (1266 lung cancer cases and 1748 controls). The overall OR was 0.63 (95% CI = 0.45–0.87).

The consistency of agreement between these studies, except for Hawaiian analysis [55], and the overall OR form the meta-analysis, suggests an important role for *MPO* in lung cancer etiology, possibly through activa-

Table 1
Lung cancer and EH3 Tyr–His polymorphism

Ethnicity	Cases/controls	OR (95% CI)	Cases (histology and smoking status)	Controls (type of control and smoking status)	Quality control of genotyping	Reference
Caucasian	50/203	1.6 (0.6–4.8)	Histology: not given Smoker: 100%	Population Smoking: not given	Sequencing	[15]
Caucasian	150/172	0.50 (0.26–1.04)	Sq: 65%, SCC: 35% Current smoker: 100%	Hospital Current smoker: 100%	?	[22]
Chinese	76/122	1.76 (0.80–3.86)[a]	Ad: 50%, SCC: 24%, Sq: 18% Smoker: 20/56, unknown: 20/76	Population Smoking: not given	?	[23]
Caucasian	175/187	0.44 (0.27–0.71)	Ad: 26%, SCC: 32%, Sq: 34%, LCC: 8% Current smoker: 98%	Population current smoker: 100%	?	[24]
African–Americans	155/242	0.08 (0.01–0.62)	Histology: not given	Population		
Caucasian	182/458	0.99 (0.46–2.14)	Smoker: 95% for all cases	Smoker: 66% for all controls	?	[25]
Chinese	84/84	1.71 (0.65–4.54)[a]	Ad: 58%, Sq: 42%	Hospital	?	
	Ad	1.38 (0.57–3.49)[a]	Smoker: 54%	Smoker: 54%		
	Sq	3.1 (0.94–13.27)[a]				[26]
African–Americans	78/72	2.0 (0.81–5.17)	Histology: not given Current smoker: 62%	Population Current smoker: 32%	?	
Mexican–Americans	60/76	1.5 (1.64–3.74)	Histology: not given Current smoker: 52%	Population Current smoker: 32%		[27]
Taiwanese	132/259	Not determined	Ad: 52%, Sq: 42%, Other: 6% Smoker: 57%	Hospital Smokers: 33%	?	[28]
Caucasian	974/1142	Not determined	Ad: 44%, Sq: 23%, Other: 18%, unknown: 15% Current smokers: 41%	Population Current smokers: 19%	?	[29]
Overall	1010/1616	0.96 (0.66–1.39)				

Smoker, prevalence of both current- and ex-smokers; Current smoker, prevalence of current-smokers; Ad, adenocarcinoma; SCC, small cell carcinoma; Sq, squamous cell carcinoma; LCC, large cell carcinoma. Variant homozygous genotype vs. wild-type genotype. Adjusted ORs were shown in table unless otherwise specified.

[a] Crude OR.

involved in both metabolic activation and detoxification of carcinogenic agents that could be involved in lung carcinogenesis. The *NQO1* gene is located on chromosome 16q22. Recently, a polymorphism of the gene encoding *NQO1* has been described. The polymorphic variant of the gene (a C to T transition at base pair 609, codon 187) is associated with reduced NQO1 activity [32–35]. The three genotypes of this gene are the homozygous wild-type C/C (normal activity), the heterozygous C/T genotype (mild activity) and the homozygous rare allele T/T genotype (2–4% of normal activity).

There have been several studies examining the relationship between the *NOQ1* polymorphism and lung cancer risk, but the conclusions have been contradictory. Rosvold et al. [36] reported that the rare allele was observed to be approximately twice as common in Caucasian cases as in controls. Lewis et al. [37] also reported that the variant allele was associated with a non-significant increased risk of lung cancer and a significant increased risk (OR = 3.80, 95% CI = 1.19–12.1) of small cell lung cancer. They concluded that the variant *NQO1* allele may play in activating genotoxins. In contrast, some studies [25,38–40] have shown that the allele is inversely associated with lung cancer risk (Table 3). Recently, a large study showed that no difference for the genotype distribution between lung cancer cases and controls [41]. However, there was a gene–environment interaction between the genotype and smoking: current smokers with the T/T genotype had a smaller cancer risk that those with C/C genotype (OR = 0.38, 95% CI = 0.19–1.00).

Therefore, it is still unclear whether variant *NQO1* genotype is associated with a decreased risk of lung