# BAX G(-248)A Gene Polymorphism and Its Association with Risk of Non-Small Cell Lung Cancer—A Case Control Study

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## Abstract

**Pro-apoptotic Bcl-2 protein BAX is an important member of mitochondrial dependent apoptosis regulation and ultimately plays a pivotal role in malignancies. A promoter G(-248)A polymorphism in the TP53 binding region of BAX results in differential binding capacity of TP53 protein there by regulating its expression, which has been found to be associated with different clinical outcomes in various malignancies. Presently we aimed to analyze the possible impact of the BAX G(-248)A polymorphism on the risk and other clinical features of non-small cell lung cancer in Indian population. The BAX promoter polymorphism was analyzed in blood samples of 320 subjects with 1:1 case/control ratio by primer-introduced restriction analysis PCR and survival curves were plotted using Kaplan-Meier analysis. It was observed that more than 3-fold increased risk of developing non-small cell lung cancer was associated with homozygous AA genotype of BAX G(-248)A promoter polymorphism in Indian population, with more predominant in smokers with pack-year > 45 (heavy) and using cigarette or huka as their smoking source than homozygous GG genotype. Significant associations was observed between TNM stage ($p$ = 0.037) and histological type (0.02), of non-small cell lung cancer patients with the polymorphism. Patients homozygous for A allele exhibited a significant poor overall survival compared with patients displaying GA + AA or GA or GG genotype [median survival 6.0 vs 9.0, 11.0, and 30.0 months, respectively ($p$ < 0.0001)]. Adenocarcinoma and advanced stage patients with AA genotype showed lower median survival time than squamous cell carcinoma and early stage non-small cell lung cancer patients (median 3.0 and 5.0 vs 8.0 and 9.0 months, respectively). We conclude that the genetic polymorphism G(-248)A in the TP53 binding promoter region of pro-apoptotic genes BAX may contribute to the risk of developing non-small cell lung cancer in Indian population and also may be an important factor for adverse clinical outcome for patients with non-small cell lung cancer.**

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## Keywords

BAX G(-248)A Polymorphism, PIRA-PCR, NSCLC Risk

## 1. Introduction

Lung cancer has become one of the most common malignancies worldwide and in India. It is the leading cause of cancer-related deaths [1] [2]. Majority of lung cancer patients are diagnosed as non-small cell type including squamous cell, adenocarcinoma and large cell lung carcinoma as the three major subtypes with 5-year survival rate of 11 to 15 percent overall, and less than 5 percent at stage IIIB/IV [3]. Exposure to smoke and environmental derived pro-carcinogens are the established risk factors for lung cancer wherein smoking accounts for 80% to 90% of cases among men and 55% to 80% of cases among women [4]. Occupational exposures in industrial facilities account for an additional 9% to 15% of lung cancer cases [5]. However, only a small fraction of smokers and workers in high-risk occupations develop this disease. This suggests that other causes, including genetic susceptibility, may contribute to the variation in individual lung cancer risk. Despite the development of new chemotherapeutic drugs and multimodal treatment strategies, the survival rate of non-small cell lung cancer remains unchanged and poor. Identification of new prognostic markers for the characterization of lung cancer biology may be helpful, as they could serve as a basis for predicting response to therapy at a molecular level [6].

Apoptosis also known as programmed cell death is an ordered and orchestrated cellular process that regulates cell death under normal physiological as well as pathological conditions [7]. Apoptosis and cell cycle progression are two intimately linked phenomena and uncontrollable cell proliferation perturbs the cellular homeostasis which may lead to malignancies. The Bcl-2 family of proteins, comprising of pro and anti-apoptotic proteins, plays a pivotal role in the regulation of apoptosis, especially via the intrinsic pathway [8]. One of the important regulators included pro-apoptotic Bcl2 associated X protein (BAX) encoded by BAX gene located on chr 19q13.3 consisting of 6 exons and 5 intervening introns. The BAX gene encodes a 21 kDa protein, named BAX-alpha, which hetero-dimerizes either with members of the BCL2 family of proteins or with tyrosine kinases for its pro-apoptotic function. Single-nucleotide polymorphism located within the 5' un-translated region of the BAX promoter -248 G/A (rs 4645878), was reported to correlate with reduced BAX expression [9] [10] and was found to be associated with various malignancies [9] [11]-[17]. In the present study, we hypothesized that BAX G(-248)A polymorphism may also be associated with risk and adverse clinical outcome of NSCLC in Indian population.

## 2. Materials and Methods

### 2.1. Study Population

Present study was approved by Institutional ethics committee of Maulana Azad Medical College & associated hospitals, New Delhi. 160 peripheral blood samples from newly diagnosed as well as therapy received NSCLC patients and gender-age (±5 years) matched 160 cancer free healthy controls were included. Patients with a history of previous cancer or metastasized cancer from other organs except lung were excluded. Both cases as well as controls belonging to north India. All subjects received a detailed description of this study and provided written informed consent. Patient follow up was obtained through of hospital records as well as by direct patient contact. The distribution of the selected characteristics for those with NSCLC and the controls are summarized in **Table 1**. Tumor stage and grade of NSCLC patients were determined using 7th edition of the Lung cancer staging system (2009) and similarities between cases and controls were maintained with respect to characteristics like age, sex, or smoking history.

### 2.2. Genotype Analysis

Blood sample (3 - 5 mL) from each participant was drawn into an EDTA vial and genomic DNA was extracted following manufacturer's protocol of DNA sure blood mini kit (Genetix Biotech Asia Pvt. Ltd, India). Primer-introduced restriction analysis (PIRA)-polymerase chain reaction (PCR) assay (18) was used to determine the G(-248)A polymorphism (rs4645878) in the promoter region of BAX gene. PIRA-PCR method is usually used where a variant or mutation does not create a new restriction site, it may be possible to perform PCR by using a

**Table 1.** Distribution of selected characteristics among the study population.

| Variable | NSCLC patients (%) | Healthy controls (%) |
|---|---|---|
| Total No. | 160 | 160 |
| **Race** | North Indians | North Indians |
| **Gender** | | |
| Males | 124 (77.5) | 124 (77.5) |
| Females | 36 (22.5) | 36 (22.5) |
| **Age at diagnosis (years )** | | |
| ≤45 Y | 32 (20.0) | 32 (20.0) |
| >45 Y | 128 (80.0) | 128 (80.0) |
| Mean ± SD age (Y) | 57.32 ± 11.9 (range, 30 - 85 years) | 56.02 ± 10.6 (range, 32 - 80 years) |
| **Smoking status** | | |
| Non smoker | 47 (29.4) | 47 (29.4) |
| Smokers | 113 (70.6) | 113 (70.6) |
| Current smokers | 48 (42.5) | 48 (42.5) |
| Ex-smokers | 65 (57.5) | 65 (57.5) |
| **Smoking level (pack year)** | | |
| Mild (≤10) | 18 (15.9) | 18 (15.9) |
| Moderate (≤40) | 49 (43.4) | 49 (43.4) |
| Heavy (>40) | 46 (40.7) | 46 (40.7) |
| **Smoking type** | | |
| Cigarette | 33 (29.2) | 33 (29.2) |
| Bidi | 16 (14.2) | 16 (14.2) |
| Huka | 27 (23.9) | 27 (23.9) |
| Cigarette + Bidi | 15 (13.3) | 15 (13.3) |
| Cigarette + Huka | 22 (19.5) | 22 (19.5) |
| **Histological type** | | |
| Squamous cell carcinoma | 79 (49.4) | |
| Adenocarcinoma | 81 (50.6) | |
| **TNM stage** | | |
| Early (I & II) | 60 (37.5) | |
| Advanced (III & IV) | 100 (62.5) | |
| **Distant metastases** | | |
| Positive | 42 (26.2) | |
| Negative | 118 (73.8) | |
| **Histological grades** | | |
| **Squamous cell carcinoma grades** | | |
| Grade 1 | 43 (54.4) | |
| Grade 2 | 22 (27.8) | |
| Grade 3 | 14 (17.7) | |
| **Adenocarcinoma grade** | | |
| Grade 1 | 14 (17.3) | |
| Grade 2 | 23 (27.2) | |
| Grade 3 | 45 (55.5) | |
| **Family history of any cancer** | | |
| Significant | 19 (11.9) | |
| Non significant | 141 (88.1) | |

primer containing a mismatch with the target DNA, such that the same base substitution is introduced into both normal and mutated sequences but a restriction site is formed only in the PCR product derived from the variant allele. The method introduces an artificial restriction site into a PCR product by the use of a primer with a single-base mismatch usually introduced near the 3' end of the primer that is close to the nucleotide change of interest. In the present study the mismatch (-2 G-to-C) was present in the sense primer shown in **Table 2** as underlined. The mismatch introduced an artificial restriction site for *MSP* I restriction enzyme in the PCR product which resulted in two band sizes (89 bp + 20 bp) when G allele is present at BAX (-248, rs 4645878) position.

PCR was performed in a final volume of 50 μL containing 50 ng genomic DNA, 5 μL of 10× polymerase buffer (200 mM Tris-HCl, pH 8.0; 500 mM KCl), 3 μL of 20 mM MgCl, 5 μL of 10 mM dNTPs, 0.5 μL of 5 U Taq polymerase (Genei, Bangalore) and 0.5 μL of 25 pmol/L of sense and anti-sense primers (**Table 2**) and the final volume was adjusted by nuclease free ddH$_2$O. PCR program was started with an initial denaturation at 95˚C for 5 minutes, followed by 40 cycles of denaturation at 95˚C for 30 s, annealing at 60˚C for 45 s, extension at 72˚C for 45 s, and completed with a final elongation step at 72˚C for 5 minutes. PCR product of 109 bp (10 μL) was verified by agarose gel electrophoresis (**Figure 1(a)**) and the remaining PCR product was then digested by the fast digest restriction enzyme MSPI (Fermentas, USA) at 37˚C for 15 min. The genotypes were assessed as follows: a single 109 bp fragment for homozygous AA genotype, two fragments of 89 bp and 20 bp for homozygous GG genotype and three fragments of 109 bp, 89 bp and 20 bp for the heterozygous GA genotype (**Figure 1(b)**). Negative control with ddH$_2$O instead of DNA template was included in each PCR run. A blind case/control analysis was performed with approximately more than 10% random samples were selected for confirmation and the results were 100% concordant.

## 2.3. Sequence Analysis

The results of the PIRA-PCR analysis were confirmed by sequencing of representative samples for each genotype (**Figure 1(c)**). PCR products were purified using NucleoSpin PCR Clean-up kit (Macherey-Nagel, Germany) and sequenced on 3730 × l DNA Analyzer (Life Technologies Corporation, California, USA) according to the manufacturer's instructions. The sequences were compared with BAX data in the GenBank (accession number U17193 for promoter).

## 2.4. Statistical Analysis

Follow up was calculated as per hospital records from the date of diagnosis to the death or till the end of the study and survival analysis was estimated using Kaplan-Meier plots and log-rank (Mantel-Cox) test. Statistical analysis was performed using the Graph Pad Prism 6.0 or SPSS 16.0 software. Assessment of the correlations between genetic carrier status and BAX polymorphism was carried out using the Chi-Squaredor Fisher Exact test. BAX variants and risk of NSCLC were estimated by computing the odds ratios (ORs), risk ratio (RR) and risk difference (RD) with 95% confidence intervals (CIs). Allele frequencies among cases as well as controls were evaluated by using the Chi-square Hardy-Weinberg equilibrium test. A *p* value < 0.05 was considered significant.

## 3. Results

### 3.1. Genotype Distribution

The observed genotype frequencies of BAX (GG, GA and AA) were statistically different among patients and healthy controls (Chi square = 15.1, *p* = 0.0005). In addition cases showed higher A allele frequency (fA = 0.43 vs 0.28) whereas controls represented with higher G allele frequency (fG = 0.72 vs 0.57) **Table 3**.

**Table 2.** Conditions of genotyping assay for the BAX (-248 G > A) polymorphism.

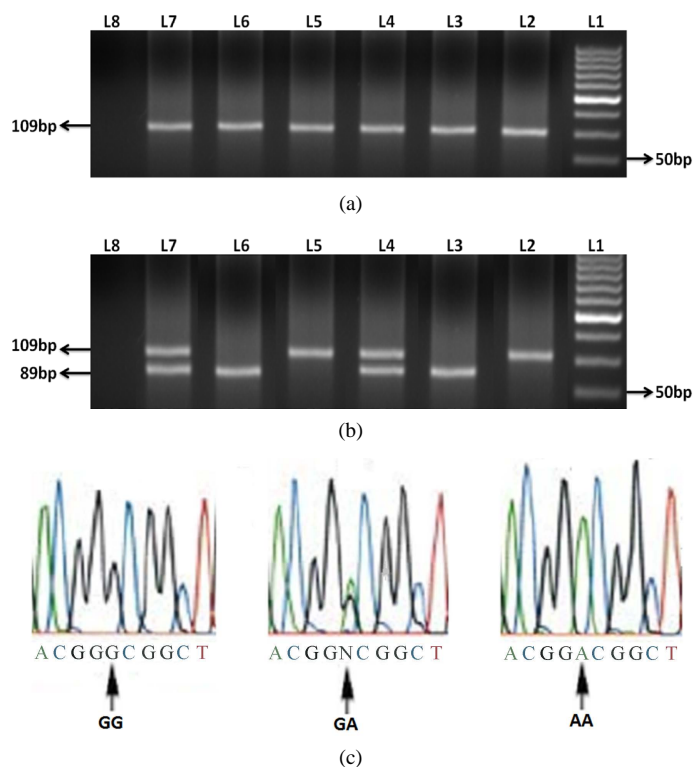| Gene polymorphism | Genotyping | Primers [18] | Restriction enzyme | Gel band pattern |
|---|---|---|---|---|
| BAX (-248 G > A rs4645878) | PIRA-PCR: mismatch, sense primer -2 G-to-C | '5-CATTAGAGCTGCGATTGGAC**C**G-3' (sense) '5-GCTCCCTCGGGAGGTTTGGT-3' (anti-sense) | *Msp* I | **A allele:** 109 bp **G allele:** 89 bp +20 bp |

**Figure 1.** PIRA-PCR analysis and sequencing of PCR products: (a) PCR amplification of BAX (-248 G > A) showing band size of 109 bp (L2-L7) (b) MSPI restriction digestion of PCR product with BAX (-248 G > A) polymorphic sites, L2, L5-homozygous AA genotype, L3, L6-homozygous GG genotype, L4, L7-heterozygous GA genotype and L8-negative control. [*]20 bp fragment not visible due to over run. (c) Sequence of the BAX promoter region showing different genotypes at -248 position indicated with the arrows.

**Table 3.** Genotype frequencies of BAX (-248 G/A) among NSCLC cases and controls.

| Genotype | GG genotype (%) | GA genotype (%) | AA genotype (%) | G allele frequency | A allele frequency | Chi-square | *p* value |
|---|---|---|---|---|---|---|---|
| Patients (n = 160) | 70 (43.8) | 43 (26.8) | 47 (29.4) | 0.57 | 0.43 | 15.1 | 0.0005 |
| Controls (n = 160) | 90 (56.2) | 51 (31.9) | 19 (11.9) | 0.72 | 0.28 | | |

## 3.2. BAX (-248 G/A) Polymorphism and Risk of NSCLC

A multivariate analysis based on logistic regression like odds ratio, risk ratio and risk difference with 95% confidence intervals were calculated for each group to estimate the association between the BAX (-248 G/A) genotypes and risk of NSCLC in Indian patients (**Table 4**). It was found that an increased risk of NSCLC was associated with the BAX (A) allele in an allele dosage-dependent manner. Compared to the GG genotype, odds ratio, risk ratio and risk difference for the heterozygous GA and homozygous AA genotypes were OR = 1.08 (0.65 - 1.80) RR = 1.04 (0.82 - 1.30) RD = 1.9 (−10.8 - 14.7) and OR = 3.18 (1.71 - 5.89) RR = 1.95 (1.31 - 2.92) RD = 27.4 (14.1 - 40.8) respectively, suggesting a possible dominant of this polymorphism with 3.2 fold increased risk associated with homozygous AA genotype. Upon comparison with the subtypes of NSCLC significantly increased risks were observed in squamous-cell carcinoma patients, OR = 5.26 (2.6 - 10.8) RR = 1.98 (1.4 - 2.9), and RD = 38.1 (22.5 - 53.8) **Table 5**. The increased risk associated with the BAX polymorphism appeared to be more pronounced in male cases and who were >45 years at diagnosis (OR 4.3 (2.09 - 8.91) and 5.4 (2.5 - 11.8 respectively) for the AA genotype (**Table 5**).

**Table 4.** Risk of NSCLC associated with the BAX G(-248)A genotypes.

| Genotypes | Control (n = 160) | Cases (n = 160) | OR* (95% CI) | RR# | RD^ | *p* value |
|---|---|---|---|---|---|---|
| GG (ref) | 90 | 70 | 1 | 1 | | |
| AA | 19 | 47 | 3.18 (1.71 - 5.89) | 1.95 (1.31 - 2.92) | 27.4 (14.1 - 40.8) | 0.0001 |
| GA | 51 | 43 | 1.08 (0.65 - 1.80) | 1.04 (0.82 - 1.30) | 1.9 (−10.7 - 14.7) | 0.75 |
| GA + AA | 70 | 90 | 1.65 (1.06 - 2.57) | 1.28 (1.09 - 1.60) | 12.5 (1.63 - 23.4) | 0.02 |

*OR: Odd ratio; #RR: Risk ratio; ^RD: Risk difference.

**Table 5.** Histological prospective of NSCLC risk associated with the BAX G (-248)A genotypes.

| Genotypes | GG (ref) | AA | GA |
|---|---|---|---|
| Control (n = 160 ) | 90 | 19 | 51 |
| Adenocarcinoma (n = 81) | 43 | 17 | 21 |
| Odd ratio (95% CI) | 1.0 | 1.9 (0.90 - 4.0), *p* = 0.098 | 0.86 (0.5 - 1.6), *p* = 0.64 |
| Squamous cell carcinoma (n = 79) | 27 | 30 | 22 |
| Odd ratio (95% CI) | 1.0 | 5.26 (2.6 - 10.8), *p* = 0.0000 | 1.44 (0.74 - 2.8), *p* = 0.27 |

## 3.3. BAX G(-248)A Genotypes and Clinical Characteristics

Among the lung cancer patients, 81 (50.6%) were diagnosed with adenocarcinoma and 79 (49.4%) with squamous-cell carcinoma. Majority of the cases were in advanced TNM stage 100 (62.5%) including more than 26% of patients with metastasis to distant organs. Grade 1 SCC 43 (54.4%) and grade 3 differentiated ADC 45 (55.5%) represented a major portion in histological grade of NSCLC cases.

Clinicopathological characteristics including histological type (*p* = 0.02), TNM Stage (*p* 0.03) and first degree family history of any cancer (*p* = 0.05) showed statistically significant associations with respect to BAX G(-248)A genotypes. SCC patients represented higher A allele frequency (0.52 vs 0.34) whereas higher G allele frequency was observed in ADC patients (0.66 vs 0.48). In addition higher prevalence of f(A) was among cases with advanced stage (0.49 vs 0.33) as well as in cases with metastasis to distant organs (0.52 vs 0.39) like brain, bone or adrenal etc. Cytological differentiation among ADC as well as SCC showed a continuous increasing trend of BAX A allele frequency from grade 1 to grade 3 differentiations. In relation to parameters like age and gender the BAX promoter polymorphism doesn't presented any significant correlation, however, males and patients of >45 years showed higher f(A) allele (**Table 6**).

## 3.4. Gene—Smoking Interaction

Healthy controls with similar smoking history as those of patients were included in the study in order to find the genetic susceptibility and risk of NSCLC in relation to BAX promoter polymorphism. Smokers constitute more than 70 percent, wherein bidi (61.3%) and huka (62.3%) were the major smoking source and about 57% had left smoking from more than 6 months among both cases as well as controls (**Table 1**). We observed that although an increased risk of NSCLC cancer was associated with BAX AA genotype in both smokers and nonsmokers, the OR for the AA genotype in smokers OR 4.3 (2.04 - 9.17, *p* = 0.0000) was significantly higher than those of nonsmokers OR 1.5 (0.49 - 4.58, *p* = 0.48). Moreover, when the risk associated with the BAX polymorphism was further evaluated within the strata of pack-years smoked, risk of development of NSCLC was increasing as the number of pack-years increases (Mild; OR 1.5, Moderate; OR 5.5 and Heavy; OR 7.2). In addition interaction between susceptible genotypes and source of smoking was mostly evident among cigarette (OR 6.7, CI: 1.6 - 28.9) and huka smokers (OR 6.5, CI: 1.1 - 36.6) (**Table 7**).

## 3.5. BAX (-248 G/A) Genotypes and Clinical Outcome of NSCLC Patients

Survival analyses were based on 160 NSCLC patients with mean follow-up time of 11.95 months (median 10.50; range, 0.5 - 127.5 months) for the overall survival. Out of 160 NSCLC patients, 87 (54.4%) died with mean

**Table 6.** BAX (-248 G/A) polymorphism with different clinical characteristics of NSCLC patients.

| Variables | GG genotype (%) | GA genotype (%) | AA genotype (%) | G allele frequency | A allele frequency | *p* value |
|---|---|---|---|---|---|---|
| **Gender** | | | | | | |
| Males | 50 (40.3) | 35 (28.2) | 39 (31.5) | 0.54 | 0.46 | 0.26 |
| Females | 20 (55.6) | 8 (22.2) | 8 (22.2) | 0.67 | 0.33 | |
| **Age at diagnosis (years)** | | | | | | |
| ≤45 Y | 16 (50.0) | 7 (21.9) | 9 (28.1) | 0.61 | 0.39 | 0.68 |
| >45 Y | 54 (42.2) | 36 (28.1) | 38 (29.7) | 0.56 | 0.44 | |
| **Histological type** | | | | | | |
| Squamous cell carcinoma | 27 (34.2) | 22 (27.8) | 30 (38.0) | 0.48 | 0.52 | 0.02 |
| Adenocarcinoma | 43 (53.1) | 21 (25.9) | 17 (21.0) | 0.66 | 0.34 | |
| **TNM stage** | | | | | | |
| Early stage (I & II) | 34 (56.6) | 13 (21.7) | 13 (21.7) | 0.68 | 0.33 | 0.03 |
| Advanced stage (III & IV) | 36 (36.0) | 30 (30.0) | 34 (34.0) | 0.51 | 0.49 | |
| **Distant metastases** | | | | | | |
| Positive | 15 (35.7) | 10 (23.8) | 17 (40.5) | 0.48 | 0.52 | 0.14 |
| Negative | 55 (46.6) | 33 (28.0) | 30 (25.4) | 0.61 | 0.39 | |
| **Squamous cell carcinoma grades** | | | | | | |
| Grade 1 | 17 (39.5) | 11 (25.6) | 15 (34.9) | 0.52 | 0.48 | 0.15 |
| Grade 2 | 7 (31.8) | 9 (40.9) | 6 (27.3) | 0.52 | 0.48 | |
| Grade 3 | 3 (21.4) | 2 (14.3) | 9 (64.3) | 0.29 | 0.71 | |
| **Adenocarcinoma grades** | | | | | | |
| Grade 1 | 11 (78.6) | 1 (7.1) | 2 (14.3) | 0.82 | 0.18 | 0.18 |
| Grade 2 | 12 (54.5) | 7 (31.8) | 3 (13.6) | 0.70 | 0.30 | |
| Grade 3 | 20 (44.4) | 13 (28.9) | 12 (26.7) | 0.59 | 0.41 | |
| **Family history of any cancer** | | | | | | |
| Yes | 13 (68.4) | 3 (15.8) | 3 (15.8) | 0.76 | 0.24 | 0.05 |
| No | 57 (40.4) | 40 (28.4) | 44 (31.2) | 0.55 | 0.45 | |

follow up time of 10.46 months ( median 10.00; range, 0.5 - 40.0 months) and for the patients who survived the follow up period (censored patients), the follow up time was 14.24 months (median 13.00; range, 0.5 - 127.5 months). Patients homozygous for A allele exhibited a significant poor overall survival ($p < 0.0001$) **Figure 2(a)**, **Figure 2(b)**. Median survival time for patients with BAX (-248) GG, GA, GA + AA and AA genotype were 30.0, 11.0, 9.0 and 6.0 months respectively. Significant poor overall survival was also observed in patients presented A allele homology with ADC ($p < 0.0001$) or SCC ($p < 0.0001$) histology (**Figure 2(c)**, **Figure 2(d)**) as well as with early ($p < 0.0001$) or advanced ($p < 0.0001$) TNM stage (**Figure 2(e)**, **Figure 2(f)**). In addition patients of ADC and advanced stage with AA genotype showed lower median survival time than SCC and Early stage NSCLC patients (Median 3.0 and 5.0 vs 8.0 and 9.0 months respectively).

## 4. Discussion

Low expression of BAX has been found to be associated with poor prognosis and reduced chemotherapy response in NSCLC patients especially with non-squamous histology [19] [20]. BAX transcription is regulated by p53 protein possessing a potent transcriptional activation domain at its amino-terminus linked to a central core domain that mediates sequence-specific direct DNA binding to the TP53-binding element in the promoter of

**Table 7.** Risk of NSCLC associated with the BAX G(-248)A polymorphism by potential risk factors in the Indian population.

| Variables | GG controls/cases | AA controls/cases | OR (95% CI) | *p* value |
|---|---|---|---|---|
| **Gender** | | | | |
| Males | 72/50 | 13/39 | 4.3 (2.09 - 8.91) | <0.0001 |
| Females | 18/20 | 6/8 | 1.2 (0.35 - 4.13) | 0.77 |
| **Age at diagnosis (years)** | | | | |
| ≤45 Y | 13/16 | 9/9 | 0.8 (0.25 - 2.6) | 0.72 |
| >45 Y | 77/54 | 10/38 | 5.4 (2.5 - 11.8) | <0.0001 |
| **Smoking status** | | | | |
| Non smokers | 24/23 | 7/10 | 1.5 (0.49 - 4.58) | 0.48 |
| Smokers | 66/47 | 12/37 | 4.3 (2.04 - 9.17) | <0.0001 |
| **Smoking level (pack year)** | | | | |
| Mild (≤10) | 11/7 | 5/5 | 1.5 (0.33 - 7.5) | 0.50 |
| Moderate (≤40) | 32/23 | 4/16 | 5.5 (1.6 - 18.8) | 0.001 |
| Heavy (>40) | 23/17 | 3/16 | 7.2 (1.8 - 28.8) | 0.04 |
| **Smoking type** | | | | |
| Cigarette | 22/12 | 3/11 | 6.7 (1.6 - 28.9) | 0.006 |
| Bidi | 6/9 | 3/5 | 1.1 (0.19 - 6.5) | 0.9 |
| Huka | 13/10 | 2/10 | 6.5 (1.1 - 36.6) | 0.02 |
| Cigarette + Bidi | 9/5 | 2/6 | 5.4 (0.78 - 37.5) | 0.07 |
| Cigarette + Huka | 16/11 | 2/5 | 3.6 (0.5 - 22.2) | 0.14 |

BAX gene and induces BAX expression [21] [22]. A polymorphism in the p53 binding element of BAX gene G(-248)A has been reported to effect the transcription of BAX gene [9] [10]. The present data explored the association of BAX G(-248)A polymorphism in the TP53 binding region with risk and prognosis of non-small cell lung cancer.

Several studies have reported that the SNP located within the untranslated region of the BAX promoter (-248 G/A) was associated with reduced expression of BAX and has been observed significantly lower among BAX (-248)AA genotype than GG genotypes carriers [9] [10]. Clinical studies on lung cancer revealed that low BAX expression was associated with unfavorable clinical outcomes and poor survival [20] [23] [24]. Our study, the first report from Indian population, suggested that homozygous BAX (-248)A allele is strongly associated with the risk of developing NSCLC particularly squamous cell carcinoma with approximately more than 5 fold increase than homozygous BAX (-248)G genotype. BAX (-248)A allele homology was also been found to be an independent prognostic marker of unfavorable clinical outcome with AA genotype at higher risk for death than GG genotype of NSCLC patients.

Histopathological correlations of BAX expression profile and NSCLC in previous studies revealed low BAX expression among patients of squamous cell carcinoma [19] [20] [23]. Present data of BAX polymorphism, shows a close correlation with the previous BAX expression studies, where in frequency of BAX (-248)AA genotype was more in SCC whereas BAX (-248)GG genotype was more among adenocarcinoma patients. A strong correlation between smoking history and low BAX expression was observed in NSCLC patients especially SCC [19] also high BAX (-248)AA frequencies were presented by our smoker patients. In addition increase in BAX (-248)AA frequencies among smokers were concordant with the increase in level of smoke exposure resulting ultimately higher risk of NSCLC among heavy smokers.

In addition we observed as the frequency BAX A allele among patients increases their occurs a transformation of cytological grade from well to poor differentiation in both SCC as well as ADC groups. Poorly differentiated NSCLC tumors showed lower BAX protein levels [25]. Low BAX expression lead to shorter overall survival in
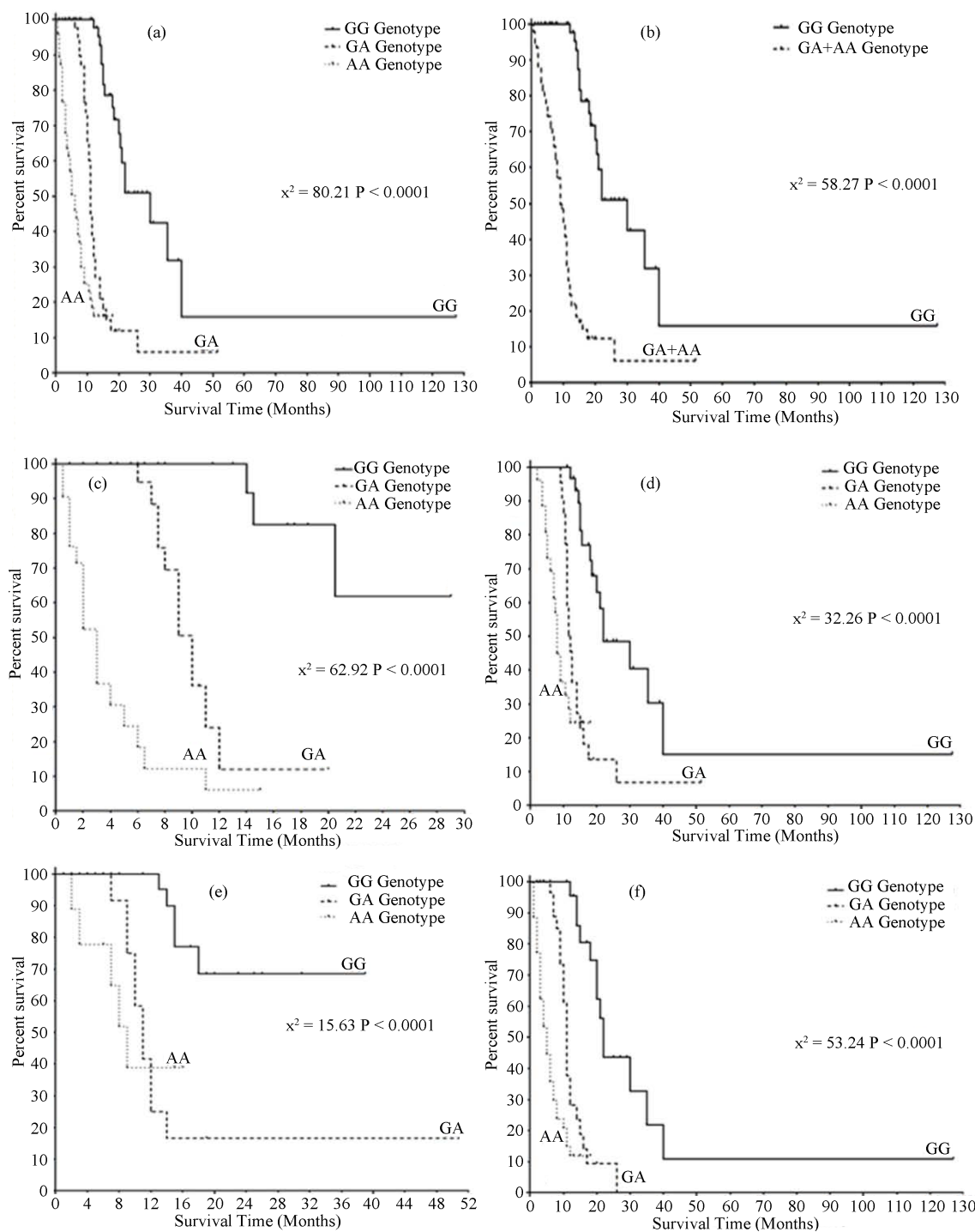
**Figure 2.** Kaplan-Meier survival analysis of NSCLC patients with respect to BAX G(-248)A polymorphism: (a), (b) Overall survival (c), (d) Survival of ADC and SCC patients, (e), (f) Survival of Early and advanced stage.

various malignancies including NSCLC [9] [19] [26] presently shorter overall survival was significantly associated with BAX (-248)AA genotype among both SCC or ADC as well as Early or Advanced stage patients. The poor outcome of the patients with BAX (-248)AA may be due its weak interaction with TP53 protein altering the transcription of BAX gene.

In relation to the role of BAX in treatment response, it has been found that over expression of BAX sensitizes

breast cancer MCF-7 cells to cisplatin and etoposide [27], reduced BAX protein was found in cisplatin resistant lung cancer cell lines [28] and increased BAX expression was observed in breast cancer cell lines following treatment with cyclophosphamide [29]. Also BAX was found to be an independent predictive factor of cisplatin response in oral cancer [30]. In case of esophageal cancer poor survival of patients with cisplatin based therapy was associated with low BAX expression [24]. Thus, genotyping ofthe BAX G(-248)A in NSCLC may be helpful to identify candidate patients for novel treatment strategies.

## 5. Conclusion

In conclusion, present study demonstrates that the promoter G(-248) A polymorphism of BAX gene may contribute to the risk of developing non-small cell lung cancer in Indian population. In addition, it may be responsible for adverse clinical outcome and differential treatment response by affecting TP53 regulation of BAX transcription in non-small cell lung cancer patients. Current findings are limited due to small sample size in the strata and results need to validate by large and preferably prospective studies on the BAX promoter polymorphism with its expression profile of NSCLC patients.

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## Abbreviations

BAX: Bcl2 associated X protein;
SCC: squamous cell carcinoma;
ADC: adenocarcinoma;
NSCLC: non-small cell lung cancer;
PIRA-PCR: primer-introduced restriction analysis PCR.