Early onset lung cancer, cigarette smoking and the SNP309 of the murine double minute-2 (MDM2) gene

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Abstract

The polymorphism SNP309 (rs2279744) in the promoter region of the MDM2 gene has been shown to alter protein expression and may play a role in the susceptibility to lung cancer. The MDM2 protein is a key inhibitor of p53 and several mechanisms of MDM2/p53 interactions are presently known: modulating DNA-repair, cell-cycle control, cell growth and apoptosis.
We used 635 Caucasian patients diagnosed with lung cancer before 51 years of age and 1300 healthy gender and age frequency matched population Caucasian controls to investigate the association between the MDM2 SNP309 and the risk of developing early onset lung cancer. Conditional logistic models were applied to assess the genotype-phenotype association, adjusted for smoking.

Compared to the GG genotype, the adjusted ORs for the TG and TT genotype were 0.9 (95% CI: 0.7–1.5) and 1.0 (95% CI: 0.7–1.5), respectively. Also no association was found for histological subtypes of lung cancer. The strength of this study is that within young cases the genetic component to develop lung cancer may be greater. Our results indicate that the MDM2 SNP309 is not significantly associated with lung carcinogenesis but point towards gender-specific differences.

Background
Human cells have developed a complex system to protect themselves from genotoxic damage where the p53 tumor suppressor gene plays an important role in protecting against such insults by serving as an integrator of the signals produced by DNA damage. The MDM2 protein inhibits p53 transactivation activity and promotes its export from the nucleus by binding to the transcriptional activation domain of p53 [1] and acts as an ubiquitin ligase upon p53 pushing fast degradation of the suppressor [2,3].

Recently, a SNP (SNP309, G2580T) at the intronic p53-response promoter of MDM2 was identified and associated with altered Sp1 binding affinity and expression levels of MDM2 RNA and protein [4]. Contradictory results were reported regarding the MDM2 SNP309 association with lung cancer [5-8]. In addition the polymorphism was correlated with a decreased age at the time of lung cancer diagnosis in Li-Fraumeni syndrome and sporadic sarcoma patients [4]. Our study is the first report on a large German case-control study of young lung cancer patients (age of onset < 51 years) investigating the MDM2 SNP 309.

Methods
Study population
The study population consists of 635 lung cancer patients with an onset of disease ≤ 50 years of age and 1300 healthy population controls, all Caucasians. Controls were recruited from the KORA S3 + S4 (Cooperative Health Research in the Augsburg Region) surveys, which are large population-based consecutive cross-sectional studies [9,10] and matched by gender and 3-years age groups to cases in a 1:2 matching design. In order to increase the sample size of early onset lung cancer patients we included cases from two existing studies. 472 cases were from the LUCY (LUng Cancer in the Young) study. Additional 163 cases were derived from the hospital-based Heidelberg Lung Cancer (HLC) study from the Thoraxklinik Heidelberg [11].

The LUCY-study is a multicenter study with 31 participating clinics all over Germany. Only newly diagnosed patients with histological or cytological confirmed primary lung cancer entered the study. Detailed epidemiologic data on family history, tobacco and smoking exposure, education and occupational exposure have been collected and blood samples were taken.

The HLC-study is an ongoing hospital based case-control study. The DKFZ has recruited over 1000 LC cases at and in collaboration with the Thoraxklinik Heidelberg. 163 of these LC cases with onset of disease under age 51, recruited between 01/1997 and 12/2003 were included in the analysis. Data on occupational exposure, tobacco smoking and educational status were assessed by a self-administered questionnaire.

The KORA-study is a population based epidemiological survey of persons living in or near the city of Augsburg, Southern Germany conducted since 1984. No major population stratification between KORA (Southwest Germany) and two other cohorts from Northern Germany could be detected in an intensive study using a genomic control approach [12].

Informed consent was obtained from all study participants and the studies were approved by the ethical committee of the Bayerische Landesärztekammer, the corresponding local ethics committees of the participating clinics and the ethical committee of the University of Heidelberg.

Genotyping
The detection of the MDM2 gene polymorphism (rs2279744; G > T; NM_002392.2) was based upon analysis of primer extension products generated from previously amplified genomic DNA using a chip-based MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry platform (SEQUENOM, Inc., San Diego, CA) as described in Weidinger et al. (2004) [13]. Genotyping was performed by laboratory personnel blinded to case-control status. Standard genotyping qual-
ity control included 10% duplicate samples, negative and positive samples and checking for Hardy-Weinberg equilibrium (HWE).

**Statistical analysis**

Hardy Weinberg Equilibrium (HWE) was tested using a log-likelihood test. Conditional logistic regression models were applied to test for a genotype-phenotype association and gene-smoking interaction, with conditioning on the matched variables age groups (≤ 40 years, 41–45 years, 46–50 years) and gender. The amount of smoking exposure (s. table 1 for definition) was considered as a covariate to adjust for a smoking-related increase in lung cancer risk. The overall level of significance was set to 5%. We investigated differences between multiple characteristics (age of onset, gender, LC subtype and MDM2 SNP309 genotype) of both cases-samples by calculating a propensity score, which is defined as the conditional probability of assignment to a particular case-sample given a vector of observed covariates [14]. We then calculated kappa as measurement of agreement between true and the assigned

### Table 1: Characteristics of lung cancer cases and healthy controls

| Category                  | Group               | LUCY-study (n = 472) | HLC-study (n = 163) | KORA-study (n = 1300) |
|---------------------------|---------------------|----------------------|---------------------|-----------------------|
| gender                    | men                 | 66% (310)            | 59% (96)            | 63% (819)             |
|                           | women               | 34% (162)            | 41% (67)            | 37% (481)             |
| age at diagnosis (mean ± SD) | men                 | 45.4 ± 4.1           | 45.4 ± 3.7          | 45.2 ± 4.3            |
|                           | women               | 44.5 ± 4.6           | 45.1 ± 4.6          | 44.7 ± 4.7            |
| age at diagnosis (men)    | ≤ 40 years          | 13% (40)             | 11% (11)            | 14% (116)             |
|                           | 41–45 years         | 32% (99)             | 33% (32)            | 31% (251)             |
|                           | 46–50 years         | 55% (171)            | 55% (53)            | 55% (451)             |
| age at diagnosis (women)  | ≤ 40 years          | 17% (28)             | 16% (11)            | 17% (83)              |
|                           | 41–45 years         | 31% (51)             | 25% (17)            | 30% (144)             |
|                           | 46–50 years         | 51% (83)             | 58% (39)            | 53% (255)             |
| LC subtypes (men)         | NSCLC-Adenocarcinoma| 26% (81)             | 35% (34)            |                       |
|                           | other NSCLC         | 40% (125)            | 43% (41)            |                       |
|                           | SCLC                | 26% (81)             | 20% (19)            |                       |
|                           | other types         | 7% (22)              | 2% (2)              |                       |
| LC subtypes (women)       | NSCLC-Adenocarcinoma| 44% (71)             | 54% (36)            |                       |
|                           | other NSCLC         | 22% (36)             | 24% (16)            |                       |
|                           | SCLC                | 25% (41)             | 16% (11)            |                       |
|                           | other types         | 9% (14)              | 6% (4)              |                       |
| smoking exposure (packyears) | minimally exposed (0–10) | 12% (57)           | 13% (21)            | 54% (712)             |
|                           | lightly exposed (11–20) | 17% (81)           | 8% (13)             | 14% (178)             |
|                           | moderately exposed (21–30) | 31% (144)          | 20% (33)            | 15% (188)             |
|                           | highly exposed (31+) | 39% (186)            | 52% (85)            | 15% (192)             |
|                           | unknown             | 0.9% (4)             | 7% (11)             | 2% (30)               |
| MDM2 SNP309 genotypes†     | TT                  | 42% (199)            | 44% (71)            | 42% (547)             |
|                           | TG                  | 47% (222)            | 44% (71)            | 46% (398)             |
|                           | GG                  | 10% (49)             | 13% (21)            | 12% (149)             |
|                           | unknown             | 0.3% (2)             | --                  | 0.5% (6)              |
| mean age at diagnosis (95% CI) | TT                  | 45.1 (44.5–45.7)     | 44.8 (43.7–45.8)    |                       |
|                           | TG                  | 45.3 (44.7–45.8)     | 45.6 (44.7–45.6)    |                       |
|                           | GG                  | 44.6 (43.3–45.9)     | 45.9 (44.2–47.6)    |                       |

LUCY-study: Lung Cancer in the Young study; HLC-Study: Heidelberg lung cancer study; Other NSCLC = other non small cell lung cancers (NSCLC excluding adenocarcinoma), SCLC = small cell lung cancer, other types = other kinds of lung cancer.
membership to a case-sample and performed a McNemar’s test for agreement between these.

All analyses were performed with SAS 9.1*. Power analyses for the single marker association was done according to the method of Slager 2001 [15] and for the single marker interaction tests it was accomplished by using Nquery Advisor 4.0.

## Results

### Population characteristics

There was no statistically significant difference in the distribution of gender (p = 0.13) and age (p = 0.80) between patients and controls. The distribution of the histology subtypes within the cases can be seen in table 1. Although one might see a higher percentage of NSCLC-Adenocarcinoma within HCL-cases, the difference between both case-populations was by far not significant (p = 0.617). In total, there are no major differences between both case-populations. Applying the propensity score approach we found no agreement between the true and the assigned membership to a case-sample (kappa 0.01; 95% CI: -0.1 to 0.1). Thus we have no evidence to assume group differences shown by the multiple characteristics considered (p = 1.00).

### Genotyping

The genotyping success rates were above 99.5%. No discordance between the duplicates was found.

### Associations of the MDM2 SNP309 with lung cancer

No deviations from HWE were found in the genotype distribution of the MDM2 polymorphism among controls (p = 0.453) or cases (p = 0.474). All but eight samples were successfully genotyped for the MDM2 SNP309. The minor allele frequency (MAF) was 0.34 (CI: 0.35–0.33). Lung cancer was not found to be associated with the MDM2 SNP309. The smoking adjusted ORs for the TG and TT genotypes were 1.0 (95% CI: 0.7–1.5) and 1.0 (95% CI: 0.7–1.5), respectively. Both case populations compared separately to the controls showed no significant associations (Table 2). Gender related subgroup analysis yielded no association of the MDM2 SNP309 and lung cancer (Table 2, 3, 4). Also no influence in lung cancer risk by the MDM2 SNP309 was observed for the diverse histological subtypes of lung cancer (Table 3, 4, 5).

Furthermore it was investigated whether an interaction existed between the MDM2 SNP309 and the smoking status. We did not find an association between the MDM2 SNP309 and lung cancer for any of the defined smoking exposure level groups (Table 3, 5).

### Table 2: Association of MDM2 SNP309 with lung cancer within both case-populations

| genotype | OR  | 95% CI  | p-value |
|----------|-----|---------|---------|
| LUCY cases (n = 470) vs. KORA controls (n = 815) |
| TG vs. GG | 1.01 | 0.7–1.6 | 0.794 |
| TT vs. GG | 1.01 | 0.7–1.6 | 0.841 |
| men: LUCY cases (n = 309) vs. KORA controls (n = 815) |
| TG vs. GG | 1.18 | 0.7–1.8 | 0.706 |
| TT vs. GG | 1.18 | 0.7–1.8 | 0.751 |
| women: LUCY cases (n = 161) vs. KORA controls (n = 815) |
| TG vs. GG | 1.01 | 0.5–2.1 | 0.937 |
| TT vs. GG | 1.01 | 0.5–2.1 | 0.966 |
| gender difference in genotypic ORs | 0.778 |

HLC cases (n = 163) vs. KORA controls (n = 479)  

| genotype | OR  | 95% CI  | p-value |
|----------|-----|---------|---------|
| TG vs. GG | 1.2 | 0.5–3.0 | 0.648 |
| TT vs. GG | 1.6 | 0.7–3.7 | 0.314 |
| men: HLC cases (n = 96) vs. KORA controls (n = 479) |
| TG vs. GG | 0.7 | 0.3–1.6 | 0.372 |
| TT vs. GG | 0.5 | 0.2–1.3 | 0.159 |
| gender difference in genotypic ORs | 0.083 |

HLC cases vs. LUCY cases (distribution of TG/GG)  

| all | 0.773 |
| men | 0.383 |
| women | 0.100 |

1 conditional to age/gender-strata (6 groups) and adjusted for smoking exposure§ conditional to age-strata (3 groups) and adjusted for smoking exposure and gender§§. Fishers Exact Test

Even though no significant difference between both case-populations could be stated statistically, we observed twice as many female GG-carriers among HCL-cases (21%) than in the control- or LUCY population (10%) (Table 5). We also observed an enrichment of NSCLC- and NSCLC adenocarcinoma-cases in the HCL-sample (78%) compared to the LUCY-sample (66%). Lind et al. (2006) [6] recently reported a gender specific risk disposing effect of the T-allele of MDM2 SNP309 for NSCLC cases. We therefore additionally performed a subgroup analysis restricted to NSCLC-cases and tested for gender differences. Compared to the GG genotype, the smoking adjusted ORs for the TG and TT genotypes for female NSCLC-cases were 0.8 (95% CI: 0.4–1.6) and 0.71 (95% CI: 0.4–4.0), respectively. For men the ORs were 1.2 (95% CI: 0.7–2.8) and 1.2 (95% CI: 0.7–2.9), respectively (Table 4). Although a possible NSCLC risk modification of MDM2 SNP309 seems to be reverse between men and women, we failed to achieve significance for this gender difference (p = 0.237). We also tested a gender specific genetic association within both case-samples separately. Neither within all HLC-cases (p = 0.083) nor within all
LUCY-cases (p = 0.778) were significant gender differences found (Table 2).

**MDM2 SNP309** was recently reported to be associated with an earlier onset of disease [4]. The average age at the time of diagnosis in our cases overall was not significantly different (p = 0.923) between the variant genotypes (Table 1) and can be assumed as homogeneous between both case-samples (p = 0.7157). The mean age of diagnosis of men was 45.2 (median = 46.0 years, range 26.0 – 50.0 years) and of women 44.7 years (median = 46.0 years, range 24.0 – 50.0 years). Women, however, with the **MDM2 SNP309** were diagnosed at a younger age (8 months) compared to males, but this was not statistically significant (p = 0.059, 95% CI: 0 to 16 months). Within the LUCY-case population this gender difference was 10 months (95% CI: 0.3 to 20 months, p = 0.044), and only 6 months (95% CI: -10 to 22 months, p = 0.496) in the much smaller HLC-case population.

**Discussion**

Reports have shown an increased risk of lung cancer for the G allele of the **MDM2 SNP309** in Korean and Chinese populations [7,16,17]. In contrast, in our study of 635 Caucasian lung cancer patients with age of onset < 51 years and 1300 controls the **MDM2 SNP309** was not associated with lung cancer. We did not observe a significant overall association either with the G allele or with the T allele as reported by Li et al. (2006) where they showed an increased lung cancer risk with the T/T genotype in a non-Hispanic white population [18]. We had a 90% power (alpha = 0.05, two sided test) to detect a minimum overall OR of 1.4 for the **MDM2 SNP309** were diagnosed at a younger age (8 months) compared to males, but this was not statistically significant (p = 0.059, 95% CI: 0 to 16 months). Within the LUCY-case population this gender difference was 10 months (95% CI: 0.3 to 20 months, p = 0.044), and only 6 months (95% CI: -10 to 22 months, p = 0.496) in the much smaller HLC-case population.

**Table 3: Association of MDM2 SNP309 with lung cancer**

| gender | Genotype | OR  | 95% CI   | p-value |
|--------|----------|-----|----------|---------|
| men*   | TG vs. GG | 1.1 | 0.7 – 2.0 | 0.630   |
| men*   | TT vs. GG | 1.2 | 0.7 – 2.2 | 0.504   |
| women* | TG vs. GG | 0.9 | 0.5 – 1.6 | 0.723   |
| women* | TT vs. GG | 0.8 | 0.5 – 1.3 | 0.565   |

**histology**

|                | Genotype | OR  | 95% CI   | p-value |
|----------------|----------|-----|----------|---------|
| NSCLC-Adenocarcinoma* | TG vs. GG | 1.0 | 0.6 – 1.7 | 0.991   |
| NSCLC-Adenocarcinoma* | TT vs. GG | 1.0 | 0.6 – 1.8 | 0.890   |
| other NSCLC**    | TG vs. GG | 1.0 | 0.6 – 1.8 | 0.873   |
| other NSCLC**    | TT vs. GG | 0.9 | 0.5 – 1.6 | 0.751   |
| SCLC**          | TG vs. GG | 1.0 | 0.5 – 1.9 | 0.980   |
| SCLC**          | TT vs. GG | 1.2 | 0.6 – 2.1 | 0.637   |

**smoking exposure (packyears)**

|                | Genotype | OR  | 95% CI   | p-value |
|----------------|----------|-----|----------|---------|
| lightly exposed (11–20 PY)* | TT vs. GG | 1.0 | 0.6 – 1.8 | 0.928   |
| moderately exposed (21–30 PY)** | TT vs. GG | 1.0 | 0.6 – 1.7 | 0.960   |
| highly exposed (31++ PY)** | TT vs. GG | 0.9 | 0.5 – 1.4 | 0.595   |

*conditional to age-strata (3 groups: ≤ 40 years, 41–45 years, 46–50 years), adjusted for smoking exposure; **conditional to age/gender-strata (6 groups) and adjusted for smoking exposure; ***conditional to age/gender-strata (6 groups)

**Table 4: Association of MDM2 SNP309 with lung cancer within NSCLC cases**

| genotype | OR  | 95% CI   | p-value |
|----------|-----|----------|---------|
| all: NSCLC cases (n = 434) vs. controls (n = 1295) | TG vs. GG | 1.1§ | 0.7–1.6 | 0.828   |
|         | TT vs. GG | 1.0 § | 0.7–1.5 | 0.841   |
| men: NSCLC cases (n = 280) vs. controls (n = 815) | TG vs. GG | 1.2 §§ | 0.7–2.9 | 0.422   |
|         | TT vs. GG | 1.2 §§ | 0.7–2.9 | 0.515   |
| women: NSCLC cases (n = 154) vs. controls (n = 479) | TG vs. GG | 0.8 §§ | 0.4–1.6 | 0.471   |
|         | TT vs. GG | 0.7 §§ | 0.4–4.0 | 0.323   |

*§ conditional to age/gender-strata (6 groups) and adjusted for smoking exposure; §§ conditional to age-strata (3 groups) and adjusted for smoking exposure and gender
Table 5: MDM2 SNP309 genotypes

| Group               | MDM2 SNP309 genotypes | Cases (n = 635) | Controls |
|---------------------|------------------------|-----------------|----------|
|                     |                        | LUCY-study (n = 472) | HLC-study (n = 163) | KORA-study (n = 1300) |
| gender              |                        |                 |          |            |
| men                 | TT                     | 128 (41%)       | 47 (49%)   | 350 (43%)  |
|                     | TG                     | 148 (48%)       | 42 (44%)   | 366 (45%)  |
|                     | GG                     | 33 (11%)        | 7 (7%)     | 99 (12%)   |
|                     | unknown                | 1 (0%)          | 3 (0%)     | 3 (0%)     |
| women               | TT                     | 71 (44%)        | 24 (36%)   | 197 (41%)  |
|                     | TG                     | 74 (46%)        | 29 (43%)   | 232 (48%)  |
|                     | GG                     | 16 (10%)        | 14 (21%)   | 50 (10%)   |
|                     | unknown                | 1 (0%)          | 3 (0%)     | 3 (0%)     |
| age at diagnosis    | ≤ 40 years             |                 |          |            |
|                     | TT                     | 30 (44%)        | 11 (50%)   | 85 (43%)   |
|                     | TG                     | 30 (44%)        | 8 (36%)    | 91 (46%)   |
|                     | GG                     | 6 (9%)          | 3 (14%)    | 21 (11%)   |
|                     | unknown                | 2 (3%)          | 0 (0%)     | 2 (1%)     |
|                     | 41–45 years            |                 |          |            |
|                     | TT                     | 58 (39%)        | 21 (43%)   | 172 (44%)  |
|                     | TG                     | 73 (49%)        | 23 (47%)   | 185 (47%)  |
|                     | GG                     | 19 (13%)        | 5 (10%)    | 37 (9%)    |
|                     | unknown                | 1 (0%)          | 0 (0%)     | 1 (0%)     |
|                     | 46–50 years            |                 |          |            |
|                     | TT                     | 111 (44%)       | 39 (42%)   | 290 (41%)  |
|                     | TG                     | 119 (47%)       | 40 (43%)   | 322 (46%)  |
|                     | GG                     | 24 (9%)         | 13 (14%)   | 91 (13%)   |
|                     | unknown                | 3 (0%)          | 0 (0%)     | 0 (0%)     |
| smoking exposure level (SEL) | minimally exposed (0–10 PY) |                 |          |            |
|                     | TT                     | 28 (49%)        | 7 (33%)    | 297 (42%)  |
|                     | TG                     | 19 (33%)        | 12 (57%)   | 332 (47%)  |
|                     | GG                     | 9 (16%)         | 2 (10%)    | 81 (11%)   |
|                     | unknown                | 1 (2%)          | 0 (0%)     | 0 (0%)     |
|                     | lightly exposed (> 10–20 PY) |                 |          |            |
|                     | TT                     | 29 (36%)        | 7 (54%)    | 72 (40%)   |
|                     | TG                     | 42 (52%)        | 5 (38%)    | 81 (46%)   |
|                     | GG                     | 9 (11%)         | 1 (8%)     | 24 (13%)   |
|                     | unknown                | 1 (1%)          | 0 (0%)     | 0 (0%)     |
|                     | moderately exposed (> 20–30 PY) |                 |          |            |
|                     | TT                     | 65 (45%)        | 13 (39%)   | 81 (44%)   |
|                     | TG                     | 70 (49%)        | 15 (45%)   | 86 (46%)   |
|                     | GG                     | 9 (6%)          | 5 (15%)    | 19 (10%)   |
|                     | unknown                | 1 (1%)          | 1 (1%)     | 0 (0%)     |
|                     | highly exposed (> 30++ PY) |                 |          |            |
|                     | TT                     | 76 (41%)        | 41 (48%)   | 84 (44%)   |
|                     | TG                     | 89 (48%)        | 33 (39%)   | 87 (45%)   |
|                     | GG                     | 21 (11%)        | 11 (13%)   | 21 (11%)   |
|                     | unknown                | 1 (1%)          | 1 (1%)     | 0 (0%)     |
| LC subtypes         | NSCLC- Adenocarcinoma   |                 |          |            |
|                     | TT                     | 63 (41%)        | 35 (50%)   |            |
|                     | TG                     | 75 (49%)        | 23 (33%)   |            |
|                     | GG                     | 13 (9%)         | 12 (17%)   |            |
|                     | unknown                | 1 (1%)          |           |            |
|                     | other NSCLC            |                 |          |            |
|                     | TT                     | 68 (43%)        | 17 (30%)   |            |
|                     | TG                     | 73 (46%)        | 34 (60%)   |            |
|                     | GG                     | 19 (12%)        | 6 (11%)    |            |
|                     | SCLC                   |                 |          |            |
|                     | TT                     | 55 (45%)        | 15 (50%)   |            |
|                     | TG                     | 55 (45%)        | 12 (40%)   |            |
|                     | GG                     | 13 (11%)        | 3 (10%)    |            |
|                     | other types            |                 |          |            |
|                     | TT                     | 13 (27%)        | 4 (40%)    |            |
|                     | TG                     | 19 (39%)        | 2 (20%)    |            |
|                     | GG                     | 4 (8%)          |           |            |
|                     | unknown                | 13 (27%)        | 4 (40%)    |            |
the analysis. They did not find any significant association of patients could also not confirm the results of this meta-
that the study populations is the age of cases. Therefore one possi-
95% CI: 1.1–1.4) [20]. A main difference between the two data of the smoking. A combined analysis of the available genotype
observe an interaction between the MDM2 SNP309 and lung cancer in a Chinese population. Our results were similar to the report of Pine et al. (2006) [8]. They also did not find any overall association between the MDM2 SNP309 and lung cancer in a Caucasian (USA) population. Similarly, we did not observe an interaction between the MDM2 SNP309 and SNP309 and lung cancer in a
search of Pine et al. (2006) [8]. Our sample size is large enough to detect a shift of two years for the time of diagnosis with 90% power (alpha = 0.05) for heterozygous as well as for homozygous allele carriers. Recently Bond et al. 2006 [22] reviewed the possible interaction between the SNP309 and the oestrogen receptor status in women. Within women of the LUCY case population we could observe an earlier onset of disease of 10 months (95% CI: 0.3 to 20 months, p = 0.044), but only 6 months (95% CI: -10 to 22 months, p = 0.496) in the by far smaller HLC-case population. Recent data suggest that the promoter polymorphism in the MDM2 gene may influence the age of cancer onset in a gender specific way [23-25].

Conclusion
Given that lung cancer is a result of gene-environmental interaction, the genetic component may be a particularly strong risk factor among early onset lung cancer patients. In our sample, after controlling for gender and other characteristics, the overall data suggest that the MDM2 SNP309 might not be a sufficient risk factor of lung carcinogenesis not even in young cases where the genetic component might have a larger contribution to the risk of disease. It might modify the time of tumour onset, especially in women, supporting the model, that female-specific hormones, such as oestrogen, could allow the SNP309 to accelerate tumour formation. Our investigation provides no significance for gender specific associations by itself; it points only towards gender specific differences. Therefore further studies have to be conducted to explicitly study these gender-specific effects.

Abbreviations
CI: confidence interval; HLCS: Heidelberg lung cancer study; KORA: Cooperative Health Research in the Augsburg Region; LC: lung cancer; LUCY: lung cancer in the young; MALDI-TOF: matrix-assisted laser desorption/ionization time-of-flight; NSCLC: none small cell lung cancer; OR: odds ratio; PY: packyear; SCLC: small cell lung cancer.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
KM carried out the molecular genetic study and drafted the manuscript. WS, NK carried out the molecular genetic study.
study. AR, JCC performed the statistical analysis. HEW, HB, ARi, TI conceived the study, and participated in its design and coordination. GW proband recruitment and data acquisitions. MT data acquisitions. NK, HD, EM, GS, MC, MD, HM, PD, AG, KG, KH, GH, CS, BJ, WS, YK, DT recruited the lung cancer cases in different hospitals. All authors read and approved the final manuscript.

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