Two-stage study of lung cancer risk modification by a functional variant in the 3'-untranslated region of SMAD5 based on the bone morphogenetic protein pathway

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Abstract. Increasing evidence supports a key role for the bone morphogenetic protein (BMP) signaling pathway in lung vasculogenesis and angiogenesis. Genetic variations in BMP genes have been found to be correlated with cancer risk. In particular, the mutation in the 3'-untranslated region of BMPs may significantly affect gene function, leading to cancer susceptibility. The aim of the present study was to determine whether genetic variations in the components of the BMP family are associated with lung cancer risk. A total of 314 tag single-nucleotide polymorphisms were identified in 18 genes, which are considered to either compose or regulate BMPs, and their association with lung cancer risk was evaluated in a two-stage case-control study with 4,680 cases and controls. A consistently significant association of SMAD5 rs12719482 with elevated lung cancer risk was observed in the three types of sources of populations (adjusted additive model in the combined population: Odds ratio=1.32, 95% confidence interval: 1.16-1.51). Consistent evidence was identified for a significant interaction between rs12719482 and cigarette smoking, performed as either a continuous or discrete variable. These findings indicated that SMAD5 rs12719482 may be a possible candidate marker for susceptibility to lung cancer in the Chinese population.

Introduction

Lung cancer is one of the most common malignant tumors worldwide. Non-small-cell lung cancer (NSCLC) constitutes ~80% of lung cancers, with a 5-year survival of only 15%. Further study on the pathogenic mechanism of lung cancer is required to establish novel diagnostic or treatment strategies for this lethal disease.

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor-β (TGF-β) superfamily. Thus far, >20 BMPs have been identified in humans. Six of the type I and three of the type II serine/threonine kinase receptors have been shown to mediate BMP signaling (1). Both type I and type II receptors consist of a N-terminal extracellular ligand-binding domain and a C-terminal serine/threonine kinase domain (2). When the BMP ligand binds to preformed hetero-oligomeric complexes, the SMAD-dependent pathway may be activated (3). The pathway-restricted SMADs (such as SMAD1, 2, 3, 5 or 8) are recruited and translocated into the nucleus with the assistance of SMAD4 and regulate the transcription of target genes, referred to as the SMAD-dependent pathway. The activated BMP signaling may then enhance invasion and bone metastasis of cancer cells via the SMAD pathway (4). BMPs significantly affect embryonic and postnatal development and maintenance of homeostasis in organs and tissues; they are also associated with cell proliferation, differentiation, motility and survival (5) and they are involved in the development and progression of certain malignant tumors, such as lung, prostate and breast cancer (6). Recently, accumulating evidence demonstrated that BMPs also participate in tumor-related angiogenesis (7,8).

It was hypothesized that the polymorphisms in the BMP component and regulatory genes may contribute to the
genetic susceptibility to lung cancer. To test this hypothesis, a two-stage case-control study was performed, including a total of 2,340 patients and 2,340 cancer-free controls, with the specific aim of evaluating the effects of gene polymorphisms in the 18 selected genes related to the BMP pathway [BMP2, 4, 6, 7 and 9, SMAD1, 4, 5, 6, 7 and 8, SMURF1 and 2, ACTR2, ALK2 (ACVR1), ALK3 (BMPR-1A), ALK6 (BMPR-1B) and BMP2] on lung cancer. To the best of our knowledge, this is the first study to evaluate the associations between a comprehensive panel of genetic variants related to the BMP pathway and lung cancer, and to identify certain patient subgroups that may be at high risk of developing lung cancer.

Patients and methods

Study population and design. The study design and subject recruitment have been previously described (9). Briefly, two independent case-control projects were conducted. The ‘stage I’ setting (discovery) included 1,422 patients and 1,422 controls mainly from Guangzhou, Guangdong, China (9). The ‘stage II’ setting (replication) included 918 cases and 918 controls from Xianyang, Hubei, China, to test the results of stage I. A total of 2,340 cases with histopathologically diagnosed lung cancer were enrolled from September, 2009 onwards. The 2,340 cancer-free controls were recruited during the same time period. The characteristics of the cases and controls are summarized in Table I. Structured questionnaires were conducted by experienced interviewers using a standardized protocol. The inclusive criteria and definition of cigarette smoking were previously detailed (9). This project was approved by the Institutional Ethics Committee of each participating center. All the participants provided written informed consent prior to enrolment.

Blood sampling, SNP selection and genotyping. Blood samples were provided by all the participants. The genomic DNA was extracted with the Qiagen Blood DNA kit (Qiagen, Valencia, CA, USA). A total of 18 genes associated with the BMP pathway were selected. For each of those genes, the tagSNPs were selected using Haploview 4.2 software (http://haploview.software.informer.com/4.2/). The genotypes of 314 selected tagSNPs and their associations with lung cancer were detailed in Fig 1 (P<1.6x10^-4) and Table II (P<0.05). The Illumina high-throughput genotyping platform and the Illumina Beadstudio software were used (Illumina, San Diego, CA, USA). To ensure quality control, genotyping was performed using blinded methods and the analysis was conducted separately by two researchers. Over 15% of the samples were used for confirmation, and the outcomes were 100% concordant. The genotyping call rates were all >95%.

Statistical analysis. The χ² test was used to evaluate the qualitative data and the Hardy-Weinberg equilibrium (HWE) applied. The Akaike Information Criteria (AIC) (10) were employed to select a genetic model for each SNP. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated by an unconditional logistic regression model adjusted for confounding factors. Stratification analyses were performed with the variables of interest. The pairwise linkage disequilibrium among the SNPs and haplotype blocks/frequencies were measured accordingly (11-13). Homogeneity between stage I and II populations was assessed by the Breslow-Day test. Statistical power was assessed by Quanto 1.2. software (http://biostats.usc.edu/Quanto.html) and corrected by the Bonferroni test for each of the 314 SNPs at a significance level of 0.05/314=1.6x10^-4 (Fig 1). All the analyses were performed using SAS 9.2 software (SAS Institute Inc., NC, USA).

Results

Characteristics of the populations. As summarized in Table I, consistent results were found in stages I and II, with significant differences in smoking status, pack-years and gender-smoking (P<0.001 for all); there was no statistically significant deviation in the distributions of gender and age between the case and control groups (P>0.05 for all). The frequency distribution of smoking status was heterogeneous (Breslow-Day test P<0.0001). The two groups were then combined to increase the study power, and almost identical change tendencies were identified separately for stages I and II.

Genetic variants in SMAD5 and lung cancer risk. As mentioned above, 314 tagSNPs were selected from 18 genes associated with the BMP pathway; those with P<0.05 in stage I, stage II, or the combined populations, are shown in Table II. Among these tagSNPs, consistently significant associations were found between SMAD5 rs12719482 and lung cancer risk in the three types of sources of populations (P<0.05 for all). In particular, when combining stages I and II, the differences were more significant compared with any individual stage in the additive genetic model (P=4.39x10^-5). All observed genotype distributions among these groups were in HWE (P=0.05 for all). The frequency distributions of the genotypes of rs12719482 are also summarized in Table III. In the stage I set, significant associations were found between rs12719482 T>C genotypes, as well as alleles, and lung cancer risk. Compared with zero-risk genotype carriers, the one- or two-risk genotype was associated with increased lung cancer risk in a dose-dependent manner (adjusted OR=1.27, 95% CI: 1.07-1.50, P=0.0060), while the rs12719482 CT/CC genotype was associated with a significantly increased risk in the dominant genetic model (OR=1.26; 95% CI: 1.05-1.52; P=0.0133). Allele C increased the risk of lung cancer by 23% compared with the wild-type T. Concordant with the results of rs12719482 T>C and lung cancer risk analysis in stage I, the variant genotype analyses revealed almost similar change tendencies in the stage II setting. Importantly, the association remained statistically significant when all the participants were combined.

Stratification analysis of rs12719482 T>C in the combined study. The associations of the rs12719482 (T>C) with lung cancer risk stratified by selected variables using the additive model was then assessed according to the AIC pattern. As shown in Table IV, individuals exhibited a significantly increased lung cancer risk as the number of variant alleles increased, in the ages of ≤60 as well as >60 years (P=0.0039 and P=0.0087, respectively), in both genders (P=0.0155 for men and P=0.0006 for women), in smokers as well as non-smokers (P=0.0135 and P=0.0012, respectively), in ≥20 and 0 pack-years (P=0.0407...
Table I. Frequency distributions of selected variables in lung cancer patients and cancer-free controls.

| Variables            | Discovery                  | Replication                | Combined                  |
|----------------------|----------------------------|----------------------------|----------------------------|
|                      | Cases, n (%)               | Controls, n (%)            | Cases, n (%)               | Controls, n (%)            | Cases, n (%)               | Controls, n (%)            |
|                      | (n=1,422)                  | (n=1,422)                  | (n=918)                    | (n=918)                    | (n=2,340)                  | (n=2,340)                  |
|                      | P-value^a                  | P-value^b                  | P-value                    | P-value                    | P-value^a                  | P-value^b                  |
| Age, years           | 0.9855                     | 0.0108                     | 0.0448                     | 0.3494                     |
| ≤60                  | 688 (50.00)                | 688 (50.00)                | 459 (53.19)                | 404 (46.81)                | 1,146 (49.31)              | 1,178 (50.69)              |
| >60                  | 734 (50.00)                | 734 (50.00)                | 459 (47.22)                | 514 (52.78)                | 1,194 (50.68)              | 1,162 (49.32)              |
| Gender               | 0.9904                     | 0.8757                     | 0.9277                     | 0.9471                     |
| Male                 | 1,007 (50.00)              | 1,007 (50.00)              | 714 (49.93)                | 716 (50.07)                | 1,721 (49.97)              | 1,723 (50.03)              |
| Female               | 415 (50.00)                | 415 (50.00)                | 204 (50.37)                | 202 (49.63)                | 619 (50.08)                | 617 (49.92)                |
| Smoking status       | <0.0001                    | <0.0001                    | <0.0001                    | <0.0001                    |
| Smoker               | 702 (54.49)                | 519 (42.51)                | 555 (59.36)                | 380 (40.64)                | 1,258 (57.63)              | 925 (42.37)                |
| Former smoker        | 168 (68.85)                | 76 (31.15)                 | 86 (44.79)                 | 106 (55.21)                | 254 (57.34)                | 189 (42.66)                |
| Non-smoker           | 551 (39.99)                | 827 (60.01)                | 277 (39.12)                | 432 (60.88)                | 828 (40.31)                | 1,226 (59.69)              |
| Pack years           | <0.0001                    | <0.0001                    | 0.7312                     | <0.0001                    |
| ≥25                  | 650 (65.46)                | 343 (34.54)                | 483 (61.12)                | 306 (38.88)                | 1,274 (61.49)              | 798 (38.51)                |
| <25                  | 220 (46.61)                | 252 (53.39)                | 94 (40.52)                 | 138 (59.48)                | 238 (42.96)                | 316 (57.04)                |
| 0                    | 551 (39.99)                | 827 (60.01)                | 277 (39.12)                | 432 (60.88)                | 828 (40.31)                | 1,226 (59.69)              |
| Gender and smoking   | <0.0001                    | <0.0001                    | 0.6112                     | <0.0001                    |
| Male smokers         | 832 (58.47)                | 591 (41.53)                | 610 (56.53)                | 469 (43.47)                | 1,443 (56.90)              | 1,093 (43.10)              |
| Male non-smokers     | 174 (29.49)                | 416 (70.51)                | 104 (29.63)                | 247 (70.37)                | 278 (30.62)                | 630 (69.38)                |
| Female smokers       | 38 (90.48)                 | 4 (9.52)                   | 31 (64.58)                 | 17 (35.42)                 | 69 (76.67)                 | 21 (23.33)                 |
| Female non-smokers   | 377 (47.84)                | 411 (52.16)                | 173 (48.46)                | 185 (51.54)                | 549 (47.99)                | 595 (52.01)                |

Bold print indicates statistical significance. ^aP-values with the two-sided χ² test; ^bP-values with the Breslow Day test for homogeneity.
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and \( P=0.0017 \), respectively) and in both male smokers and female non-smokers \( (P_{\text{max}}=0.0350) \) following adjustment for confounding factors. There were also statistically significant multiplicative interactions among gender, smoking status, pack-years and allele genes \( (P<0.0001 \text{ for all}) \).

Discussion

Lung cancer remains a major health concern, due to its 5-year survival of only 15% and associated high medical costs. Genetic biomarkers may help identify susceptible subgroups for screening, diagnosis and even therapy in earlier stages, and it may also be beneficial for clinical outcomes. A total of 314 tagSNPs were identified in the 18 pivotal genes from the BMP pathway and evaluated for their association with lung cancer risk. In the present study, a significant role was indicated for SMAD5 in TGF-\( \beta \)-mediated lung cancer. The findings were concordant for a significant association between the SMAD5 rs12719482 and cigarette smoking. In addition, using Function Prediction websites (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html), it was demonstrated that SMAD5 rs12719482 may be a susceptibility marker by decreasing the expression of SMAD5 through binding of one of hsa-miR-1270, hsa-miR-571 or hsa-miR-920 to the polymorphic site in the Chinese population. To the best of our knowledge, the present study is the first to estimate the associations between a comprehensive panel of genetic polymorphism of BMP genes and lung cancer risk, and to investigate the potential susceptible subgroups. A series of functional experiences are required in the future, as this is the first epidemiological study supporting an association between SMAD5 rs12719482 and lung cancer risk.

Identification of SMAD proteins has been helpful in promoting the understanding of TGF-\( \beta \) signaling. BMPs may signal through both canonical and non-canonical pathways. In particular, in the canonical pathway, the BMP functions through BMP ligand (BMPR1)-binding membrane-bound receptors, resulting in the phosphorylation of intracellular mediators, known as the receptor-regulated SMADs (R-SMADs). SMADs are crucial intracellular signaling transmitters of the TGF-\( \beta \) superfamily of peptide growth factors that regulate a series of biological processes (5). Controlling SMAD activity and protein levels are crucial for proper signaling by TGF-\( \beta \) and its related factors. Due to their ubiquitous expression and various functions as regulators in various organs of the body, BMPs were referred to as body morphogenetic proteins (14), as disruptions of BMP signaling are associated with a wide variety of defects or severe pathologies, such as vascular diseases, skeletal diseases and cancer.

SMAD5, one of the MAD-homologues, is situated on chromosome 5q31 in humans and acts downstream of the TGF-\( \beta \) receptors. It is an important component of R-SMADs (SMAD1/5/8) and the closest homolog of SMAD1, which plays a role in the BMP-2 signaling pathway (15). Accumulating evidence indicates that the SMAD5 gene functions in the signaling pathway involving the inhibitory effect of TGF-\( \beta \) on
Table II. Genotyping of the 314 tagSNPs in the BMP pathway and their associations with lung cancer (P<0.05).

| CHR | SNP    | Gene    | Allele<sup>a</sup> | Cases/controls (n=1,422/1,422) | P<sub>add</sub> | P<sub>HWE</sub> | Cases/controls (n=918/918) | P<sub>add</sub> | P<sub>HWE</sub> | Cases/controls (n=2,340/2,340) | P<sub>add</sub> | P<sub>HWE</sub> |
|-----|--------|---------|--------------------|-------------------------------|----------------|--------------|----------------------------|----------------|--------------|-------------------------------|----------------|--------------|
| 2   | rs7589059 | BMP2   | A>G                | 0.12/0.10                     | 0.0216         | 0.6569       | 0.11/0.11                  | 0.7100         | 0.1501       | 0.11/0.10                     | 0.1237         | 0.5779       |
| 2   | rs6747756 | BMP2   | A>G                | 0.14/0.11                     | 0.0101         | 0.8424       | 0.12/0.14                  | 0.3826         | 0.081        | 0.13/0.12                     | 0.1589         | 0.3288       |
| 4   | rs10516957 | ALK6 | T>C                | 0.84/0.94                     | 0.0411         | 0.1689       | 0.87/0.82                  | 0.3901         | 0.7904       | 0.85/0.89                     | 0.2955         | 0.3682       |
| 5   | rs12719482 | SMAD5 | T>C                | 0.14/0.11                     | 0.0060         | 0.7201       | 0.14/0.10                  | 0.0018         | 0.9762       | 0.14/0.11                     | 4.39E-5        | 0.7971       |
| 10  | rs7095804 | ALK3   | T>A                | 0.46/0.41                     | 0.0360         | 0.4681       | 0.48/0.47                  | 0.9265         | 0.2704       | 0.47/0.43                     | 0.0936         | 0.1967       |
| 20  | rs230194  | BMP7   | A>G                | 0.24/0.27                     | 0.0454         | 0.6498       | 0.28/0.26                  | 0.4705         | 0.4835       | 0.25/0.27                     | 0.2778         | 0.9208       |
| 20  | rs230198  | BMP7   | G>A                | 0.23/0.27                     | 0.0423         | 0.6011       | 0.28/0.26                  | 0.4954         | 0.5107       | 0.25/0.27                     | 0.2573         | 0.9831       |
| 20  | rs4811823 | BMP7   | G>A                | 0.24/0.27                     | 0.0492         | 0.6004       | 0.28/0.26                  | 0.5102         | 0.5261       | 0.25/0.27                     | 0.2713         | 0.9974       |
| 20  | rs6126377 | BMP7   | G>A                | 0.24/0.28                     | 0.0324         | 0.5969       | 0.27/0.26                  | 0.6336         | 0.6184       | 0.25/0.27                     | 0.1768         | 0.9296       |

Bold print indicates statistical significance. <sup>a</sup>Major > minor alleles. MAF, minor allele frequency; CHR, chromosome; SNP, single nucleotide polymorphism; P<sub>HWE</sub>, P-value calculated by the Hardy-Weinberg equilibrium among the control subjects; P<sub>add</sub>, P-value calculated by the additive model; BMP, bone morphogenetic protein; BMPR, BMP receptor; SMAD5, mothers against decapentaplegic homolog 5.

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Table III. Distribution of genotypes in *SMAD5* and associations with risk of lung cancer.

| Genotypes/alleles | Discovery | Replication | Combined |
|-------------------|-----------|-------------|----------|
|                   | Cases/controls | OR (95% CI) | P-value | Cases/controls | OR (95% CI) | P-value |
| Total no. of subjects | 1,422/1,422 | 0.0921 | 918/918 | 2,340/2,340 | 4,680/4,680 |
| Total no. of alleles | 2,844/2,844 | - | 1,836/1,836 | - |
| rs12719482 T>C | Co-dominant model | | | |
| TT | 1,091/1,147 | 1.00 (ref.) | 711/762 | 1.00 (ref.) | 1,802/1,909 | 1.00 (ref.) |
| CT | 308/263 | 1.23 (1.02-1.48) | 0.0344 | 194/148 | 1.38 (1.08-1.76) | 0.0097 |
| CC | 22/12 | 2.10 (1.02-4.32) | 0.0437 | 13/7 | 1.88 (0.72-4.93) | 0.1989 |
| Trend test P-value | 0.0060 | - | - | 0.0046 | - |
| Additive model | 1.27 (1.07-1.50) | 0.0060 | 1.38 (1.10-1.72) | 0.0046 | 1.32 (1.16-1.51) | 4.9e-5 |
| Allele model | T | 2,490/2,557 | 1.00 (ref.) | 1616/1672 | 1.00 (ref.) | 4,106/4,229 | 1.00 (ref.) |
| C | 352/287 | 1.23 (1.06-1.49) | 0.0087 | 220/162 | 1.35 (1.08-1.70) | 0.0080 | 572/449 | 1.31 (1.14-1.50) | 1.1e-4 |
| Dominant model | CT+CC | 330/275 | 1.26 (1.05-1.52) | 0.0133 | 207/155 | 1.40 (1.10-1.78) | 0.0056 | 537/430 | 1.33 (1.15-1.53) | 1.5e-4 |
| Recessive model | TT+CT | 1,399/1,410 | 1.00 (ref.) | 905/910 | 1.00 (ref.) | 2,304/2,320 | 1.00 (ref.) | 35/19 | 1.98 (1.11-3.52) | 0.0203 |
| CC | 22/12 | 2.02 (0.98-4.14) | 0.0565 | 13/7 | 1.77 (0.68-4.63) | 0.2467 |

Bold print indicates statistical significance. *aAdjusted in a logistic regression model that included age, gender and pack-years of smoking. *bPHWE, P-value calculated by the Hardy-Weinberg equilibrium among the control subjects. AIC of additive = 6,288; co-dominant = 6,289; dominant = 6,290; recessive = 6,299, allele = 12,286 in the combined set. Power of additive = 0.99, dominant = 0.98, recessive = 0.80 in the combined set. *SMAD5*, mothers against decapentaplegic homolog 5; AIC, akaike's information criterion. OR, odds ratio; CI, confidence interval.
Table IV. Stratification analysis on rs12719482 T>C in BMP.

| Variables                        | Cases<sup>a</sup> | Controls<sup>a</sup> | OR (95% CI)<sup>b</sup> | P-value<sup>b</sup> | P-value<sup>c</sup> |
|----------------------------------|-------------------|----------------------|-------------------------|---------------------|---------------------|
|                                  | TT, n (%)         | CT, n (%)            | CC, n (%)                |                     |                     |
|                                  |                   |                      |                         |                     |                     |
| Age, years                       |                   |                      |                         |                     |                     |
| ≤60                              | 881 (76.88)       | 245 (21.38)          | 20 (1.75)                | 961 (81.58)         | 205 (17.40)         | 12 (1.02)           | 1.32 (1.09-1.59)    | 0.0039              |
| >60                              | 921 (77.20)       | 257 (21.54)          | 15 (1.26)                | 948 (81.65)         | 206 (17.74)         | 7 (0.60)            | 1.30 (1.07-1.59)    | 0.0087              |
| Gender                           |                   |                      |                         |                     |                     |
| Male                             | 1,332 (77.40)     | 362 (21.03)          | 27 (1.57)                | 1,392 (80.79)       | 313 (18.17)         | 18 (1.04)           | 1.21 (1.04-1.42)    | 0.0155              |
| Female                           | 470 (76.05)       | 140 (22.65)          | 8 (1.29)                 | 517 (83.93)         | 98 (15.91)          | 1 (0.16)            | 1.63 (1.23-2.15)    | 0.0006              |
| Smoking status                   |                   |                      |                         |                     |                     |
| Yes                              | 1,169 (77.31)     | 319 (21.10)          | 24 (1.59)                | 900 (80.79)         | 206 (18.49)         | 8 (0.72)            | 1.25 (1.05-1.50)    | 0.0135              |
| No                               | 633 (76.54)       | 183 (22.13)          | 11 (1.33)                | 1,009 (82.37)       | 205 (16.73)         | 11 (0.90)           | 1.41 (1.14-1.73)    | 0.0012              |
| Pack years                       |                   |                      |                         |                     |                     |
| ≥20                              | 979 (76.84)       | 275 (21.59)          | 20 (1.57)                | 645 (80.83)         | 149 (18.67)         | 4 (0.50)            | 1.26 (1.01-1.58)    | 0.0407              |
| 0-20                             | 190 (79.83)       | 44 (18.49)           | 4 (1.68)                 | 255 (80.70)         | 57 (18.04)          | 4 (1.27)            | 1.08 (0.70-1.65)    | 0.7301              |
| 0                                | 633 (76.54)       | 183 (22.13)          | 11 (1.33)                | 1,009 (82.37)       | 205 (16.73)         | 11 (0.90)           | 1.43 (1.14-1.79)    | 0.0017              |
| Group by gender and smoking      |                   |                      |                         |                     |                     |
| Male smoker                      | 1,118 (77.48)     | 302 (20.93)          | 23 (1.59)                | 881 (80.60)         | 204 (18.66)         | 8 (0.73)            | 1.22 (1.01-1.46)    | 0.0350              |
| Male non-smoker                  | 214 (76.98)       | 60 (21.58)           | 4 (1.44)                 | 511 (81.11)         | 109 (17.30)         | 10 (1.59)           | 1.21 (0.89-1.65)    | 0.2176              |
| Female smoker                    | 51 (73.91)        | 17 (24.64)           | 1 (1.45)                 | 19 (90.48)          | 2 (9.52)            | 0 (0)               | 2.76 (0.58-13.1)    | 0.2017              |
| Female non-smoker                | 419 (76.32)       | 123 (22.40)          | 7 (1.28)                 | 498 (83.70)         | 96 (16.13)          | 1 (0.17)            | 1.62 (1.23-2.15)    | 0.0007              |
| Source                           |                   |                      |                         |                     |                     |
| Discovery                        | 1,091 (76.78)     | 308 (21.67)          | 22 (1.55)                | 1,147 (80.66)       | 263 (18.50)         | 12 (0.84)           | 1.26 (1.06-1.49)    | 0.0086              |
| Replication                      | 711 (77.45)       | 194 (21.13)          | 13 (1.42)                | 762 (83.10)         | 148 (16.14)         | 7 (0.76)            | 1.36 (1.08-1.70)    | 0.0080              |

Bold print indicates statistical significance. <sup>a</sup>Additive model: Wild-type (TT)/heterozygote (CT)/variant homozygote (CC). <sup>b</sup>Adjusted in a logistic regression model that included age, gender and pack years of smoking. <sup>c</sup>P-value calculated by gene-environment interaction. BMP, bone morphogenetic protein. OR, odds ratio; CI, confidence interval.
cancer. For example, SMAD5 was considered to be a candidate tumor suppressor gene in myelodysplastic syndrome and acute myeloid leukemia and is found in human hematopoietic progenitor cells.

Animal studies have also revealed specific functions of SMAD5. In the absence of SMAD5, mice die between embryonic day (E)9.5 and E10.5 from impaired circulation. Difficulties in angiogenesis and diminished number of vascular smooth muscle cells (VSMCs) have been reported in SMAD5-deficient mice (16,17). Mice lacking SMAD5 exhibited increased apoptosis of cardiac myocytes and craniofacial abnormalities. At the cellular level, previous studies demonstrated that conditional knockout of SMAD1 and SMAD5 in granulosa cells results in the formation of vascularized granulosa cell tumors with complete penetrance and with an elevated incidence of peritoneal metastases and hemorrhagic ascites with increasing age (18). In addition, SMAD5-deficient embryos exhibited defects in angiogenesis leading to decreased VSMC numbers and enlarged dilated vessels. A disorganized allantois was formed, primordial germ cells were largely diminished, with left-right asymmetry and impaired embryo turning, all resulting from SMAD5 deficiency (19,20).

Furthermore, the SMAD5+ yolk sac may lead to abnormalities in the vitelline network (17). However, the potential molecular mechanism underlying SMAD5-dependent function remains to be further elucidated.

The results of the present study also indicated that the effect of SMAD5 rs12719482 (T>C) on humans was detrimental, particularly among heavy smokers and light smokers. In particular, the effect of SMAD5 rs12719482 (T>C) was more prominent among light smokers (0 pack-years) and non-smokers compared with the wild-type TT. For example, the heavy smoking group (≥20 pack-years) exhibited a 1.26-fold increased risk for rs12719482 (T>C), whereas its effect was 1.43 among light smokers when wild-type TT was used as the reference genotype in the additive model. When stratified by smoking status, non-smokers showed a 1.41-fold increased risk for rs12719482 (T>C) compared with the TT genotype. Therefore, there may be a modified association between cigarette smoking and the genotype variants of SMAD5 rs12719482 (T>C) of the potential mechanism underlying the interaction of SMAD5 and smoking is not clear. However, the role of SMAD5 rs12719482 (T>C) was highlighted among non-smokers and light smokers. Therefore, additional evidence and functional studies are required. In addition, in the present study the frequency distribution of smoking status was not homogeneous between cases and controls in stage I as well as stage II, reflecting the differences in lifestyle.

Several inherent biases of this study should be elucidated. First, patients were mainly selected from hospitals and the controls were selected from the center of health examination during a routine health check; therefore, there was an inherent selection bias. However, by matching the controls to the patients according to age, gender and residential area, the underlying confounding factors may be minimized. Second, risk factors other than smoking status, such as occupational exposure and nutrition, which may interact with BMP genotypes or function as potential confounding factors, were not included in our study. Possible interactions between those factors should be thoroughly investigated in future studies. Finally, the functional relevance of rs12719482 remains unknown, and further related investigations should be performed.

In conclusion, we herein provided evidence indicating that the SMAD5 rs12719482 polymorphism and its interactions with smoking status may be a potential etiology of lung cancer in Chinese patients. These findings remain to be tested by larger scale studies in different ethnic groups.

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