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Impact of passive smoking, cooking with solid fuel exposure, and MBL/MASP-2 gene polymorphism upon susceptibility to tuberculosis

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SUMMARY

Background: To explore the impact of passive smoking, cooking with solid fuel, mannose-binding lectin (MBL) gene, MBL-associated serine proteases 2 (MASP-2) gene, and gene–environment interactions on the susceptibility to tuberculosis (TB) in non-smokers.

Methods: A total of 205 TB patients and 216 healthy controls were recruited to participate in this case–control study. PCR with sequence-specific primers (PCR-SSP) technology was leveraged to genotype rs7096206 of MBL genes and rs2273346 and rs6695096 of MASP-2 genes. Demographic data and information on exposures of participants were collected. Unconditioned logistic regression analysis was conducted to identify associations between the various factors and TB, and marginal structural linear odds models were used to estimate the interactions.

Results: Passive smoking and cooking with solid fuel were associated with the risk of TB, with odds ratios (OR) of 1.58 and 2.93, respectively ($p < 0.05$). Genotype CG at rs7096206 of MBL genes (OR 2.02) and genotype TC at rs6695096 of MASP-2 genes (OR 1.67) were more prevalent in the TB patients than in healthy controls ($p < 0.05$). The relative excess risk of interaction (RERI) between rs7096206 of MBL genes and passive smoking or cooking with solid fuel exposure was 1.86 (95% confidence interval (CI) 0.59–3.16) and 2.66 (95% CI 1.85–3.47), respectively. The RERI between rs6695096 of MASP-2 genes and cooking with solid fuel exposure was 3.70 (95% CI 2.63–4.78), which was also a positive interaction. However, the RERI between rs6695096 of MASP-2 genes and passive smoking was not statistically significant.

Conclusions: Passive smoking, cooking with solid fuel, and polymorphisms of MBL (rs7096206) and MASP-2 (rs6695096) genes were associated with susceptibility to TB in non-smokers, and there were gene–environment interactions among them. Further studies are needed to explore details of the mechanisms of association.

1. Introduction

More and more studies have revealed that after controlling for a series of potential confounding factors, smoking remains an independent risk factor for tuberculosis (TB), and that the risk of TB increases with increments of smoking.[1,2] Both passive smokers and smokers are exposed to tobacco smoke, but there is a difference in the amount of exposure. According to a meta-analysis conducted by Lin et al.,[3] there is poor consistency for the association between passive smoking and TB among different populations. Additionally, adolescents might be particularly susceptible to the effects of secondhand smoke.[4] However, some studies hold that passive smoking may increase the risk of *Mycobacterium tuberculosis* infection among children only when one or more family members suffer from TB in a household.[5] Currently, there are only a few studies reporting an increasing TB

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incidence due to passive smoking,[4,6] which are not adequate to identify passive smoking as a risk factor for TB. Further studies need to be conducted.

Recently, the impact of indoor air pollution on TB has attracted public attention.[7–10] There are two main types of indoor air pollution: indoor secondhand smoke and kitchen smoke. For the second type, current attention has been drawn to the impact of heating fuel smoke on TB, while the impact of cooking with solid fuel on TB has largely been ignored. Further, research into fuel smoke exposure has generally neglected the contribution of the location of fuel combustion and the structure of the building to indoor air pollution. Also, time spent indoors has a significant impact on fuel fume exposure. Therefore, inaccuracies are inevitable when estimating the actual exposure of different family members.

Mannose-binding lectin (MBL) and MBL-associated serine proteases 2 (MASP-2) are important proteins in the lectin pathway of the immune system. Studies have suggested that polymorphism of MBL gene rs7096206 (−221, Y/X) affects the serum MBL concentration.[11–13] Mutation of MBL gene rs7096206 (−221, Y/X) can lower the serum MBL level.[11] Conclusions are contradictory in terms of the impact of low levels of serum MBL on tubercle bacilli infections. Some studies have found that low levels of serum MBL could reduce tubercle bacilli infections,[14,15] while another has suggested the opposite.[16] The results of association studies on rs7096206 and the risk of TB are also inconsistent among studies.[16,17] Therefore it remains necessary to investigate its relationship with TB in the Chinese population.

MASP-2 gene mutation also facilitates changes in the serum concentration of MASP-2, and results in impaired binding with MBL and ficolin molecules, consequently blocking the lectin pathway of complement activation and resulting in impaired non-specific immune system functioning.[18,19] Mutation of MASP-2 gene rs2273346 (p.V377A) can also lower the serum MASP-2 level.[18] Polymorphism of MASP-2 genes is associated with susceptibility to hepatitis C virus infection and cardiomyopathy in chronic Chagas disease.[20–22] It is not clear whether mutation of MASP-2 gene rs2273346 can lead to increased susceptibility to TB. rs6695096 is located at intron 7 of the MASP-2 gene. Its mutation will not affect the amino acid sequence. However, mutation of the intron may affect gene regulation and selective splicing regulation.[23,24] Consequently rs6695096 may also affect the serum concentration of MASP-2 and influence the susceptibility to TB.

This study explored the impact of passive smoking, cooking with solid fuel, rs7096206 of MBL genes, rs2273346 and rs6695096 of MASP-2 genes, and gene–environment interactions on the susceptibility to TB in non-smokers.

2. Materials and methods

2.1. Sources of cases

A two-step stratified sampling was conducted to randomly select cases. First, four county-level centers for disease control (CDCs) (i.e., Qidong County CDC, Yueyanglou District CDC, Yueyang County CDC, and Hongjiang City CDC) were chosen using a random number table containing 122 counties/cities/districts in Hunan Province. Next, cases were selected randomly from all new TB patients registered in 2009 in the four county-level CDCs. All cases were TB patients with disease confirmed using the TB diagnosis criteria developed by the Chinese Ministry of Health[25]. All smoking patients were excluded from participation in the study.

2.2. Sources of healthy controls

Controls were selected following a stratified sampling strategy similar to that used for cases. First, one community health service center (i.e., Xingang Community Health Service Center) was selected using a random number table containing 14 community health service centers in Kaifu District, Changsha City. Next, one of the six communities (i.e., Xin'ansi Community) covered by the Xingang Community Health Service Center was selected randomly. Finally, all eligible controls were selected randomly from the permanent residents of the Xin'ansi Community. Eligible controls were those who had never smoked, who had a history of *M. tuberculosis* contact (with a bacille Calmette–Guérin (BCG) scar and average diameter of purified protein derivative (PPD) induration ≥10 mm; for those without a BCG scar and without a history of BCG vaccination, the average diameter of PPD induration was ≥5 mm), and no abnormalities on chest X-ray.

After each subject had signed a written informed consent form, an in-house questionnaire was used to collect data. Ethylenediaminetetraacetic acid (EDTA) anticoagulant tubes were used to collect 5-ml venous blood samples, which were stored in a refrigerator at 4 °C. A blood DNA small kit was used to extract the peripheral white blood cell genome (Shanghai Sangon Biotech Co., Ltd).

Exposures to secondhand smoke and solid fuel fumes from cooking were self-reported in the questionnaire. A never-smoker was defined as a person who had never smoked as much as one cigarette a day or the equivalent for the duration of 1 year.[6] The questionnaire collected information on exposure to secondhand smoke at multiple places (home, workplace, restaurant, etc.) and the frequency of exposure (number of days exposed per week). We defined passive smoking[4] as exposure to secondhand smoke at home, in the workplace, or at a restaurant, etc. With regard to fuel exposure, solid fuel (coal and biomass) included coal/lignite, charcoal, wood, straw/shrubs/grass, animal dung, and agricultural crop residue; non-solid fuel included electricity, liquefied petroleum gas, natural gas, biogas, and kerosene. An individual who was responsible for cooking in his/her household using solid fuel was classified as cooking with solid fuel.

2.3. Genotyping

PCR with sequence-specific primers (PCR-SSP) was used to genotype rs7096206 of MBL genes, as well as rs2273346 and rs6695096 of MASP-2 genes. The site sequence of rs7096206 of MBL genes and rs2273346 and rs6695096 of MASP-2 genes were identified in GenBank. Primers were designed using Primer Premier 5.0; the specificity was verified using BLAST software of the US National Center for Biotechnology Information (NCBI). All primers were synthesized by Shanghai Sangon Biotech Co. Ltd (Table 1). The PCR reaction system was 20 μl, including 10 μl mixture, 0.8 μl gDNA, 0.4 μl upstream primers, 0.4 μl downstream primers, and 8.4 μl ddH_2O. The reaction conditions were

Table 1
Primer site sequences of MBL and MASP-2 genes

Mutant	Sense primer	Anti-sense primer	Enzyme
rs7096206	5′ TGGGTTGGTGACTAAGGT 3′	5′ GGTAGGCACTATGATGAGC 3′	Btg I
rs2273346	5′ CAGTAGCAGCAGAGGGAG 3′	5′ CCAGGAGTGTCGGGATTA 3′	Sfc I
rs6695096	5′ TCTGTAAACTGCCTGTCC 3′	5′ ACTACTCCGTAATCCAAG 3′	HpyCH4 III