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Home dampness, beta-2 adrenergic receptor genetic polymorphisms, and asthma phenotypes in children

I-Jen Wang [a,b,c], Ching-Hui Tsai [d], Nai-Wei Kuo [d], Bor-Luen Chiang [e], Kuan-Yen Tung [d], Yungling L. Lee [d,*]

[a] Department of Pediatrics, Taipei Hospital, Department of Health, Taipei, Taiwan
[b] College of Public Health, China Medical University, Taichung, Taiwan
[c] College of Medicine, Fu Jen Catholic University, Taipei, Taiwan
[d] Institute of Epidemiology and Preventive Medicine, National Taiwan University College of Public Health, Taipei, Taiwan
[e] Department of Pediatrics, National Taiwan University Hospital, Taipei, Taiwan

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ABSTRACT

Background: Dampness in the home is a strong risk factor for respiratory symptoms and constitutes a significant public health issue in subtropical areas. However, little is known about the effects of dampness and genetic polymorphisms on asthma.
Methods: In 2007, 6078 schoolchildren were evaluated using a standard questionnaire with regard to information about respiratory symptoms and environmental exposure. Multiple logistic regression analyses were performed to assess the effects of home dampness and beta-2-adrenergic receptor (ADRB2) gene polymorphisms on the prevalence of asthma and selected indicators of severity of asthma.
Results: The frequency of mildewy odor, the number of walls with water stamp, and the duration of water damage were all associated with being awakened at night due to wheezing. However, no other clear-cut associations were found for any of the other indicators of asthma. Children exposed to mildewy odor with ADRB2 Arg/Arg genotype were associated with being awakened at night due to wheezing (OR=1.95, 95% CI, 1.14–3.36), compared to those without exposure and with the ADRB2 Gly allele. ADRB2 Arg16Gly showed a significant interactive effect with home dampness on being awakened at night due to wheezing and current wheezing, but no significant effect on active asthma and medication use. Frequency and degree of home dampness were also associated with the prevalence of asthma and selected indicators of severity of asthma, in an exposure–response manner among children with ADRB2 Arg/Arg genotype.
Conclusions: Home dampness prevention is one of the important steps of asthma control, especially in children carrying ADRB2 Arg/Arg genotypes.

1. Introduction

The prevalence of asthma varies in different geographic areas (Zanolin et al., 2004). In addition to genetic susceptibility, both climate and residential environment are important determinants that contribute to such differences (Zanolin et al., 2004; Wang et al., 2007). Warm year-round temperatures (18–28 °C) and high relative humidity (80%) in subtropical areas result in moist housing, with estimates ranging from 18% to 50% of buildings (Han et al., 2009; Mudarri and Fisk, 2007). In these areas, home dampness constitutes significant public health issues and related economic burden (Yang et al., 1998; Mudarri and Fisk, 2007). Evidence has also shown that the positive percentages of specific IgE antibodies to inhaled allergens leading to asthma were higher in a subtropical city than in a temperate city (Agata et al., 1994). Dampness in dwellings may cause water damage, wet spots, visible mold, mildewy odor and create conditions ideal for the growth of bacteria, fungi, mites, and even viruses (Camara et al., 2004). Furthermore, emissions from building materials caused by dampness and microbial growth may be involved in indoor air health concerns. Since the adverse effects of water damage have been found to be perennially related to childhood asthma, eliminating dampness-related agents should be the primary steps towards achieving asthma control (Han et al., 2009).

Fungi, a type of aeroallergen, are very common in our environment, and respiratory exposure to airborne spores is generally constant. When excessive moisture accumulates in buildings (relative humidity level above 60%), mold growth will often occur, particularly if the issue of moisture remains unaddressed (Storey

* Correspondence to: Institute of Epidemiology and Preventive Medicine, College of Public Health, National Taiwan University, Taipei 100, Taiwan. Fax: +886 2 2351 1955.
E-mail address: leolee@ntu.edu.tw (Y.L. Lee).

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et al., 2004). There are four types of health problems that come from exposure to mold: allergic reactions, irritation of tissues, infections, and toxic effects due to mycotoxins (Storey et al., 2004). A prospective cohort study of 1984 children from Finland demonstrated that exposure to mold influenced the development of asthma in childhood (Jaakkola et al., 2005). In our longitudinal cohort study in Taiwan, we found that fungi play a role in infantile atopic diseases (Wang et al., 2007). Other studies also indicate that early exposure to mold and dampness problems predicts the development of asthma, wheezing, and bronchial obstruction during the first 2 years of life (Wickman et al., 2003a,b). Mold may also release toxic volatile organic compounds that are associated with "Sick Building Syndrome" (Claeson et al., 2009). Therefore, assessing the health effects associated with fungal exposure in children is urgently needed.

The β_2-adrenergic receptor (β2AR) gene (ADRB2) on chromosome 5q31–q32, is expressed on the surface of airway smooth muscle cells and plays an important role in airway reactivity (Shek et al., 2001). Previous studies revealed that the two most common single-nucleotide polymorphisms in the ADRB2 gene, Arg16Gly and Glu27Gln, are related to asthma susceptibility (Liggett, 2000). However, some epidemiologic studies investigating the associations of ADRB2 polymorphisms and asthma have produced inconsistent results (Thakkinstian et al., 2005; Holloway et al., 2000; Turner et al., 2004; Hall et al., 2006). Furthermore, meta-analyses that merged data from these studies failed to demonstrate significant associations with asthma (Hall et al., 2006). Failing to take into account environmental factors that regulate receptor activity may account for the discrepancies between these studies (Wang et al., 2001).

Home dampness has been reported to be related to childhood asthma. However, there has been no relevant published literature concerning genetic modification effects on the pathogenesis pathway of dampness exposure. Since ADRB2 polymorphisms may play a role in airway reactivity and asthma susceptibility and dampness exposure may lead to respiratory tract irritation and asthma exacerbation, we sought to evaluate the gene–environment interaction between ADRB2 genetic polymorphisms and home dampness exposure on childhood asthma. To assess the joint effects of home dampness exposure and ADRB2 polymorphisms, we asked three research questions: (1) Is exposure to home dampness during childhood associated with asthma? (2) Are ADRB2 Arg16Gly and Glu27Gln polymorphisms associated with asthma? (3) Does home dampness exposure show stronger associations with asthma in children with ADRB2 polymorphisms?

2. Materials and methods

2.1. Study population

Two cohorts of children were recruited from public schools in 14 communities that were selected based on demographic similarities and a cooperative school district in Taiwan Children Health Study. In each classroom targeted for participation, every student was invited to volunteer. Classroom-level incentives were used to encourage participation. In each school, science, health, or physical education classes were targeted, excluding any special classes for gifted or learning-disabled subjects. The first cohort of 4134 seventh-grade children was enrolled in 2007, and the second cohort of 2839 fourth-grade children was enrolled in 2010. The response rate for the first cohort was 86.5% and was 75.5% for the second cohort. The parents or guardians of each participating student provided written informed consent and completed a questionnaire, which provided demographic information and characterized each participant's history of respiratory illness and associated risk factors, exposure information, and household characteristics. All of the selected subjects were Han Chinese, with the premise that they were rather homogeneous. The study protocol was approved by the institutional review board of our university hospital and complied with the principles outlined in the Helsinki Declaration.

2.2. Health effects assessment

The parents' questionnaire responses were used to categorize children's asthmatic and wheezing status as previously described (Tsai et al., 2010). Active asthma was defined as physician-diagnosed asthma with any asthma-related symptoms or illnesses in the past 12 months. Children were considered as having lifetime asthma if the answer to the question "Has a doctor ever diagnosed this child as having asthma?" was a yes. Medication use was defined as use of any inhaled, oral, or intravenous medication in the past 12 months. Current wheezing was determined by a positive response to the question "Has your child ever had wheezing or whistling in the chest at any time in the past 12 months when he/she did not have a cold or the flu?". Being awakened at night was determined by a positive response to the question "In the past 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?".

2.3. Dampness measurements

In the baseline questionnaire, we collected information about four dampness indices at home. Visible mold exposure was determined by the question "Have you had visible mold in the walls or bathroom in your house in the past 12 months?". Mildewy odor was defined as the parents having perceived mold odor in the house during the past 12 months. Water stamp on the wall was defined as parents having perceived wet stamps because of moisture in the ceilings, floors or walls in their houses during the past 12 months. Water damage was defined by the question "Have you ever had water damage in your house in the past 12 months?". The subgroup of visible mold and water stamp on the wall was divided into three categories as follows: none, one wall, and two or more than two walls with visible mold or water stamps at home. The frequency of mildewy odor was categorized as none, seldom, and monthly or more than monthly. The frequency of water damage was divided into none, 1 day, and two or more than 2 days.

2.4. Analysis of genetic polymorphisms

DNA collection and genotyping Cotton swabs containing oral mucosa were collected and were immediately maintained at $-80\ ^{\circ}C$ throughout transfer and storage. Genomic DNA was isolated using the phenol/chloroform extraction method previously described with some modifications (Gill et al., 1985). Two ADRB2 functional single-nucleotide polymorphisms were selected for genotyping in this study: rs1047213 (Arg16Gly) and rs1042714 (Gln27Glu) and were detected by real-time polymerase chain reaction using the TaqMan Allelic Discrimination assay on an ABI PRISMTM 7900 Sequence Detector (Applied Biosystems; Foster City, California). Our primer and probe sequences are listed in Table S1. All assays were performed by workers unaware of the clinical status of individual subjects, and genotype assignments were based on two consistent experimental results.

2.5. Statistical analysis

Multiple unconditional logistic regression models adjusted for major covariates were developed to examine the effects of dampness with ADRB2 genotypes on the prevalence of asthma and selected indicators of severity of asthma. To further assess gene–environmental interaction, the combined association of home dampness and ADRB2 Arg16Gly genotypes was examined by stratifying subjects into four groups: no visible mold Gly/Gly or Gly/Arg genotypes, no visible mold Arg/Arg, visible mold Gly/Gly or Gly/Arg, and visible mold Arg/Arg. With regard to ADRB2 Gln27Glu genotypes, the genotypes were also stratified into four groups: no visible mold Glu/Glu or Glu/Gln, no visible mold Gln/Gln, visible mold Glu/Glu or Glu/Gln, and visible mold Gln/Gln.

Gene–environmental interaction was tested by adding a product term in the regression model. For categorical variables with more than two categories, the gene–environmental interaction was evaluated using the likelihood ratio test, comparing the model with indicator variables for the cross-classified variables with a reduced model containing indicator variables for the main effects only. Within a genotype category, a one degree of freedom trend test was used to evaluate the possible exposure–response relationship across categories of the home dampness variables. Odds ratio (OR) and a 95% confidence interval (CI) were adjusted for important potential confounders in all analyses. Potential confounders from literature reviews, such as age, gender, cohort, parental education, family income, parental history of asthma or atopy, gestational age, residence, community, maternal smoking, environmental tobacco smoke exposure, incense burning, pets raising, carpets at home, and number of siblings were all taken into consideration (Jaakkola et al., 2005; Wang et al., 2007; Han et al., 2009). The selection of confounders that were included in the model was based on a priori consideration and standard statistical procedure of 10% change in point estimates (Tong and Lu, 2001).