BMJ Open

Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-047768
Article Type:	Original research
Date Submitted by the Author:	08-Dec-2020
Complete List of Authors:	Li, Yajia; Xiangya Hospital Central South University, Jing, Danrong; Xiangya Hospital Central South University, Dermatology Huang, Yuzhou ; Xiangya Hospital Central South University, dermatology Su, Juan; Xiangya Hospital Central South University, Dermatology Li, Ji Tao, Juan; Affiliated Union Hospital, Tongji Medical College, Huazhong University of Science and Technology Shan, Shijun; Xiang'an Hospital, Xiamen University, Department of Dermatology Wang, Xiaohui; Zhongshan Hospital Xiamen University, Department of Dermatology, Kang, Xiaojing; People's Hospital of Xinjiang Uygur Autonomous Region, Department of Dermatology Wu, Bin; People's Hospital, Inner Mongolia Medical University, Department of Dermatology Chen, Xiang; Xiangya Hospital Central South University, Xiao, Yi; Xiangya Hospital Central South University Shen, Minxue; Xiangya Hospital Central South University, Dermatology
Keywords:	Allergy < THORACIC MEDICINE, Adult dermatology < DERMATOLOGY, Eczema < DERMATOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood

Yajia Li, M.A student [1], Danrong Jing, Ph.D. student [1], Yuzhou Huang, Ph.D. student [1], Juan Su, Ph.D. [1], Ji Li, Ph.D. [1], Juan Tao, Ph.D. [2], Shijun Shan, Ph.D. [3], Xiaohui Wang, Ph.D.[4], Xiaojing Kang, Ph.D. [5], Bin Wu, Ph.D.[6], Yi Xiao, Ph.D. [1*], Xiang Chen, Ph.D.[1*], Minxue Shen, Ph.D. [1,7*]

[1]Department of Dermatology; Hunan Engineering Research Center of Skin Health and Disease; Hunan Key Laboratory of Skin Cancer and Psoriasis, Xiangya Hospital, Central South University, Changsha, China

[2]Department of Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[3]Department of Dermatology, Xiang'an Hospital, Xiamen University, Xiamen, China

[4]Department of Dermatology, Zhongshan Hospital, Xiamen University, Xiamen, China

[5]Department of Dermatology, People's Hospital of Xinjiang Uygur Autonomous Region, Urumchi, Xinjiang, China

[6]Department of Dermatology, People's Hospital, Inner Mongolia Medical University, Hohhot, China

[7]Department of Social Medicine and Health Management, Xiangya School of Public Health, Central South University, Changsha, China

***Correspondence:**
Minxue Shen, Xiang Chen and Yi Xiao

Address: Xiangya Hospital, 87 Xiangya Road, Changsha, Hunan, China 410008

Email: shenmx1988@csu.edu.cn (M.S.), chenxiangck@126.com (X.C.) and xiaoyixy@csu.edu.cn (Y.X.)

Keywords: antibiotics, atopic and allergic skin diseases, preschool and young adulthood.

Abstract

Background: Overuse and misuse of antibiotics is a public health problem in low- and middle-income countries. Although the association of antibiotics with atopic and allergic diseases has been established, most studies focused on prenatal exposure and the occurrence of disease in infants or young children.

Objective: To investigate the association of preschool use of antibiotics with atopic and allergic skin diseases in young adulthood for the association of antibiotics use with eczema.

Design: Population-based retrospective cohort.

Setting and participants: The first-year college students (n=20138) from five universities were investigated. The sampled universities are located in Changsha, Wuhan, Xiamen, Urumqi, and Hohhot, respectively.

Methods: We conducted a dermatological field examination and a questionnaire survey inquiring the participants about frequency of upper respiratory tract infection (URTI) and the preschool antibiotics use (prior to 7 years old). The two-level Probit model was used to estimate the associations, and adjusted risk ratio (aRR) and 95% confidence interval (CI) were presented as the effect size.

Results: A total of 20138 participants with complete information was included in the final analysis. The frequent antibiotics use intravenously (aRR 1.36, 95% CI 1.14–1.62) and orally (aRR 1.18, 95% CI 1.01–1.38) prior to 7 years old was significantly associated with atopic dermatitis in young adulthood. Similar trends could be observed in allergic skin diseases among those who use antibiotics orally and intravenously, with RRs of 1.16 (1.01, 1.34) and 1.33 (1.13, 1.57), respectively.

Conclusions: Preschool URTI and antibiotics use significantly increases the risk of atopic and allergic skin diseases in young adulthood.

Strengths and limitations of this study

- The main outcomes were diagnosed by specialists instead of self-report.
- This study provides a relatively large and representative sample, and sufficient variations in geographic regions and sociodemographic subgroups, as well as the random effect at the university level was fitted by the 2-level models, resulting in an unbiased estimation of associations.
- Recall bias in the measurement of exposure to antibiotics might have been introduced, which could not be ignored in most retrospective studies.
- We lacked the information about the type and dose of antibiotics, and there might be a reversed causal relationship because antibiotics could be used in the treatment of AD and other conditions accompanied by a bacterial infection.

1 Introduction

The incidence and prevalence of atopic and allergic conditions such as asthma, allergic rhinitis, food allergies, and atopic dermatitis (AD) among the worldwide population have significantly increased during the past several decades.[1-3] An area of environmental change that may be responsible for the increase of allergic and atopic diseases is the growing use of medications which may alter the development of human microbiome.[4] It also seems that use of some antibiotics, which can directly cause intestinal dysbiosis and affect human microbiome and increase the risk for allergy development, is of particular concern in light of accumulating evidence.[5-7]

Furthermore, overuse and misuse of antibiotics is a severe public health problem worldwide, especially in low- and middle-income countries. In the last decade, prescriptions of broad-spectrum antibiotics increased by 49% in children under five years and doubled in children aged 5–17 years, concomitant with the increasing prevalence of allergic diseases.[8, 9] In China, 70% of outpatients attending primary care facilities with colds were inappropriately treated with antibiotics, often by intravenous infusion. The situation is even worse in children because many parents demand treatment with antibiotics[10]. However, most upper respiratory tract infections (URTI) in children are viral, for which antibiotics are unnecessary.[11-13]

The association between the use of antibiotics and atopic and allergic diseases has been observed in longitudinal studies. But most studies focused on antibiotics use during pregnancy or infancy when early colonization is initiated by maternal microbes.[14-17] With the childhood microbiome transition owing to alterations in food and exposure to more diverse microbes in external environments, children in preschool age (<7 yrs) are at higher risks of URTI infection and antibiotics treatment. However, the effect of antibiotics used during this period on atopic and allergic skin diseases that occurred in their young adulthood is not clear. The objective of this study was to evaluate the hypothesis that exposure to antibiotics in preschool age is associated with an increased risk of allergic and atopic skin diseases in young adulthood. We tested this hypothesis by conducting a retrospective study in college students.

2 Materials and Methods

2.1 Study setting and design

This was a retrospective cohort study based on the data from the China College Student Skin Health Study (CCSSHS)[18]. The first-year college students from five universities were investigated. They underwent a health examination and completed a questionnaire survey. The sampled universities are located in Changsha, Wuhan, Xiamen, Urumqi, and Hohhot, respectively. The medical ethics committee of Xiangya Hospital, Central South University, approved the study (#201709993).

2.2 Exposure assessment

Two semi-quantitative questions served as the proxy measures of the frequency of antibiotics exposure in preschool age, with detailed explanations for the definitions of URTI and antibiotics. In our study, the definition of URTI refers to a series of acute illnesses that have an effect on the upper respiratory system including the common cold, acute otitis media, tonsillitis/tonsillopharyngitis, sinusitis and recurrent sinusitis.[19] The first question was “How often did you have URTI in your preschool age or before 7 years old”, with three potential responses: ⩽1 time/year, 2-3 times/year”, and “4 or more

times/year". The second question was "How often did you receive antibiotics treatment when you had a URTI", with four responses: "rare", "occasional", "often, orally; and "often, intravenously".

2.3 Outcome assessment

Diagnosis of skin diseases and inquiry of disease history were performed by dermatologists during the field survey. All subjects underwent skin examination by resident doctors in dermatology, and the diagnoses were further validated by senior dermatologists. Clinical manifestation, disease history, and family history were inquired about, and inspections were conducted to diagnose skin diseases. For recurrent skin diseases, only those with current symptoms and cutaneous lesions were diagnosed as cases. AD was diagnosed according to the Williams criteria.[20] Hand eczema was diagnosed according to eczema (rash) on the fingers, finger webs, palms, or back of hands, which had appeared once and continued for at least two weeks or had appeared several times or had been persistent. Allergies and urticaria were diagnosed by clinical manifestations, potential triggers, and histories. Asthma, allergic rhinitis, and allergic conjunctivitis were self-reported according to doctors' diagnoses. We also combined some of the outcomes. Atopic march is an apparent progression of allergic diseases from AD, to allergic asthma (AA) to allergic rhinitis (AR) and allergic conjunctivitis.[21, 22] We include the conditions of AD, AA, AR, and allergic conjunctivitis for the outcome of the atopic march. Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria. Participants with a history of atopic/allergic conditions but without the current disease are excluded.

2.4 Covariates

Demographic characteristics, socioeconomic status (annual family income and parental highest educational level), family history, behavioral factors (dietary and bathing habits) were inquired by the questionnaire.

2.5 Statistical analysis

Categorical data were presented as number (%), and the between-group difference was tested using the chi-square test. Two-level Probit regression models (individual as level 1 and university as level 2) were used to estimate the associations of preschool exposure to antibiotics with atopic skin diseases in young adulthood, adjusting for level-1 covariates (gender, ethnicity, annual household income and parental education) and level-2 random effects. The effect size was presented as relative risk (RR) and 95% confidence interval (CI). $P< 0.05$ was considered statistically significant for all tests. Statistical analysis was performed in SAS 9.4 (SAS Institute Inc., Cary, USA).

3 Results

A total of 27144 registries for new enrolment was identified; among them, 21086 (77.7%) consented to participate, and 20138 (74.2%) who underwent the health examination and completed the online questionnaire survey were included in the final analyses. The mean age was 18.3± 0.8 years and 10289 (51.1%) were men. The characteristics of participants in the study are shown in the Table 1. The prevalence rates of chronic urticaria, allergic reactions to food/drug/light, hand eczema, and AD were 1.89%, 2.27%, 3.35%, and 3.86%, respectively. The prevalence rates of AD and allergic skin disease were 3.86 % and 4.14%, respectively (Figure 1 and Table S1).

In general, URTI and the use of antibiotics were significantly associated with atopic and allergic diseases dose-dependently (Figure 1 and Table S1). For example, the prevalence of AD increased from 3.39% to 4.11% and 4.79% in participants who reported rare or occasional use, frequent oral use, and

frequent intravenous use of antibiotics, respectively. Consistent trends could be observed in all atopic and allergic diseases and their combinations.

After adjustments for sociodemographic factors (Figure 2 and Table S2), URTI and the use of antibiotics were significantly associated with atopic/allergic skin diseases in dose-response manners. For instance, compared to those reporting rare or occasional use of antibiotics, the RRs for AD in participants who reported frequent oral administration and intravenous injection of antibiotics was 1.18 (95% CI: 1.01, 1.39) and 1.36 (95% CI: 1.14, 1.62), respectively. For other allergies or atopic disease of skin or beyond skin, the correlations were highly consistent despite some variations in the magnitude of the association.

Because our study was not a prospective cohort, it was difficult to know if antibiotic use was ahead of suffering from AD. We further evaluated the joint effect of URTI and antibiotics on AD by including an interaction term in the model. As shown in Figure 3, in each category of antibiotic use, the frequency of URTI was positively associated with the risk of AD. Vice versa, in each category of URTI, antibiotic use was positively associated with AD according to the effect size, despite some insignificant results in categories with a small sample size.

4 Discussion

This retrospective cohort study demonstrated that preschool exposure to antibiotics, either through oral administration or intravenous infusion, was associated with increased risks of having allergic and atopic skin diseases in young adulthood. Participants who reported frequent URTI in preschool-age also had higher risks of allergies and atopy.

Similar trends were identified in previous observational studies that early-life antibiotic use was associated with an increased risk of eczema, but there were still some inconsistent results.[23, 24] Meta-analysis including 22 studies with 394,517 patients concluded that children with antibiotics exposure in the first two years had increased odds of atopic eczema with an OR of 1.26 (95%CI: 1.15-1.37). Notably, the onset age of the outcomes in the included studies was the period of childhood (<12 years old). [7] A large population-based retrospective cohort study in twins showed that antibiotic use was also associated with an increased risk of eczema; however, it is likely that the relationship between early-life antibiotic use and eczema was confounded by shared familial environment and genetic factors.[25] However, current data lacked the information regarding the atopic and allergic skin diseases occurred in late adolescence to early adulthood, while we firstly investigated the effects of preschool antibiotic in a retrospective study and revealed a positive association.

Evidence showed that AD was the first manifestation of an atopic phenotype which begins in early childhood and the progression from AD to the diseases such as food allergy, asthma, and allergic rhinitis were more likely to be shown in adolescence.[22, 26, 27] The mechanisms behind the march from AD to allergic airway diseases and allergic conjunctivitis likely arise from initial epicutaneous allergen sensitization inducing robust local and systemic type 2 immune responses with increased production of type 2 cytokines including interleukin (IL)-4, IL-13, IL-31, and thymic stromal lymphopoietin.[28, 29] Most studies were prone to make these responses responsible for the commonly shared pathogenesis of cutaneous, airway, and conjunctiva inflammation, supporting the view that AD is not merely a disease confined to the skin, but is in fact, a systemic disease.[30, 31] Therefore, it could be explained that the increased risks beyond skin manifestations in young adulthood were consistent with those of skin diseases and some with even greater effect sizes. While not fully understood, the underlying mechanism of the association between antibiotics and atopic and allergic

diseases can be elucidated by microbial diversity. The gut microbial community is dynamic and variable during the first 3 years of life, before stabilizing to an adult-like state.[32] Studies have demonstrated continued development through childhood into the teenage years.[33, 34] Dietary intake plays a key role in the development period of the gut microbiome. Breast-fed infants have microbiota enriched in *Lactobacillus*, *Staphylococcus*, and *Bifidobacterium*. Studies have shown that human milk isolates contain symbiotic and potentially probiotic microbes, and supplementation of *Bifidobacteria* was found to be effective in primary preventing allergic diseases.[35-37] But among children of preschool age, the dominance of *Bifidobacterium* diminishes with the alteration in dietary intake.[15] On the other hand, the high prevalence of antibiotic use may also lead to a concurrent increase in antibiotic-resistant bacteria.[38, 39] Antibiotic-treated children have a less diverse gut microbiota and less stable communities. Antibiotic therapy affects microbiome variety and thus may increase the risk of atopic diseases.[40, 41]

In our study, increased risks of atopic march or allergic diseases were observed in students who reported frequent URTI. Another potential explanation was related to the infections which could also affect microbial conditions. Except for the change in diet, children of preschool age tend to be exposed to more diverse microbes and infectious diseases including URTI in kindergarten or external environments. Respiratory viral infections, in particular, have been shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut,[42] which could further shape atopic microenvironments.

Several limitations should be noted, and the results should be interpreted with caution. First, recall bias in the measurement of exposure to antibiotics might have been introduced. While recall bias on the frequency of antibiotics use and URTIs should not be ignored, this is a limitation in most retrospective studies. Second, there was a lack of information about the type and dose of antibiotics, and we could not attribute the association to specific antibiotics. Third, there might be a reversed causal relationship, because antibiotics could be used in the treatment of AD and other conditions accompanied by bacterial infection. Last but not least, we assessed the role of URTI and antibiotics separately, because the two variables were significantly correlated (contingency coefficient=0.4, $P<0.001$) and were therefore not included in the same model to avoid collinearity and biased estimation of parameters. It is possible that the association of URTI with AD and allergies is confounded by antibiotics, and vice versa.

However, both infection and antibiotics may be correlated with allergies/atopies, with different mechanisms. Although under the hygiene hypothesis, exposure to pathogens during infancy and early childhood has been proposed to explain the lower prevalence of asthma and other atopic diseases among children in developing countries,[43, 44] some studies showed that early respiratory infections could not protect against atopic eczema or recurrent wheezing, but could drive the development of atopic disease. [45-49] Atopic sensitization, a process of generation of specific immunoglobulin E (sIgE) when exposed to an innocuous antigen, was common to all allergic diseases. As preschool URTI most probably represent viral infections in the majority of cases, some studies investigated a potential mechanistic explanation for how a respiratory viral infection could drive the development of atopic sensitization and disease. Martorano et al. used a Sendai virus to establish the mouse model mimicking a human limited respiratory syncytial virus infection and found that Sendai virus infection could promote the crosslinking of high-affinity IgE receptor (FcεRI) on the lung conventional dendritic cells (cDC), which led to the production of the chemokine CCL28, recruiting IL-13 and driving the development of mucous cell metaplasia and airway hyperreactivity.[50] Another animal study found that except for the increase of sIgE against Sendai virus in mice, there was also a large increase in total IgE and it remained elevated long after the viral infection being resolved.[51] This notion has further been fuelled by findings that mice infected with flu virus developed virus-specific mast cell

degranulation in the skin, indicating a possible pathway of viral infections that could mediate allergic symptoms.[52] Besides, the respiratory viral infections were shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut.[42, 53] However, we cannot ignore that infections that do not require antibiotics are not captured in our design, such that it is difficult to assess whether the observed association is caused by a specific infection or antibiotics because they occur simultaneously in many cases.

Our study also has strengths. The primary strength is that the sample size for the retrospective cohort study was large. Second, the outcomes were diagnosed by specialists. In contrast, some previous studies used self-reported diagnoses that might introduce misclassification bias. Third, the study had a relatively large and representative sample, and sufficient variations in geographic regions and sociodemographic subgroups. The random effect at the university level was fitted by the 2-level models, resulting in an unbiased estimation of associations.

To conclude, preschool children exposed to URTI or antibiotics may be at higher risks of atopic and allergic skin diseases in their young adulthood, especially among those who frequently had URTI or received antibiotics by intravenous infusion. Our study implies that unnecessary antibiotics treatment in children should be avoided to prevent the occurrence of atopic and allergic diseases in their later life. Prospective studies that consider the type and dose of antibiotics are warranted.

5 Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

6 Ethics statement

This study was conducted according to the guidelines established in the Declaration of Helsinki. All procedures involving patients were approved by the institutional research ethics boards of Xiangya Hospital, Central South University (Changsha, China). Informed consent was obtained from all the students before the investigation.

7 Author contributions

Dr. Minxue Shen, Dr. Xiang Chen, and Dr. Yi Xiao are joint corresponding authors and have full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Shen, Chen, and Xiao.

Acquisition, analysis, or interpretation of data: Y.J. Li, Jing, and Huang

Drafting of the manuscript: Y.J. Li

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Shen, Xiao, and Y.J. Li.

Administrative, technical, or material support: Su, J. Li, Tao, Shan, Wang, Kang, and Wu.

Supervision: Chen

8 Conflict of interest

The authors declare no conflict of interest.

9 Funding

This work was supported by the National Key Research & Development Project "Precision Medicine Initiative" of China (2016YFC0900802) and the Program of Introducing Talents of Discipline to Universities (111 Project, No. B20017).

10 Acknowledgment

The authors would like to thank the following individuals and institutions who have made substantial contribution to the establishment of the cohort: Central South University (Lei Cai, Duling Cao, Qin Cao, Chao Chen, Liping Chen, Menglin Chen, Mengting Chen, Xiang Chen, Qing Deng, Xin Gao, Yihuan Gong, Jia Guo, Yeye Guo, Rui Hu, Xin Hu, Chuchu Huang, Huining Huang, Kai Huang, Xiaoyan Huang, Yuzhou Huang, Danrong Jing, Xinwei Kuang, Li Lei, Jia Li, Jiaorui Li, Jie Li, Keke Li, Peiyao Li, Yajia Li, Yayun Li, Yangfan Li, Dan Liu, Dihui Liu, Fangfen Liu, Nian Liu, Panoan Liu, Runqiu Liu, Hui Lu, Wenhua Lu, Yan Luo, Zhongling Luo, Manyun Mao, Mengping Mao, Yuyan Ouyang, Shiyao Pei, Qunshi Qin, Ke Sha, Lirong Tan, Ling Tang, Ni Tang, Yan Tang, Ben Wang, Yaling Wang, Tianhao Wu, Yun Xie, Siyu Yan, Sha Yan, Bei Yan, Xizhao Yang, Lin Ye, Hu Yuan, Taolin Yu, Yan Yuan, Yi Yu, Rui Zhai, Jianghua Zhang, Jianglin Zhang, Mi Zhang, Xingyu Zhang, Zhibao Zhang, Shuang Zhao, Yaqian Zhao, Kuangbiao Zhong, Lei Zhou, Youyou Zhou, Zhe Zhou, Susi Zhu); Huazhong University of Science and Technology (Xiangjie An, Siqi Da, Yaqi Dong, Yangxue Fu, Lixie Gao, Han Han, Biling Jiang, Jiajia Lan, Jun Li, Xiaonan Li, Yan Li, Liquan Liu, Yuchen Lou, Pu Meng, Yingli Nie, Gong Rao, Shanshan Sha, Xingyu Su, Huinan Suo, Rongying Wang, Jun Xie, Yuanxiang Yi, Jia Zhang, Qiao Zhang, Li Zhu, Yanming Zhu); Xiamen University (Zhiming Cai, Lina Chen, Xiaozhu Fu, Hongjun Jiang, Guihua Luo, Jianbing Xiahou, Binxiang Zheng); People's Hospital of Xinjiang Uygur Autonomous Region (Jianxia Chen, Xiaomin Chen, Xinqi Chen, Li Dai, Yanyan Feng, Fanhe Jiang, Lan Jin, Qingyu Ma, Qun Shi, Hongbo Tang, Fang Wang, Zhen Wang, Xiujuan Wu, Kunjie Zhang, Yu Zhang); Xinjiang Medical University (Huagui Li, Jianguang Li, Lei Shi); Inner Mongolia Medical University (Chao Tian, Wei Wang, Rina Wu, Hongjun Xing, Baogui Yang).

11 References

[1]. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action[J]. World Allergy Organ J. 2014,7(1):12.

[2]. Platts-Mills TA. The allergy epidemics: 1870-2010[J]. J Allergy Clin Immunol. 2015,136(1):3-13.

[3]. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys[J]. Lancet. 2006,368(9537):733-43.

[4]. Mitre E, Susi A, Kropp LE, Schwartz DJ, Gorman GH, Nylund CM. Association Between Use of Acid-Suppressive Medications and Antibiotics During Infancy and Allergic Diseases in Early Childhood[J]. JAMA Pediatr. 2018,172(6):e180315.

[5]. Wang JY, Liu LF, Chen CY, Huang YW, Hsiung CA, Tsai HJ. Acetaminophen and/or antibiotic use in early life and the development of childhood allergic diseases[J]. Int J Epidemiol. 2013,42(4):1087-99.

[6]. Kim DH, Han K, Kim SW. Effects of Antibiotics on the Development of Asthma and Other Allergic Diseases in Children and Adolescents[J]. Allergy Asthma Immunol Res. 2018,10(5):457-65.

[7]. Ahmadizar F, Vijverberg SJH, Arets HGM, de Boer A, Lang JE, Garssen J, et al. Early-life antibiotic exposure increases the risk of developing allergic symptoms later in life: A meta-analysis[J]. Allergy. 2018,73(5):971-86.

[8]. Lee GC, Reveles KR, Attridge RT, Lawson KA, Mansi IA, Lewis JS, 2nd, et al. Outpatient antibiotic prescribing in the United States: 2000 to 2010[J]. BMC Med. 2014,12:96.

[9]. Versporten A, Bielicki J, Drapier N, Sharland M, Goossens H. The Worldwide Antibiotic Resistance and Prescribing in European Children (ARPEC) point prevalence survey: developing hospital-quality indicators of antibiotic prescribing for children[J]. J Antimicrob Chemother. 2016,71(4):1106-17.

[10]. Biezen R, Grando D, Mazza D, Brijnath B. Dissonant views - GPs' and parents' perspectives on antibiotic prescribing for young children with respiratory tract infections[J]. BMC Fam Pract. 2019,20(1):46.

[11]. Wei X, Zhang Z, Walley JD, Hicks JP, Zeng J, Deng S, et al. Effect of a training and educational intervention for physicians and caregivers on antibiotic prescribing for upper respiratory tract infections in children at primary care facilities in rural China: a cluster-randomised controlled trial[J]. Lancet Glob Health. 2017,5(12):e1258-e67.

[12]. Li J, Song X, Yang T, Chen Y, Gong Y, Yin X, et al. A Systematic Review of Antibiotic Prescription Associated With Upper Respiratory Tract Infections in China[J]. Medicine (Baltimore). 2016,95(19):e3587.

[13]. Ding L, Sun Q, Sun W, Du Y, Li Y, Bian X, et al. Antibiotic use in rural China: a cross-sectional survey of knowledge, attitudes and self-reported practices among caregivers in Shandong province[J]. BMC Infect Dis. 2015,15:576.

[14]. McKeever TM, Lewis SA, Smith C, Collins J, Heatlie H, Frischer M, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database[J]. J Allergy Clin Immunol. 2002,109(1):43-50.

[15]. Kundu P, Blacher E, Elinav E, Pettersson S. Our Gut Microbiome: The Evolving Inner Self[J]. Cell. 2017,171(7):1481-93.

[16]. Yoshida S, Ide K, Takeuchi M, Kawakami K. Prenatal and early-life antibiotic use and risk of childhood asthma: A retrospective cohort study[J]. Pediatr Allergy Immunol. 2018,29(5):490-5.

[17]. Kusel MM, de Klerk N, Holt PG, Sly PD. Antibiotic use in the first year of life and risk of atopic disease in early childhood[J]. Clin Exp Allergy. 2008,38(12):1921-8.

[18]. S M, X Y, S J. Prevalence and patient-reported outcomes of noncommunicable skin diseases among college students in China.[J]. JAAD Int. 2020,1(1):23-30.

[19]. Eccles MP, Grimshaw JM, Johnston M, Steen N, Pitts NB, Thomas R, et al. Applying psychological theories to evidence-based clinical practice: identifying factors predictive of managing upper respiratory tract infections without antibiotics[J]. Implement Sci. 2007,2:26.

[20]. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation[J]. Br J Dermatol. 1994,131(3):406-16.

[21]. Spergel JM, Paller AS. Atopic dermatitis and the atopic march[J]. The Journal of allergy and clinical immunology. 2003,112(6 Suppl):S118-27.

[22]. Hill DA, Spergel JM. The atopic march: Critical evidence and clinical relevance[J]. Ann Allergy Asthma Immunol. 2018,120(2):131-7.

[23]. Metzler S, Frei R, Schmaußer-Hechfellner E, von Mutius E, Pekkanen J, Karvonen AM, et al. Association between antibiotic treatment during pregnancy and infancy and the development of allergic diseases[J]. Pediatr Allergy Immunol. 2019,30(4):423-33.

[24]. Tsakok T, McKeever TM, Yeo L, Flohr C. Does early life exposure to antibiotics increase the risk of eczema? A systematic review[J]. Br J Dermatol. 2013,169(5):983-91.

[25]. Slob EMA, Brew BK, Vijverberg SJH, Kats C, Longo C, Pijnenburg MW, et al. Early-life antibiotic use and risk of asthma and eczema: results of a discordant twin study[J]. Eur Respir J. 2020,55(4).

[26]. Johansson E, Hershey GKK. Contribution of an impaired epithelial barrier to the atopic march[J]. Ann Allergy Asthma Immunol. 2018,120(2):118-9.

[27]. Belgrave DC, Granell R, Simpson A, Guiver J, Bishop C, Buchan I, et al. Developmental profiles of eczema, wheeze, and rhinitis: two population-based birth cohort studies[J]. PLoS Med. 2014,11(10):e1001748.

[28]. Akei HS, Brandt EB, Mishra A, Strait RT, Finkelman FD, Warrier MR, et al. Epicutaneous aeroallergen exposure induces systemic TH2 immunity that predisposes to allergic nasal responses[J]. J Allergy Clin Immunol. 2006,118(1):62-9.

[29]. Cianferoni A, Spergel J. The importance of TSLP in allergic disease and its role as a potential therapeutic target[J]. Expert Rev Clin Immunol. 2014,10(11):1463-74.

[30]. Asada Y, Nakae S, Ishida W, Hori K, Sugita J, Sudo K, et al. Roles of Epithelial Cell-Derived Type 2-Initiating Cytokines in Experimental Allergic Conjunctivitis[J]. Invest Ophthalmol Vis Sci. 2015,56(9):5194-202.

[31]. Han H, Xu W, Headley MB, Jessup HK, Lee KS, Omori M, et al. Thymic stromal lymphopoietin (TSLP)-mediated dermal inflammation aggravates experimental asthma[J]. Mucosal Immunol. 2012,5(3):342-51.

[32]. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography[J]. Nature. 2012,486(7402):222-7.

[33]. Hollister EB, Riehle K, Luna RA, Weidler EM, Rubio-Gonzales M, Mistretta TA, et al. Structure and function of the healthy pre-adolescent pediatric gut microbiome[J]. Microbiome. 2015,3:36.

[34]. Agans R, Rigsbee L, Kenche H, Michail S, Khamis HJ, Paliy O. Distal gut microbiota of adolescent children is different from that of adults[J]. FEMS Microbiol Ecol. 2011,77(2):404-12.

[35]. Heikkilä MP, Saris PE. Inhibition of Staphylococcus aureus by the commensal bacteria of human milk[J]. J Appl Microbiol. 2003,95(3):471-8.

[36]. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life[J]. Cell Host Microbe. 2015,17(6):852.

[37]. Enomoto T, Sowa M, Nishimori K, Shimazu S, Yoshida A, Yamada K, et al. Effects of bifidobacterial supplementation to pregnant women and infants in the prevention of allergy development in infants and on fecal microbiota[J]. Allergol Int. 2014,63(4):575-85.

[38]. Hiltunen T, Virta M, Laine AL. Antibiotic resistance in the wild: an eco-evolutionary perspective[J]. Philos Trans R Soc Lond B Biol Sci. 2017,372(1712).

[39]. Hawkey PM, Jones AM. The changing epidemiology of resistance[J]. J Antimicrob Chemother. 2009,64 Suppl 1:i3-10.

[40]. Penders J, Stobberingh EE, van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders[J]. Allergy. 2007,62(11):1223-36.

[41]. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema[J]. J Allergy Clin Immunol. 2012,129(2):434-40, 40.e1-2.

[42]. Hanada S, Pirzadeh M, Carver KY, Deng JC. Respiratory Viral Infection-Induced Microbiome Alterations and Secondary Bacterial Pneumonia[J]. Front Immunol. 2018,9:2640.

[43]. Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis[J]. Nat Immunol. 2017,18(10):1076-83.

[44]. Haspeslagh E, Heyndrickx I, Hammad H, Lambrecht BN. The hygiene hypothesis: immunological mechanisms of airway tolerance[J]. Curr Opin Immunol. 2018,54:102-8.

[45]. Kramer MS, Guo T, Platt RW, Sevkovskaya Z, Dzikovich I, Collet JP, et al. Does previous infection protect against atopic eczema and recurrent wheeze in infancy?[J]. Clin Exp Allergy. 2004,34(5):753-6.

[46]. Peebles RS, Jr. Viral infections, atopy, and asthma: is there a causal relationship?[J]. The Journal of allergy and clinical immunology. 2004,113(1 Suppl):S15-8.

[47]. Martinez FD. Viruses and atopic sensitization in the first years of life[J]. Am J Respir Crit Care Med. 2000,162(3 Pt 2):S95-9.

[48]. Nafstad P, Brunekreef B, Skrondal A, Nystad W. Early respiratory infections, asthma, and allergy: 10-year follow-up of the Oslo Birth Cohort[J]. Pediatrics. 2005,116(2):e255-62.

[49]. Dharmage SC, Lodge CJ, Lowe AJ, Allen KJ. Antibiotics and risk of asthma: a debate that is set to continue[J]. Clin Exp Allergy. 2015,45(1):6-8.

[50]. Martorano LM, Grayson MH. Respiratory viral infections and atopic development: From possible mechanisms to advances in treatment[J]. Eur J Immunol. 2018,48(3):407-14.

[51]. Cheung DS, Ehlenbach SJ, Kitchens T, Riley DA, Grayson MH. Development of atopy by severe paramyxoviral infection in a mouse model[J]. Ann Allergy Asthma Immunol. 2010,105(6):437-43.e1.

[52]. Grunewald SM, Hahn C, Wohlleben G, Teufel M, Major T, Moll H, et al. Infection with influenza a virus leads to flu antigen-induced cutaneous anaphylaxis in mice[J]. The Journal of investigative dermatology. 2002,118(4):645-51.

[53]. Culley FJ, Pennycook AM, Tregoning JS, Hussell T, Openshaw PJ. Differential chemokine expression following respiratory virus infection reflects Th1- or Th2-biased immunopathology[J]. J Virol. 2006,80(9):4521-7.

Figure legends

Figure 1. The prevalence of atopic and allergic diseases in exposure vs. non-exposure group of antibiotics and URTI.

Figure 2. Association of early-life exposure to antibiotics with the risk of atopic/allergic diseases later in life. RR: risk ratio; CI: confidence interval.

Figure 3. The joint effect of URTI and antibiotics on AD after including an interaction term in the model. RR: risk ratio; CI: confidence interval.

Supplementary file captions

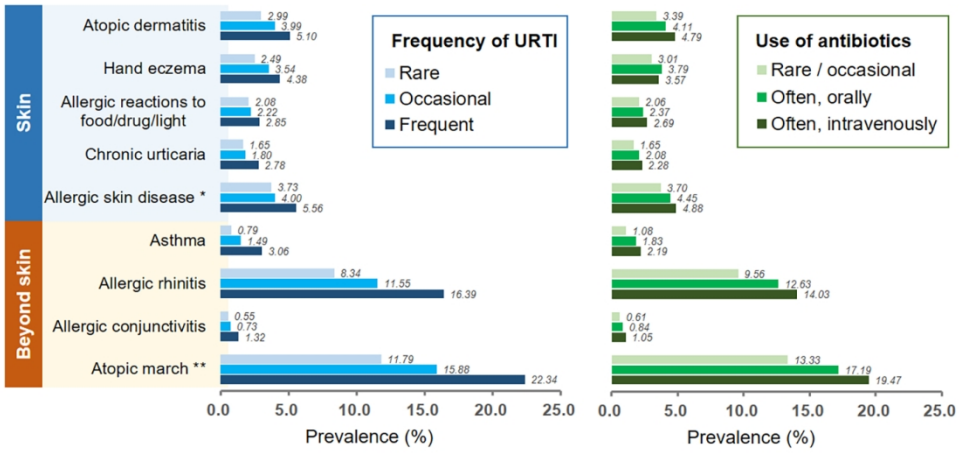
Table S1. The prevalence of atopic and allergic diseases in college students.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students.

446 **Table1. Characteristics of participants in the Present Study (N=20138)**

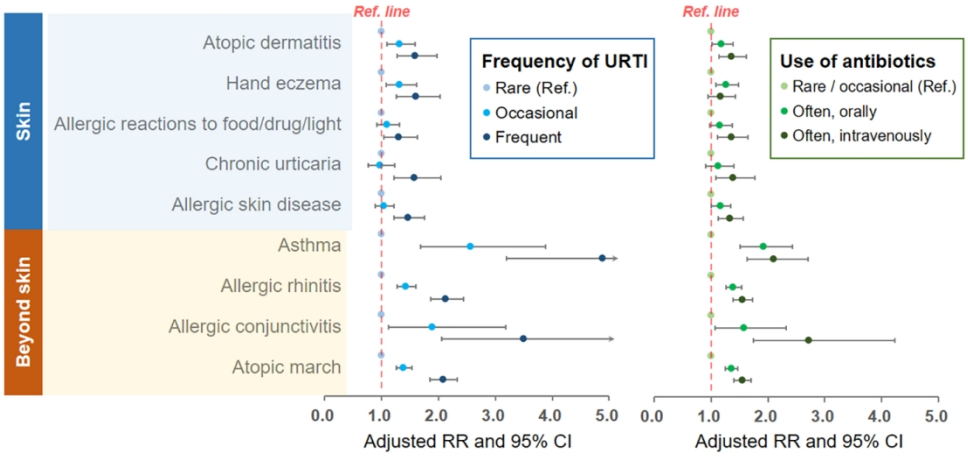
Characteristics	N	%
Gender		
Male	10289	51.1
Female	9849	48.9
Income (CNY)		
<10,000	2169	10.8
10,000–29,999	4378	21.7
30,000–49,999	3470	17.2
50,000–99,999	4419	21.9
100,000–199,999	4065	20.2
≥200,000	1637	8.1
Parental highest education		
Primary school	2288	11.5
Middle school	5557	28
High school	4782	24.1
College and above	7233	36.4
Ethnicity		
Han Chinese	16230	80.6
Other ethnicities	3908	19.4

447



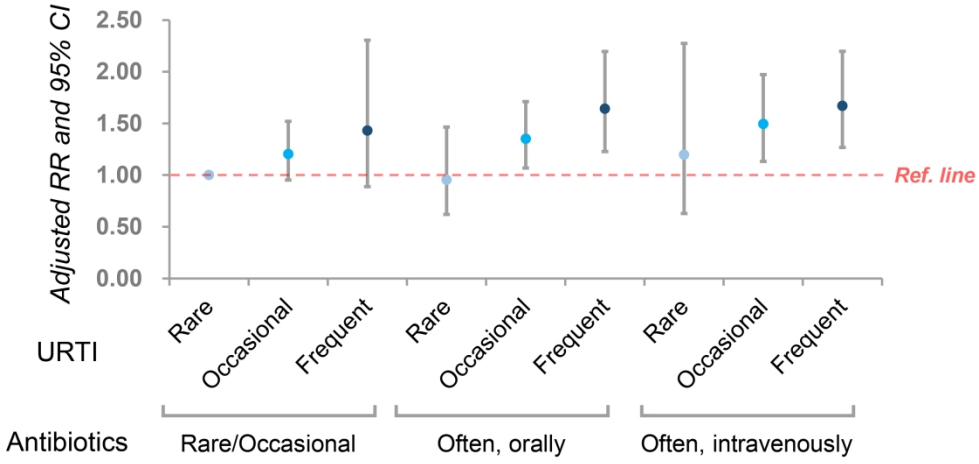
The prevalence of atopic and allergic diseases in exposure vs. non-exposure group of antibiotics and URTI.

127x71mm (300 x 300 DPI)



Association of early-life exposure to antibiotics with the risk of atopic/allergic diseases later in life. RR: risk ratio; CI: confidence interval.

127x71mm (300 x 300 DPI)



The joint effect of URTI and antibiotics on AD after including an interaction term in the model. RR: risk ratio; CI: confidence interval.

236x115mm (300 x 300 DPI)

Supplementary file captions

Table S1. The prevalence of atopic and allergic diseases in college students.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students.

Table S1. The prevalence of atopic and allergic diseases in college students

Disease	Prevalence[a]	URTI, %			Antibiotics, %		
		Rare	Occasional	Frequent	Rare, occasional	Often,orally	Often, intravenously
Skin							
Atopic dermatitis	3.86	2.99	3.99	5.1	3.39	4.11	4.79
Hand eczema	3.35	2.49	3.54	4.38	3.01	3.79	3.57
Allergic reactions to food/drug/light	2.27	2.08	2.22	2.85	2.06	2.37	2.69
Chronic urticaria	1.89	1.65	1.8	2.78	1.65	2.08	2.28
Allergic skin disease [b]	4.14	3.73	4	5.56	3.7	4.45	4.88
Beyond skin							
Atopic march [c]	15.6	11.79	15.88	22.34	13.33	17.19	19.47
Allergic conjunctivitis	0.76	0.55	0.73	1.32	0.61	0.84	1.05
Allergic rhinitis	15.6	8.34	11.55	16.39	9.56	12.63	14.03
Asthma	1.51	0.79	1.49	3.06	1.08	1.83	2.19

[a] The total prevalence of atopic and allergic diseases in our study population.

[b] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.

[c] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students

Disease	URTI, aRR (95%CI) [a]			Antibiotics, aRR (95%CI) [a]		
	Rare	Occasional	Frequent	Rare/Occasional	Often, orally	Often, intravenously
Skin						
Atopic dermatitis	Reference	1.32 (1.09, 1.54)	1.59 (1.27, 1.98)	Reference	1.18 (1.01, 1.39)	1.36 (1.14, 1.62)
Hand eczema	Reference	1.32 (1.08, 1.56)	1.60 (1.26, 2.02)	Reference	1.27 (1.08, 1.49)	1.17 (0.95, 1.44)
Allergic reactions to food/drug/light	Reference	1.10 (0.92, 1.28)	1.30 (1.04, 1.63)	Reference	1.15 (0.97, 1.37)	1.36 (1.12, 1.65)
Chronic urticaria	Reference	0.97 (0.76, 1.17)	1.58 (1.21, 2.05)	Reference	1.13 (0.90, 1.40)	1.39 (1.09, 1.78)
Allergic skin disease [b]	Reference	1.04 (0.89, 1.20)	1.46 (1.22, 1.76)	Reference	1.16 (1.01, 1.34)	1.33 (1.13, 1.57)
Beyond skin						
Atopic march [c]	Reference	1.39 (1.26, 1.52)	2.08 (1.85, 2.34)	Reference	1.35 (1.24, 1.48)	1.55 (1.40, 1.71)
Allergic conjunctivitis	Reference	1.89 (1.13, 2.66)	3.49 (2.05, 5.96)	Reference	1.58 (1.08, 2.32)	2.72 (1.75, 4.23)
Allergic rhinitis	Reference	1.43 (1.27, 1.59)	2.13 (1.86, 2.43)	Reference	1.39 (1.26, 1.53)	1.56 (1.39, 1.74)
Asthma	Reference	2.56 (1.69, 3.43)	4.89 (3.20, 7.47)	Reference	1.92 (1.52, 2.43)	2.10 (1.64, 2.70)

[a] Adjusted for the fixed effects of gender, income, education, and ethnicity and the random effect of university.
[b] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.
[c] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.

BMJ Open

Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood: A population-based retrospective cohort study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-047768.R1
Article Type:	Original research
Date Submitted by the Author:	08-Apr-2021
Complete List of Authors:	Li, Yajia; Xiangya Hospital Central South University, Jing, Danrong; Xiangya Hospital Central South University, Dermatology Huang, Yuzhou ; Xiangya Hospital Central South University, dermatology Su, Juan; Xiangya Hospital Central South University, Dermatology Li, Ji Tao, Juan; Affiliated Union Hospital, Tongji Medical College, Huazhong University of Science and Technology Shan, Shijun; Xiang'an Hospital, Xiamen University, Department of Dermatology Wang, Xiaohui; Zhongshan Hospital Xiamen University, Department of Dermatology, Kang, Xiaojing; People's Hospital of Xinjiang Uygur Autonomous Region, Department of Dermatology Wu, Bin; People's Hospital, Inner Mongolia Medical University, Department of Dermatology Xiao, Yi; Xiangya Hospital Central South University Chen, Xiang; Xiangya Hospital Central South University, Shen, Minxue; Xiangya Hospital Central South University, Dermatology
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Dermatology, Epidemiology
Keywords:	Allergy < THORACIC MEDICINE, Adult dermatology < DERMATOLOGY, Eczema < DERMATOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood: A population-based retrospective cohort study.

Yajia Li, M.A student [1], Danrong Jing, Ph.D. student [1], Yuzhou Huang, Ph.D. student [1], Juan Su, Ph.D. [1], Ji Li, Ph.D. [1], Juan Tao, Ph.D. [2], Shijun Shan, Ph.D. [3], Xiaohui Wang, Ph.D.[4], Xiaojing Kang, Ph.D. [5], Bin Wu, Ph.D.[6], Yi Xiao, Ph.D. [1*], Xiang Chen, Ph.D.[1*], Minxue Shen, Ph.D. [1,7*]

[1]Department of Dermatology; Hunan Engineering Research Center of Skin Health and Disease; Hunan Key Laboratory of Skin Cancer and Psoriasis, Xiangya Hospital, Central South University, Changsha, China

[2]Department of Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[3]Department of Dermatology, Xiang'an Hospital, Xiamen University, Xiamen, China

[4]Department of Dermatology, Zhongshan Hospital, Xiamen University, Xiamen, China

[5]Department of Dermatology, People's Hospital of Xinjiang Uygur Autonomous Region, Urumchi, Xinjiang, China

[6]Department of Dermatology, People's Hospital, Inner Mongolia Medical University, Hohhot, China

[7]Department of Social Medicine and Health Management, Xiangya School of Public Health, Central South University, Changsha, China

***Correspondence:**
Minxue Shen, Xiang Chen and Yi Xiao

Address: Xiangya Hospital, 87 Xiangya Road, Changsha, Hunan, China 410008

Email: shenmx1988@csu.edu.cn (M.S.), chenxiangck@126.com (X.C.) and xiaoyixy@csu.edu.cn (Y.X.)

Keywords: antibiotics, atopic and allergic skin diseases, preschool and young adulthood.

Abstract

Background: Overuse and misuse of antibiotics is a public health problem in low- and middle-income countries. Although the association of antibiotics with atopic and allergic diseases has been established, most studies focused on prenatal exposure and the occurrence of disease in infants or young children.

Objective: To investigate the association of preschool use of antibiotics with atopic and allergic skin diseases in young adulthood for the association of antibiotics use with eczema.

Design: Population-based retrospective cohort.

Setting and participants: The first-year college students (n=20123) from five universities were investigated. The sampled universities are located in Changsha, Wuhan, Xiamen, Urumqi, and Hohhot, respectively.

Methods: We conducted a dermatological field examination and a questionnaire survey inquiring the participants about the frequency of upper respiratory tract infection (URTI) and the preschool antibiotics use (before 7 years old). The two-level Probit model was used to estimate the associations, and adjusted risk ratio (aRR) and 95% confidence interval (CI) were presented as the effect size.

Results: A total of 20123 participants with complete information was included in the final analysis. The frequent antibiotics use intravenously (aRR 1.36, 95% CI 1.14–1.62) and orally (aRR 1.18, 95% CI 1.01–1.39) before 7 years old was significantly associated with atopic dermatitis in young adulthood. Similar trends could be observed in allergic skin diseases among those who use antibiotics orally and intravenously, with RRs of 1.16 (1.01, 1.34) and 1.33 (1.13, 1.57), respectively.

Conclusions: Preschool URTI and antibiotics use significantly increases the risk of atopic and allergic skin diseases in young adulthood.

Strengths and limitations of this study

- The main outcomes were diagnosed by specialists instead of self-report.
- This study provides a relatively large and representative sample, and sufficient variations in geographic regions and sociodemographic subgroups, as well as the random effect at the university level, was fitted by the 2-level models, resulting in an unbiased estimation of associations.
- Recall bias in the measurement of exposure to antibiotics might have been introduced, which could not be ignored in most retrospective studies.
- We lacked the information about the type and dose of antibiotics, and there might be a reversed causal relationship because antibiotics could be used in the treatment of AD and other conditions accompanied by a bacterial infection.

1 Introduction

The incidence and prevalence of atopic and allergic conditions such as asthma, allergic rhinitis, food allergies, and atopic dermatitis (AD) among the worldwide population have significantly increased during the past several decades.[1-3] An area of environmental change that may be responsible for the increase of allergic and atopic diseases is the growing use of medications that may alter the development of the human microbiome.[4] It also seems that the use of some antibiotics, which can directly cause intestinal dysbiosis and affect the human microbiome and increase the risk for allergy development, is of particular concern in light of accumulating evidence.[5-7]

Furthermore, overuse and misuse of antibiotics is a severe public health problem worldwide, especially in low- and middle-income countries. In the last decade, prescriptions of broad-spectrum antibiotics increased by 49% in children under five years and doubled in children aged 5–17 years, concomitant with the increasing prevalence of allergic diseases.[8, 9] In China, 70% of outpatients attending primary care facilities with colds were inappropriately treated with antibiotics, often by intravenous infusion. The situation is even worse in children because many parents demand treatment with antibiotics[10]. However, most upper respiratory tract infections (URTI) in children are viral, for which antibiotics are unnecessary.[11-13]

The association between the use of antibiotics and atopic and allergic diseases has been observed in longitudinal studies. But most studies focused on antibiotics use during pregnancy or infancy when early colonization is initiated by maternal microbes.[14-17] With the childhood microbiome transition owing to alterations in food and exposure to more diverse microbes in external environments, children in preschool age (<7 yrs) are at higher risks of URTI infection and antibiotics treatment. However, the effect of antibiotics used during this period on atopic and allergic skin diseases that occurred in their young adulthood is not clear. The objective of this study was to evaluate the hypothesis that exposure to antibiotics in preschool age is associated with an increased risk of allergic and atopic skin diseases in young adulthood. We tested this hypothesis by conducting a retrospective study on college students.

2 Materials and Methods

2.1 Study setting and design

This was a retrospective cohort study based on the data from the China College Student Skin Health Study (CCSSHS)[18]. The first-year college students from five universities were investigated. They underwent a health examination and completed a questionnaire survey. The sampled universities are located in Changsha, Wuhan, Xiamen, Urumqi, and Hohhot, respectively. The medical ethics committee of Xiangya Hospital, Central South University, approved the study (#201709993).

2.2 Exposure assessment

Two semi-quantitative questions served as the proxy measures of the frequency of antibiotics exposure in preschool age, with detailed explanations for the definitions of URTI and antibiotics. In our study,

the definition of URTI refers to a series of acute illnesses that have an effect on the upper respiratory system including the common cold, acute otitis media, tonsillitis/tonsillopharyngitis, sinusitis, and recurrent sinusitis.[19] The first question was "How often did you have URTI in your preschool-age or before 7 years old", with three potential responses:⩽1 time/year, 2-3 times/year", and "4 or more times/year". The second question was "How often did you receive antibiotics treatment when you had a URTI", with four responses: "rare", "occasional", "often, orally; and "often, intravenously".

2.3 Outcome assessment

Diagnosis of skin diseases and inquiry of disease history were performed by dermatologists during the field survey. All subjects underwent skin examination by resident doctors in dermatology, and the diagnoses were further validated by senior dermatologists. Clinical manifestation, disease history, and family history were inquired about, and inspections were conducted to diagnose skin diseases. For recurrent skin diseases, only those with current symptoms and cutaneous lesions were diagnosed as cases. AD was diagnosed according to the Williams criteria.[20] Hand eczema was diagnosed according to eczema (rash) on the fingers, finger webs, palms, or back of hands, which had appeared once and continued for at least two weeks or had appeared several times or had been persistent. Allergies and urticaria were diagnosed by clinical manifestations, potential triggers, and histories. Asthma, allergic rhinitis, and allergic conjunctivitis were self-reported according to doctors' diagnoses. We also combined some of the outcomes. Atopic march is an apparent progression of allergic diseases from AD, to allergic asthma (AA) to allergic rhinitis (AR) and allergic conjunctivitis.[21, 22] We include the conditions of AD, AA, AR, and allergic conjunctivitis for the outcome of the atopic march. Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria. Participants with a history of atopic/allergic conditions but without the current disease are excluded.

2.4 Covariates

Demographic characteristics, socioeconomic status (annual family income and parental highest educational level), family history, behavioral factors (dietary, passive smoking, and bathing habits) were enquired by the questionnaire.

2.5 Statistical analysis

Categorical data were presented as number (%), and the between-group difference was tested using the chi-square test. Two-level Probit regression models (individual as level 1 and university as level 2) were used to estimate the associations of preschool exposure to antibiotics with atopic skin diseases in young adulthood, adjusting for level-1 covariates (gender, ethnicity, annual household income, and parental education) and level-2 random effects. The effect size was presented as relative risk (RR) and 95% confidence interval (CI). $P< 0.05$ was considered statistically significant for all tests. Statistical analysis was performed in SAS 9.4 (SAS Institute Inc., Cary, USA).

3 Results

A total of 27144 registries for new enrolment was identified; among them, 21086 (77.7%) consented to participate, and 20123 (74.1%) who underwent the health examination and completed the online

questionnaire survey were included in the final analyses. The mean age was 18.3± 0.8 years and 10283 (51.1%) were men. The characteristics of participants in the study are shown in Table 1. The prevalence rates of chronic urticaria, allergic reactions to food/drug/light, hand eczema, and AD were 1.89%, 2.27%, 3.35%, and 3.86%, respectively. The prevalence rates of AD and allergic skin disease were 3.86 % and 4.14%, respectively (Figure 1 and Table S1).

In general, URTI and the use of antibiotics were significantly associated with atopic and allergic diseases (Figure 1 and Table S1). For example, the prevalence of AD increased from 3.39% to 4.11% and 4.79% in participants who reported rare or occasional use, frequent oral use, and frequent intravenous use of antibiotics, respectively. Consistent trends could be observed in all atopic and allergic diseases and their combinations.

After adjustments for sociodemographic factors (Figure 2 and Table S2), URTI and the use of antibiotics were significantly associated with atopic/allergic skin diseases. For instance, compared to those reporting rare or occasional use of antibiotics, the RRs for AD in participants who reported frequent oral administration and intravenous injection of antibiotics were 1.18 (95% CI: 1.01, 1.39) and 1.36 (95% CI: 1.14, 1.62), respectively. For other allergies or atopic diseases of skin or beyond the skin, the correlations were highly consistent despite some variations in the magnitude of the association.

Because our study was not a prospective cohort, it was difficult to know if antibiotic use was ahead of suffering from AD. We further evaluated the joint effect of URTI and antibiotics on AD by including an interaction term in the model. As shown in Figure 3, in each category of antibiotic use, the frequency of URTI was positively associated with the risk of AD. Vice versa, in each category of URTI, antibiotic use was positively associated with AD according to the effect size, despite some insignificant results in categories with small sample size.

4 Discussion

This retrospective cohort study demonstrated that preschool exposure to antibiotics, either through oral administration or intravenous infusion, was associated with increased risks of having allergic and atopic skin diseases in young adulthood. Participants who reported frequent URTI in preschool-age also had higher risks of allergies and atopy.

Similar trends were identified in previous observational studies that early-life antibiotic use was associated with an increased risk of eczema, but there were still some inconsistent results.[23, 24] Meta-analysis including 22 studies with 394,517 patients concluded that children with antibiotics exposure in the first two years had increased odds of atopic eczema with an OR of 1.26 (95% CI: 1.15-1.37). Notably, the onset age of the outcomes in the included studies was the period of childhood (<12 years old). [7] A large population-based retrospective cohort study in twins showed that antibiotic use was also associated with an increased risk of eczema; however, it is likely that the relationship between early-life antibiotic use and eczema was confounded by shared familial environment and genetic factors.[25] However, current data lacked the information regarding the atopic and allergic skin diseases occurred in late adolescence to early adulthood, while we firstly investigated the effects of

preschool antibiotic in a retrospective study and revealed a positive association. Furthermore, we have provided the data on the health seeking behavior dealing with a cold or fever in preschool age (shown in Table S3), and we found there were 66.2% in participants with the atopic march and 64.7% in participants allergic skin disease showed that they/their parents would like to receive antibiotics treatment when the participants had a cold/fever in their preschool age. In those without atopic/allergic diseases, this proportion ratio was 61.5%. We did not observe a significant difference in health seeking behavior in our study, as most of the Chinese parents could pay close attention to the preschool health of children, and keep a non-exclusion attitude to antibiotics use. Keeping antibiotics at home for children was pervasive in China, as well as the parents sought medical care and use antibiotics in dealing with respiratory tract infections.

Evidence showed that AD was the first manifestation of an atopic phenotype which begins in early childhood and the progression from AD to the diseases such as food allergy, asthma, and allergic rhinitis were more likely to be shown in adolescence.[22, 26, 27] The mechanisms behind the march from AD to allergic airway diseases and allergic conjunctivitis likely arise from initial epicutaneous allergen sensitization inducing robust local and systemic type 2 immune responses with increased production of type 2 cytokines including interleukin (IL)-4, IL-13, IL-31, and thymic stromal lymphopoietin.[28, 29] Most studies were prone to make these responses responsible for the commonly shared pathogenesis of cutaneous, airway, and conjunctiva inflammation, supporting the view that AD is not merely a disease confined to the skin, but is in fact, a systemic disease.[30, 31] Therefore, it could be explained that the increased risks beyond skin manifestations in young adulthood were consistent with those of skin diseases and some with even greater effect sizes. While not fully understood, the underlying mechanism of the association between antibiotics and atopic and allergic diseases can be elucidated by microbial diversity. The gut microbial community is dynamic and variable during the first 3 years of life, before stabilizing to an adult-like state.[32] Studies have demonstrated continued development through childhood into the teenage years.[33, 34] Dietary intake plays a key role in the development period of the gut microbiome. Breast-fed infants have microbiota enriched in *Lactobacillus*, *Staphylococcus*, and *Bifidobacterium*. Studies have shown that human milk isolates contain symbiotic and potentially probiotic microbes, and supplementation of *Bifidobacteria* was found to be effective in primary preventing allergic diseases.[35-37] But among children of preschool age, the dominance of *Bifidobacterium* diminishes with the alteration in dietary intake.[15] On the other hand, the high prevalence of antibiotic use may also lead to a concurrent increase in antibiotic-resistant bacteria.[38, 39] Antibiotic-treated children have a less diverse gut microbiota and less stable communities. Antibiotic therapy affects microbiome variety and thus may increase the risk of atopic diseases.[40, 41]

In our study, increased risks of atopic march or allergic diseases were observed in students who reported frequent URTI. Another potential explanation was related to the infections which could also affect microbial conditions. Except for the change in diet, children of preschool age tend to be exposed to more diverse microbes and infectious diseases including URTI in kindergarten or external environments. Respiratory viral infections, in particular, have been shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut,[42] which could further shape atopic microenvironments.

Several limitations should be noted, and the results should be interpreted with caution. First, recall bias in the measurement of exposure to antibiotics might have been introduced. While recall bias on the frequency of antibiotics use and URTIs should not be ignored, this is a limitation in most retrospective studies. We are not able to validate the medical records because China does not have a registry system for primary care, and a large number of patients with mild conditions also visit doctors in secondary and tertiary hospitals. While participants could obtain the information from their parents, but unfortunately, we could not evaluate the extent of recall bias. This will be in our further consideration in future studies.

Second, there was a lack of information about the type and dose of antibiotics, and we could not attribute the association to specific antibiotics. Third, there might be a reversed causal relationship, because antibiotics could be used in the treatment of AD and other conditions accompanied by bacterial infection. Last but not least, we assessed the role of URTI and antibiotics separately, because the two variables were significantly correlated (contingency coefficient=0.4, $P<0.001$) and were therefore not included in the same model to avoid collinearity and biased estimation of parameters. The association of URTI with AD and allergies may be confounded by antibiotics, and vice versa.

However, both infection and antibiotics may be correlated with allergies/atopies, with different mechanisms. Although under the hygiene hypothesis, exposure to pathogens during infancy and early childhood has been proposed to explain the lower prevalence of asthma and other atopic diseases among children in developing countries,[43, 44] some studies showed that early respiratory infections could not protect against atopic eczema or recurrent wheezing, but could drive the development of atopic disease. [45-49] Atopic sensitization, a process of generation of specific immunoglobulin E (sIgE) when exposed to an innocuous antigen, was common to all allergic diseases. As preschool URTI most probably represent viral infections in the majority of cases, some studies investigated a potential mechanistic explanation for how a respiratory viral infection could drive the development of atopic sensitization and disease. Martorano et al. used a Sendai virus to establish the mouse model mimicking a human limited respiratory syncytial virus infection and found that Sendai virus infection could promote the crosslinking of high-affinity IgE receptor (FcεRI) on the lung conventional dendritic cells (cDC), which led to the production of the chemokine CCL28, recruiting IL-13 and driving the development of mucous cell metaplasia and airway hyperreactivity.[50] Another animal study found that except for the increase of sIgE against Sendai virus in mice, there was also a large increase in total IgE and it remained elevated long after the viral infection being resolved.[51] This notion has further been fuelled by findings that mice infected with the flu virus developed virus-specific mast cell degranulation in the skin, indicating a possible pathway of viral infections that could mediate allergic symptoms.[52] Besides, the respiratory viral infections were shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut.[42, 53] However, we cannot ignore that infections that do not require antibiotics are not captured in our design, such that it is difficult to assess whether the observed association is caused by a specific infection or antibiotics because they occur simultaneously in many cases.

Our study also has strengths. The primary strength is that the sample size for the retrospective cohort study was large. Second, the outcomes were diagnosed by specialists. In contrast, some previous

studies used self-reported diagnoses that might introduce misclassification bias. Third, the study had a relatively large and representative sample, and sufficient variations in geographic regions and sociodemographic subgroups. The random effect at the university level was fitted by the 2-level models, resulting in an unbiased estimation of associations.

To conclude, preschool children exposed to URTI or antibiotics may be at higher risks of atopic and allergic skin diseases in their young adulthood, especially among those who frequently had URTI or received antibiotics by intravenous infusion. Our study implies that unnecessary antibiotics treatment in children should be avoided to prevent the occurrence of atopic and allergic diseases in their later life. Prospective studies that consider the type and dose of antibiotics are warranted.

5 Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

6 Ethics statement

This study was conducted according to the guidelines established in the Declaration of Helsinki. All procedures involving patients were approved by the institutional research ethics boards of Xiangya Hospital, Central South University (Changsha, China). Informed consent was obtained from all the students before the investigation.

7 Patient and Public Involvement Statement

This is a retrospective cohort study based on the data from the China College Student Skin Health Study (CCSSHS). The first-year college students from five universities were recruited and investigated. They underwent a health examination and completed a questionnaire survey, and the results will be disseminated to study participants by a medical examination report. Participants were not involved in the design and implementation of the study.

8 Author contributions

Dr. Minxue Shen, Dr. Xiang Chen, and Dr. Yi Xiao are joint corresponding authors and have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Shen, Chen, and Xiao.

Acquisition, analysis, or interpretation of data: Y.J. Li, Jing, and Huang

Drafting of the manuscript: Y.J. Li

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Shen, Xiao, and Y.J. Li.

Administrative, technical, or material support: Su, J. Li, Tao, Shan, Wang, Kang, and Wu.

Supervision: Chen

9 Conflict of interest

The authors declare no conflict of interest.

10 Funding

This work was supported by the National Key Research & Development Project "Precision Medicine Initiative" of China (2016YFC0900802) and the Program of Introducing Talents of Discipline to Universities (111 Project, No. B20017).

11 Acknowledgment

The authors would like to thank the following individuals and institutions who have made substantial contribution to the establishment of the cohort: Central South University (Lei Cai, Duling Cao, Qin Cao, Chao Chen, Liping Chen, Menglin Chen, Mengting Chen, Xiang Chen, Qing Deng, Xin Gao, Yihuan Gong, Jia Guo, Yeye Guo, Rui Hu, Xin Hu, Chuchu Huang, Huining Huang, Kai Huang, Xiaoyan Huang, Yuzhou Huang, Danrong Jing, Xinwei Kuang, Li Lei, Jia Li, Jiaorui Li, Jie Li, Keke Li, Peiyao Li, Yajia Li, Yayun Li, Yangfan Li, Dan Liu, Dihui Liu, Fangfen Liu, Nian Liu, Panoan Liu, Runqiu Liu, Hui Lu, Wenhua Lu, Yan Luo, Zhongling Luo, Manyun Mao, Mengping Mao, Yuyan Ouyang, Shiyao Pei, Qunshi Qin, Ke Sha, Lirong Tan, Ling Tang, Ni Tang, Yan Tang, Ben Wang, Yaling Wang, Tianhao Wu, Yun Xie, Siyu Yan, Sha Yan, Bei Yan, Xizhao Yang, Lin Ye, Hu Yuan, Taolin Yu, Yan Yuan, Yi Yu, Rui Zhai, Jianghua Zhang, Jianglin Zhang, Mi Zhang, Xingyu Zhang, Zhibao Zhang, Shuang Zhao, Yaqian Zhao, Kuangbiao Zhong, Lei Zhou, Youyou Zhou, Zhe Zhou, Susi Zhu); Huazhong University of Science and Technology (Xiangjie An, Siqi Da, Yaqi Dong, Yangxue Fu, Lixie Gao, Han Han, Biling Jiang, Jiajia Lan, Jun Li, Xiaonan Li, Yan Li, Liquan Liu, Yuchen Lou, Pu Meng, Yingli Nie, Gong Rao, Shanshan Sha, Xingyu Su, Huinan Suo, Rongying Wang, Jun Xie, Yuanxiang Yi, Jia Zhang, Qiao Zhang, Li Zhu, Yanming Zhu); Xiamen University (Zhiming Cai, Lina Chen, Xiaozhu Fu, Hongjun Jiang, Guihua Luo, Jianbing Xiahou, Binxiang Zheng); People's Hospital of Xinjiang Uygur Autonomous Region (Jianxia Chen, Xiaomin Chen, Xinqi Chen, Li Dai, Yanyan Feng, Fanhe Jiang, Lan Jin, Qingyu Ma, Qun Shi, Hongbo Tang, Fang Wang, Zhen Wang, Xiujuan Wu, Kunjie Zhang, Yu Zhang); Xinjiang Medical University (Huagui Li, Jianguang Li, Lei Shi); Inner Mongolia Medical University (Chao Tian, Wei Wang, Rina Wu, Hongjun Xing, Baogui Yang).

12 References

[1]. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action[J]. World Allergy Organ J. 2014,7(1):12.

[2]. Platts-Mills TA. The allergy epidemics: 1870-2010[J]. J Allergy Clin Immunol. 2015,136(1):3-13.

[3]. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys[J]. Lancet. 2006,368(9537):733-43.

[4]. Mitre E, Susi A, Kropp LE, Schwartz DJ, Gorman GH, Nylund CM. Association Between Use of Acid-Suppressive Medications and Antibiotics During Infancy and Allergic Diseases in Early Childhood[J]. JAMA Pediatr. 2018,172(6):e180315.

[5]. Wang JY, Liu LF, Chen CY, Huang YW, Hsiung CA, Tsai HJ. Acetaminophen and/or antibiotic use in early life and the development of childhood allergic diseases[J]. Int J Epidemiol. 2013,42(4):1087-99.

[6]. Kim DH, Han K, Kim SW. Effects of Antibiotics on the Development of Asthma and Other Allergic Diseases in Children and Adolescents[J]. Allergy Asthma Immunol Res. 2018,10(5):457-65.

[7]. Ahmadizar F, Vijverberg SJH, Arets HGM, de Boer A, Lang JE, Garssen J, et al. Early-life antibiotic exposure increases the risk of developing allergic symptoms later in life: A meta-analysis[J]. Allergy. 2018,73(5):971-86.

[8]. Lee GC, Reveles KR, Attridge RT, Lawson KA, Mansi IA, Lewis JS, 2nd, et al. Outpatient antibiotic prescribing in the United States: 2000 to 2010[J]. BMC Med. 2014,12:96.

[9]. Versporten A, Bielicki J, Drapier N, Sharland M, Goossens H. The Worldwide Antibiotic Resistance and Prescribing in European Children (ARPEC) point prevalence survey: developing hospital-quality indicators of antibiotic prescribing for children[J]. J Antimicrob Chemother. 2016,71(4):1106-17.

[10]. Biezen R, Grando D, Mazza D, Brijnath B. Dissonant views - GPs' and parents' perspectives on antibiotic prescribing for young children with respiratory tract infections[J]. BMC Fam Pract. 2019,20(1):46.

[11]. Wei X, Zhang Z, Walley JD, Hicks JP, Zeng J, Deng S, et al. Effect of a training and educational intervention for physicians and caregivers on antibiotic prescribing for upper respiratory tract infections in children at primary care facilities in rural China: a cluster-randomised controlled trial[J]. Lancet Glob Health. 2017,5(12):e1258-e67.

[12]. Li J, Song X, Yang T, Chen Y, Gong Y, Yin X, et al. A Systematic Review of Antibiotic Prescription Associated With Upper Respiratory Tract Infections in China[J]. Medicine (Baltimore). 2016,95(19):e3587.

[13]. Ding L, Sun Q, Sun W, Du Y, Li Y, Bian X, et al. Antibiotic use in rural China: a cross-sectional survey of knowledge, attitudes and self-reported practices among caregivers in Shandong province[J]. BMC Infect Dis. 2015,15:576.

[14]. McKeever TM, Lewis SA, Smith C, Collins J, Heatlie H, Frischer M, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database[J]. J Allergy Clin Immunol. 2002,109(1):43-50.

[15]. Kundu P, Blacher E, Elinav E, Pettersson S. Our Gut Microbiome: The Evolving Inner Self[J]. Cell. 2017,171(7):1481-93.

[16]. Yoshida S, Ide K, Takeuchi M, Kawakami K. Prenatal and early-life antibiotic use and risk of childhood asthma: A retrospective cohort study[J]. Pediatr Allergy Immunol. 2018,29(5):490-5.

[17]. Kusel MM, de Klerk N, Holt PG, Sly PD. Antibiotic use in the first year of life and risk of atopic disease in early childhood[J]. Clin Exp Allergy. 2008,38(12):1921-8.

[18]. S M, X Y, S J. Prevalence and patient-reported outcomes of noncommunicable skin diseases among college students in China.[J]. JAAD Int. 2020,1(1):23-30.

[19]. Eccles MP, Grimshaw JM, Johnston M, Steen N, Pitts NB, Thomas R, et al. Applying psychological theories to evidence-based clinical practice: identifying factors predictive of managing upper respiratory tract infections without antibiotics[J]. Implement Sci. 2007,2:26.

[20]. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation[J]. Br J Dermatol. 1994,131(3):406-16.

[21]. Spergel JM, Paller AS. Atopic dermatitis and the atopic march[J]. The Journal of allergy and clinical immunology. 2003,112(6 Suppl):S118-27.

[22]. Hill DA, Spergel JM. The atopic march: Critical evidence and clinical relevance[J]. Ann Allergy Asthma Immunol. 2018,120(2):131-7.

[23]. Metzler S, Frei R, Schmaußer-Hechfellner E, von Mutius E, Pekkanen J, Karvonen AM, et al. Association between antibiotic treatment during pregnancy and infancy and the development of allergic diseases[J]. Pediatr Allergy Immunol. 2019,30(4):423-33.

[24]. Tsakok T, McKeever TM, Yeo L, Flohr C. Does early life exposure to antibiotics increase the risk of eczema? A systematic review[J]. Br J Dermatol. 2013,169(5):983-91.

[25]. Slob EMA, Brew BK, Vijverberg SJH, Kats C, Longo C, Pijnenburg MW, et al. Early-life antibiotic use and risk of asthma and eczema: results of a discordant twin study[J]. Eur Respir J. 2020,55(4).

[26]. Johansson E, Hershey GKK. Contribution of an impaired epithelial barrier to the atopic march[J]. Ann Allergy Asthma Immunol. 2018,120(2):118-9.

[27]. Belgrave DC, Granell R, Simpson A, Guiver J, Bishop C, Buchan I, et al. Developmental profiles of eczema, wheeze, and rhinitis: two population-based birth cohort studies[J]. PLoS Med. 2014,11(10):e1001748.

[28]. Akei HS, Brandt EB, Mishra A, Strait RT, Finkelman FD, Warrier MR, et al. Epicutaneous aeroallergen exposure induces systemic TH2 immunity that predisposes to allergic nasal responses[J]. J Allergy Clin Immunol. 2006,118(1):62-9.

[29]. Cianferoni A, Spergel J. The importance of TSLP in allergic disease and its role as a potential therapeutic target[J]. Expert Rev Clin Immunol. 2014,10(11):1463-74.

[30]. Asada Y, Nakae S, Ishida W, Hori K, Sugita J, Sudo K, et al. Roles of Epithelial Cell-Derived Type 2-Initiating Cytokines in Experimental Allergic Conjunctivitis[J]. Invest Ophthalmol Vis Sci. 2015,56(9):5194-202.

[31]. Han H, Xu W, Headley MB, Jessup HK, Lee KS, Omori M, et al. Thymic stromal lymphopoietin (TSLP)-mediated dermal inflammation aggravates experimental asthma[J]. Mucosal Immunol. 2012,5(3):342-51.

[32]. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography[J]. Nature. 2012,486(7402):222-7.

[33]. Hollister EB, Riehle K, Luna RA, Weidler EM, Rubio-Gonzales M, Mistretta TA, et al. Structure and function of the healthy pre-adolescent pediatric gut microbiome[J]. Microbiome. 2015,3:36.

[34]. Agans R, Rigsbee L, Kenche H, Michail S, Khamis HJ, Paliy O. Distal gut microbiota of adolescent children is different from that of adults[J]. FEMS Microbiol Ecol. 2011,77(2):404-12.

[35]. Heikkilä MP, Saris PE. Inhibition of Staphylococcus aureus by the commensal bacteria of human milk[J]. J Appl Microbiol. 2003,95(3):471-8.

[36]. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life[J]. Cell Host Microbe. 2015,17(6):852.

[37]. Enomoto T, Sowa M, Nishimori K, Shimazu S, Yoshida A, Yamada K, et al. Effects of bifidobacterial supplementation to pregnant women and infants in the prevention of allergy development in infants and on fecal microbiota[J]. Allergol Int. 2014,63(4):575-85.

[38]. Hiltunen T, Virta M, Laine AL. Antibiotic resistance in the wild: an eco-evolutionary perspective[J]. Philos Trans R Soc Lond B Biol Sci. 2017,372(1712).

[39]. Hawkey PM, Jones AM. The changing epidemiology of resistance[J]. J Antimicrob Chemother. 2009,64 Suppl 1:i3-10.

[40]. Penders J, Stobberingh EE, van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders[J]. Allergy. 2007,62(11):1223-36.

[41]. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema[J]. J Allergy Clin Immunol. 2012,129(2):434-40, 40.e1-2.

[42]. Hanada S, Pirzadeh M, Carver KY, Deng JC. Respiratory Viral Infection-Induced Microbiome Alterations and Secondary Bacterial Pneumonia[J]. Front Immunol. 2018,9:2640.

[43]. Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis[J]. Nat Immunol. 2017,18(10):1076-83.

[44]. Haspeslagh E, Heyndrickx I, Hammad H, Lambrecht BN. The hygiene hypothesis: immunological mechanisms of airway tolerance[J]. Curr Opin Immunol. 2018,54:102-8.

[45]. Kramer MS, Guo T, Platt RW, Sevkovskaya Z, Dzikovich I, Collet JP, et al. Does previous infection protect against atopic eczema and recurrent wheeze in infancy?[J]. Clin Exp Allergy. 2004,34(5):753-6.

[46]. Peebles RS, Jr. Viral infections, atopy, and asthma: is there a causal relationship?[J]. The Journal of allergy and clinical immunology. 2004,113(1 Suppl):S15-8.

[47]. Martinez FD. Viruses and atopic sensitization in the first years of life[J]. Am J Respir Crit Care Med. 2000,162(3 Pt 2):S95-9.

[48]. Nafstad P, Brunekreef B, Skrondal A, Nystad W. Early respiratory infections, asthma, and allergy: 10-year follow-up of the Oslo Birth Cohort[J]. Pediatrics. 2005,116(2):e255-62.

[49]. Dharmage SC, Lodge CJ, Lowe AJ, Allen KJ. Antibiotics and risk of asthma: a debate that is set to continue[J]. Clin Exp Allergy. 2015,45(1):6-8.

[50]. Martorano LM, Grayson MH. Respiratory viral infections and atopic development: From possible mechanisms to advances in treatment[J]. Eur J Immunol. 2018,48(3):407-14.

[51]. Cheung DS, Ehlenbach SJ, Kitchens T, Riley DA, Grayson MH. Development of atopy by severe paramyxoviral infection in a mouse model[J]. Ann Allergy Asthma Immunol. 2010,105(6):437-43.e1.

[52]. Grunewald SM, Hahn C, Wohlleben G, Teufel M, Major T, Moll H, et al. Infection with influenza a virus leads to flu antigen-induced cutaneous anaphylaxis in mice[J]. The Journal of investigative dermatology. 2002,118(4):645-51.

[53]. Culley FJ, Pennycook AM, Tregoning JS, Hussell T, Openshaw PJ. Differential chemokine expression following respiratory virus infection reflects Th1- or Th2-biased immunopathology[J]. J Virol. 2006,80(9):4521-7.

Figure legends

Figure 1. The prevalence of atopic and allergic diseases in exposure vs. non-exposure group of antibiotics and URTI.

Figure 2. Association of early-life exposure to antibiotics with the risk of atopic/allergic diseases later in life. RR: risk ratio; CI: confidence interval.

Figure 3. The joint effect of URTI and antibiotics on AD after including an interaction term in the model. RR: risk ratio; CI: confidence interval.

Supplementary file captions

Table S1. The prevalence of atopic and allergic diseases in college students.

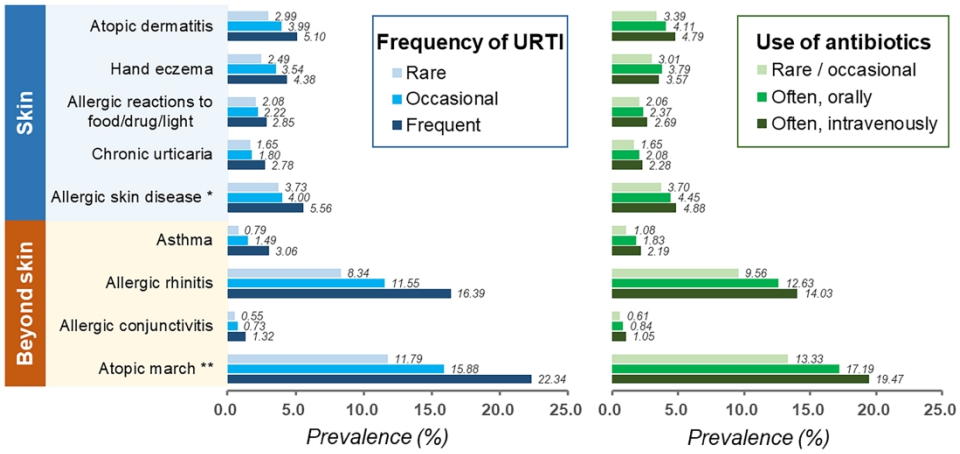
Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students.

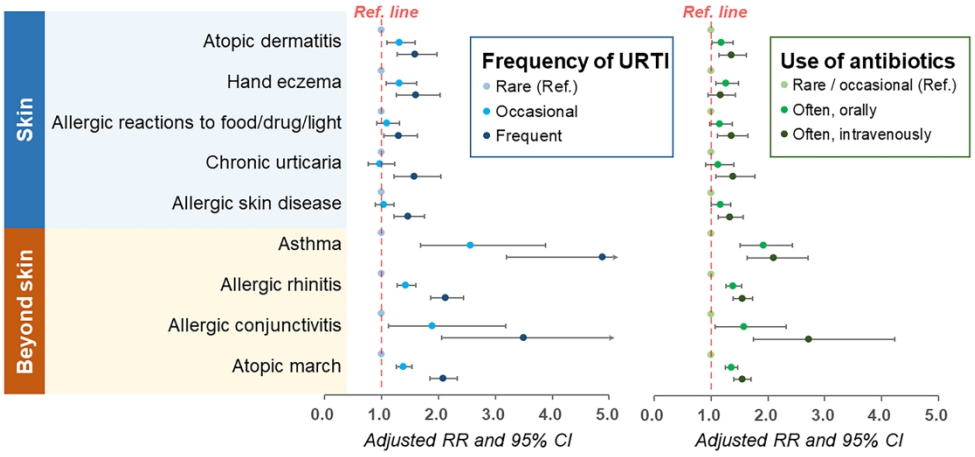
Table S3. Health seeking behavior when dealing with a cold or fever in the preschool age.

Material S1. Chinese college students health survey questionaire (Translation version in English).

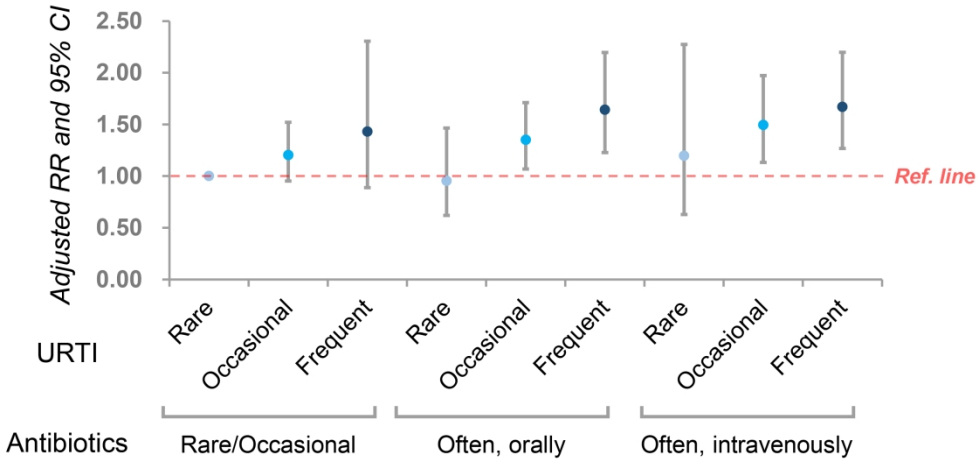
Table1. Characteristics of participants in the Present Study (N=20123).

Characteristics	N	%
Gender		
Male	10283	51.1
Female	9840	48.9
Income (CNY)		
<10,000	2168	10.8
10,000–29,999	4376	21.7
30,000–49,999	3465	17.2
50,000–99,999	4417	22.0
100,000–199,999	4063	20.2
≥200,000	1634	8.1
Parental highest education		
Primary school	1320	6.6
Middle school	5316	26.4
High school	5021	24.9
College and above	8466	42.1
Ethnicity		
Han Chinese	16218	80.6
Other ethnicities	3905	19.4
Passive smoke exposure		
Hardly	15883	78.9
Often	4240	21.1





338x170mm (300 x 300 DPI)



236x115mm (300 x 300 DPI)

Supplementary file captions

Table S1. The prevalence of atopic and allergic diseases in college students.
Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students.
Table S3. Health seeking behavior when dealing with a cold or fever in the preschool age.
Material S1. Chinese college students health survey questionnaire (Translation version in English)

Table S1. The prevalence of atopic and allergic diseases in college students

Disease	N, Prevalence (%)[a]	URTI, n (%)			Antibiotics, n (%)		
		Rare	Occasional	Frequent	Rare, occasional	Often,orally	Often, intravenously
Skin							
Atopic dermatitis	776 (3.86)	174 (2.99)	459 (3.99)	143 (5.1)	2[illegible] (3.39)	263 (4.11)	164 (4.79)
Hand eczema	675 (3.35)	145 (2.49)	407 (3.54)	123 (4.38)	[illegible]3 (3.01)	243 (3.79)	122 (3.57)
Allergic reactions to food/drug/light	456 (2.27)	121 (2.08)	255 (2.22)	80 (2.85)	[illegible]2 (2.06)	152 (2.37)	92 (2.69)
Chronic urticaria	381 (1.89)	96 (1.65)	207 (1.8)	78 (2.78)	[illegible]1 (1.65)	133 (2.08)	78 (2.28)
Allergic skin disease [b]	833 (4.14)	217 (3.73)	460 (4)	156 (5.56)	3[illegible]1 (3.7)	285 (4.45)	167 (4.88)
Beyond skin							
Atopic march [c]	3139 (15.6)	687 (11.79)	1825 (15.88)	627 (22.34)	1372 (13.33)	1101 (17.19)	666 (19.47)
Allergic conjunctivitis	153 (0.76)	32 (0.55)	84 (0.73)	37 (1.32)	6[illegible] (0.61)	54 (0.84)	36 (1.05)
Allergic rhinitis	2273 (15.6)	486 (8.34)	1327 (11.55)	460 (16.39)	98[illegible] (9.56)	809 (12.63)	480 (14.03)
Asthma	303 (1.51)	46 (0.79)	171 (1.49)	86 (3.06)	11[illegible] (1.08)	117 (1.83)	75 (2.19)

[a] The total prevalence of atopic and allergic diseases in our study population.

[b] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.

[c] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students

Disease	URTI, aRR (95%CI) [a]			Antibiotics, aRR (95%CI) [a]		
	Rare	Occasional	Frequent	Rare/Occasional	Often, orally	Often, intravenously
Skin						
Atopic dermatitis	Reference	1.32 (1.09, 1.54)	1.59 (1.27, 1.98)	Reference	1.18 (1.01, 1.39)	1.36 (1.14, 1.62)
Hand eczema	Reference	1.32 (1.08, 1.56)	1.60 (1.26, 2.02)	Reference	1.27 (1.08, 1.49)	1.17 (0.95, 1.44)
Allergic reactions to food/drug/light	Reference	1.10 (0.92, 1.28)	1.30 (1.04, 1.63)	Reference	1.15 (0.97, 1.37)	1.36 (1.12, 1.65)
Chronic urticaria	Reference	0.97 (0.76, 1.17)	1.58 (1.21, 2.05)	Reference	1.13 (0.90, 1.40)	1.39 (1.09, 1.78)
Allergic skin disease [b]	Reference	1.04 (0.89, 1.20)	1.46 (1.22, 1.76)	Reference	1.16 (1.01, 1.34)	1.33 (1.13, 1.57)
Beyond skin						
Atopic march [c]	Reference	1.39 (1.26, 1.52)	2.08 (1.85, 2.34)	Reference	1.35 (1.24, 1.48)	1.55 (1.40, 1.71)
Allergic conjunctivitis	Reference	1.89 (1.13, 2.66)	3.49 (2.05, 5.96)	Reference	1.58 (1.08, 2.32)	2.72 (1.75, 4.23)
Allergic rhinitis	Reference	1.43 (1.27, 1.59)	2.13 (1.86, 2.43)	Reference	1.39 (1.26, 1.53)	1.56 (1.39, 1.74)
Asthma	Reference	2.56 (1.69, 3.43)	4.89 (3.20, 7.47)	Reference	1.92 (1.52, 2.43)	2.10 (1.64, 2.70)

[a] Adjusted for the fixed effects of gender, income, education, passive smoking, and ethnicity and the random effect of the university.

[b] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.

[c] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.

Table S3. Health seeking behavior when dealing with a cold or fever in the preschool age.[a]

Disease condition	Total	Health seeking behavior, n(%)[b]				
		Ignore them	Drink water or have a rest	Receive antibiotics orally or intravenously	Receive Chinese traditional medicine orally	Unknown
Skin						
Atopic dermatitis	776	20 (2.6)	370 (47.6)	512 (65.9)	194 (25.0)	2 (0.3)
Hand eczema	675	32 (4.7)	343 (50.8)	430 (63.7)	149 (22.0)	3 (0.5)
Allergic reactions to food/drug/light	456	9 (2.0)	227 (49.7)	286 (62.7)	95 (20.8)	1 (0.2)
Chronic urticaria	381	8 (2.1)	158 (41.4)	257 (67.4)	100 (26.2)	1 (0.3)
Allergic skin disease [c]	833	17 (2.0)	384 (46.0)	539 (64.7)	194 (23.2)	2 (0.2)
Beyond skin						
Atopic march [d]	3139	66 (2.1)	1542 (49.1)	2081 (66.2)	728 (23.1)	9 (0.3)
Allergic conjunctivitis	153	4 (2.6)	83 (54.2)	107 (69.9)	36 (23.5)	0 (0)
Allergic rhinitis	2273	42 (1.8)	1135 (49.9)	1515 (66.6)	532 (23.4)	9 (0.4)
Asthma	303	7 (2.3)	154 (50.8)	206 (67.9)	70 (23.1)	2 (0.6)
Without atopic/allergic diseases	16343	597 (3.7)	7761 (47.4)	10057 (61.5)	3058 (18.7)	74 (0.4)

[a] Multiple selections are allowed in the questionnaire.

[b] Proportion ratios in populations.

[c] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria

[d] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis

Material S1. Chinese college students health survey questionnaire (English version)

Chinese college students health survey questionnaire

Informed consent

Welcome to participate in the Chinese University Student Health Survey. In order to promte a better health management for Chinese college freshmen, you are invited to fill out a questionnaire including several parts: (A) General information; (B) Medical history, (C) Lifestyle habits, (D) Skin health, which take about 15 minutes to answer. We will provide you with reasonable health education and health management based on this information, combined with the results of the health checkup. All the information you fill in will be kept strictly confidential. Continue to fill in the following content, indicating that you and your guardian have understood and are willing to continue to cooperate with our work. Thanks you!

A. General information

A01. Before you went to university, please provide your address. _____
(*Specific to districts and counties*)

A02. Have you moved to other places (cross-city) in the past 10 years
(*Single selection, if "No" jump to A04*)

O Never O Ever

A03. If so, the previous address is?_____ (*Specific to districts and counties*)

A04. Sex (*Single selection*)

O Male O Female

A05. Your Ethnicity:_____

A06. Annual household income. (*Single selection*)

O <10,000 O 10,000 – 29,999 O 30,000 – 49,999
O 50,000 – 99,999 O 100,000 – 199,999 O ⩾200,000

A07. Your father's highest education (*Single selection*)

O Primary school or O Middle school O High school

blow ○ College ○ Postgraduate or above ○ Unclear
A08. Your mother's highest education (*Single selection*) ○ Primary school or blow ○ Middle school ○ High school ○ College ○ Postgraduate or above ○ Unclear

B. Medical History

B01. Have you ever been specifically diagnosed with any of the following cardiovascular or metabolic diseases? (*Multiple selections are allowed*) ☐ Hypertension ☐ Coronary heart disease ☐ Hyperlipidemia ☐ Obesity ☐ Fatty liver ☐ Gout ☐ Psoriasis ☐ None of above
B02. Have you ever been diagnosed with any of the following allergic diseases? (*Multiple selections are allowed*) ☐ Asthma ☐ Allergic Rhinitis ☐ Allergic conjunctivitis ☐ Eczema ☐ Urticaria ☐ None of above
B03. Have you ever been diagnosed with any of the following infectious diseases? (*Multiple selections are allowed*) ☐ Tuberculosis or other forms of tuberculosis ☐ Hepatitis B ☐ Hepatitis C ☐ Helicobacter pylori infection ☐ HIV infection ☐ None of above
B04. Have you ever been diagnosed with any of the following endocrine diseases? (*Multiple selections are allowed*)

☐ Type 1 diabetes	☐ Type 2 diabetes	☐ Polycystic ovarian syndrome
☐ Hypertrichosis	☐ Hypothyroidism	☐ Hyperthyroidism
☐ Graves disease	☐ Hashimoto thyroiditis	☐ None of above

B05.Have you ever been diagnosed with any of the following immune diseases? (*Multiple selections are allowed*)

☐ SLE	☐ Scleroderma	☐ Sjogren syndrome
☐ Uveitis	☐ Rheumatoid arthritis	☐ Dermatomyositis
☐ None of above		

B06. Have you ever been diagnosed with any of the following hematologic diseases? (*Multiple selections are allowed*)

☐ Iron-deficiency anemia	☐ Megaloblastic anemia	☐ Thalassemia
☐ Anemia	☐ Hemophilia	☐ leucocythemia
☐ Lymphadenoma	☐ None of above	

B07. Have you ever been diagnosed with any of the following mental or neurological disorders? (*Multiple selections are allowed*)

☐ ADHD and attention deficit disorder	☐ Depressive disorder	☐ Anxiety
☐ Schizophrenia	☐ None of above	

B08. Have you ever had any of the following food allergies? (*Multiple selections are allowed*)

☐ None	☐ Milk	☐ Egg
☐ Wheat	☐ Soybean	☐ Fish
☐ Nuts	☐ Fruit	☐ Crustaceans (e.g., shrimp, crab, etc.)
☐ Others	☐ Unclear	

B09. Have you ever been allergic to any of the following medications? (*Multiple*

selections are allowed) ☐ None ☐ Antibiotics ☐ Non-steroidal anti-inflammatory drugs ☐ Contrast medium ☐ Anesthetic analgesics (e.g., aspirin) ☐ Anticonvulsant ☐ Chemotherapy drugs ☐ Others ☐ Unclear
B10. Have you ever been allergic to any of the following environmental substances? (*Multiple selections are allowed*) ☐ None ☐ Dust mite ☐ Mycete ☐ Animal skins ☐ Pollen ☐ House dust ☐ Cockroach ☐ Others ☐ Unclear
B11. Do you have an excessive bug bite response? (*Single selection*) ○ No ○ Yes
B12. In the past year, have you had a dog, cat or other small stuffed animal such as rabbit, guinea pig, hamster, etc.? (*Multiple selections are allowed*) ☐ No ☐ Dog ☐ Cat ☐ Other stuffed animals ☐ Unclear
B13. Do you have a long history of close exposure to chemicals? (*Multiple selections are allowed*) ☐ None ☐ Formaldehyde ☐ Gasoline ☐ Oil varnish ☐ Others ☐ Unclear
B14. Have you used or taken any medication almost every day for the last 2 weeks? (*Multiple selections are allowed*) ☐ None ☐ Antibiotics ☐ Nonsteroidal anti-inflammatory drugs and analgesics (NASIDS) ☐ Hormone ☐ Antituberculosis drugs ☐ Others ☐ Clear

B15. Whether breastfed after birth or not. (*Single selection*) O No O Yes O Unclear
B16. How often do you have "colds and fevers" in your early school years (before age of 7 years old)? (*Single selection*) O Rare O Occasional (Several times a year) O Often (almost every month)
B17. Which way did you (or your parents) usually deal with your "cold or fever" in the early school age years? (*Multiple selections are allowed*) ☐ Ignore them ☐ Drink more water or have a rest ☐ Receive antibiotics orally ☐ Oral Chinese Traditional medicine ☐ Receive antibiotics intravenously ☐ Others
B18. **Which of the following methods can improve or cure your "Cold or fever" in the early school age years? (*Single selection*)** O It usually cured without O By oral antibiotics occasionally O Often by antibiotics orally O Often by antibiotics intravenously

C. Lifestyle Habits

C01. Do you smoke (refers to smoking at least one cigarette a day for more than six months) (*Single selection, jump to C3 if you choose "hardly"*) O Hardly O < 1 packet /day (about 20 cigarettes in a packect) O 1-2 packets /day O > 2 packs/day
C02. If you smoke, how many years have you smoked in total so far? (*Single selection*) O < 1 year O 1-3 years O > 3 years

C03. In the last month, your frequency of passive smoking is (the involuntary inhalation of smoke caused by other people's smoking in your living or working environment) (*Single selection, jump to C5 if you choose "hardly"*)

- O Hardly
- O < 1 day/week
- O 1-2 days/week
- O 3-5 days/week
- O 6-7 days/week

C04. How many years have you been second-hand smoking? (*Single selection*)

- O < 2 years
- O 2-3 years
- O 4-6 years
- O > 6 years

C05. How often do you drink alcohol? (referring to once a week for at least six months) (*Single selection, jump to C9 if you choose "hardly"*)

- O Hardly
- O Once a week
- O 2-4 times/week
- O 5-7 times/week
- O 8-10 times/week
- O > 10 times/week

C06. How many years have you been drinking? (*Single selection*)

- O < 1year
- O 1-3years
- O 4-5years
- O > 5 years

C07. What's your main drink? (*Single selection*)

- O Beer
- O Liqueur
- O Red wine
- O Sweet wine
- O Chinese rice wine

C08. How much do you drink on average each time? (*Single selection*)

- O Less (1 bottle of beer, or 50-100g other types of alcohol)
- O Medium (2 bottles of beer, or 100g other types of alcohol)
- O Much (three bottles of beer, or 150g other types of alcohol)
- O A lot (more than 3 bottles of beer, or more than 150g of other types of alcohol)

D. Skin Health

D01.How many showers do you take per week in the spring and fall? (*Single selection*)

- O ≤1 time per week
- O 2~4 times per week
- O 5~7 times per week
- O 8~10 times per week
- O >10 times per week

D02. How long do you take a shower in spring and fall? (*Single selection*)

○ < 5 minutes ○ 5-10 minutes ○ 11-20 minutes ○ 21-30 minutes ○ > 30 minutes
D03. What kind of toiletries do you use most? (*Single selection*) ○ None ○ Soap ○ Shower gel ○ Others
D04. In spring and fall, the temperature of your bath is (*Single selection*) ○ Low temperature(<35 centigrade) ○ Close to the body temperature(35-40 centigrade) ○ High temperature(> 40 centigrade)
D05. Do you use moisturizers all over your body almost daily in fall and winter? (*Single selection*) ○ No ○ Yes
D06. How often do you use facial cleansing products (e.g. cleanser, soap)? (*Single selection*) ○ Hardly ○ Usually ○ Once per day ○ ≥twice per day
D07. How often have you washed your hair in the past two years? *(Single selection)* ○ ≥ Twice per day ○ Once per day ○ Once on alternate days ○ Once every 2-6 days ○ Once a week or more
D08. In the past two years, what kind of toiletries you use is? *(Single selection)* ○ None ○ Shampoo ○ Shampoo + conditioner ○ Others
D09. Have you used hair care/hair products in the past two years? (*multiple selections*) ☐ None ☐ Essential oil, elastin and other hair care products ☐ Hair gel ☐ Others

BMJ Open

Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood: A population-based retrospective cohort study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-047768.R2
Article Type:	Original research
Date Submitted by the Author:	10-Jun-2021
Complete List of Authors:	Li, Yajia; Xiangya Hospital Central South University, Jing, Danrong; Xiangya Hospital Central South University, Dermatology Huang, Yuzhou ; Xiangya Hospital Central South University, dermatology Su, Juan; Xiangya Hospital Central South University, Dermatology Li, Ji Tao, Juan; Affiliated Union Hospital, Tongji Medical College, Huazhong University of Science and Technology Shan, Shijun; Xiang'an Hospital, Xiamen University, Department of Dermatology Wang, Xiaohui; Zhongshan Hospital Xiamen University, Department of Dermatology, Kang, Xiaojing; People's Hospital of Xinjiang Uygur Autonomous Region, Department of Dermatology Wu, Bin; People's Hospital, Inner Mongolia Medical University, Department of Dermatology Xiao, Yi; Xiangya Hospital Central South University Chen, Xiang; Xiangya Hospital Central South University, Shen, Minxue; Xiangya Hospital Central South University, Dermatology
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Dermatology, Epidemiology
Keywords:	Allergy < THORACIC MEDICINE, Adult dermatology < DERMATOLOGY, Eczema < DERMATOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood: A population-based retrospective cohort study.

Yajia Li, M.A student [1], Danrong Jing, Ph.D. student [1], Yuzhou Huang, Ph.D. student [1], Juan Su, Ph.D. [1], Ji Li, Ph.D. [1], Juan Tao, Ph.D. [2], Shijun Shan, Ph.D. [3], Xiaohui Wang, Ph.D.[4], Xiaojing Kang, Ph.D. [5], Bin Wu, Ph.D.[6], Yi Xiao, Ph.D. [1*], Xiang Chen, Ph.D.[1*], Minxue Shen, Ph.D. [1,7*]

[1]Department of Dermatology; Hunan Engineering Research Center of Skin Health and Disease; Hunan Key Laboratory of Skin Cancer and Psoriasis, Xiangya Hospital, Central South University, Changsha, China

[2]Department of Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[3]Department of Dermatology, Xiang'an Hospital, Xiamen University, Xiamen, China

[4]Department of Dermatology, Zhongshan Hospital, Xiamen University, Xiamen, China

[5]Department of Dermatology, People's Hospital of Xinjiang Uygur Autonomous Region, Urumchi, Xinjiang, China

[6]Department of Dermatology, People's Hospital, Inner Mongolia Medical University, Hohhot, China

[7]Department of Social Medicine and Health Management, Xiangya School of Public Health, Central South University, Changsha, China

***Correspondence:**
Minxue Shen, Xiang Chen and Yi Xiao

Address: Xiangya Hospital, 87 Xiangya Road, Changsha, Hunan, China 410008

Email: shenmx1988@csu.edu.cn (M.S.), chenxiangck@126.com (X.C.) and xiaoyixy@csu.edu.cn (Y.X.)

Keywords: antibiotics, atopic and allergic skin diseases, preschool and young adulthood.

Abstract

Background: Overuse and misuse of antibiotics is a public health problem in low- and middle-income countries. Although the association of antibiotics with atopic and allergic diseases has been established, most studies focused on prenatal exposure and the occurrence of disease in infants or young children.

Objective: To investigate the association of preschool use of antibiotics with atopic and allergic skin diseases in young adulthood.

Design: Population-based retrospective cohort.

Setting and participants: The first-year college students (n=20123) from five universities were investigated. The sampled universities are located in Changsha, Wuhan, Xiamen, Urumqi, and Hohhot, respectively.

Methods: We conducted a dermatological field examination and a questionnaire survey inquiring the participants about frequency of upper respiratory tract infection (URTI) and the preschool antibiotics use (prior to 7 years old). The two-level Probit model was used to estimate the associations, and adjusted risk ratio (aRR) and 95% confidence interval (CI) were presented as the effect size.

Results: A total of 20123 participants with complete information was included in the final analysis. The frequent antibiotics use intravenously (aRR 1.36, 95% CI 1.14–1.62) and orally (aRR 1.18, 95% CI 1.01–1.38) prior to 7 years old was significantly associated with atopic dermatitis in young adulthood. Similar trends could be observed in allergic skin diseases among those who use antibiotics orally and intravenously, with RRs of 1.16 (1.01, 1.34) and 1.33 (1.13, 1.57), respectively.

Conclusions: Preschool URTI and antibiotics use significantly increases the risk of atopic and allergic skin diseases in young adulthood.

Strengths and limitations of this study

- The main outcomes were diagnosed by specialists instead of self-report.
- This study provides a relatively large and representative sample, and sufficient variations in geographic regions and sociodemographic subgroups, as well as the random effect at the university level was fitted by the 2-level models, resulting in an unbiased estimation of associations.
- Recall bias in the measurement of exposure to antibiotics might have been introduced, which could not be ignored in most retrospective studies.
- We lacked the information about the type and dose of antibiotics, and there might be a reversed causal relationship because antibiotics could be used in the treatment of AD and other conditions accompanied by a bacterial infection.

1 Introduction

The incidence and prevalence of atopic and allergic conditions such as asthma, allergic rhinitis, food allergies, and atopic dermatitis (AD) among the worldwide population have significantly increased during the past several decades.[1-3] An area of environmental change that may be responsible for the increase of allergic and atopic diseases is the growing use of medications which may alter the development of human microbiome.[4] It also seems that use of some antibiotics, which can directly cause intestinal dysbiosis and affect human microbiome and increase the risk for allergy development, is of particular concern in light of accumulating evidence.[5-7]

Furthermore, overuse and misuse of antibiotics is a severe public health problem worldwide, especially in low- and middle-income countries. In the last decade, prescriptions of broad-spectrum antibiotics increased by 49% in children under five years and doubled in children aged 5–17 years, concomitant with the increasing prevalence of allergic diseases.[8, 9] In China, 70% of outpatients attending primary care facilities with colds were inappropriately treated with antibiotics, often by intravenous infusion. The situation is even worse in children because many parents demand treatment with antibiotics[10]. However, most upper respiratory tract infections (URTI) in children are viral, for which antibiotics are unnecessary.[11-13]

The association between the use of antibiotics and atopic and allergic diseases has been observed in longitudinal studies. But most studies focused on antibiotics use during pregnancy or infancy when early colonization is initiated by maternal microbes.[14-17] With the childhood microbiome transition owing to alterations in food and exposure to more diverse microbes in external environments, children in preschool age (<7 yrs) are at higher risks of URTI infection and antibiotics treatment. However, the effect of antibiotics used during this period on atopic and allergic skin diseases that occurred in their young adulthood is not clear. The objective of this study was to evaluate the hypothesis that exposure to antibiotics in preschool age is associated with an increased risk of allergic and atopic skin diseases in young adulthood. We tested this hypothesis by conducting a retrospective study in college students.

2 Materials and Methods

2.1 Study setting and design

This was a retrospective cohort study based on the data from the China College Student Skin Health Study (CCSSHS)[18]. The first-year college students from five universities were investigated. They underwent a health examination and completed a questionnaire survey. The sampled universities are located in Changsha, Wuhan, Xiamen, Urumqi, and Hohhot, respectively. The medical ethics committee of Xiangya Hospital, Central South University, approved the study (#201709993).

2.2 Exposure assessment

Two semi-quantitative questions served as the proxy measures of the frequency of antibiotics exposure in preschool age, with detailed explanations for the definitions of URTI and antibiotics. In our study,

the definition of URTI refers to a series of acute illnesses that have an effect on the upper respiratory system including the common cold, acute otitis media, tonsillitis/tonsillopharyngitis, sinusitis and recurrent sinusitis.[19] The first question was "How often did you have URTI in your preschool age or before 7 years old", with three potential responses: ⩽1 time/year, 2-3 times/year", and "4 or more times/year". The second question was "How often did you receive antibiotics treatment when you had a URTI", with four responses: "rare", "occasional", "often, orally; and "often, intravenously".

2.3 Outcome assessment

Diagnosis of skin diseases and inquiry of disease history were performed by dermatologists during the field survey. All subjects underwent skin examination by resident doctors in dermatology, and the diagnoses were further validated by senior dermatologists. Clinical manifestation, disease history, and family history were inquired about, and inspections were conducted to diagnose skin diseases. For recurrent skin diseases, only those with current symptoms and cutaneous lesions were diagnosed as cases. AD was diagnosed according to the Williams criteria.[20] Hand eczema was diagnosed according to eczema (rash) on the fingers, finger webs, palms, or back of hands, which had appeared once and continued for at least two weeks or had appeared several times or had been persistent. Allergies and urticaria were diagnosed by clinical manifestations, potential triggers, and histories. Asthma, allergic rhinitis, and allergic conjunctivitis were self-reported according to doctors' diagnoses. We also combined some of the outcomes. Atopic march is an apparent progression of allergic diseases from AD, to allergic asthma (AA) to allergic rhinitis (AR) and allergic conjunctivitis.[21, 22] We include the conditions of AD, AA, AR, and allergic conjunctivitis for the outcome of the atopic march. Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria. Participants with a history of atopic/allergic conditions but without the current disease are excluded.

2.4 Covariates

Demographic characteristics, socioeconomic status (annual family income and parental highest educational level), family history, behavioral factors (dietary, passive smoking, and bathing habits) were inquired by the questionnaire. The questionnaire used in the study was shown in Material S1.

2.5 Statistical analysis

Categorical data were presented as number (%), and the between-group difference was tested using the chi-square test. Two-level Probit regression models (individual as level 1 and university as level 2) were used to estimate the associations of preschool exposure to antibiotics with atopic skin diseases in young adulthood, adjusting for level-1 covariates (gender, ethnicity, annual household income and parental education) and level-2 random effects. The effect size was presented as relative risk (RR) and 95% confidence interval (CI). $P < 0.05$ was considered statistically significant for all tests. Statistical analysis was performed in SAS 9.4 (SAS Institute Inc., Cary, USA).

3 Results

A total of 27144 registries for new enrolment was identified; among them, 21086 (77.7%) consented to participate, and 20123 (74.1%) who underwent the health examination and completed the online

questionnaire survey were included in the final analyses. The mean age was 18.3± 0.8 years and 10283 (51.1%) were men. The characteristics of participants in the study are shown in the Table 1. The prevalence rates of chronic urticaria, allergic reactions to food/drug/light, hand eczema, and AD were 1.89%, 2.27%, 3.35%, and 3.86%, respectively. The prevalence rates of AD and allergic skin disease were 3.86 % and 4.14%, respectively (Figure 1 and Table S1).

In general, URTI and the use of antibiotics were significantly associated with atopic and allergic diseases dose-dependently (Figure 1 and Table S1). For example, the prevalence of AD increased from 3.39% to 4.11% and 4.79% in participants who reported rare or occasional use, frequent oral use, and frequent intravenous use of antibiotics, respectively. Consistent trends could be observed in all atopic and allergic diseases and their combinations.

After adjustments for sociodemographic factors (Figure 2 and Table S2), URTI and the use of antibiotics were significantly associated with atopic/allergic skin diseases in dose-response manners. For instance, compared to those reporting rare or occasional use of antibiotics, the RRs for AD in participants who reported frequent oral administration and intravenous injection of antibiotics was 1.18 (95% CI: 1.01, 1.39) and 1.36 (95% CI: 1.14, 1.62), respectively. For other allergies or atopic disease of skin or beyond skin, the correlations were highly consistent despite some variations in the magnitude of the association.

Furthermore, we have provided the data on the health seeking behavior dealing with a cold or fever in preschool age (shown in the Table 2), and we found there were 66.2% among participants with the atopic march and 64.7% among participants with allergic skin disease showed that they/their parents would like to seek antibiotics treatment when they had a cold/fever in their preschool age. In those without atopic/allergic diseases, this proportion ratio was 61.5%. Indeed, we found a moderate but significant difference in the seeking behavior of antibiotic treatment between those with allergic skin disease/atopic march and healthy participants (P=0.001). Similar trends could be seen in AD patients and non-patients (P=0.034).

Besides, because our study was not a prospective cohort, it was difficult to know if antibiotic use was ahead of suffering from AD. We further evaluated the joint effect of URTI and antibiotics on AD by including an interaction term in the model. As shown in Figure 3, in each category of antibiotic use, the frequency of URTI was positively associated with the risk of AD. Vice versa, in each category of URTI, antibiotic use was positively associated with AD according to the effect size, despite some insignificant results in categories with small sample size.

4 Discussion

This retrospective cohort study demonstrated that preschool exposure to antibiotics, either through oral administration or intravenous infusion, was associated with increased risks of having allergic and atopic skin diseases in young adulthood. Participants who reported frequent URTI in preschool-age also had higher risks of allergies and atopy.

Similar trends were identified in previous observational studies that early-life antibiotic use was associated with an increased risk of eczema, but there were still some inconsistent results.[23, 24] Meta-analysis including 22 studies with 394,517 patients concluded that children with antibiotics exposure in the first two years had increased odds of atopic eczema with an OR of 1.26 (95%CI: 1.15-1.37). Notably, the onset age of the outcomes in the included studies was the period of childhood (<12 years old). [7] A large population-based retrospective cohort study in twins showed that antibiotic use was also associated with an increased risk of eczema; however, it is likely that the relationship between early-life antibiotic use and eczema was confounded by shared familial environment and genetic factors.[25] However, current data lacked the information regarding the atopic and allergic skin diseases occurred in late adolescence to early adulthood, while we firstly investigated the effects of preschool antibiotic in a retrospective study and revealed a positive association. We did not observe significant difference in health seeking behavior in our study, as most of the Chinese parents could pay a close attention to prschool health of children, and keep a non-exclusion attitude to antibiotics use. Using antibiotics at home, seeking medical care, and use antibiotics in the hospital for children was pervasive in China when parents dealing with children's respiratory tract infections. However, the results should be discussed with caution as doctor seeking behavior varies a lot in populations. In this setting, children with AD will seek a doctor frequently and more likely get a URTI diagnosis if they also have a cold. Equally, children with asthma and wheezing will more frequently get a URTI diagnosis and are more likely to get an antibiotic.

Evidence showed that AD was the first manifestation of an atopic phenotype which begins in early childhood and the progression from AD to the diseases such as food allergy, asthma, and allergic rhinitis were more likely to be shown in adolescence.[22, 26, 27] The mechanisms behind the march from AD to allergic airway diseases and allergic conjunctivitis likely arise from initial epicutaneous allergen sensitization inducing robust local and systemic type 2 immune responses with increased production of type 2 cytokines including interleukin (IL)-4, IL-13, IL-31, and thymic stromal lymphopoietin.[28, 29] Most studies were prone to make these responses responsible for the commonly shared pathogenesis of cutaneous, airway, and conjunctiva inflammation, supporting the view that AD is not merely a disease confined to the skin, but is in fact, a systemic disease.[30, 31] Therefore, it could be explained that the increased risks beyond skin manifestations in young adulthood were consistent with those of skin diseases and some with even greater effect sizes. While not fully understood, the underlying mechanism of the association between antibiotics and atopic and allergic diseases can be elucidated by microbial diversity. The gut microbial community is dynamic and variable during the first 3 years of life, before stabilizing to an adult-like state.[32] Studies have demonstrated continued development through childhood into the teenage years.[33, 34] Dietary intake plays a key role in the development period of the gut microbiome. Breast-fed infants have microbiota enriched in *Lactobacillus*, *Staphylococcus*, and *Bifidobacterium*. Studies have shown that human milk isolates contain symbiotic and potentially probiotic microbes, and supplementation of *Bifidobacteria* was found to be effective in primary preventing allergic diseases.[35-37] But among children of preschool age, the dominance of *Bifidobacterium* diminishes with the alteration in dietary intake.[15] On the other hand, the high prevalence of antibiotic use may also lead to a concurrent increase in antibiotic-resistant bacteria.[38, 39] Antibiotic-treated children have a less diverse gut

microbiota and less stable communities. Antibiotic therapy affects microbiome variety and thus may increase the risk of atopic diseases.[40, 41]

In our study, increased risks of atopic march or allergic diseases were observed in students who reported frequent URTI. Another potential explanation was related to the infections which could also affect microbial conditions. Except for the change in diet, children of preschool age tend to be exposed to more diverse microbes and infectious diseases including URTI in kindergarten or external environments. Respiratory viral infections, in particular, have been shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut,[42] which could further shape atopic microenvironments.

Several limitations should be noted, and the results should be interpreted with caution. First, recall bias in the measurement of exposure to antibiotics might have been introduced. While recall bias on the frequency of antibiotics use and URTIs should not be ignored, this is a limitation in most retrospective studies. Besides, selection bias might also be introduced as students with skin conditions might be more interested to participate and recall carefully. We are not able to validate the medical records because China does not have a registry system for primary care, and a large number of patients with mild conditions also visit doctors in secondary and tertiary hospitals. While participants could obtain the information from their parents, but unfortunately, we could not evaluate the extent of recall bias. Similarly, for the conditions of the disease including asthma, allergic rhinitis and allergic conjunctivitis, which we collected based on both clinical diagnosis and questionnaire data, we could not ignore the atopic conditions in the past that might not be existing anymore, though in previous studies, the 'atopic march' referred to the sequential development of symptoms and was considered to be not strictly limited by the occurring time. [43,44] Those will be in our further consideration in future studies.

Second, there was a lack of information about the type and dose of antibiotics, as well as some antibiotic use for the treatment of low respiratory tract infection, skin infections, and other infections, which was not collected while could also affect the microbiome. So we could not attribute the associations to specific antibiotics use. Third, we noticed that some factors related to the allergic/atopic conditions in preschool age could be ignored, such as prematurity, the doctor seeking behavior, etc., and we had difficulty in fully collecting of related information and need to explain the results with caution. Last but not least, there might be a reversed causal relationship because antibiotics could be used in the treatment of AD and other conditions accompanied by bacterial infection. We assessed the role of URTI and antibiotics separately, because the two variables were significantly correlated (contingency coefficient=0.4, $P<0.001$) and were therefore not included in the same model to avoid collinearity and biased estimation of parameters. It is possible that the association of URTI with AD and allergies is confounded by antibiotics, and vice versa.

However, both infection and antibiotics may be correlated with allergies/atopies, with different mechanisms. Although under the hygiene hypothesis, exposure to pathogens during infancy and early childhood has been proposed to explain the lower prevalence of asthma and other atopic diseases among children in developing countries,[45, 46] some studies showed that early respiratory infections

could not protect against atopic eczema or recurrent wheezing, but could drive the development of atopic disease. [47-51] Atopic sensitization, a process of generation of specific immunoglobulin E (sIgE) when exposed to an innocuous antigen, was common to all allergic diseases. As preschool URTI most probably represent viral infections in the majority of cases, some studies investigated a potential mechanistic explanation for how a respiratory viral infection could drive the development of atopic sensitization and disease. Martorano et al. used a Sendai virus to establish the mouse model mimicking a human limited respiratory syncytial virus infection and found that Sendai virus infection could promote the crosslinking of high-affinity IgE receptor (FcεRI) on the lung conventional dendritic cells (cDC), which led to the production of the chemokine CCL28, recruiting IL-13 and driving the development of mucous cell metaplasia and airway hyperreactivity.[52] Another animal study found that except for the increase of sIgE against Sendai virus in mice, there was also a large increase in total IgE and it remained elevated long after the viral infection being resolved.[53] This notion has further been fuelled by findings that mice infected with flu virus developed virus-specific mast cell degranulation in the skin, indicating a possible pathway of viral infections that could mediate allergic symptoms.[54] Besides, the respiratory viral infections were shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut.[42, 55] However, we cannot ignore that infections that do not require antibiotics are not captured in our design, such that it is difficult to assess whether the observed association is caused by a specific infection or antibiotics because they occur simultaneously in many cases.

Our study also has strengths. The primary strength is that the sample size for the retrospective cohort study was large. Second, the outcomes were diagnosed by specialists. In contrast, some previous studies used self-reported diagnoses that might introduce misclassification bias. Third, the study had a relatively large and representative sample, and sufficient variations in geographic regions and sociodemographic subgroups. The random effect at the university level was fitted by the 2-level models, resulting in an unbiased estimation of associations.

To conclude, preschool children exposed to URTI or antibiotics may be at higher risks of atopic and allergic skin diseases in their young adulthood, especially among those who frequently had URTI or received antibiotics by intravenous infusion. Our study implies that unnecessary antibiotics treatment in children should be avoided to prevent the occurrence of atopic and allergic diseases in their later life. Prospective studies that consider the type and dose of antibiotics are warranted.

5 Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

6 Ethics statement

This study was conducted according to the guidelines established in the Declaration of Helsinki. All procedures involving patients were approved by the institutional research ethics boards of Xiangya Hospital, Central South University (Changsha, China). Informed consent was obtained from all the students before the investigation.

7 Patient and Public Involvement Statement

This is a retrospective cohort study based on the data from the China College Student Skin Health Study (CCSSHS).The first-year college students from five universities were recuited and investigated. They underwent a health examination and completed a questionnaire survey, and the results will be disseminated to study participants by a medical examination report. Participants were not involved in the design and implementation of the study.

8 Author contributions

Dr. Minxue Shen, Dr. Xiang Chen, and Dr. Yi Xiao are joint corresponding authors and have full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Shen, Chen, and Xiao.

Acquisition, analysis, or interpretation of data: Y.J. Li, Jing, and Huang

Drafting of the manuscript: Y.J. Li

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Shen, Xiao, and Y.J. Li.

Administrative, technical, or material support: Su, J. Li, Tao, Shan, Wang, Kang, and Wu.

Supervision: Chen

9 Conflict of interest

The authors declare no conflict of interest.

10 Funding

This work was supported by the National Key Research & Development Project "Precision Medicine Initiative" of China (2016YFC0900802) and the Program of Introducing Talents of Discipline to Universities (111 Project, No. B20017).

11 Acknowledgment

The authors would like to thank the following individuals and institutions who have made substantial contribution to the establishment of the cohort: Central South University (Lei Cai, Duling Cao, Qin Cao, Chao Chen, Liping Chen, Menglin Chen, Mengting Chen, Xiang Chen, Qing Deng, Xin Gao, Yihuan Gong, Jia Guo, Yeye Guo, Rui Hu, Xin Hu, Chuchu Huang, Huining Huang, Kai Huang, Xiaoyan Huang, Yuzhou Huang, Danrong Jing, Xinwei Kuang, Li Lei, Jia Li, Jiaorui Li, Jie Li, Keke Li, Peiyao Li, Yajia Li, Yayun Li, Yangfan Li, Dan Liu, Dihui Liu, Fangfen Liu, Nian Liu, Panoan Liu, Runqiu Liu, Hui Lu, Wenhua Lu, Yan Luo, Zhongling Luo, Manyun Mao, Mengping Mao, Yuyan Ouyang, Shiyao Pei, Qunshi Qin, Ke Sha, Lirong Tan, Ling Tang, Ni Tang, Yan Tang, Ben Wang, Yaling Wang, Tianhao Wu, Yun Xie, Siyu Yan, Sha Yan, Bei Yan, Xizhao Yang, Lin Ye, Hu Yuan, Taolin Yu, Yan Yuan, Yi Yu, Rui Zhai, Jianghua Zhang, Jianglin Zhang, Mi Zhang, Xingyu Zhang, Zhibao Zhang, Shuang Zhao, Yaqian Zhao, Kuangbiao Zhong, Lei Zhou, Youyou Zhou, Zhe Zhou, Susi Zhu); Huazhong University of Science and Technology (Xiangjie An, Siqi Da, Yaqi Dong, Yangxue Fu, Lixie Gao, Han Han, Biling Jiang, Jiajia Lan, Jun Li, Xiaonan Li, Yan Li, Liquan Liu, Yuchen Lou, Pu Meng, Yingli Nie, Gong Rao, Shanshan Sha, Xingyu Su, Huinan Suo, Rongying Wang, Jun Xie, Yuanxiang Yi, Jia Zhang, Qiao Zhang, Li Zhu, Yanming Zhu); Xiamen University (Zhiming Cai, Lina Chen, Xiaozhu Fu, Hongjun Jiang, Guihua Luo, Jianbing Xiahou, Binxiang Zheng); People's Hospital of Xinjiang Uygur Autonomous Region (Jianxia Chen, Xiaomin Chen, Xinqi Chen, Li Dai, Yanyan Feng, Fanhe Jiang, Lan Jin, Qingyu Ma, Qun Shi, Hongbo Tang, Fang Wang, Zhen Wang, Xiujuan Wu, Kunjie Zhang, Yu Zhang); Xinjiang Medical University (Huagui Li, Jianguang Li, Lei Shi); Inner Mongolia Medical University (Chao Tian, Wei Wang, Rina Wu, Hongjun Xing, Baogui Yang).

12 References

[1]. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action[J]. World Allergy Organ J. 2014,7(1):12.

[2]. Platts-Mills TA. The allergy epidemics: 1870-2010[J]. J Allergy Clin Immunol. 2015,136(1):3-13.

[3]. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys[J]. Lancet. 2006,368(9537):733-43.

[4]. Mitre E, Susi A, Kropp LE, Schwartz DJ, Gorman GH, Nylund CM. Association Between Use of Acid-Suppressive Medications and Antibiotics During Infancy and Allergic Diseases in Early Childhood[J]. JAMA Pediatr. 2018,172(6):e180315.

[5]. Wang JY, Liu LF, Chen CY, Huang YW, Hsiung CA, Tsai HJ. Acetaminophen and/or antibiotic use in early life and the development of childhood allergic diseases[J]. Int J Epidemiol. 2013,42(4):1087-99.

[6]. Kim DH, Han K, Kim SW. Effects of Antibiotics on the Development of Asthma and Other Allergic Diseases in Children and Adolescents[J]. Allergy Asthma Immunol Res. 2018,10(5):457-65.

[7]. Ahmadizar F, Vijverberg SJH, Arets HGM, de Boer A, Lang JE, Garssen J, et al. Early-life antibiotic exposure increases the risk of developing allergic symptoms later in life: A meta-analysis[J]. Allergy. 2018,73(5):971-86.

[8]. Lee GC, Reveles KR, Attridge RT, Lawson KA, Mansi IA, Lewis JS, 2nd, et al. Outpatient antibiotic prescribing in the United States: 2000 to 2010[J]. BMC Med. 2014,12:96.

[9]. Versporten A, Bielicki J, Drapier N, Sharland M, Goossens H. The Worldwide Antibiotic Resistance and Prescribing in European Children (ARPEC) point prevalence survey: developing hospital-quality indicators of antibiotic prescribing for children[J]. J Antimicrob Chemother. 2016,71(4):1106-17.

[10]. Biezen R, Grando D, Mazza D, Brijnath B. Dissonant views - GPs' and parents' perspectives on antibiotic prescribing for young children with respiratory tract infections[J]. BMC Fam Pract. 2019,20(1):46.

[11]. Wei X, Zhang Z, Walley JD, Hicks JP, Zeng J, Deng S, et al. Effect of a training and educational intervention for physicians and caregivers on antibiotic prescribing for upper respiratory tract infections in children at primary care facilities in rural China: a cluster-randomised controlled trial[J]. Lancet Glob Health. 2017,5(12):e1258-e67.

[12]. Li J, Song X, Yang T, Chen Y, Gong Y, Yin X, et al. A Systematic Review of Antibiotic Prescription Associated With Upper Respiratory Tract Infections in China[J]. Medicine (Baltimore). 2016,95(19):e3587.

[13]. Ding L, Sun Q, Sun W, Du Y, Li Y, Bian X, et al. Antibiotic use in rural China: a cross-sectional survey of knowledge, attitudes and self-reported practices among caregivers in Shandong province[J]. BMC Infect Dis. 2015,15:576.

[14]. McKeever TM, Lewis SA, Smith C, Collins J, Heatlie H, Frischer M, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database[J]. J Allergy Clin Immunol. 2002,109(1):43-50.

[15]. Kundu P, Blacher E, Elinav E, Pettersson S. Our Gut Microbiome: The Evolving Inner Self[J]. Cell. 2017,171(7):1481-93.

[16]. Yoshida S, Ide K, Takeuchi M, Kawakami K. Prenatal and early-life antibiotic use and risk of childhood asthma: A retrospective cohort study[J]. Pediatr Allergy Immunol. 2018,29(5):490-5.

[17]. Kusel MM, de Klerk N, Holt PG, Sly PD. Antibiotic use in the first year of life and risk of atopic disease in early childhood[J]. Clin Exp Allergy. 2008,38(12):1921-8.

[18]. S M, X Y, S J. Prevalence and patient-reported outcomes of noncommunicable skin diseases among college students in China.[J]. JAAD Int. 2020,1(1):23-30.

[19]. Eccles MP, Grimshaw JM, Johnston M, Steen N, Pitts NB, Thomas R, et al. Applying psychological theories to evidence-based clinical practice: identifying factors predictive of managing upper respiratory tract infections without antibiotics[J]. Implement Sci. 2007,2:26.

[20]. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation[J]. Br J Dermatol. 1994,131(3):406-16.

[21]. Spergel JM, Paller AS. Atopic dermatitis and the atopic march[J]. The Journal of allergy and clinical immunology. 2003,112(6 Suppl):S118-27.

[22]. Hill DA, Spergel JM. The atopic march: Critical evidence and clinical relevance[J]. Ann Allergy Asthma Immunol. 2018,120(2):131-7.

[23]. Metzler S, Frei R, Schmaußer-Hechfellner E, von Mutius E, Pekkanen J, Karvonen AM, et al. Association between antibiotic treatment during pregnancy and infancy and the development of allergic diseases[J]. Pediatr Allergy Immunol. 2019,30(4):423-33.

[24]. Tsakok T, McKeever TM, Yeo L, Flohr C. Does early life exposure to antibiotics increase the risk of eczema? A systematic review[J]. Br J Dermatol. 2013,169(5):983-91.

[25]. Slob EMA, Brew BK, Vijverberg SJH, Kats C, Longo C, Pijnenburg MW, et al. Early-life antibiotic use and risk of asthma and eczema: results of a discordant twin study[J]. Eur Respir J. 2020,55(4).

[26]. Johansson E, Hershey GKK. Contribution of an impaired epithelial barrier to the atopic march[J]. Ann Allergy Asthma Immunol. 2018,120(2):118-9.

[27]. Belgrave DC, Granell R, Simpson A, Guiver J, Bishop C, Buchan I, et al. Developmental profiles of eczema, wheeze, and rhinitis: two population-based birth cohort studies[J]. PLoS Med. 2014,11(10):e1001748.

[28]. Akei HS, Brandt EB, Mishra A, Strait RT, Finkelman FD, Warrier MR, et al. Epicutaneous aeroallergen exposure induces systemic TH2 immunity that predisposes to allergic nasal responses[J]. J Allergy Clin Immunol. 2006,118(1):62-9.

[29]. Cianferoni A, Spergel J. The importance of TSLP in allergic disease and its role as a potential therapeutic target[J]. Expert Rev Clin Immunol. 2014,10(11):1463-74.

[30]. Asada Y, Nakae S, Ishida W, Hori K, Sugita J, Sudo K, et al. Roles of Epithelial Cell-Derived Type 2-Initiating Cytokines in Experimental Allergic Conjunctivitis[J]. Invest Ophthalmol Vis Sci. 2015,56(9):5194-202.

[31]. Han H, Xu W, Headley MB, Jessup HK, Lee KS, Omori M, et al. Thymic stromal lymphopoietin (TSLP)-mediated dermal inflammation aggravates experimental asthma[J]. Mucosal Immunol. 2012,5(3):342-51.

[32]. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography[J]. Nature. 2012,486(7402):222-7.

[33]. Hollister EB, Riehle K, Luna RA, Weidler EM, Rubio-Gonzales M, Mistretta TA, et al. Structure and function of the healthy pre-adolescent pediatric gut microbiome[J]. Microbiome. 2015,3:36.

[34]. Agans R, Rigsbee L, Kenche H, Michail S, Khamis HJ, Paliy O. Distal gut microbiota of adolescent children is different from that of adults[J]. FEMS Microbiol Ecol. 2011,77(2):404-12.

[35]. Heikkilä MP, Saris PE. Inhibition of Staphylococcus aureus by the commensal bacteria of human milk[J]. J Appl Microbiol. 2003,95(3):471-8.

[36]. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life[J]. Cell Host Microbe. 2015,17(6):852.

[37]. Enomoto T, Sowa M, Nishimori K, Shimazu S, Yoshida A, Yamada K, et al. Effects of bifidobacterial supplementation to pregnant women and infants in the prevention of allergy development in infants and on fecal microbiota[J]. Allergol Int. 2014,63(4):575-85.

[38]. Hiltunen T, Virta M, Laine AL. Antibiotic resistance in the wild: an eco-evolutionary perspective[J]. Philos Trans R Soc Lond B Biol Sci. 2017,372(1712).

[39]. Hawkey PM, Jones AM. The changing epidemiology of resistance[J]. J Antimicrob Chemother. 2009,64 Suppl 1:i3-10.

[40]. Penders J, Stobberingh EE, van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders[J]. Allergy. 2007,62(11):1223-36.

[41]. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema[J]. J Allergy Clin Immunol. 2012,129(2):434-40, 40.e1-2.

[42]. Hanada S, Pirzadeh M, Carver KY, Deng JC. Respiratory Viral Infection-Induced Microbiome Alterations and Secondary Bacterial Pneumonia[J]. Front Immunol. 2018,9:2640.

[43]. Wahn U. What drives the allergic march?[J]. Allergy. 2000 Jul;55(7):591-9.

[44]. Spergel JM. From atopic dermatitis to asthma: the atopic march[J]. Ann Allergy Asthma Immunol. 2010 Aug;105(2):99-106; quiz 107-9, 117.

[45]. Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis[J]. Nat Immunol. 2017,18(10):1076-83.

[46]. Haspeslagh E, Heyndrickx I, Hammad H, Lambrecht BN. The hygiene hypothesis: immunological mechanisms of airway tolerance[J]. Curr Opin Immunol. 2018,54:102-8.

[47]. Kramer MS, Guo T, Platt RW, Sevkovskaya Z, Dzikovich I, Collet JP, et al. Does previous infection protect against atopic eczema and recurrent wheeze in infancy?[J]. Clin Exp Allergy. 2004,34(5):753-6.

[48]. Peebles RS, Jr. Viral infections, atopy, and asthma: is there a causal relationship?[J]. The Journal of allergy and clinical immunology. 2004,113(1 Suppl):S15-8.

[49]. Martinez FD. Viruses and atopic sensitization in the first years of life[J]. Am J Respir Crit Care Med. 2000,162(3 Pt 2):S95-9.

[50]. Nafstad P, Brunekreef B, Skrondal A, Nystad W. Early respiratory infections, asthma, and allergy: 10-year follow-up of the Oslo Birth Cohort[J]. Pediatrics. 2005,116(2):e255-62.

[51]. Dharmage SC, Lodge CJ, Lowe AJ, Allen KJ. Antibiotics and risk of asthma: a debate that is set to continue[J]. Clin Exp Allergy. 2015,45(1):6-8.

[52]. Martorano LM, Grayson MH. Respiratory viral infections and atopic development: From possible mechanisms to advances in treatment[J]. Eur J Immunol. 2018,48(3):407-14.

[53]. Cheung DS, Ehlenbach SJ, Kitchens T, Riley DA, Grayson MH. Development of atopy by severe paramyxoviral infection in a mouse model[J]. Ann Allergy Asthma Immunol. 2010,105(6):437-43.e1.

[54]. Grunewald SM, Hahn C, Wohlleben G, Teufel M, Major T, Moll H, et al. Infection with influenza a virus leads to flu antigen-induced cutaneous anaphylaxis in mice[J]. The Journal of investigative dermatology. 2002,118(4):645-51.

[55]. Culley FJ, Pennycook AM, Tregoning JS, Hussell T, Openshaw PJ. Differential chemokine expression following respiratory virus infection reflects Th1- or Th2-biased immunopathology[J]. J Virol. 2006,80(9):4521-7.

Figure legends

Figure 1. The prevalence of atopic and allergic diseases in exposure vs. non-exposure group of antibiotics and URTI.

Figure 2. Association of early-life exposure to antibiotics with the risk of atopic/allergic diseases later in life. RR: risk ratio; CI: confidence interval.

Figure 3. The joint effect of URTI and antibiotics on AD after including an interaction term in the model. RR: risk ratio; CI: confidence interval.

Supplementary file captions

Material S1. Chinese college students health survey questionaire (Translation version in English).

Table S1. The prevalence of atopic and allergic diseases in college students.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students.

Table1. Characteristics of participants in the Present Study (N=20123)

Characteristics	N	%
Gender		
Male	10283	51.1
Female	9840	48.9
Income (CNY)		
<10,000	2168	10.8
10,000–29,999	4376	21.7
30,000–49,999	3465	17.2
50,000–99,999	4417	22.0
100,000–199,999	4063	20.2
≥200,000	1634	8.1
Parental highest education		
Primary school	1320	6.6
Middle school	5316	26.4
High school	5021	24.9
College and above	8466	42.1
Ethnicity		
Han Chinese	16218	80.6
Other ethnicities	3905	19.4

Table 2. Health seeking behavior when dealing with a cold or fever in the preschool age.[a]

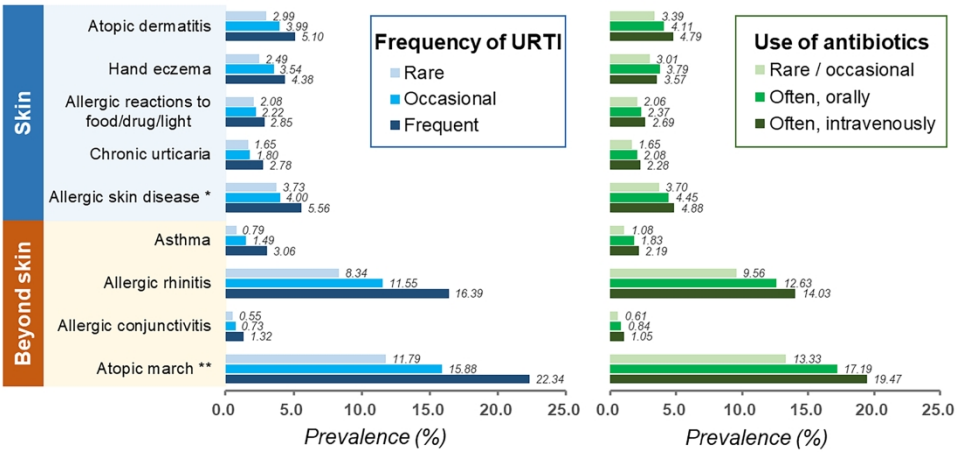
Disease condition	Total	Health seeking behavior, n (%)[b]				
		Ignore them	Drink water or have a rest	Receive antibiotics orally or intravenously	Receive Chinese traditional medicine orally	Unknown
Skin						
Atopic dermatitis	776	20 (2.6)	370 (47.6)	512 (65.9)	194 (25.0)	3 (0.3)
Hand eczema	675	32 (4.7)	343 (50.8)	430 (63.7)	149 (22.0)	4 (0.5)
Allergic reactions to food/drug/light	456	9 (2.0)	227 (49.7)	286 (62.7)	95 (20.8)	1 (0.2)
Chronic urticaria	381	8 (2.1)	158 (41.4)	257 (67.4)	100 (26.2)	1 (0.3)
Allergic skin disease [c]	833	17 (2.0)	384 (46.0)	539 (64.7)	194 (23.2)	2 (0.2)
Beyond skin						
Atopic march [d]	3139	66 (2.1)	1542 (49.1)	2081 (66.2)	728 (23.1)	12 (0.3)
Allergic conjunctivitis	153	4 (2.6)	83 (54.2)	107 (69.9)	36 (23.5)	0 (0)
Allergic rhinitis	2273	42 (1.8)	1135 (49.9)	1515 (66.6)	532 (23.4)	8 (0.4)
Asthma	303	7 (2.3)	154 (50.8)	206 (67.9)	70 (23.1)	2 (0.6)
Without atopic/allergic diseases	16343	597 (3.7)	7761 (47.4)	10057 (61.5)	3058 (18.7)	76 (0.4)

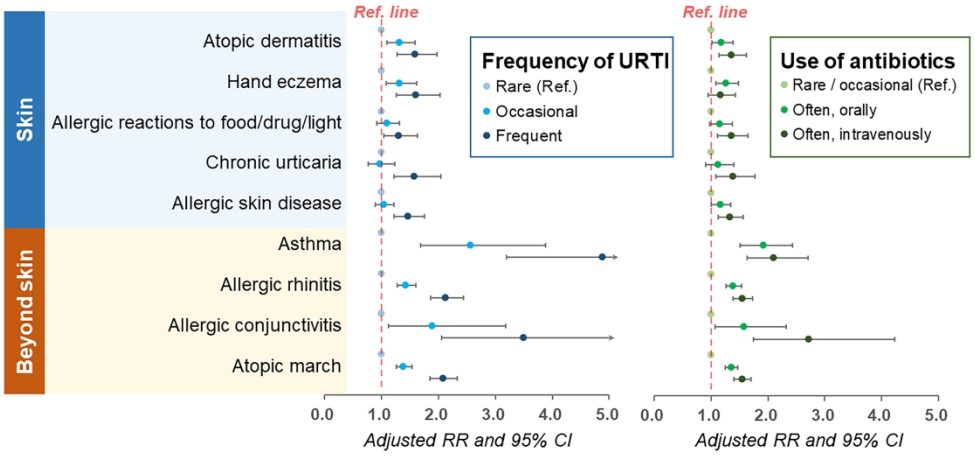
[a] Multiple selections are allowed in the questionnaire.

[b] Proportion ratios in populations.

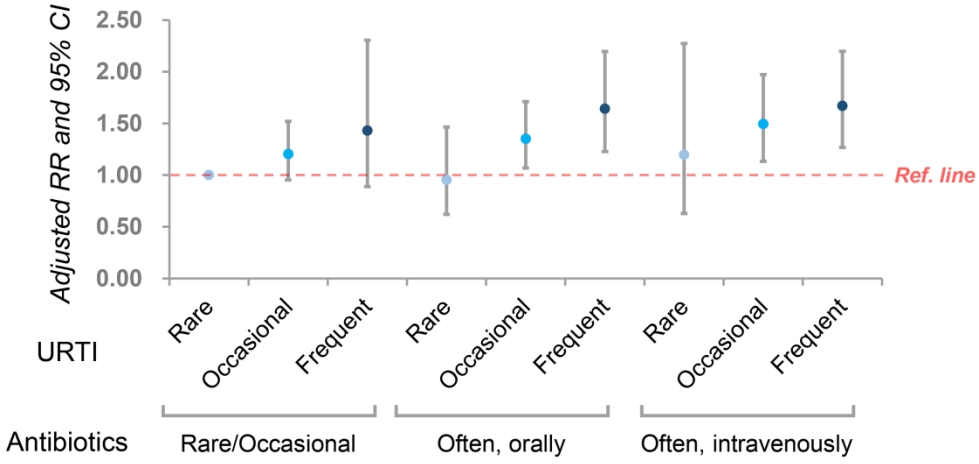
[c] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.

[d] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.





338x170mm (300 x 300 DPI)



236x115mm (300 x 300 DPI)

Supplementary file captions

Material S1. Chinese college students health survey questionaire (Translation version in English)

Table S1. The prevalence of atopic and allergic diseases in college students.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students.

Material S1. Chinese college students health survey questionaire (English version)

Chinese college students health survey questionaire

Informed consent

Welcome to participate in the Chinese University Student Health Survey. In order to promte a better health management for Chinese college freshmen, you are invited to fill out a questionnaire including several parts: (A) General information; (B) Medical history, (C) Lifestyle habits, (D) Skin health, which take about 15 minutes to answer. We will provide you with reasonable health education and health management based on this information, combined with the results of the health checkup. All the information you fill in will be kept strictly confidential. Continue to fill in the following content, indicating that you and your guardian have understood and are willing to continue to cooperate with our work. Thanks you!

A. General information

A01. Before you went to university, please provide your address. _____

(*Specific to districts and counties*)

A02. Have you moved to other places (cross-city) in the past 10 years

(*Single selection, if "No" jump to A04*)

O Never O Ever

A03. If so, the previous address is?_____ (*Specific to districts and counties*)

A04. Sex (*Single selection*)

O Male O Female

A05. Your Ethnicity:_____

A06. Annual household income. (*Single selection*)

O <10,000 O 10,000 – 29,999 O 30,000 – 49,999

O 50,000 – 99,999 O 100,000 – 199,999 O ⩾200,000

A07. Your father's highest education (*Single selection*)

O Primary school or O Middle school O High school

blow ○ College ○ Postgraduate or above ○ Unclear
A08. Your mother's highest education (*Single selection*) ○ Primary school or blow ○ Middle school ○ High school ○ College ○ Postgraduate or above ○ Unclear

B. Medical History

B01. Have you ever been specifically diagnosed with any of the following cardiovascular or metabolic diseases? (*Multiple selections are allowed*) ☐ Hypertension ☐ Coronary heart disease ☐ Hyperlipidaemia ☐ Obesity ☐ Fatty liver ☐ Gout ☐ Psoriasis ☐ None of above
B02. Have you ever been diagnosed with any of the following allergic diseases? (*Multiple selections are allowed*) ☐ Asthma ☐ Allergic Rhinitis ☐ Allergic conjunctivitis ☐ Eczema ☐ Urticaria ☐ None of above
B03. Have you ever been diagnosed with any of the following infectious diseases? (*Multiple selections are allowed*) ☐ Tuberculosis or other forms of tuberculosis ☐ Hepatitis B ☐ Hepatitis C ☐ Helicobacter pylori infection ☐ HIV infection ☐ None of above
B04. Have you ever been diagnosed with any of the following endocrine diseases? (*Multiple selections are allowed*)

☐ Type 1 diabetes ☐ Type 2 diabetes ☐ Polycystic ovarian syndrome ☐ Hypertrichosis ☐ Hypothyroidism ☐ Hyperthyroidism ☐ Graves disease ☐ Hashimoto thyroiditis ☐ None of above
B05.Have you ever been diagnosed with any of the following immune diseases? (*Multiple selections are allowed*) ☐ SLE ☐ Scleroderma ☐ Sjogren syndrome ☐ Uveitis ☐ Rheumatoid arthritis ☐ Dermatomyositis ☐ None of above
B06. Have you ever been diagnosed with any of the following hematologic diseases? (*Multiple selections are allowed*) ☐ Iron-deficiency anemia ☐ Megaloblastic anemia ☐ Thalassemia ☐ Anemia ☐ Hemophilia ☐ leucocythemia ☐ Lymphadenoma ☐ None of above
B07. Have you ever been diagnosed with any of the following mental or neurological disorders? (*Multiple selections are allowed*) ☐ ADHD and attention deficit ☐ Depressive disorder ☐ Anxiety disorder ☐ Schizophrenia ☐ None of above
B08. Have you ever had any of the following food allergies？ (*Multiple selections are allowed*) ☐ None ☐ Milk ☐ Egg ☐ Wheat ☐ Soybean ☐ Fish ☐ Nuts ☐ Fruit ☐ Crustaceans (e.g., shrimp, crab, etc.) ☐ Others ☐ Unclear
B09. Have you ever been allergic to any of the following medications? (*Multiple*

selections are allowed) ☐ None ☐ Antibiotics ☐ Non-steroidal anti-inflammatory drugs analgesics (e.g., aspirin) ☐ Contrast medium ☐ Anesthetic ☐ Anticonvulsant ☐ Chemotherapy drugs ☐ Others ☐ Unclear
B10. Have you ever been allergic to any of the following environmental substances? (*Multiple selections are allowed*) ☐ None ☐ Dust mite ☐ Mycete ☐ Animal skins ☐ Pollen ☐ House dust ☐ Cockroach ☐ Others ☐ Unclear
B11. Do you have an excessive bug bite response? (*Single selection*) ○ No ○ Yes
B12. In the past year, have you had a dog, cat or other small stuffed animal such as rabbit, guinea pig, hamster, etc.? (*Multiple selections are allowed are allowed*) ☐ No ☐ Dog ☐ Cat ☐ Other stuffed animals ☐ Unclear
B13. Do you have a long history of close exposure to chemicals? (*Multiple selections are allowed*) ☐ None ☐ Formaldehyde ☐ Gasoline ☐ Oil varnish ☐ Others ☐ Unclear
B14. Have you used or taken any medication almost every day for the last 2 weeks? (*Multiple selections are allowed*) ☐ None ☐ Antibiotics ☐ Nonsteroidal anti-inflammatory drugs and analgesics (NASIDS) ☐ Hormone ☐ Antituberculosis drugs ☐ Others ☐ Clear

B15. Whether breastfed after birth or not. (*Single selection*)

O No O Yes O Unclear

B16. How often do you have "colds and fevers" in your early school years (before age of 7 years old)? (*Single selection*)

O Rare (⩽1 time/year)	O Occasional (2-3 times/year)	O Often (4 or more times/year)

B17. Which way did you (or your parents) usually deal with your "cold or fever" in the early school age years? (*Multiple selections are allowed*)

☐ Ignore them ☐ Drink more water or have a rest ☐ Receive antibiotics orally

☐ Oral Chinese Traditional medicine ☐ Receive antibiotics intravenously ☐ Others

B18. **Which of the following frequency of antibiotic use can improve or cure your "Cold or fever" in the early school age years? (*Single selection*)**

O Rarely, it usually cured without antibiotic treatment O occasional

O Often by antibiotics orally O Often by antibiotics intravenously

C. Lifestyle Habits

C01.Do you smoke (refers to smoking at least one cigarette a day for more than six months) (*Single selection, jump to C3 if you choose "hardly"*)

O Hardly O < 1 packet /day (about 20 cigarettes in a packect) O 1-2 packets /day O > 2 packs/day

C02. If you smoke, how many years have you smoked in total so far? (*Single selection*)

○ < 1 year ○ 1-3 years ○ > 3 years
C03. In the last month, your frequency of passive smoking is (the involuntary inhalation of smoke caused by other people's smoking in your living or working environment) (*Single selection, jump to C5 if you choose "hardly"*) ○ Hardly ○ < 1 day/week ○ 1-2 days/week ○ 3-5 days/week ○ 6-7 days/week
C04. How many years have you been second hand smoking? (*Single selection*) ○ < 2 years ○ 2-3 years ○ 4-6 years ○ > 6 years
C05. How often do you drink alcohol? (refering to once a week for at least six months) (*Single selection, jump to C9 if you choose "hardly"*) ○ Hardly ○ Once a week ○ 2-4 times/week ○ 5-7 times/week ○ 8-10 times/week ○ > 10 times/week
C06. How many years have you been drinking? (*Single selection*) ○ < 1year ○ 1-3years ○ 4-5years ○ > 5 years
C07. What's your main drink? (*Single selection*) ○ Beer ○ Liqueur ○ Red wine ○ Sweet wine ○ Chinese rice wine
C08. How much do you drink on average each time? (*Single selection*) ○ Less (1 bottle of beer, or 50-100g other types of alchohol) ○ Medium (2 bottles of beer, or 100g other types of alchohol) ○ Much (three bottles of beer, or 150g other types of alchohol) ○ A lot (more than 3 bottles of beer, or more than 150g other types of alchohol)

D. Skin Health

D01.How many showers do you take per week in the spring and fall? (*Single selection*) ○ ≤1 time per week ○ 2~4 times per week ○ 5~7 times per week

O 8~10 times per week O >10 time per week
D02. How long do you take a shower in spring and fall? (*Single selection*) O < 5 minutes O 5-10 minutes O 11-20 minutes O 21-30 minutes O > 30 minutes
D03. What kind of toiletries do you use most? (*Single selection*) O None O Soap O Shower gel O Others
D04. In spring and fall, the temperature of your bath is (*Single selection*) O Low temperature(<35 centigrade) O Close to the body temperature(35-40 centigrade) O High temperature(> 40 centigrade)
D05. Do you use moisturizer all over your body almost daily in fall and winter? (*Single selection*) O No O Yes
D06. How often do you use facial cleansing products (e.g. cleanser, soap)? (*Single selection*) O Hardly O Usually O Once per day O ≥twice per day
D07. How often have you washed your hair in the past two years? *(Single selection)* O ≥ Twice per day O Once per day O Once on alternate days O Once every 2-6 days O Once a week or more
D08. In the past two years, what kind of toiletries you use is? *(Single selection)* O None O Shampoo O Shampoo + conditioner O Others
D09. Have you used hair care/hair products in the past two years? (*multiple selections*) ☐ None ☐ Essential oil, elastin and other hair

care products ☐ Hair gel ☐ Others
D10. In the last two years, how often have you dyed your hair? *(Single selection)* O Never O Less than once a year O Once every 2 to 6 months O Once every 7 to 11 months O More than once a month
D11. In the past two years, your perm frequency is *(Single selection)* O Never O Less than once a year O Once every 2 to 6 months O Once every 7 to 11 months O More than once a month
D12. Do you have frequent itchy skin, and to what extent (0 is not itchy at all and 10 is extremely itchy)? 0 – 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10
D13. Do you have regular skin pain, and to what extent (0 is no pain at all and 10 is extreme pain)? 0 – 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10

Table S1. The prevalence of atopic and allergic diseases in college students

Disease	N, Prevalence (%)[a]	URTI, n (%)			Antibiotics, n (%)		
		Rare	Occasional	Frequent	Rare, Occasional	Often,orally	Often, intravenously
Skin							
Atopic dermatitis	776 (3.86)	174 (2.99)	459(3.99)	143(5.1)	176(3.39)	263(4.11)	164(4.79)
Hand eczema	675(3.35)	145 (2.49)	407(3.54)	123(4.38)	156(3.01)	243(3.79)	122(3.57)
Allergic reactions to food/drug/light	456(2.27)	121(2.08)	255(2.22)	80(2.85)	107(2.06)	152(2.37)	92(2.69)
Chronic urticaria	381(1.89)	96(1.65)	207(1.8)	78(2.78)	85(1.65)	133(2.08)	78(2.28)
Allergic skin disease [b]	833(4.14)	217(3.73)	460(4)	156(5.56)	192(3.7)	285(4.45)	167(4.88)
Beyond skin							
Atopic march [c]	3139(15.6)	687(11.79)	1825(15.88)	627(22.34)	687(13.33)	1101(17.19)	666(19.47)
Allergic conjunctivitis	153(0.76)	32(0.55)	84(0.73)	37(1.32)	32(0.61)	54(0.84)	36(1.05)
Allergic rhinitis	2273(15.6)	486(8.34)	1327(11.55)	460(16.39)	494(9.56)	809(12.63)	480(14.03)
Asthma	303(1.51)	46(0.79)	171(1.49)	86(3.06)	111(1.08)	117(1.83)	75(2.19)

[a] The total prevalence of atopic and allergic diseases in our study population.

[b] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.

[c] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students

Disease	URTI, aRR (95%CI) [a]			Antibiotics, aRR (95%CI) [a]		
	Rare	Occasional	Frequent	Rare / occasional	Often, orally	Often, intravenously
Skin						
Atopic dermatitis	Reference	1.32 (1.09, 1.54)	1.59 (1.27, 1.98)	Reference	1.18 (1.01, 1.39)	1.36 (1.14, 1.62)
Hand eczema	Reference	1.32 (1.08, 1.56)	1.60 (1.26, 2.02)	Reference	1.27 (1.08, 1.49)	1.17 (0.95, 1.44)
Allergic reactions to food/drug/light	Reference	1.10 (0.92, 1.28)	1.30 (1.04, 1.63)	Reference	1.15 (0.97, 1.37)	1.36 (1.12, 1.65)
Chronic urticaria	Reference	0.97 (0.76, 1.17)	1.58 (1.21, 2.05)	Reference	1.13 (0.90, 1.40)	1.39 (1.09, 1.78)
Allergic skin disease [b]	Reference	1.04 (0.89, 1.20)	1.46 (1.22, 1.76)	Reference	1.16 (1.01, 1.34)	1.33 (1.13, 1.57)
Beyond skin						
Atopic march [c]	Reference	1.39 (1.26, 1.52)	2.08 (1.85, 2.34)	Reference	1.35 (1.24, 1.48)	1.55 (1.40, 1.71)
Allergic conjunctivitis	Reference	1.89 (1.13, 2.66)	3.49 (2.05, 5.96)	Reference	1.58 (1.08, 2.32)	2.72 (1.75, 4.23)
Allergic rhinitis	Reference	1.43 (1.27, 1.59)	2.13 (1.86, 2.43)	Reference	1.39 (1.26, 1.53)	1.56 (1.39, 1.74)
Asthma	Reference	2.56 (1.69, 3.43)	4.89 (3.20, 7.47)	Reference	1.92 (1.52, 2.43)	2.10 (1.64, 2.70)

[a] Adjusted for the fixed effects of gender, income, education, passive smoking, and ethnicity and the random effect of university.

[b] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.

[c] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.

BMJ Open

Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood: A population-based retrospective cohort study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-047768.R3
Article Type:	Original research
Date Submitted by the Author:	14-Jul-2021
Complete List of Authors:	Li, Yajia; Xiangya Hospital Central South University, Jing, Danrong; Xiangya Hospital Central South University, Dermatology Huang, Yuzhou ; Xiangya Hospital Central South University, dermatology Su, Juan; Xiangya Hospital Central South University, Dermatology Li, Ji Tao, Juan; Affiliated Union Hospital, Tongji Medical College, Huazhong University of Science and Technology Shan, Shijun; Xiang'an Hospital, Xiamen University, Department of Dermatology Wang, Xiaohui; Zhongshan Hospital Xiamen University, Department of Dermatology, Kang, Xiaojing; People's Hospital of Xinjiang Uygur Autonomous Region, Department of Dermatology Wu, Bin; People's Hospital, Inner Mongolia Medical University, Department of Dermatology Xiao, Yi; Xiangya Hospital Central South University Chen, Xiang; Xiangya Hospital Central South University, Shen, Minxue; Xiangya Hospital Central South University, Dermatology
Primary Subject Heading:	Dermatology
Secondary Subject Heading:	Dermatology, Epidemiology
Keywords:	Allergy < THORACIC MEDICINE, Adult dermatology < DERMATOLOGY, Eczema < DERMATOLOGY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

Association of antibiotics use in preschool age with atopic and allergic skin diseases in young adulthood: A population-based retrospective cohort study.

Yajia Li, M.A student [1], Danrong Jing, Ph.D. student [1], Yuzhou Huang, Ph.D. student [1], Juan Su, Ph.D. [1], Ji Li, Ph.D. [1], Juan Tao, Ph.D. [2], Shijun Shan, Ph.D. [3], Xiaohui Wang, Ph.D.[4], Xiaojing Kang, Ph.D. [5], Bin Wu, Ph.D.[6], Yi Xiao, Ph.D. [1*], Xiang Chen, Ph.D.[1*], Minxue Shen, Ph.D. [1,7*]

[1]Department of Dermatology; Hunan Engineering Research Center of Skin Health and Disease; Hunan Key Laboratory of Skin Cancer and Psoriasis, Xiangya Hospital, Central South University, Changsha, China

[2]Department of Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

[3]Department of Dermatology, Xiang'an Hospital, Xiamen University, Xiamen, China

[4]Department of Dermatology, Zhongshan Hospital, Xiamen University, Xiamen, China

[5]Department of Dermatology, People's Hospital of Xinjiang Uygur Autonomous Region, Urumchi, Xinjiang, China

[6]Department of Dermatology, People's Hospital, Inner Mongolia Medical University, Hohhot, China

[7]Department of Social Medicine and Health Management, Xiangya School of Public Health, Central South University, Changsha, China

***Correspondence:**
Minxue Shen, Xiang Chen and Yi Xiao

Address: Xiangya Hospital, 87 Xiangya Road, Changsha, Hunan, China 410008

Email: shenmx1988@csu.edu.cn (M.S.), chenxiangck@126.com (X.C.) and xiaoyixy@csu.edu.cn (Y.X.)

Keywords: antibiotics, atopic and allergic skin diseases, preschool and young adulthood.

Abstract

Background: Overuse and misuse of antibiotics is a public health problem in low- and middle-income countries. Although the association of antibiotics with atopic and allergic diseases has been established, most studies focused on prenatal exposure and the occurrence of disease in infants or young children.

Objective: To investigate the association of preschool use of antibiotics with atopic and allergic skin diseases in young adulthood.

Design: Population-based retrospective cohort.

Setting and participants: The first-year college students (n=20123) from five universities were investigated. The sampled universities are located in Changsha, Wuhan, Xiamen, Urumqi, and Hohhot, respectively.

Methods: We conducted a dermatological field examination and a questionnaire survey inquiring the participants about the frequency of upper respiratory tract infection (URTI) and the preschool antibiotics use (prior to 7 years old). The two-level Probit model was used to estimate the associations, and adjusted risk ratio (aRR) and 95% confidence interval (CI) were presented as the effect size.

Results: A total of 20123 participants with complete information was included in the final analysis. The frequent antibiotics use intravenously (aRR 1.36, 95% CI 1.14–1.62) and orally (aRR 1.18, 95% CI 1.01–1.38) prior to 7 years old was significantly associated with atopic dermatitis in young adulthood. Similar trends could be observed in allergic skin diseases among those who use antibiotics orally and intravenously, with RRs of 1.16 (1.01, 1.34) and 1.33 (1.13, 1.57), respectively.

Conclusions: Preschool URTI and antibiotics use significantly increases the risk of atopic and allergic skin diseases in young adulthood.

Strengths and limitations of this study

- The main outcomes were diagnosed by specialists instead of self-report.
- This study provides a relatively large and representative sample, and sufficient variations in geographic regions and sociodemographic subgroups, as well as the random effect at the university level, was fitted by the 2-level models, resulting in an unbiased estimation of associations.
- Recall bias in the measurement of exposure to antibiotics might have been introduced, which could not be ignored in most retrospective studies.
- We lacked the information about the type and dose of antibiotics, and there might be a reversed causal relationship because antibiotics could be used in the treatment of AD and other conditions accompanied by a bacterial infection.

1 Introduction

The incidence and prevalence of atopic and allergic conditions such as asthma, allergic rhinitis, food allergies, and atopic dermatitis (AD) among the worldwide population have significantly increased during the past several decades.[1-3] An area of environmental change that may be responsible for the increase of allergic and atopic diseases is the growing use of medications that may alter the development of the human microbiome.[4] It also seems that the use of some antibiotics, which can directly cause intestinal dysbiosis and affect the human microbiome and increase the risk for allergy development, is of particular concern in light of accumulating evidence.[5-7]

Furthermore, overuse and misuse of antibiotics is a severe public health problem worldwide, especially in low- and middle-income countries. In the last decade, prescriptions of broad-spectrum antibiotics increased by 49% in children under five years and doubled in children aged 5–17 years, concomitant with the increasing prevalence of allergic diseases.[8, 9] In China, 70% of outpatients attending primary care facilities with colds were inappropriately treated with antibiotics, often by intravenous infusion. The situation is even worse in children because many parents demand treatment with antibiotics[10]. However, most upper respiratory tract infections (URTI) in children are viral, for which antibiotics are unnecessary.[11-13]

The association between the use of antibiotics and atopic and allergic diseases has been observed in longitudinal studies. But most studies focused on antibiotics use during pregnancy or infancy when early colonization is initiated by maternal microbes.[14-17] With the childhood microbiome transition owing to alterations in food and exposure to more diverse microbes in external environments, children in preschool age (<7 yrs) are at higher risks of URTI infection and antibiotics treatment. However, the effect of antibiotics used during this period on atopic and allergic skin diseases that occurred in their young adulthood is not clear. The objective of this study was to evaluate the hypothesis that exposure to antibiotics in preschool age is associated with an increased risk of allergic and atopic skin diseases in young adulthood. We tested this hypothesis by conducting a retrospective study in college students.

2 Materials and Methods

2.1 Study setting and design

This was a retrospective cohort study based on the data from the China College Student Skin Health Study (CCSSHS)[18]. The first-year college students from five universities were investigated. They underwent a health examination and completed a questionnaire survey. The sampled universities are located in Changsha, Wuhan, Xiamen, Urumqi, and Hohhot, respectively. The medical ethics committee of Xiangya Hospital, Central South University, approved the study (#201709993).

2.2 Exposure assessment

Two semi-quantitative questions served as the proxy measures of the frequency of antibiotics exposure in preschool age, with detailed explanations for the definitions of URTI and antibiotics. In our study, the definition of URTI refers to a series of acute illnesses that have an effect on the upper respiratory system including the common cold, acute otitis media, tonsillitis/tonsillopharyngitis, sinusitis, and recurrent sinusitis.[19] The first question was “How often did you have URTI in your preschool-age or before 7 years old”, with three potential responses:⩽1 time/year, 2-3 times/year”, and “4 or more

times/year". The second question was "How often did you receive antibiotics treatment when you had a URTI", with four responses: "rare", "occasional", "often, orally; and "often, intravenously".

2.3 Outcome assessment

Diagnosis of skin diseases and inquiry of disease history were performed by dermatologists during the field survey. All subjects underwent skin examination by resident doctors in dermatology, and the diagnoses were further validated by senior dermatologists. Clinical manifestation, disease history, and family history were inquired about, and inspections were conducted to diagnose skin diseases. For recurrent skin diseases, only those with current symptoms and cutaneous lesions were diagnosed as cases. AD was diagnosed according to the Williams criteria.[20] Hand eczema was diagnosed according to eczema (rash) on the fingers, finger webs, palms, or back of hands, which had appeared once and continued for at least two weeks or had appeared several times or had been persistent. Allergies and urticaria were diagnosed by clinical manifestations, potential triggers, and histories. Asthma, allergic rhinitis, and allergic conjunctivitis were self-reported according to doctors' diagnoses. We also combined some of the outcomes. Atopic march is an apparent progression of allergic diseases from AD, to allergic asthma (AA) to allergic rhinitis (AR) and allergic conjunctivitis.[21, 22] We include the conditions of AD, AA, AR, and allergic conjunctivitis for the outcome of the atopic march. Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria. Participants with a history of atopic/allergic conditions but without the current disease are excluded.

2.4 Covariates

Demographic characteristics, socioeconomic status (annual family income and parental highest educational level), family history, behavioral factors (dietary, passive smoking, and bathing habits) were inquired by the questionnaire. The questionnaire used in the study was shown in Material S1.

2.5 Statistical analysis

Categorical data were presented as number (%), and the between-group difference was tested using the chi-square test. Two-level Probit regression models (individual as level 1 and university as level 2) were used to estimate the associations of preschool exposure to antibiotics with atopic skin diseases in young adulthood, adjusting for level-1 covariates (gender, ethnicity, annual household income, and parental education) and level-2 random effects. The effect size was presented as relative risk (RR) and 95% confidence interval (CI). $P< 0.05$ was considered statistically significant for all tests. Statistical analysis was performed in SAS 9.4 (SAS Institute Inc., Cary, USA).

3 Results

A total of 27144 registries for new enrolment was identified; among them, 21086 (77.7%) consented to participate, and 20123 (74.1%) who underwent the health examination and completed the online questionnaire survey were included in the final analyses. The mean age was 18.3± 0.8 years and 10283 (51.1%) were men. The characteristics of participants in the study are shown in Table 1. The prevalence rates of chronic urticaria, allergic reactions to food/drug/light, hand eczema, and AD were 1.89%, 2.27%, 3.35%, and 3.86%, respectively. The prevalence rates of AD and allergic skin disease were 3.86 % and 4.14%, respectively (Figure 1 and Table S1).

In general, URTI and the use of antibiotics were significantly associated with atopic and allergic diseases dose-dependently (Figure 1 and Table S1). For example, the prevalence of AD increased from 3.39% to 4.11% and 4.79% in participants who reported rare or occasional use, frequent oral use, and

frequent intravenous use of antibiotics, respectively. Consistent trends could be observed in all atopic and allergic diseases and their combinations.

After adjustments for sociodemographic factors (Figure 2 and Table S2), URTI and the use of antibiotics were significantly associated with atopic/allergic skin diseases in dose-response manners. For instance, compared to those reporting rare or occasional use of antibiotics, the RRs for AD in participants who reported frequent oral administration and intravenous injection of antibiotics was 1.18 (95% CI: 1.01, 1.39) and 1.36 (95% CI: 1.14, 1.62), respectively. For other allergies or atopic diseases of skin or beyond skin, the correlations were highly consistent despite some variations in the magnitude of the association.

Furthermore, we have provided the data on the health seeking behavior dealing with a cold or fever in preschool age (shown in Table 2), and we found there were 66.2% among participants with the atopic march and 64.7% among participants with allergic skin disease showed that they/their parents would like to seek antibiotics treatment when they had a cold/fever in their preschool age. In those without atopic/allergic diseases, this proportion ratio was 61.5%. Indeed, we found a moderate but significant difference in the seeking behavior of antibiotic treatment between those with allergic skin disease/atopic march and healthy participants (P=0.001). Similar trends could be seen in AD patients and non-patients (P=0.034).

Besides, because our study was not a prospective cohort, it was difficult to know if antibiotic use was ahead of suffering from AD. We further evaluated the joint effect of URTI and antibiotics on AD by including an interaction term in the model. As shown in Figure 3, in each category of antibiotic use, the frequency of URTI was positively associated with the risk of AD. Vice versa, in each category of URTI, antibiotic use was positively associated with AD according to the effect size, despite some insignificant results in categories with a small sample size.

4 Discussion

This retrospective cohort study demonstrated that preschool exposure to antibiotics, either through oral administration or intravenous infusion, was associated with increased risks of having allergic and atopic skin diseases in young adulthood. Participants who reported frequent URTI in preschool-age also had higher risks of allergies and atopy.

Similar trends were identified in previous observational studies that early-life antibiotic use was associated with an increased risk of eczema, but there were still some inconsistent results.[23, 24] Meta-analysis including 22 studies with 394,517 patients concluded that children with antibiotics exposure in the first two years had increased odds of atopic eczema with an OR of 1.26 (95%CI: 1.15-1.37). Notably, the onset age of the outcomes in the included studies was the period of childhood (<12 years old). [7] A large population-based retrospective cohort study in twins showed that antibiotic use was also associated with an increased risk of eczema. However, the relationship between early-life antibiotic use and eczema was likely to be confounded by shared familial environment and genetic factors.[25] However, current data lacked the information regarding the atopic and allergic skin diseases occurred in late adolescence to early adulthood, while we firstly investigated the effects of preschool antibiotic in a retrospective study and revealed a positive association. Chinese parents were found to pay close attention to the preschool health of children and keep a non-exclusion attitude to antibiotics use. Using antibiotics at home, seeking medical care, and use antibiotics in the hospital for children was pervasive in China when parents dealing with children's respiratory tract infections.

However, the results should be discussed with caution as a doctor seeking behavior varies a lot in populations. In this setting, children with AD will seek a doctor frequently and more likely get a URTI diagnosis if they also have a cold. Equally, children with asthma and wheezing will more frequently get a URTI diagnosis and are more likely to get an antibiotic.

Evidence showed that AD was the first manifestation of an atopic phenotype that begins in early childhood and the progression from AD to the diseases such as food allergy, asthma, and allergic rhinitis were more likely to be shown in adolescence.[22, 26, 27] The mechanisms behind the march from AD to allergic airway diseases and allergic conjunctivitis likely arise from initial epicutaneous allergen sensitization inducing robust local and systemic type 2 immune responses with increased production of type 2 cytokines including interleukin (IL)-4, IL-13, IL-31, and thymic stromal lymphopoietin.[28, 29] Most studies were prone to make these responses responsible for the commonly shared pathogenesis of cutaneous, airway, and conjunctiva inflammation, supporting the view that AD is not merely a disease confined to the skin, but is in fact, a systemic disease.[30, 31] Therefore, it could be explained that the increased risks beyond skin manifestations in young adulthood were consistent with those of skin diseases and some with even greater effect sizes. While not fully understood, the underlying mechanism of the association between antibiotics and atopic and allergic diseases can be elucidated by microbial diversity. The gut microbial community is dynamic and variable during the first 3 years of life, before stabilizing to an adult-like state.[32] Studies have demonstrated continued development through childhood into the teenage years.[33, 34] Dietary intake plays a key role in the development period of the gut microbiome. Breast-fed infants have microbiota enriched in *Lactobacillus*, *Staphylococcus*, and *Bifidobacterium*. Studies have shown that human milk isolates contain symbiotic and potentially probiotic microbes, and supplementation of *Bifidobacteria* was found to be effective in the primary prevention of allergic diseases.[35-37] But among children of preschool age, the dominance of *Bifidobacterium* diminishes with the alteration in dietary intake.[15] On the other hand, the high prevalence of antibiotic use may also lead to a concurrent increase in antibiotic-resistant bacteria.[38, 39] Antibiotic-treated children have a less diverse gut microbiota and less stable communities. Antibiotic therapy affects microbiome variety and thus may increase the risk of atopic diseases.[40, 41]

In our study, increased risks of atopic march or allergic diseases were observed in students who reported frequent URTI. Another potential explanation was related to the infections which could also affect microbial conditions. Except for the change in diet, children of preschool age tend to be exposed to more diverse microbes and infectious diseases including URTI in kindergarten or external environments. Respiratory viral infections, in particular, have been shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut,[42] which could further shape atopic microenvironments.

Several limitations should be noted, and the results should be interpreted with caution. First, recall bias in the measurement of exposure to antibiotics might have been introduced. While recall bias on the frequency of antibiotics use and URTIs should not be ignored, this is a limitation in most retrospective studies. Besides, selection bias might also be introduced as students with skin conditions might be more interested to participate and recall carefully. We are not able to validate the medical records because China does not have a registry system for primary care, and a large number of patients with mild conditions also visit doctors in secondary and tertiary hospitals. While participants could obtain the information from their parents, but unfortunately, we could not evaluate the extent of recall bias. Similarly, for the conditions of the disease including asthma, allergic rhinitis, and allergic conjunctivitis, which we collected based on both clinical diagnosis and questionnaire data, we could not ignore the atopic conditions in the past that might not be existing anymore, though in

previous studies, the 'atopic march' referred to the sequential development of symptoms and was considered to be not strictly limited by the occurring time. [43,44] Those will be in our further consideration in future studies.

Second, there was a lack of information about the type and dose of antibiotics, as well as some antibiotic use for the treatment of low respiratory tract infection, skin infections, and other infections, which was not collected while could also affect the microbiome. So we could not attribute the associations to specific antibiotics use. Third, we noticed that some factors related to the allergic/atopic conditions in preschool age could be ignored, such as prematurity, the doctor seeking behavior, etc., and we had difficulty in fully collecting of related information and need to explain the results with caution. Last but not least, there might be a reversed causal relationship because antibiotics could be used in the treatment of AD and other conditions accompanied by bacterial infection. We assessed the role of URTI and antibiotics separately, because the two variables were significantly correlated (contingency coefficient=0.4, $P<0.001$) and were therefore not included in the same model to avoid collinearity and biased estimation of parameters. It is possible that the association of URTI with AD and allergies is confounded by antibiotics, and vice versa.

However, both infection and antibiotics may be correlated with allergies/atopies, with different mechanisms. Although under the hygiene hypothesis, exposure to pathogens during infancy and early childhood has been proposed to explain the lower prevalence of asthma and other atopic diseases among children in developing countries,[45, 46] some studies showed that early respiratory infections could not protect against atopic eczema or recurrent wheezing, but could drive the development of atopic disease. [47-51] Atopic sensitization, a process of generation of specific immunoglobulin E (sIgE) when exposed to an innocuous antigen, was common to all allergic diseases. As preschool URTI most probably represents viral infections in the majority of cases, some studies investigated a potential mechanistic explanation for how a respiratory viral infection could drive the development of atopic sensitization and disease. Martorano et al. used a Sendai virus to establish the mouse model mimicking a human limited respiratory syncytial virus infection and found that Sendai virus infection could promote the crosslinking of high-affinity IgE receptor (FcεRI) on the lung conventional dendritic cells (cDC), which led to the production of the chemokine CCL28, recruiting IL-13 and driving the development of mucous cell metaplasia and airway hyperreactivity.[52] Another animal study found that except for the increase of sIgE against Sendai virus in mice, there was also a large increase in total IgE and it remained elevated long after the viral infection being resolved.[53] This notion has further been fuelled by findings that mice infected with the flu virus developed virus-specific mast cell degranulation in the skin, indicating a possible pathway of viral infections that could mediate allergic symptoms.[54] Besides, the respiratory viral infections were shown to initiate a cascade of host immune responses altering microbial growth in the respiratory tract and gut.[42, 55] However, we cannot ignore that infections that do not require antibiotics are not captured in our design, such that it is difficult to assess whether the observed association is caused by a specific infection or antibiotics because they occur simultaneously in many cases.

Our study also has strengths. The primary strength is that the sample size for the retrospective cohort study was large. Second, the outcomes were diagnosed by specialists. In contrast, some previous studies used self-reported diagnoses that might introduce misclassification bias. Third, the study had a relatively large and representative sample, and sufficient variations in geographic regions and sociodemographic subgroups. The random effect at the university level was fitted by the 2-level models, resulting in an unbiased estimation of associations.

To conclude, preschool children exposed to URTI or antibiotics may be at higher risks of atopic and allergic skin diseases in their young adulthood, especially among those who frequently had URTI or received antibiotics by intravenous infusion. Our study implies that unnecessary antibiotics treatment in children should be avoided to prevent the occurrence of atopic and allergic diseases in their later life. Prospective studies that consider the type and dose of antibiotics are warranted.

5 Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

6 Ethics statement

This study was conducted according to the guidelines established in the Declaration of Helsinki. All procedures involving patients were approved by the institutional research ethics boards of Xiangya Hospital, Central South University (Changsha, China). Informed consent was obtained from all the students before the investigation.

7 Patient and Public Involvement Statement

This is a retrospective cohort study based on the data from the China College Student Skin Health Study (CCSSHS). The first-year college students from five universities were recruited and investigated. They underwent a health examination and completed a questionnaire survey, and the results will be disseminated to study participants by a medical examination report. Participants were not involved in the design and implementation of the study.

8 Author contributions

Dr. Minxue Shen, Dr. Xiang Chen, and Dr. Yi Xiao are joint corresponding authors and have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Concept and design: Shen, Chen, and Xiao.

Acquisition, analysis, or interpretation of data: Y.J. Li, Jing, and Huang

Drafting of the manuscript: Y.J. Li

Critical revision of the manuscript for important intellectual content: All authors.

Statistical analysis: Shen, Xiao, and Y.J. Li.

Administrative, technical, or material support: Su, J. Li, Tao, Shan, Wang, Kang, and Wu.

Supervision: Chen

9 Conflict of interest

The authors declare no conflict of interest.

10 Funding

This work was supported by the National Key Research & Development Project "Precision Medicine Initiative" of China (2016YFC0900802) and the Program of Introducing Talents of Discipline to Universities (111 Project, No. B20017).

11 Acknowledgment

The authors would like to thank the following individuals and institutions who have made substantial contribution to the establishment of the cohort: Central South University (Lei Cai, Duling Cao, Qin Cao, Chao Chen, Liping Chen, Menglin Chen, Mengting Chen, Xiang Chen, Qing Deng, Xin Gao, Yihuan Gong, Jia Guo, Yeye Guo, Rui Hu, Xin Hu, Chuchu Huang, Huining Huang, Kai Huang, Xiaoyan Huang, Yuzhou Huang, Danrong Jing, Xinwei Kuang, Li Lei, Jia Li, Jiaorui Li, Jie Li, Keke Li, Peiyao Li, Yajia Li, Yayun Li, Yangfan Li, Dan Liu, Dihui Liu, Fangfen Liu, Nian Liu, Panoan Liu, Runqiu Liu, Hui Lu, Wenhua Lu, Yan Luo, Zhongling Luo, Manyun Mao, Mengping Mao, Yuyan Ouyang, Shiyao Pei, Qunshi Qin, Ke Sha, Lirong Tan, Ling Tang, Ni Tang, Yan Tang, Ben Wang, Yaling Wang, Tianhao Wu, Yun Xie, Siyu Yan, Sha Yan, Bei Yan, Xizhao Yang, Lin Ye, Hu Yuan, Taolin Yu, Yan Yuan, Yi Yu, Rui Zhai, Jianghua Zhang, Jianglin Zhang, Mi Zhang, Xingyu Zhang, Zhibao Zhang, Shuang Zhao, Yaqian Zhao, Kuangbiao Zhong, Lei Zhou, Youyou Zhou, Zhe Zhou, Susi Zhu); Huazhong University of Science and Technology (Xiangjie An, Siqi Da, Yaqi Dong, Yangxue Fu, Lixie Gao, Han Han, Biling Jiang, Jiajia Lan, Jun Li, Xiaonan Li, Yan Li, Liquan Liu, Yuchen Lou, Pu Meng, Yingli Nie, Gong Rao, Shanshan Sha, Xingyu Su, Huinan Suo, Rongying Wang, Jun Xie, Yuanxiang Yi, Jia Zhang, Qiao Zhang, Li Zhu, Yanming Zhu); Xiamen University (Zhiming Cai, Lina Chen, Xiaozhu Fu, Hongjun Jiang, Guihua Luo, Jianbing Xiahou, Binxiang Zheng); People's Hospital of Xinjiang Uygur Autonomous Region (Jianxia Chen, Xiaomin Chen, Xinqi Chen, Li Dai, Yanyan Feng, Fanhe Jiang, Lan Jin, Qingyu Ma, Qun Shi, Hongbo Tang, Fang Wang, Zhen Wang, Xiujuan Wu, Kunjie Zhang, Yu Zhang); Xinjiang Medical University (Huagui Li, Jianguang Li, Lei Shi); Inner Mongolia Medical University (Chao Tian, Wei Wang, Rina Wu, Hongjun Xing, Baogui Yang).

12 References

[1]. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action[J]. World Allergy Organ J. 2014,7(1):12.

[2]. Platts-Mills TA. The allergy epidemics: 1870-2010[J]. J Allergy Clin Immunol. 2015,136(1):3-13.

[3]. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys[J]. Lancet. 2006,368(9537):733-43.

[4]. Mitre E, Susi A, Kropp LE, Schwartz DJ, Gorman GH, Nylund CM. Association Between Use of Acid-Suppressive Medications and Antibiotics During Infancy and Allergic Diseases in Early Childhood[J]. JAMA Pediatr. 2018,172(6):e180315.

[5]. Wang JY, Liu LF, Chen CY, Huang YW, Hsiung CA, Tsai HJ. Acetaminophen and/or antibiotic use in early life and the development of childhood allergic diseases[J]. Int J Epidemiol. 2013,42(4):1087-99.

[6]. Kim DH, Han K, Kim SW. Effects of Antibiotics on the Development of Asthma and Other Allergic Diseases in Children and Adolescents[J]. Allergy Asthma Immunol Res. 2018,10(5):457-65.

[7]. Ahmadizar F, Vijverberg SJH, Arets HGM, de Boer A, Lang JE, Garssen J, et al. Early-life antibiotic exposure increases the risk of developing allergic symptoms later in life: A meta-analysis[J]. Allergy. 2018,73(5):971-86.

[8]. Lee GC, Reveles KR, Attridge RT, Lawson KA, Mansi IA, Lewis JS, 2nd, et al. Outpatient antibiotic prescribing in the United States: 2000 to 2010[J]. BMC Med. 2014,12:96.

[9]. Versporten A, Bielicki J, Drapier N, Sharland M, Goossens H. The Worldwide Antibiotic Resistance and Prescribing in European Children (ARPEC) point prevalence survey: developing hospital-quality indicators of antibiotic prescribing for children[J]. J Antimicrob Chemother. 2016,71(4):1106-17.

[10]. Biezen R, Grando D, Mazza D, Brijnath B. Dissonant views - GPs' and parents' perspectives on antibiotic prescribing for young children with respiratory tract infections[J]. BMC Fam Pract. 2019,20(1):46.

[11]. Wei X, Zhang Z, Walley JD, Hicks JP, Zeng J, Deng S, et al. Effect of a training and educational intervention for physicians and caregivers on antibiotic prescribing for upper respiratory tract infections in children at primary care facilities in rural China: a cluster-randomised controlled trial[J]. Lancet Glob Health. 2017,5(12):e1258-e67.

[12]. Li J, Song X, Yang T, Chen Y, Gong Y, Yin X, et al. A Systematic Review of Antibiotic Prescription Associated With Upper Respiratory Tract Infections in China[J]. Medicine (Baltimore). 2016,95(19):e3587.

[13]. Ding L, Sun Q, Sun W, Du Y, Li Y, Bian X, et al. Antibiotic use in rural China: a cross-sectional survey of knowledge, attitudes and self-reported practices among caregivers in Shandong province[J]. BMC Infect Dis. 2015,15:576.

[14]. McKeever TM, Lewis SA, Smith C, Collins J, Heatlie H, Frischer M, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database[J]. J Allergy Clin Immunol. 2002,109(1):43-50.

[15]. Kundu P, Blacher E, Elinav E, Pettersson S. Our Gut Microbiome: The Evolving Inner Self[J]. Cell. 2017,171(7):1481-93.

[16]. Yoshida S, Ide K, Takeuchi M, Kawakami K. Prenatal and early-life antibiotic use and risk of childhood asthma: A retrospective cohort study[J]. Pediatr Allergy Immunol. 2018,29(5):490-5.

[17]. Kusel MM, de Klerk N, Holt PG, Sly PD. Antibiotic use in the first year of life and risk of atopic disease in early childhood[J]. Clin Exp Allergy. 2008,38(12):1921-8.

[18]. S M, X Y, S J. Prevalence and patient-reported outcomes of noncommunicable skin diseases among college students in China.[J]. JAAD Int. 2020,1(1):23-30.

[19]. Eccles MP, Grimshaw JM, Johnston M, Steen N, Pitts NB, Thomas R, et al. Applying psychological theories to evidence-based clinical practice: identifying factors predictive of managing upper respiratory tract infections without antibiotics[J]. Implement Sci. 2007,2:26.

[20]. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation[J]. Br J Dermatol. 1994,131(3):406-16.

[21]. Spergel JM, Paller AS. Atopic dermatitis and the atopic march[J]. The Journal of allergy and clinical immunology. 2003,112(6 Suppl):S118-27.

[22]. Hill DA, Spergel JM. The atopic march: Critical evidence and clinical relevance[J]. Ann Allergy Asthma Immunol. 2018,120(2):131-7.

[23]. Metzler S, Frei R, Schmaußer-Hechfellner E, von Mutius E, Pekkanen J, Karvonen AM, et al. Association between antibiotic treatment during pregnancy and infancy and the development of allergic diseases[J]. Pediatr Allergy Immunol. 2019,30(4):423-33.

[24]. Tsakok T, McKeever TM, Yeo L, Flohr C. Does early life exposure to antibiotics increase the risk of eczema? A systematic review[J]. Br J Dermatol. 2013,169(5):983-91.

[25]. Slob EMA, Brew BK, Vijverberg SJH, Kats C, Longo C, Pijnenburg MW, et al. Early-life antibiotic use and risk of asthma and eczema: results of a discordant twin study[J]. Eur Respir J. 2020,55(4).

[26]. Johansson E, Hershey GKK. Contribution of an impaired epithelial barrier to the atopic march[J]. Ann Allergy Asthma Immunol. 2018,120(2):118-9.

[27]. Belgrave DC, Granell R, Simpson A, Guiver J, Bishop C, Buchan I, et al. Developmental profiles of eczema, wheeze, and rhinitis: two population-based birth cohort studies[J]. PLoS Med. 2014,11(10):e1001748.

[28]. Akei HS, Brandt EB, Mishra A, Strait RT, Finkelman FD, Warrier MR, et al. Epicutaneous aeroallergen exposure induces systemic TH2 immunity that predisposes to allergic nasal responses[J]. J Allergy Clin Immunol. 2006,118(1):62-9.

[29]. Cianferoni A, Spergel J. The importance of TSLP in allergic disease and its role as a potential therapeutic target[J]. Expert Rev Clin Immunol. 2014,10(11):1463-74.

[30]. Asada Y, Nakae S, Ishida W, Hori K, Sugita J, Sudo K, et al. Roles of Epithelial Cell-Derived Type 2-Initiating Cytokines in Experimental Allergic Conjunctivitis[J]. Invest Ophthalmol Vis Sci. 2015,56(9):5194-202.

[31]. Han H, Xu W, Headley MB, Jessup HK, Lee KS, Omori M, et al. Thymic stromal lymphopoietin (TSLP)-mediated dermal inflammation aggravates experimental asthma[J]. Mucosal Immunol. 2012,5(3):342-51.

[32]. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography[J]. Nature. 2012,486(7402):222-7.

[33]. Hollister EB, Riehle K, Luna RA, Weidler EM, Rubio-Gonzales M, Mistretta TA, et al. Structure and function of the healthy pre-adolescent pediatric gut microbiome[J]. Microbiome. 2015,3:36.

[34]. Agans R, Rigsbee L, Kenche H, Michail S, Khamis HJ, Paliy O. Distal gut microbiota of adolescent children is different from that of adults[J]. FEMS Microbiol Ecol. 2011,77(2):404-12.

[35]. Heikkilä MP, Saris PE. Inhibition of Staphylococcus aureus by the commensal bacteria of human milk[J]. J Appl Microbiol. 2003,95(3):471-8.

[36]. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, et al. Dynamics and Stabilization of the Human Gut Microbiome during the First Year of Life[J]. Cell Host Microbe. 2015,17(6):852.

[37]. Enomoto T, Sowa M, Nishimori K, Shimazu S, Yoshida A, Yamada K, et al. Effects of bifidobacterial supplementation to pregnant women and infants in the prevention of allergy development in infants and on fecal microbiota[J]. Allergol Int. 2014,63(4):575-85.

[38]. Hiltunen T, Virta M, Laine AL. Antibiotic resistance in the wild: an eco-evolutionary perspective[J]. Philos Trans R Soc Lond B Biol Sci. 2017,372(1712).

[39]. Hawkey PM, Jones AM. The changing epidemiology of resistance[J]. J Antimicrob Chemother. 2009,64 Suppl 1:i3-10.

[40]. Penders J, Stobberingh EE, van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders[J]. Allergy. 2007,62(11):1223-36.

[41]. Abrahamsson TR, Jakobsson HE, Andersson AF, Björkstén B, Engstrand L, Jenmalm MC. Low diversity of the gut microbiota in infants with atopic eczema[J]. J Allergy Clin Immunol. 2012,129(2):434-40, 40.e1-2.

[42]. Hanada S, Pirzadeh M, Carver KY, Deng JC. Respiratory Viral Infection-Induced Microbiome Alterations and Secondary Bacterial Pneumonia[J]. Front Immunol. 2018,9:2640.

[43]. Wahn U. What drives the allergic march?[J]. Allergy. 2000 Jul;55(7):591-9.

[44]. Spergel JM. From atopic dermatitis to asthma: the atopic march[J]. Ann Allergy Asthma Immunol. 2010 Aug;105(2):99-106; quiz 107-9, 117.

[45]. Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis[J]. Nat Immunol. 2017,18(10):1076-83.

[46]. Haspeslagh E, Heyndrickx I, Hammad H, Lambrecht BN. The hygiene hypothesis: immunological mechanisms of airway tolerance[J]. Curr Opin Immunol. 2018,54:102-8.

[47]. Kramer MS, Guo T, Platt RW, Sevkovskaya Z, Dzikovich I, Collet JP, et al. Does previous infection protect against atopic eczema and recurrent wheeze in infancy?[J]. Clin Exp Allergy. 2004,34(5):753-6.

[48]. Peebles RS, Jr. Viral infections, atopy, and asthma: is there a causal relationship?[J]. The Journal of allergy and clinical immunology. 2004,113(1 Suppl):S15-8.

[49]. Martinez FD. Viruses and atopic sensitization in the first years of life[J]. Am J Respir Crit Care Med. 2000,162(3 Pt 2):S95-9.

[50]. Nafstad P, Brunekreef B, Skrondal A, Nystad W. Early respiratory infections, asthma, and allergy: 10-year follow-up of the Oslo Birth Cohort[J]. Pediatrics. 2005,116(2):e255-62.

[51]. Dharmage SC, Lodge CJ, Lowe AJ, Allen KJ. Antibiotics and risk of asthma: a debate that is set to continue[J]. Clin Exp Allergy. 2015,45(1):6-8.

[52]. Martorano LM, Grayson MH. Respiratory viral infections and atopic development: From possible mechanisms to advances in treatment[J]. Eur J Immunol. 2018,48(3):407-14.

[53]. Cheung DS, Ehlenbach SJ, Kitchens T, Riley DA, Grayson MH. Development of atopy by severe paramyxoviral infection in a mouse model[J]. Ann Allergy Asthma Immunol. 2010,105(6):437-43.e1.

[54]. Grunewald SM, Hahn C, Wohlleben G, Teufel M, Major T, Moll H, et al. Infection with influenza a virus leads to flu antigen-induced cutaneous anaphylaxis in mice[J]. The Journal of investigative dermatology. 2002,118(4):645-51.

[55]. Culley FJ, Pennycook AM, Tregoning JS, Hussell T, Openshaw PJ. Differential chemokine expression following respiratory virus infection reflects Th1- or Th2-biased immunopathology[J]. J Virol. 2006,80(9):4521-7.

Figure legends

Figure 1. The prevalence of atopic and allergic diseases in exposure vs. non-exposure group of antibiotics and URTI.

Figure 2. Association of early-life exposure to antibiotics with the risk of atopic/allergic diseases later in life. RR: risk ratio; CI: confidence interval.

Figure 3. The joint effect of URTI and antibiotics on AD after including an interaction term in the model. RR: risk ratio; CI: confidence interval.

Supplementary file captions

Material S1. Chinese college students health survey questionnaire (Translation version in English).

Table S1. The prevalence of atopic and allergic diseases in college students.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students.

Table1. Characteristics of participants in the Present Study (N=20123)

Characteristics	N	%
Gender		
Male	10283	51.1
Female	9840	48.9
Income (CNY)		
<10,000	2168	10.8
10,000–29,999	4376	21.7
30,000–49,999	3465	17.2
50,000–99,999	4417	22.0
100,000–199,999	4063	20.2
≥200,000	1634	8.1
Parental highest education		
Primary school	1320	6.6
Middle school	5316	26.4
High school	5021	24.9
College and above	8466	42.1
Ethnicity		
Han Chinese	16218	80.6
Other ethnicities	3905	19.4

Table 2. Health seeking behavior when dealing with a cold or fever in the preschool age.[a]

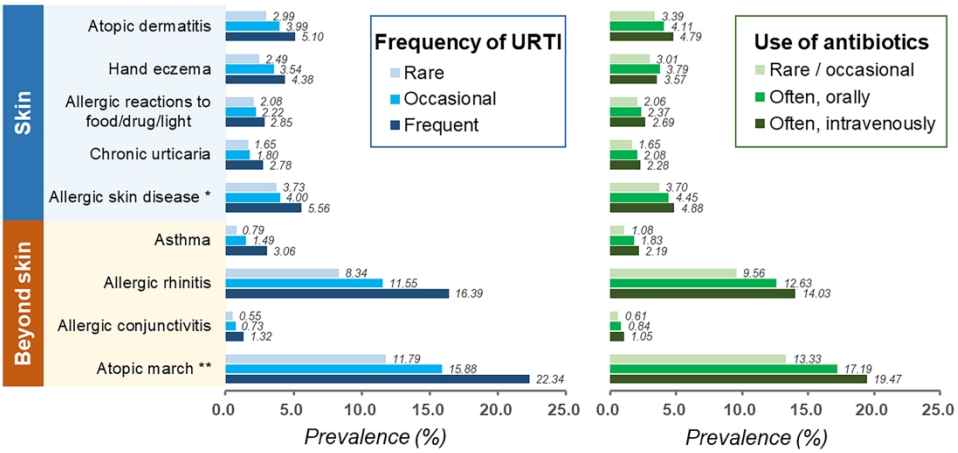
Disease condition	Total	Health seeking behavior, n (%)[b]				
		Ignore them	Drink water or have a rest	Receive antibiotics orally or intravenously	Receive Chinese traditional medicine orally	Unknown
Skin						
Atopic dermatitis	776	20 (2.6)	370 (47.6)	512 (65.9)	194 (25.0)	3 (0.3)
Hand eczema	675	32 (4.7)	343 (50.8)	430 (63.7)	149 (22.0)	4 (0.5)
Allergic reactions to food/drug/light	456	9 (2.0)	227 (49.7)	286 (62.7)	95 (20.8)	1 (0.2)
Chronic urticaria	381	8 (2.1)	158 (41.4)	257 (67.4)	100 (26.2)	1 (0.3)
Allergic skin disease [c]	833	17 (2.0)	384 (46.0)	539 (64.7)	194 (23.2)	2 (0.2)
Beyond skin						
Atopic march [d]	3139	66 (2.1)	1542 (49.1)	2081 (66.2)	728 (23.1)	12 (0.3)
Allergic conjunctivitis	153	4 (2.6)	83 (54.2)	107 (69.9)	36 (23.5)	0 (0)
Allergic rhinitis	2273	42 (1.8)	1135 (49.9)	1515 (66.6)	532 (23.4)	8 (0.4)
Asthma	303	7 (2.3)	154 (50.8)	206 (67.9)	70 (23.1)	2 (0.6)
Without atopic/allergic diseases	16343	597 (3.7)	7761 (47.4)	10057 (61.5)	3058 (18.7)	76 (0.4)

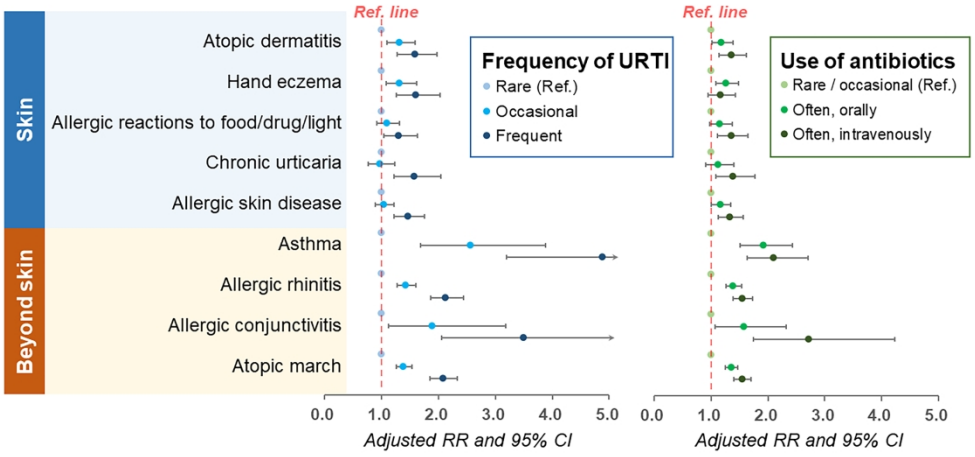
[a] Multiple selections are allowed in the questionnaire.

[b] Proportion ratios in populations.

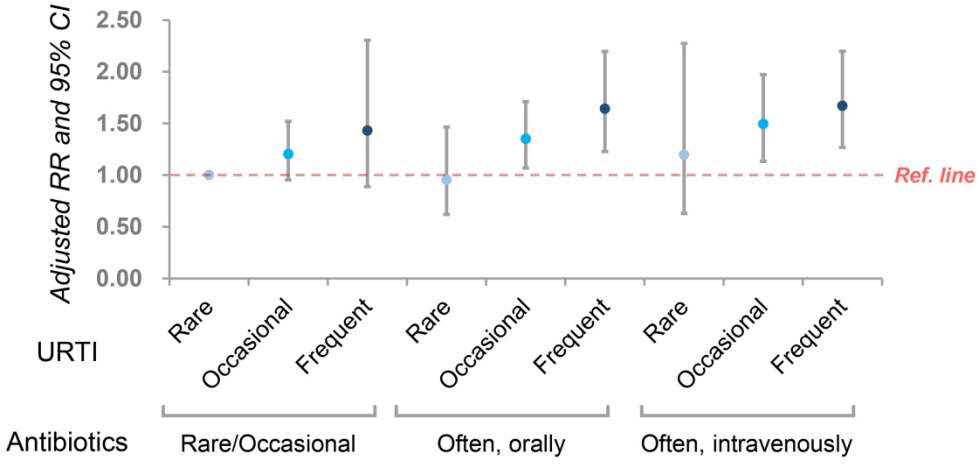
[c] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.

[d] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.





338x170mm (300 x 300 DPI)



236x115mm (300 x 300 DPI)

Supplementary file captions

Material S1. Chinese college students health survey questionnaire (Translation version in English)

Table S1. The prevalence of atopic and allergic diseases in college students.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students.

Material S1. Chinese college students health survey questionnaire (English version)

Chinese college students health survey questionnaire

Informed consent

Welcome to participate in the Chinese University Student Health Survey. To promote better health management for Chinese college freshmen, you are invited to fill out a questionnaire including several parts: (A) General information; (B) Medical history, (C) Lifestyle habits, (D) Skin health, which take about 15 minutes to answer. We will provide you with reasonable health education and health management based on this information, combined with the results of the health checkup. All the information you fill in will be kept strictly confidential. Continue to fill in the following content, indicating that you and your guardian have understood and are willing to continue to cooperate with our work. Thank you!

A. General information

A01. Before you went to university, please provide your address. _____

(*Specific to districts and counties*)

A02. Have you moved to other places (cross-city) in the past 10 years

(*Single selection, if "No" jump to A04*)

O Never O Ever

A03. If so, the previous address is?_____ (*Specific to districts and counties*)

A04. Sex (*Single selection*)

O Male O Female

A05. Your Ethnicity:_____

A06. Annual household income. (*Single selection*)

O <10,000 O 10,000 – 29,999 O 30,000 – 49,999

O 50,000 – 99,999 O 100,000 – 199,999 O ⩾200,000

A07. Your father's highest education (*Single selection*)

O Primary school or O Middle school O High school

blow ○ College ○ Postgraduate or above ○ Unclear
A08. Your mother's highest education (*Single selection*) ○ Primary school or blow ○ Middle school ○ High school ○ College ○ Postgraduate or above ○ Unclear

B. Medical History

B01. Have you ever been specifically diagnosed with any of the following cardiovascular or metabolic diseases? (*Multiple selections are allowed*) ☐ Hypertension ☐ Coronary heart disease ☐ Hyperlipidemia ☐ Obesity ☐ Fatty liver ☐ Gout ☐ Psoriasis ☐ None of above
B02. Have you ever been diagnosed with any of the following allergic diseases? (*Multiple selections are allowed*) ☐ Asthma ☐ Allergic Rhinitis ☐ Allergic conjunctivitis ☐ Eczema ☐ Urticaria ☐ None of above
B03. Have you ever been diagnosed with any of the following infectious diseases? (*Multiple selections are allowed*) ☐ Tuberculosis or other forms of tuberculosis ☐ Hepatitis B ☐ Hepatitis C ☐ Helicobacter pylori infection ☐ HIV infection ☐ None of above
B04. Have you ever been diagnosed with any of the following endocrine diseases? (*Multiple selections are allowed*)

☐ Type 1 diabetes ☐ Type 2 diabetes ☐ Polycystic ovarian syndrome ☐ Hypertrichosis ☐ Hypothyroidism ☐ Hyperthyroidism ☐ Graves disease ☐ Hashimoto thyroiditis ☐ None of above
B05.Have you ever been diagnosed with any of the following immune diseases? (*Multiple selections are allowed*) ☐ SLE ☐ Scleroderma ☐ Sjogren syndrome ☐ Uveitis ☐ Rheumatoid arthritis ☐ Dermatomyositis ☐ None of above
B06. Have you ever been diagnosed with any of the following hematologic diseases? (*Multiple selections are allowed*) ☐ Iron-deficiency anemia ☐ Megaloblastic anemia ☐ Thalassemia ☐ Anemia ☐ Hemophilia ☐ leucocythemia ☐ Lymphadenoma ☐ None of above
B07. Have you ever been diagnosed with any of the following mental or neurological disorders? (*Multiple selections are allowed*) ☐ ADHD and attention deficit ☐ Depressive disorder ☐ Anxiety disorder ☐ Schizophrenia ☐ None of above
B08. Have you ever had any of the following food allergies？ (*Multiple selections are allowed*) ☐ None ☐ Milk ☐ Egg ☐ Wheat ☐ Soybean ☐ Fish ☐ Nuts ☐ Fruit ☐ Crustaceans (e.g., shrimp, crab, etc.) ☐ Others ☐ Unclear
B09. Have you ever been allergic to any of the following medications? (*Multiple*

selections are allowed) ☐ None ☐ Antibiotics ☐ Non-steroidal anti-inflammatory drugs analgesics (e.g., aspirin) ☐ Contrast medium ☐ Anesthetic ☐ Anticonvulsant ☐ Chemotherapy drugs ☐ Others ☐ Unclear
B10. Have you ever been allergic to any of the following environmental substances? (*Multiple selections are allowed*) ☐ None ☐ Dust mite ☐ Mycete ☐ Animal skins ☐ Pollen ☐ House dust ☐ Cockroach ☐ Others ☐ Unclear
B11. Do you have an excessive bug bite response? (*Single selection*) ○ No ○ Yes
B12. In the past year, have you had a dog, cat, or other small stuffed animal such as rabbit, guinea pig, hamster, etc.? (*Multiple selections are allowed*) ☐ No ☐ Dog ☐ Cat ☐ Other stuffed animals ☐ Unclear
B13. Do you have a long history of close exposure to chemicals? (*Multiple selections are allowed*) ☐ None ☐ Formaldehyde ☐ Gasoline ☐ Oil varnish ☐ Others ☐ Unclear
B14. Have you used or taken any medication almost every day for the last 2 weeks? (*Multiple selections are allowed*) ☐ None ☐ Antibiotics ☐ Nonsteroidal anti-inflammatory drugs and analgesics (NSAIDS) ☐ Hormone ☐ Antituberculosis drugs ☐ Others ☐ Clear

B15. Whether breastfed after birth or not. (*Single selection*)

O No O Yes O Unclear

B16. How often do you have "colds and fevers" in your early school years (before age of 7 years old)? (*Single selection*)

O Rare (⩽1 time/year) O Occasional (2-3 times/year) O Often (4 or more times/year)

B17. Which way did you (or your parents) usually deal with your "cold or fever" in the early school age years? (*Multiple selections are allowed*)

☐ Ignore them ☐ Drink more water or have a rest ☐ Receive antibiotics orally

☐ Oral Chinese Traditional medicine ☐ Receive antibiotics intravenously ☐ Others

B18. **Which of the following frequency of antibiotic use can improve or cure your "Cold or fever" in the early school age years? (*Single selection*)**

O Rarely, it usually cured without antibiotic treatment O occasional

O Often by antibiotics orally O Often by antibiotics intravenously

C. Lifestyle Habits

C01.Do you smoke (refers to smoking at least one cigarette a day for more than six months) (*Single selection, jump to C3 if you choose "hardly"*)

O Hardly O < 1 packet /day (about 20 cigarettes in a packect) O 1-2 packets /day O > 2 packs/day

C02. If you smoke, how many years have you smoked in total so far? (*Single selection*)

○ < 1 year ○ 1-3 years ○ > 3 years
C03. In the last month, your frequency of passive smoking is (the involuntary inhalation of smoke caused by other people's smoking in your living or working environment) (*Single selection, jump to C5 if you choose "hardly"*) ○ Hardly ○ < 1 day/week ○ 1-2 days/week ○ 3-5 days/week ○ 6-7 days/week
C04. How many years have you been second-hand smoking? (*Single selection*) ○ < 2 years ○ 2-3 years ○ 4-6 years ○ > 6 years
C05. How often do you drink alcohol? (referring to once a week for at least six months) (*Single selection, jump to C9 if you choose "hardly"*) ○ Hardly ○ Once a week ○ 2-4 times/week ○ 5-7 times/week ○ 8-10 times/week ○ > 10 times/week
C06. How many years have you been drinking? (*Single selection*) ○ < 1year ○ 1-3years ○ 4-5years ○ > 5 years
C07. What's your main drink? (*Single selection*) ○ Beer ○ Liqueur ○ Red wine ○ Sweet wine ○ Chinese rice wine
C08. How much do you drink on average each time? (*Single selection*) ○ Less (1 bottle of beer, or 50-100g other types of alcohol) ○ Medium (2 bottles of beer, or 100g other types of alcohol) ○ Much (three bottles of beer, or 150g other types of alcohol) ○ A lot (more than 3 bottles of beer, or more than 150g of other types of alcohol)

D. Skin Health

D01.How many showers do you take per week in the spring and fall? (*Single selection*) ○ ≤1 time per week ○ 2~4 times per week ○ 5~7 times per week

O 8~10 times per week O >10 times per week
D02. How long do you take a shower in spring and fall? (*Single selection*) O < 5 minutes O 5-10 minutes O 11-20 minutes O 21-30 minutes O > 30 minutes
D03. What kind of toiletries do you use most? (*Single selection*) O None O Soap O Shower gel O Others
D04. In spring and fall, the temperature of your bath is (*Single selection*) O Low temperature(<35 centigrade) O Close to the body temperature(35-40 centigrade) O High temperature(> 40 centigrade)
D05. Do you use moisturizer all over your body almost daily in fall and winter? (*Single selection*) O No O Yes
D06. How often do you use facial cleansing products (e.g. cleanser, soap)? (*Single selection*) O Hardly O Usually O Once per day O ≥twice per day
D07. How often have you washed your hair in the past two years? *(Single selection)* O ≥ Twice per day O Once per day O Once on alternate days O Once every 2-6 days O Once a week or more
D08. In the past two years, what kind of toiletries you use is? *(Single selection)* O None O Shampoo O Shampoo + conditioner O Others
D09. Have you used hair care/hair products in the past two years? (*multiple selections*) ☐ None ☐ Essential oil, elastin and other hair

care products ☐ Hair gel ☐ Others
D10. In the last two years, how often have you dyed your hair? *(Single selection)* O Never O Less than once a year O Once every 2 to 6 months O Once every 7 to 11 months O More than once a month
D11. In the past two years, your perm frequency is *(Single selection)* O Never O Less than once a year O Once every 2 to 6 months O Once every 7 to 11 months O More than once a month
D12. Do you have frequent itchy skin, and to what extent (0 is not itchy at all and 10 is extremely itchy)? 0 – 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10
D13. Do you have regular skin pain, and to what extent (0 is no pain at all and 10 is extreme pain)? 0 – 1 – 2 – 3 – 4 – 5 – 6 – 7 – 8 – 9 – 10

Table S1. The prevalence of atopic and allergic diseases in college students

Disease	N, Prevalence (%)[a]	URTI, n (%)			Antibiotics, n (%)		
		Rare	Occasional	Frequent	Rare, Occasional	Often,orally	Often, intravenously
Skin							
Atopic dermatitis	776 (3.86)	174 (2.99)	459(3.99)	143(5.1)	172(3.39)	263(4.11)	164(4.79)
Hand eczema	675(3.35)	145 (2.49)	407(3.54)	123(4.38)	153(3.01)	243(3.79)	122(3.57)
Allergic reactions to food/drug/light	456(2.27)	121(2.08)	255(2.22)	80(2.85)	105(2.06)	152(2.37)	92(2.69)
Chronic urticaria	381(1.89)	96(1.65)	207(1.8)	78(2.78)	84(1.65)	133(2.08)	78(2.28)
Allergic skin disease [b]	833(4.14)	217(3.73)	460(4)	156(5.56)	382(3.7)	285(4.45)	167(4.88)
Beyond skin							
Atopic march [c]	3139(15.6)	687(11.79)	1825(15.88)	627(22.34)	1371(13.33)	1101(17.19)	666(19.47)
Allergic conjunctivitis	153(0.76)	32(0.55)	84(0.73)	37(1.32)	63(0.61)	54(0.84)	36(1.05)
Allergic rhinitis	2273(15.6)	486(8.34)	1327(11.55)	460(16.39)	984(9.56)	809(12.63)	480(14.03)
Asthma	303(1.51)	46(0.79)	171(1.49)	86(3.06)	111(1.08)	117(1.83)	75(2.19)

[a] The total prevalence of atopic and allergic diseases in our study population.

[b] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.

[c] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.

Table S2. Association of antibiotic and URTI exposure with atopic and allergic diseases in college students

Disease	URTI, aRR (95%CI) [a]			Antibiotics, aRR (95%CI) [a]		
	Rare	Occasional	Frequent	Rare / occasional	Often, orally	Often, intravenously
Skin						
Atopic dermatitis	Reference	1.32 (1.09, 1.54)	1.59 (1.27, 1.98)	Reference	1.18 (1.01, 1.39)	1.36 (1.14, 1.62)
Hand eczema	Reference	1.32 (1.08, 1.56)	1.60 (1.26, 2.02)	Reference	1.27 (1.08, 1.49)	1.17 (0.95, 1.44)
Allergic reactions to food/drug/light	Reference	1.10 (0.92, 1.28)	1.30 (1.04, 1.63)	Reference	1.15 (0.97, 1.37)	1.36 (1.12, 1.65)
Chronic urticaria	Reference	0.97 (0.76, 1.17)	1.58 (1.21, 2.05)	Reference	1.13 (0.90, 1.40)	1.39 (1.09, 1.78)
Allergic skin disease [b]	Reference	1.04 (0.89, 1.20)	1.46 (1.22, 1.76)	Reference	1.16 (1.01, 1.34)	1.33 (1.13, 1.57)
Beyond skin						
Atopic march [c]	Reference	1.39 (1.26, 1.52)	2.08 (1.85, 2.34)	Reference	1.35 (1.24, 1.48)	1.55 (1.40, 1.71)
Allergic conjunctivitis	Reference	1.89 (1.13, 2.66)	3.49 (2.05, 5.96)	Reference	1.58 (1.08, 2.32)	2.72 (1.75, 4.23)
Allergic rhinitis	Reference	1.43 (1.27, 1.59)	2.13 (1.86, 2.43)	Reference	1.39 (1.26, 1.53)	1.56 (1.39, 1.74)
Asthma	Reference	2.56 (1.69, 3.43)	4.89 (3.20, 7.47)	Reference	1.92 (1.52, 2.43)	2.10 (1.64, 2.70)

[a] Adjusted for the fixed effects of gender, income, education, passive smoking, and ethnicity and the random effect of university.

[b] Allergic skin disease includes allergic reactions to food/drug/light, contact dermatitis, and urticaria.

[c] Atopic march refers to atopic dermatitis, allergic asthma, allergic rhinitis, and allergic conjunctivitis.