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Gradient disparities in allergy and the gut microbiome among rural, migrant, and urban populations across China

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ABSTRACT

Background: While much of the evidence linking the rapid urbanization and the increasing prevalence of allergen sensitization, but little is known regarding rural-to-urban migrants. The aim of this study was to identify the disparities in allergy, the gut microbiome and factors among native urban, migrating, and native rural Chinese.

Methods: We redesigned the dataset of the China Alliance of Research on Respiratory Allergic Disease secondary survey, and after stratified sampling, a subsample of 2422 subjects were enrolled for the analysis of a questionnaire, skin prick tests (SPT), and specific immunoglobulin E (sIgE) titer measurements against 8 common allergens. Fecal microbiotal composition was also sequenced by 16S rRNA and regression-based analyses with covariate adjustment applied.

Results: From urban to migrant and rural populations, IgE sensitization was predominantly directed against Dermatophagoides pteronyssinus (Der p). The titers of Der p-sIgE decreased sequentially across the 3 respective populations and co-sensitization to other allergens also showed a sequential decrease. Rural-to-urban migrants showed a low prevalence of Der p-SPT and Der p-sIgE initially, but developed substantial IgE titers and their gut microbiotal diversity, as well as species richness, appeared to change along with residential time spent in the urban area. High-fat diet, using a mattress, an SPT wheal size from Der p $\geq$ 6 mm, and duration of immigration $>$5 years were significantly associated with sIgE positivity in the migrants.

Conclusion: The Der p-sIgE responses and the composition of gut microbiota differs synchronously with extended living time in an urban area. Studies in immigrants provide a unique opportunities to evaluate the effects of environmental factors in the pathogenesis of allergic disorders.

Keywords: Allergens, Environment, Gut microbiota, Immigrant, Immunoglobulin E

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INTRODUCTION

The prevalence of inflammatory disease conditions, including allergy, asthma, and autoimmune disorders, increased during the latter half of the twentieth century, as societies transitioned from rural to urban lifestyles.[1] Though allergy have traditionally been associated with industrial countries, they are also endemic in developing nations. Recent reports indicate that in the Asia-Pacific region, allergic disease prevalence has reached the highest levels in decades due to rapid urbanization and changes in lifestyle.[2,3] Subsequently, many studies have demonstrated the urban-rural disparity in the prevalence of allergic diseases.[4,5] One reason may be that persons raised in rural environments in proximity to farm animals develop an enriched human microbiome, which can promote immune balance and protect against allergic disorders.[6] Previous studies have also reported that children raised on traditional European and Asian farms are protected from asthma.[7,8] In China, preliminary data suggest that the population residing on farms had a lower prevalence of allergic diseases.[9] However, these epidemiological studies mainly focus on comparisons of school-aged populations in urban native and rural native areas, but seldom address rural-to-urban migrating subjects. Rural-urban migration is associated with a high burden of asthma in some urban areas.[10] The risk of mite allergy is lower in the rural than in the native urban population, and this protective effect may disappear among rural-to-urban migrants.[11] Significant rural-to-urban and urban-to-urban migration in developing China presents a natural epidemiological model to better understand population-level trends in allergy prevalence without confounding genetic factors. To assess whether the dominance of Dermatophagoides pteronyssinus (Der p)-specific IgE (sIgE) is an environmental phenomenon, we compared the sIgE titers of urban native individuals with those of migrants to cities. Furthermore, whether the migrant sensitization and gut microbial characteristics gradually changed with increasing years has not been reported in China, so the objective of the present study was to identify the disparity of sensitization patterns, factors, and gut microbiome in native urban, migrating, and native rural Chinese.

METHODS

Study design and participants

The China Alliance of Research on Respiratory Allergic Disease second stage survey was conducted in 26 cities with 36 hospitals from northern, eastern, central, and southern coastal regions. Between January 2018 and March 2019, 6442 individuals from the attending outpatient clinics of the pulmonology, ENT, and allergology departments were selected and invited to participate in the survey. We initially enrolled 5752 people who completed the questionnaire and skin-prick test, and after excluding individuals with disqualifying blood test results, 5176 participants (2535 native urban individuals, 1939 rural-to-urban migrants, and 702 native rural individuals) were included in the final analysis. Urban, migrant, and rural regions were comprehensively divided according to their administrative level, city size, population number, economic development level, and total GDP standards from the National Bureau of Statistics of China (https://www.stats.gov.cn). Generally, rural zone refers to agricultural area, including market town, and village. Villager mainly engaged in agricultural production in various farms (eg, animal husbandry and aquaculture), forest farms, horticulture, and vegetable production. The population in rural areas is scattered, with specific natural landscape and socioeconomic conditions. More densely populated area refers to urban zone (city), which includes residential, industrial and commercial areas and have administrative jurisdiction functions.The administrative jurisdiction of the city may involve buildings, streets, hospitals, schools, commercial stores, squares, parks and other public facilities. According to the distributions in age groups of migrant and rural individuals, the population in the native urban region was randomly sampled and adjusted for 8 age groups (5–7, 8–10, 11–14, 15–24, 25–34, 35–44, 45–54, and 55–65 years). Similarly, according to the distributions in the age groups of rural individuals, the migrants were also stratified for 8 age groups as above. We ultimately enrolled in the survey a total of 914 native urban individuals, 806 migrant individuals, and 702 native rural individuals aged 5–65 years and who attended outpatient clinics at the aforementioned hospitals (Appendix S1). A uniform study protocol and

sample operating procedures were used among the hospitals and the results were double-checked by the principal investigator and transmitted back to each center. All data were coded and inputted into a programmed database by 2 individuals independently, and evaluated for outliers and logic errors.

Skin-prick test (SPT)

We tested sensitization toward 8 common aeroallergens and the extracts concentration were labeled in mg/ml: Dermatophagoides pteronyssinus (0.057 mg/mL), cat dander (0.086 mg/mL), cockroach (0.549 mg/mL), timothy grass (1.036 mg/mL), Aspergillus (0.069 mg/mL), Ambrosia artemisiifolia (1.043 mg/mL), Artemisia vulgaris (0.113 mg/mL), and *Alternaria alternata* (0.078 mg/mL). A positive control of 10 mg/mL of histamine and a negative control solution (0.9% sodium chloride, 4 mg/mL of phenol, and 563 mg/mL of glycerol) were obtained from ALK (Horsholm, Denmark). SPT was performed on the volar side of the forearm, and the wheal reaction after 15 min was measured as the mean of the longest diameter and the length of the perpendicular line through its midpoint. A positive skin reaction was defined as a wheal size of 3 mm, after subtraction of the negative control.

Serum IgE measurement

All participants were additionally asked to sign a consent form to provide blood for the measurement of serum IgE levels. A peripheral blood sample of 10 mL was taken from each subject, coagulated at room temperature, centrifuged, and stored at 4 °C; then sent to the central laboratory at Guangzhou monthly. The specific IgE (sIgE) levels against Dermatophagoides pteronyssinus, cat dander, cockroach, timothy grass, Aspergillus, Ambrosia artemisiifolia, Artemisia vulgaris, and *Alternaria alternata* were measured on an ADVIA Centaur immunoassay system (Siemens AG, Erlangen, Germany). The analysis of sIgE was conducted only on the patients in whom the SPT was also performed. Results were then categorized into the following groups: grade 0 (<0.35 IU/ml), grade 1 (0.35–0.70 IU/ml), grade 2 (0.70–3.50 IU/ml), grade 3 (3.5–17.5 IU/ml), grade 4 (17.5–50 IU/ml), grade 5 (50–100 IU/ml), and grade 6 (>100 IU/ml). The sIgE cutoff value was set at 0.35 IU/ml, and a response was defined as positive if the sIgE level was ≥0.35 IU/ml.

Questionnaire

The questionnaire used in the survey was adopted from a version of the standardized questionnaire from the International Study of Asthma and Sensitizations in Childhood Phase II (ISAAC) (http://isaac.auckland.ac.nz). We used the ISAAC questionnaire as validated in the written Chinese version (Appendix S6).[12,13] The questionnaire was administered by physicians or research nurses face to face, and contained questions on baseline demographic characteristics such as family history of atopy; physical allergic symptoms; pet ownership; and dietary habits. Questions regarding a participant's registered permanent residence and resident conditions were also asked. The individuals of urban and rural populations in this study both lived in their original household as registered from birth, while the migrants were rural residents who later went to live and work in urban areas for various reasons. The home typology was based on visual inspection and characterized by significant differences in living environment. Urban dwellings usually contained a living room, bedroom, and kitchen in a western style. However, the rural lifestyle was much simpler, and individuals were often in contact with farms and livestock and participated in agricultural activities.

Stool sample collection, DNA extraction and sequencing

Stool specimens were collected either by a researcher during a study visit or by the subjects themselves at home using sterile collection kits. Collection instructions included requests not to contaminate the sample with urine or water and to store the sample at room temperature until the study visit. The stool samples were immediately transferred on ice packs to the hospital, where the samples were placed in 2-mL Eppendorf tubes (250 mg/tube) and immediately frozen at −80 °C upon receipt until processing.

Fecal samples were incubated with lysis buffer and proteinase K. Glass beads (0.1 mm, Sigma, St. Louis, MO) were added to the lysed samples, which were then homogenized for 60 s in a FastPrep bead

	Urban	Migrant	Rural	P value[b]	P value[c]	P value[d]
Total participants	914	806	702			
Subjects, n (%)						
Asymptomatic[a]	306 (33.5)	*212 (26.3)*	241 (34.3)	0.058	0.712	0.062
Symptomatic	608 (66.5)	594 (73.7)	461 (65.7)			
Sex, n (%)						
Male	485 (53.1)	414 (51.4)	370 (52.8)	0.482	0.91	0.583
Female	429 (46.9)	392 (48.6)	331 (47.2)			
Mean age (SD)	28.7(14.9)	32.4 (16.8)	30.2 (15)	0.144	0.216	0.284
Ethnicity, n (%)						
Han Chinese	914 (100)	806 (100)	702 (100)	–	–	–
Diagnosis, n (%)						
Asthma alone	137 (22.5)	140 (23.6)	125 (27.1)	0.67	0.085	0.188
Rhinitis alone	320 (52.6)	326 (54.9)	239 (51.9)	0.434	0.798	0.326
Asthma with rhinitis	151 (24.8)	128 (21.5)	97 (21.0)	0.177	0.146	0.842
Region, n (%)						
North	318 (34.8)	265 (32.9)	213 (30.3)	0.486	0.139	0.431
East	146 (16.0)	135 (16.7)	128 (18.3)	0.756	0.35	0.538
Western south	167 (18.3)	142 (17.6)	144 (20.5)	0.636	0.397	0.203
Southern coast	283 (30.9)	264 (32.8)	217 (30.9)	0.4	0.913	0.493
Lifestyle						
Use of an air-conditioner	825 (90.3)	689 (85.8)	312 (44.4)	0.002[e]	< 0.001[e]	< 0.001[e]
Use of a mattress	776 (84.9)	624 (77.4)	399 (56.8)	< 0.001[e]	< 0.001[e]	< 0.001[e]
Raising pets	567 (62)	448 (55.6)	468 (66.7)	0.007[e]	0.054	< 0.001[e]
Building height <6 m	296 (32.4)	453 (56.2)	536 (76.3)	< 0.001[e]	< 0.001[e]	< 0.001[e]
Using carpet as the material of the household floor	462 (50.5)	320 (39.7)	108 (15.3)	< 0.001[e]	< 0.001[e]	< 0.001[e]
Using natural gas in cooking	859 (94)	735 (91.2)	238 (34)	0.027	< 0.001[e]	< 0.001[e]
Diet (≥2 times/week)						
Fresh fruits or vegetable	696 (76.1)	612 (75.9)	584 (83.2)	0.916	0.001[e]	0.001[e]
Red meat	708 (77.5)	691 (85.7)	592 (84.3)	< 0.001[e]	< 0.001[e]	0.446
Fried fish	307 (33.6)	288 (35.7)	139 (19.8)	0.351	< 0.001[e]	< 0.001[e]
Ice cream	470 (51.4)	517 (64.1)	181 (25.8)	< 0.001[e]	< 0.001[e]	< 0.001[e]

Table 1. Description of the study population. *[a]Asymptomatic group includes atopic and healthy individuals. [b]P-values were calculated between urban and migrant individuals. [c]P-values were calculated between urban and rural individuals. [d]P-values were calculated between migrant and rural individuals. Percentages may not total 100 because of rounding. [e]Indicates statistically significant difference using critical value after Bonferroni's correction: 0.017*

beater (BD Biosciences, San Jose, CA) at a speed of 4 m/s. The homogenized samples were purified using spin columns and then eluted with 200 mL of buffer AE. Microbial DNA was quantified using spectrophotometry (NanoDrop ND-1000; eBioscience, Waltham, MA), and 1.0% agarose gel electrophoresis was performed for quality assessment. We performed DNA amplification and next-generation sequencing of the V3-V4 region of the 16S rRNA gene by using the NEXTflexTM 16S AmpliconSeq Kit 2.0 (Genomic DNA Screen Tape; Agilent Technologies, Santa Clara, CA), and the following 2 sets of primers were used: F, 5′-CCTACGGGNGGCWGCAG-3′; R, 5′GACTACHV GGGTATCTAATCC-3′). Each sample's amplicon was mixed at the same concentration, and the amplicons were then sequenced using high-throughput sequencing (Illumina, San Diego, CA) according to Illumina's 16S Metagenomics Sequencing Library Preparation Guide. Bacterial α-

diversity was measured using the Chao1, observed species, and Shannon indices. Sequencing data was finally processed through the QIIME pipeline.[14,15]

Statistical analysis

Categorical variables are presented as numerical values and percentages, and were analyzed using Fisher's exact-probability test. Continuous variables are presented as mean ± standard deviation and were analyzed using the Wilcoxon rank-sum test. We evaluated bivariate analysis results using Pearson's correlation coefficients. Logistic regression analyses were conducted to test for any association between explanatory variables and clinical outcomes. Baseline variables that were considered clinically relevant or that showed a univariate relationship with outcomes (i.e., an overall P value for the explanatory variable of <0.05) were introduced into a stepwise multivariate logistic regression model. All variables for inclusion were carefully chosen given the number of events available so as to ensure parsimony of the final model. The results are presented with adjusted odds ratios (ORs) and 95% confidence intervals (95% CI). A non-parametric Mann-Whitney *U* test was applied to perform the abundance analyses from phylum to species levels. Differences in detection rate of taxa (i.e., detected, >0%; or not detected, 0% in a given sample) between groups were identified using a χ^2 test. Critical values for statistical significance were determined by Bonferroni's correction. Statistical Package for the Social Sciences for Windows, release 21.0 (SPSS, Inc.,Chicago, IL) was used.

RESULTS

General demographic characteristics

This study included 3 groups of participants: 914 individuals who were born and raised in urban areas (urban individuals), 806 individuals who had migrated to urban areas over time (migrants), and 702 individuals who were born and raised in rural areas (rural individuals). These 3 groups were equally distributed with respect to age, sex, geographic location, and disease distribution, without any significant differences among the groups. There were significant differences in lifestyle, such as the use of air conditioners, mattresses, carpets, and natural gas among the 3 groups. In terms of diets, rural individuals ate fresh fruits and vegetables more often than urban individuals and migrants, and fewer rural individuals chose to eat ice cream (Table 1). The allergen induced wheal size in 4 regions of China are listed in Appendix S5. The skin reactivity to Dermatophagoides pteronyssinus was the highest in the southern coast and the lowest in the northern regions. Subjects in the north had significantly higher response to pollens (Timothy grass, Artemisia vulgaris, Ambrosia artemisifolia) than the other 3 regions.

Characteristics of allergen-specific IgE in urban individuals, those from rural areas, and in migrants were dominated by Der p

We examined the overall profiles of 8 inhaled allergens among urban individuals, rural individuals, and migrants. Allergic reactions to Der p constituted the commonest and strongest, in terms of both titer and positivity rate. The positivity rates of sensitization to Der p were 46.3%, 42%, and 39.8% in urban individuals, migrants, and rural individuals, respectively. Mugwort pollen was the most common pollen allergen, with positivity rates of 10.5%, 7.6%, and 5.4% in urban individuals, migrants, and rural individuals, respectively. Among the other allergens tested, cat dander and Timothy grass showed a significant gradient in titers from high to low, with the titers of IgE to cockroach and ragweed higher in urban individuals than in either migrants or rural individuals, without a significant difference between migrants and rural individuals. Allergic reactions to *Alternaria* were stronger in urban individuals than in migrants or rural individuals, with higher titers in rural individuals than in migrants. We noted no differences in the titer or positivity rate of sensitization to *Aspergillus fumigatus* among the 3 groups (Fig. 1).

Comparison of serum total IgE (tIgE) levels of Der p-sIgE-positive and Der p-sIgE-negative individuals among urban, migrant, and rural area

Atopy was more prominent in urban individuals than in migrants, and more prominent in migrants than in rural individuals (301.8 kU/ml *vs* 248.6 kU/ml, 301.8 kU/ml *vs.* 128.9 kU/ml, and 248.6 kU/ml *vs* 128.9 kU/ml, respectively; $P < 0.001$) (Fig. 2A). Among urban individuals, the median titer of tIgE for individuals with sensitization to Der p was

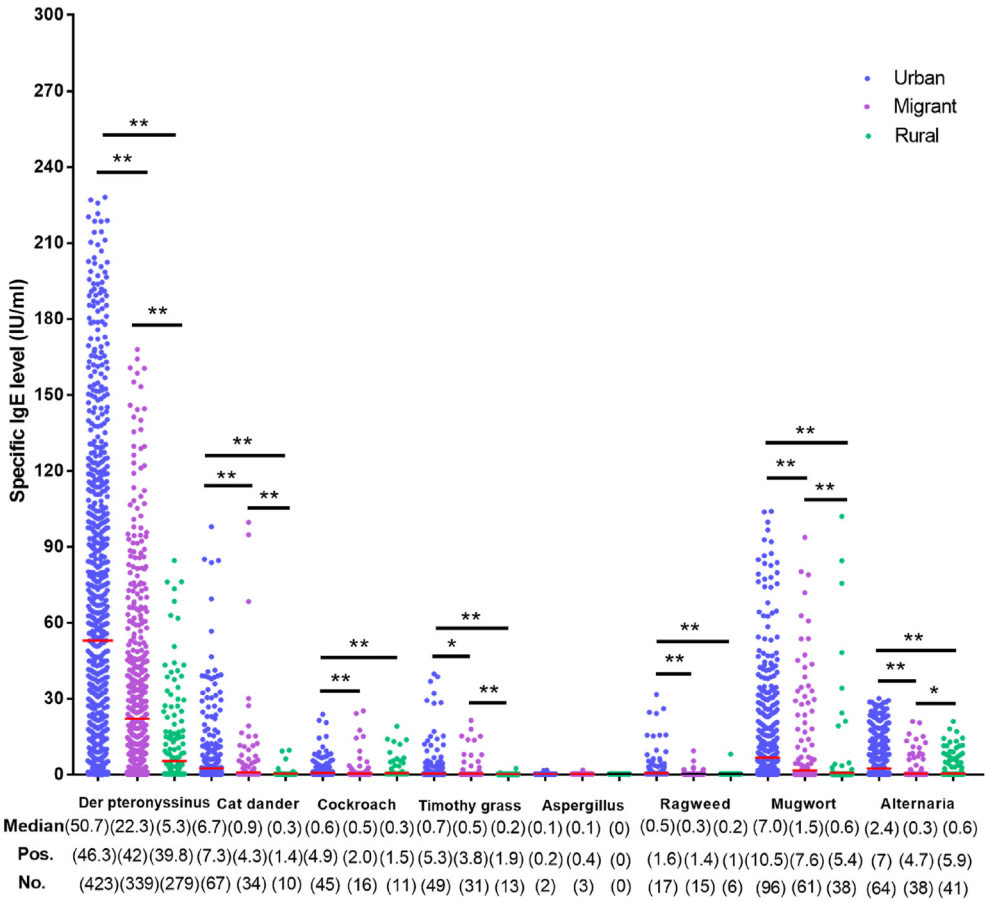


Fig. 1 Comparison of serum specific IgE levels of 8 common allergens among urban individuals, migrants, and rural individuals. Red bar indicates the median value. Pos. and No. represent positivity of allergen sensitization and number of subjects, respectively. *Indicates statistically significant difference using critical value after Bonferroni's correction: 0.017. *P < 0.017, **P < 0.001.

three-fold higher than for those without sensitization to Der p (294.8 kU/ml *vs* 91.8 kU/ml, P < 0.001). Among migrants, the median titer of tIgE with sensitization to Der p was five-fold higher than for individuals without sensitization (208.4 kU/ml *vs*. 40.9 kU/ml, P < 0.001). Among the rural individuals, the median titer of tIgE with sensitization to Der p was three-fold higher than in those without sensitization to Der p (132.7 kU/ml *vs*. 38.1 kU/ml, P < 0.001). For both individuals with sensitization to Der p and those without, atopy was more prominent in urban individuals than in migrants and rural individuals, and atopy was more prominent in migrants relative to rural individuals (Fig. 2B).

Comparison of polysensitization among urban individuals, migrants, and rural individuals

Overall, individuals with positive Der p-sIgE demonstrated higher rates of multiple sensitization when compared to individuals with negative Der p-sIgE. In urban areas, more than a quarter of individuals (29.4%) with sensitization to Der p were also sensitized to other allergens, while the proportion was lower in migrants (24.3%) and even lower in rural individuals (19.5%) (Fig. 2C).

Prolonged exposure to urban environments was linked to Der p sensitization and Der p-sIgE response

In the overall population, Der p sensitization in migrants was seriously affected by the length of stay in the urban environments. According to the SPT results, 19.5%, 28.9%, and 39.6% of migrants developed sensitization to Der p after residing in urban areas for 0–5 years, 5–10 years, and >10 years, respectively. In addition, the Der p-sIgE titer increased in a pattern similar to the increasing trend observed for Der p-SPT (Fig. 3A and B).

This same trend was also present among migrant patients with allergic airway diseases. According to the SPT results, 25.5%, 35.8%, and 41.5% of migrants with rhinitis developed

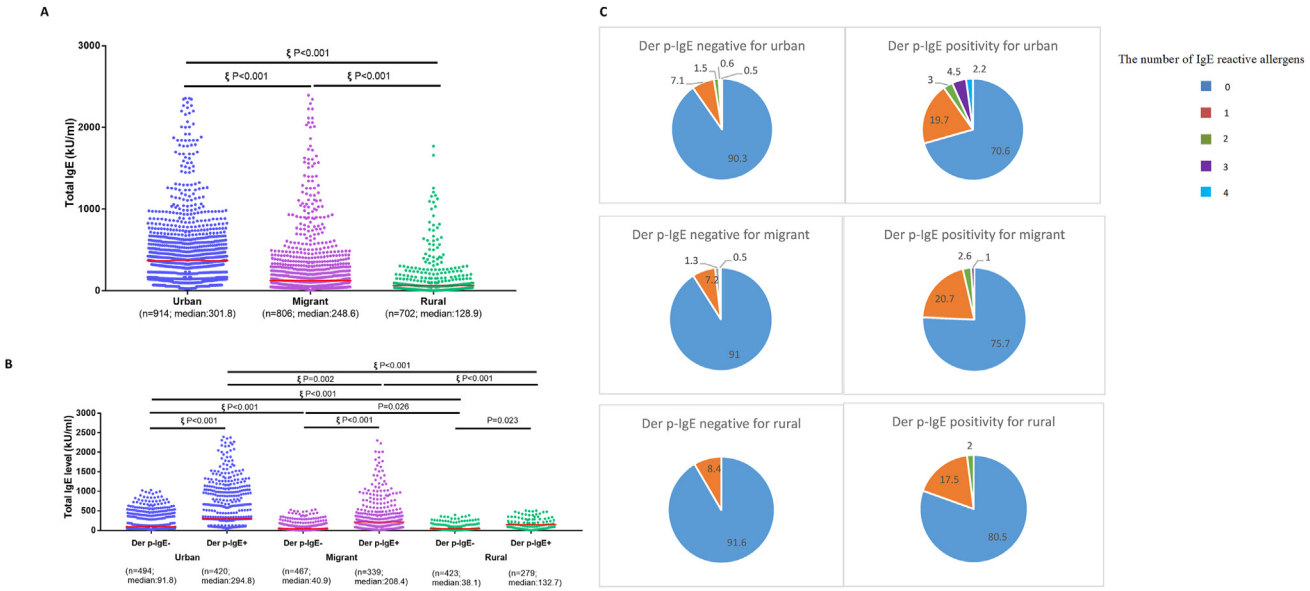


Fig. 2 Association of Der p sensitization with "atopy" and serum total IgE among urban individuals, migrants, and rural individuals. (A) Serum total IgE levels in urban individuals, immigrants, and rural individuals. (B) Serum total IgE levels of Der p-sIgE-positive and Der p-sIgE-negative individuals in urban, migrant, and rural areas. (C) Fraction (%) of individuals with sIgE against other non Der p allergens among either Der p-sIgE-positive or Der p-sIgE-negative individuals in urban, migrant, and rural areas. Red bar indicates the median value. 2A ξ indicates statistically significant difference using critical value after Bonferroni's correction: 0.017; 2B ξ indicates statistically significant difference using critical value after Bonferroni's correction: 0.003.

sensitization to Der p after residing in urban areas for 0–5 years, 5–10 years, and >10 years, respectively; the titer of Der p-sIgE was significantly increased in a similar pattern (Fig. 3C and D). We observed the same trend in migrants with asthma, although to a lesser degree (Fig. 3E and F). After residing in urban areas for 0–5 years, 5–10 years, and >10 years, 38%, 41.2%, and 47.2%, of the individuals developed Der p sensitization, respectively; and the Der p-sIgE titer was also significantly elevated (Fig. 3G and H).

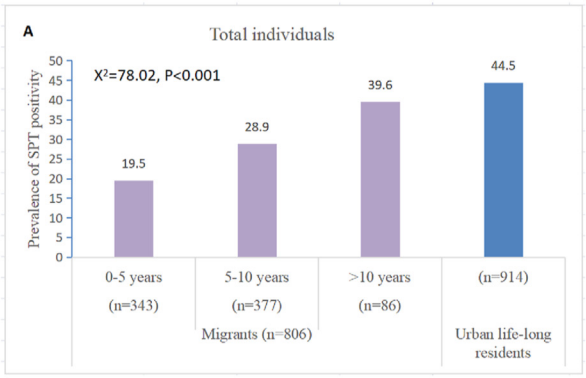
Comparison of fecal flora among urban individuals, migrants, and rural individuals

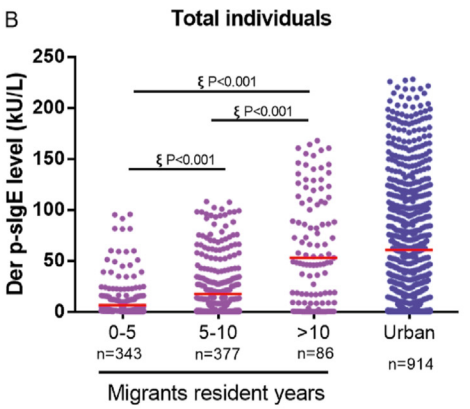
The number of observed species, and Chao1 and Shannon indices of migrants (>10 years) were significantly lower than those of rural individuals and migrants (0–5 years) ($P < 0.001$, Fig. 4A, B, C). The numbers of observed bacterial species in migrants >10 years were significantly lower than that of migrants 0–5 years, and the Chao1 index of migrants at 5–10 years was significantly lower than that of migrants at 0–5 years.

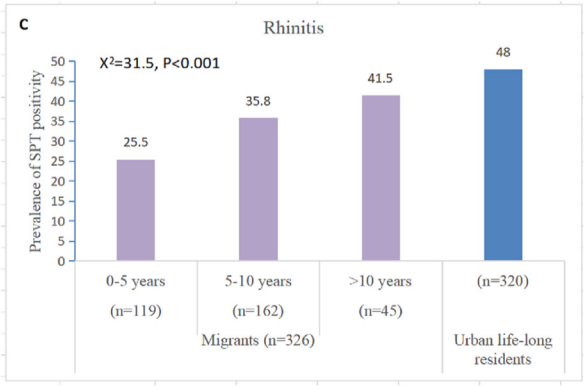
Analysis of the overall composition of the gut microbiota showed significant differences between any 2 groups. Firmicutes and Bacteroidetes were the dominant bacterial phyla in all groups. The average relative abundance of Bacteroidetes was the highest among urban individuals (59.29%), and the lowest among rural individuals (33.14%). Among migrants, the average relative abundance of Bacteroidetes of migrants >10 years and 5–10 years were significantly higher than that of migrants 0–5 years (54.66% *vs.* 36.23%; 43.36% *vs.* 36.23%, $P < 0.001$, respectively). In contrast, the average relative abundance of Firmicutes was the lowest in urban individuals (30.22%) and the highest in rural individuals (50.74%).

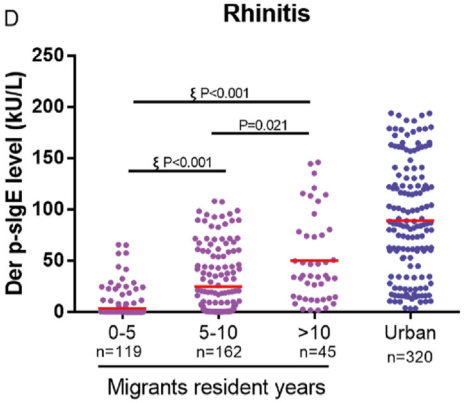
Factors associated with sIgE positivity among urban individuals, migrants, and rural individuals

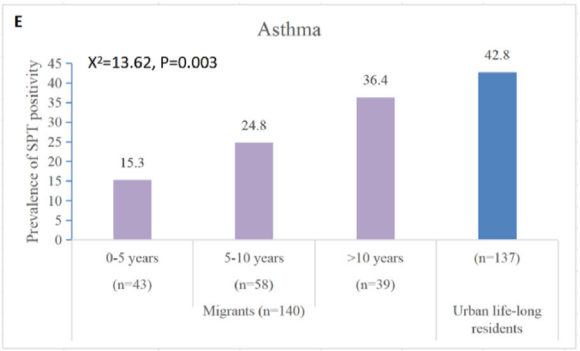
The univariate analysis of each of the explanatory variables is presented in Appendix S2. Table 2 shows the mutually adjusted OR estimates for all variables selected in the final models. The multivariate regression analysis showed that an SPT wheal of Der p $\geq$ 6 mm, the use of mattresses, >5 years of residency in urban areas, and consumption of high-fat diets were also related to the increased risk of sensitization in migrants. Of these characteristics, the consumption of high-fat diets resulted in the highest risk of sensitization in migrants (OR, 1.89 [CI, 1.17–3.32], $P < 0.001$).

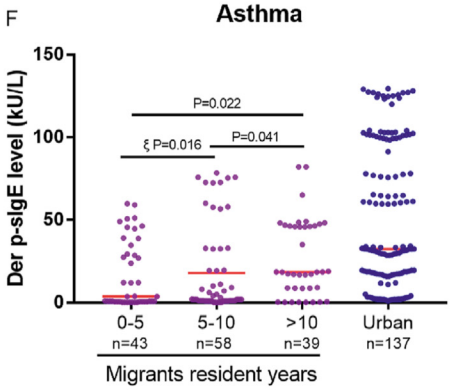


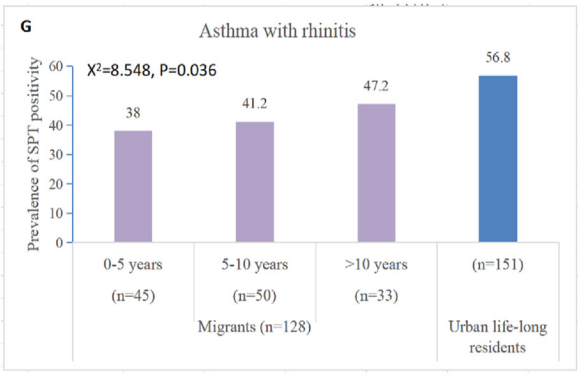












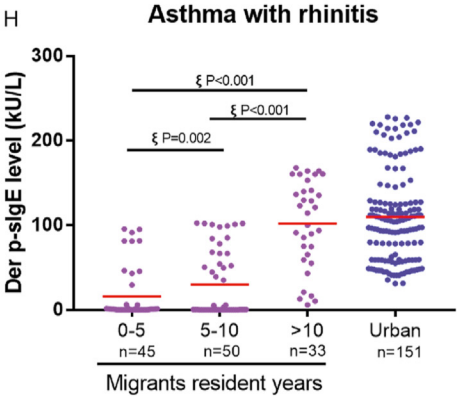


Fig. 3 Influence of time of exposure to urban environments on the prevalence of allergic airway diseases in the migrants. 3B,D,F,H ξ indicates statistically significant difference using critical value after Bonferroni's correction: 0.017. Red bar indicates the median value.

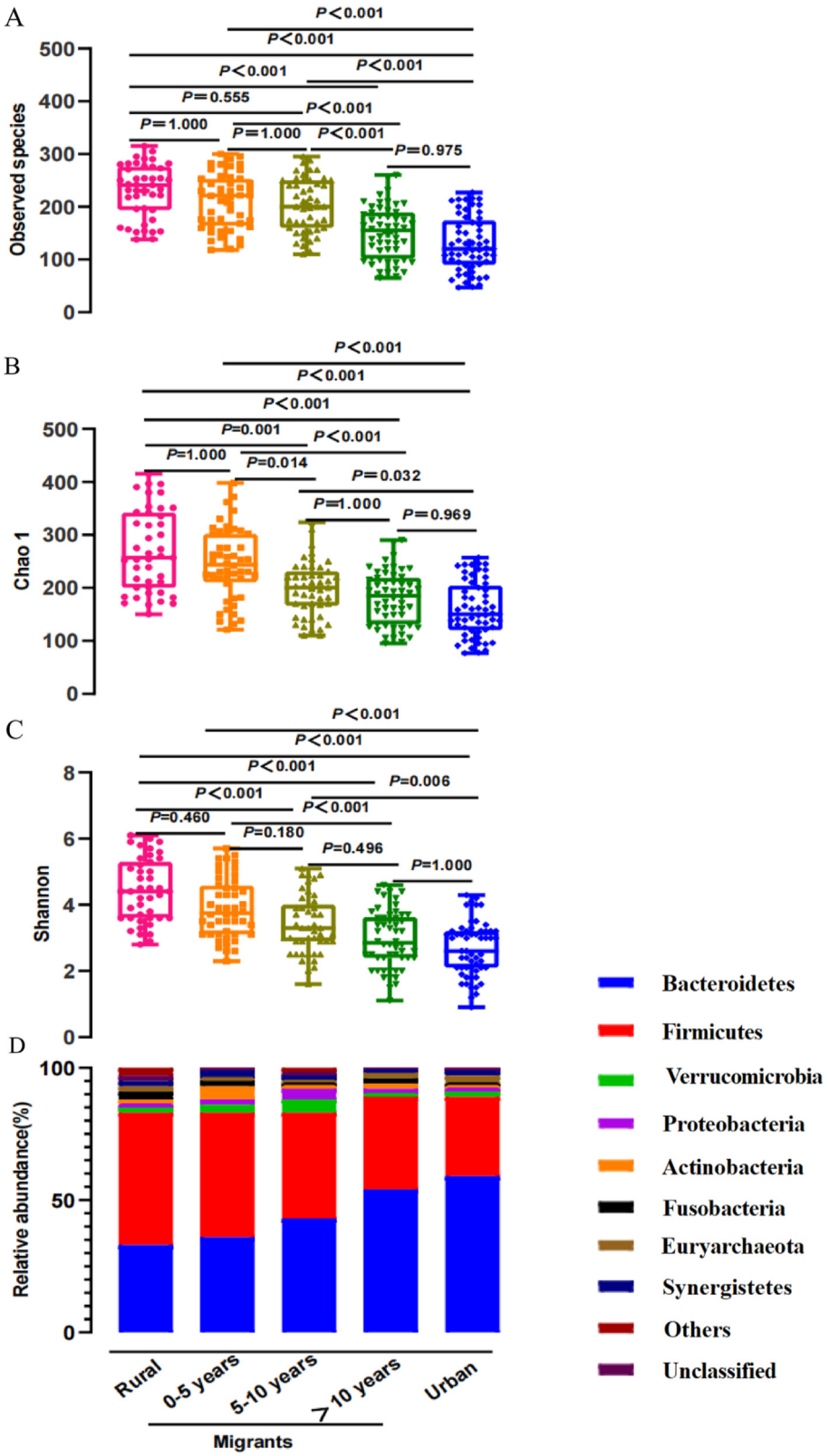


Fig. 4 Altered gut microflora in individuals in rural-to-urban migrations. The sample size was rural individuals (n = 68); migrant individuals 0–5years (n = 58), 5–10 years (n = 56), >10 years (n = 59); and urban individuals (n = 76). (A) Total number of observed species. (B–C) Chao1 and Shannon indices for alpha-diversity. (D) The relative abundances of bacteria at the phylum level in the urban group, the migrant group, and the rural group; the top 10 genera according to the relative abundances were included. 4A,B,C P-value indicates statistically significant difference using critical value after Bonferroni's correction: 0.005.

Exposure	Specific IgE positivity to any allergen (odds ratio)[b]		
	Odds ratio[a]	95% CI	P value
		Urban n = 914	
SPT wheal of Der p ≥ 6 mm	1.58	1.31–2.43	0.001
Use of an air-conditioner	2.67	1.4–3.41	0.006
Using mattress	1.76	1.45–2.27	<0.001
Air conditioner installed in both the living room and bedroom	1.42	1.08–2.48	0.022
Carpet as the material used for the bedroom floor	1.76	1.46–2.71	0.003
High-fat diet	1.86	1.5–2.97	<0.001
	Migrant n = 806		
SPT wheal of Der p ≥ 6 mm	1.54	1.13–2.38	0.02
Using mattress	1.73	1.38–2.6	<0.001
Duration of immigration >5 years	1.49	1.04–2.32	0.037
High-fat diet	1.89	1.17–3.32	<0.001
	Rural n = 702		
SPT wheal of Der p ≥ 6 mm	1.73	1.22–2.4	0.001

Table 2. Multivariate logistic regression analyses between selected factors and sIgE sensitizations. *[a]Adjusted for age, sex, and region. [b]A variety of factors were selected from those with P < 0.05 in univariate logistic regression analyses and were then entered in the multivariable logistic analyses. Only variables with a P < 0.05 were ultimately shown in the final model. SPT, skin prick test*

DISCUSSION

This study demonstrated a influence of time of exposure to urban environment on the prevalence of Der p-sensitization and gut microflora in individuals of rural-to-urban migrations. Although the prevalence rates of allergy and atopy have increased significantly over the past several decades,[16] the rapid increase cannot be explained only by changes in genetic factors; it has become apparent that environmental factors also play a key role in the development of allergy.[17] The increase in dust mite sensitization in patients with asthma and/or rhinitis in China over the past decade had been attributed to increased environmental humidity,[18] which was a climatic factor, but sensitization to *Aspergillus fumigatus* had not increased. Previous studies had shown that lifestyle might be the principal cause of house dust mite sensitization,[19] and this was consistent with the results of our questionnaire: ie, the rates of air conditioner usage, mattress usage, and carpet usage in urban individuals and migrants were significantly higher than rural individuals. Thus, it could explain why new immigrants displayed a time-dependent increase in the number of sensitized allergens and the Der p sensitization patterns in migrants likewise bore greater similarity to that of the local general population in urban areas.

Over time, first-generation immigrants acquire the sensitization profiles of the host country, which has been described as the assimilation or acculturation effect.[20] These effect was consistent with the study conducted in Italy and Vietnam.[11,21] Some study found an inverse correlation between immigration age and asthma prevalence.[22] Most recruiters in our study were adolescents and adults, which supported that an later age at the time of migration associated with increased risks of developing atopy. Besides, all individuals in

our study were from the Han ethnic group, thus obviating a primary role of genetics in our observations. The length of stay in urban areas was a strong confounding factor, as the rate of Der p sensitization for urban residents with >10 years of residency was 39.6%, denoting a significant increasing tendency. Exposure to Der p not only led to alarming titer levels of sIgE, but also seriously impacted disease progression, as the results showed that patients with asthma and rhinitis possessed the strongest allergic reactions to Der p after residing for more than 10 years in urban areas. These migrants, developed an increased prevalence of allergic rhinitis and/or asthma over time, gradually acquiring a pattern of sensitization typical of their host city. These observations were consistent with the findings of the Singaporean and Australia cohort study.[23,24]

In general, the majority of migrants in our study moved from rural to urban areas in the same province, few migrated across the provinces. Therefore, they were less affected by the climate change, but pollution, lifestyle factors, diet, and microbial exposures may influence allergic risk among migrant populations. We showed differences in the diversity of gut microbiota between migrants with >10 years residency in urban areas and migrants with 0–5 years residency in urban areas. The change in the ratio of Bacteroidetes to Firmicutes might be the cause of gut microbiota imbalance, similar to results reported in some foreign studies.[25,26] Bacteroidetes are the dominant type of bacteria in individuals who consume high-fat westernized diets.[27] We founded that consumption of ice-cream (high-fat diet) increased the risk of allergic diseases in migrant populations. The types of gut microbes in migrants may be related to the adaptation to urban diet pattern (not only ice-cream, but also other high-fat foods). On the other hand, endotoxins in farm dust regulate gut microbiota.[28] Studies have shown that they are important supplementary sources of gut microbiota in mammals,[29,30] even urban air pollutants may alter gut microbiota.[31] Animal study has confirmed that animals and plants from traditional farms produce proteins which transport hydrophobic microorganisms and plant metabolites and modulate airway responses to dust once they are delivered to the mucosal surfaces of the airway.[32] Thus, we inferred that microorganisms in the respiratory environment may be transported to the oropharynx and the gut through the mucociliary clearance, affecting gut microbiota. This suggests that natural exposure to urban environments and changes in dietary patterns among migrants may subtly contribute to the imbalance of the gut microbiota.

Sanitary conditions in Chinese rural areas were poor, and rural individuals were prone to chronic helminth infection. However, these infections also suppressed the development of cell-mediated immune responses to unrelated antigens.[33] In the study, the proportion of rural individuals who had positive reaction to *Schistosoma japonicum* was higher than migrant and urban individuals, but there was no statistical significance (rural individuals *vs.* migrant individuals *vs.* urban individuals: 22.4% *vs.* 20.5% *vs.* 16.8%, Appendix S3). Chronic infection with *Schistosoma* had a specific protective effect on Der p sensitization.[34] The difference in helminth infection rates might be a factor in sensitization among migrants, urban, and rural individuals in the study.

In addition, our sampling results showed that the endotoxin levels in the residences of urban and migrant populations were significantly lower than in residences of the rural population, regardless of whether in living rooms (rural *vs.* urban: 1133.8 EU/mg vs. 483.5 EU/mg; migrant *vs.* urban: 1133.8 EU/mg *vs* 489.4 EU/mg, $P < 0.001$) or bedrooms (rural *vs.* urban: 1407.4 EU/mg *vs*. 524.3 EU/mg; migrant *vs.* urban: 1407.4 EU/mg *vs* 513.2 EU/mg, $P < 0.001$) (Appendix S4). This were consistent with the findings of previous studies.[35] Farm environments often contain high loads of commensal bacteria, which are Gram-negative bacteria, resulting in elevated endotoxin levels.[36,37] Switching on regulatory T cells through early-life exposure to farming-emitted endotoxins may protect rural individuals from bronchial asthma.[38] We founded that the prevalence of allergic asthma in immigrants increased over time, further supporting the evidence.

Our study had several limitations. First, the study was not a prospective follow-up study, it could not establish a cause-effect relationship between prolonged exposure to urban and sensitization as well as gut microbial dysbiosis. Second, we did not

analyze fecal total IgE, so the association of gut microbiota related to fecal and serum IgE could not be addressed. Furthermore, measure Der p 1/Der p 2 levels dust samples as a marker of exposure to mite allergens was recommended in further study.

In conclusion, our study demonstrated the correlations between exposure to westernized environments over time and aggravation of Der p sensitization, elevation in polysensitizations, and increase in allergic disorders. Meanwhile, the etiologies (e.g., lifestyle, diet, helminth infection and environmental endotoxin) might exert an impact on the sensitization and gut microbiota. This study suggested that the immunoregulatory mechanisms of the migrant population gradually changed to susceptibility to allergic disease, which providing a vital intervention strategy that we expect would reduce future allergy to them.

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Data availability statement
To protect study participant privacy, the data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Author's consent for publication
All the authors have contributed to the conception and revision of the manuscript and have approved the submitted version.

Ethics statement
The study protocol was approved by the ethics review board and informed consent was taken from all the patients.

Declaration of competing interest
The authors declare they have nothing to disclose.

Appendix A. Supplementary data
Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2024.101018.

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