

Original article

The EuroPrevall-INCO surveys on the prevalence of food allergies in children from China, India and Russia: the study methodology

Background: Very little is known regarding the global variations in the prevalence of food allergies. The EuroPrevall-INCO project has been developed to evaluate the prevalence of food allergies in China, India and Russia using the standardized methodology of the EuroPrevall protocol used for studies in the European Union. The epidemiological surveys of the project were designed to estimate variations in the prevalence of food allergy and exposure to known or suspected risk factors for food allergy and to compare the data with different European countries.

Methods: Random samples of primary schoolchildren were recruited from urban and rural regions of China, Russia and India for screening to ascertain possible adverse reactions to foods. Cases and controls were then selected to answer a detailed questionnaire designed to evaluate the possible risk factors of food allergies. Objective evidence of sensitisation including skin-prick test and serum specific IgE measurement was also collected.

Results: More than 37 000 children from the three participating countries have been screened. The response rates for the screening phase ranged from 83% to 95%. More than 3000 cases and controls were studied in the second phase of the study. Further confirmation of food allergies by double blind food challenge was conducted.

Conclusions: This will be the first comparative study of the epidemiology of food allergies in China, India, and Russia using the same standardized methodology. The findings of these surveys will complement the data obtained from Europe and provide insights into the development of food allergy.

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Key words: epidemiology; food allergy; paediatrics.

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Accepted for publication 23 August 2009

IgE mediated food allergy is common affecting children and adults in many developed countries, and there is evidence of a possible increase in the prevalence of this condition (1–5). However, the exact pathophysiology of food allergy remains poorly understood and currently the only preventive treatment for this potentially fatal condition is avoidance of foods. The stress and anxiety about possible reactions in patients with food allergies can significantly affect their quality of life as the clinical manifestations of food allergy can range from a mild oral itch to a life-threatening anaphylactic reaction (6, 7). It has been estimated that about 30 000 food-induced anaphylactic episodes occurred each year in the United States, resulting in more than 150 deaths (8). Despite the availability of many published reports from Europe and America (9, 10), it has been difficult to make any firm conclusion regarding the prevalence and aetiology of

food allergy because of the lack of objective measures in many of these studies. The diagnosis of food allergy can be difficult because there are no simple and reliable diagnostic tests. Because of the concern over food allergy and its effect on public health, improved understanding and measures of prevention, diagnosis and treatment of food allergy are important priorities for the European Community. It was for this purpose that the EU-funded the multidisciplinary Integrated Project, EuroPrevall. This was launched in June 2005 (11). The aim of the EuroPrevall project is to evaluate the prevalence, basis and costs of food allergy in different European countries using the same standardized and validated methodology.

Despite many studies in Europe and elsewhere, the determinants of food allergy are still largely unknown. It is possible that some of the risk factors for food allergy may be so widespread in Europe that it is difficult for

epidemiological studies to identify them. On the other hand, food allergy appears to be relatively less common in developing or underdeveloped countries (12–15). Therefore, careful comparative studies in these communities may reveal possible risk factors that may explain the higher prevalence of food allergy in developed countries. This is why the EuroPrevall project was extended to study food allergies in China, India, and Russia, countries that are emerging economies where lifestyle in many parts is changing from traditional to a modern one.

Epidemiological studies in high income countries suggested that a big proportion of the population in Europe and America reports adverse reactions to food. For example, data from the European Community Respiratory Health Survey (ECRHS) (16) revealed that 12% of the population reported adverse reactions caused by eating certain foods (17), though this ranged from 4.6% in Spain to 19.1% in Melbourne, Australia. The reasons for such variations are not clear. Furthermore, there have been very few published data regarding the prevalence of food allergies outside the developed market economies. The limited published data suggested that both the prevalence of food allergy and the food most commonly causing problems are different from those of the Europe and North America (12–15).

Epidemiological studies have also shown that children from rural areas have much lower prevalence of asthma and related atopic conditions (18–21). Early exposure to microorganisms and parasites, and modulation of the immature immune system have been thought to be the important contributing protective factors in the rural environment (22, 23). It is conceivable that similar protection against the development of food allergy may occur in the rural environment as the development of food allergy is frequently the first manifestation of the 'allergic march' (24, 25). There is also evidence that food allergens likely differ significantly depending on the region as well as ethnic background. For example, shellfish appears to be one of the most common food allergens in the Chinese population (12). Edible bird's nest considered to be a delicacy in Asia has been reported to be one of the most common causes of anaphylaxis in Singapore (26). Peanut is one of the most common food allergens in Europe and America, but allergy to peanut is rather uncommon, despite heavy consumption, in many Asian countries (12, 27). Comparative study of children from Western Europe and other developing countries using standardized methods may reveal important risk factors responsible for the higher prevalence of food allergies in the west.

Methods

EuroPrevall-INCO community surveys

The EuroPrevall-INCO survey was a multi-centre, cross-sectional study in random samples of children from the general populations

with a nested case-control design conducted in China, India, and Russia. Primary school children aged 7–10 years were screened for the presence of adverse reactions to foods in seven centers from the three countries. These centers include Hong Kong, urban Beijing and rural Beijing from China, Mysore and Bangalore from India, Tomsk city and rural Tomsk Oblast from Russia (Fig. 1). In rural Tomsk Oblast region, areas with high and low prevalence of an endemic fish parasite, *Opisthorchis felinus*, were included. The main objective of the study was to estimate the prevalence of food allergies in urban and rural areas of China, India, and Russia and the specific objectives of the study are:

1. To determine the prevalence of food allergies among urban and rural communities from the three countries and compare with European data collected by the same standardized fashion
2. To determine the possible risk factors for food allergies and the extent to which they may explain possible variations of prevalence of food allergies between these three countries and other European communities

Identification of children with food allergies

To obtain accurate estimates of the food allergies among children from the three participating countries, a multi-staged approach has been adopted. In line with studies carried out in Europe, we will focus on IgE-mediated allergies to a panel of foods most commonly reported to result in Type I allergic reactions. The published EuroPrevall priority foods ($n = 24$) were assessed in the three countries (28). These foods were hen's egg, cow's milk, peanut, soy, hazelnut, walnut, celery, kiwi, apple, peach, sesame, mustard, wheat, fish, shrimp, buckwheat, corn, carrot, tomato, melon, banana, lentils, sunflower seeds and poppy seeds. Furthermore, additional foods of local importance were included in the panel of foods for assessment. The additional foods of interest were mango and crab for China, jackfruit, eggplant, orange and chickpea for India, and carrot for Russia. The aim of our study was to identify children who (i) report adverse reactions to any of the listed foods above, (ii) evidence of specific food sensitization as determined by skin-prick test or the presence of serum specific IgE. Double-blind placebo-controlled food challenge tests (DBPCFC) to confirm the specificity of reactions to a subset of foods were performed using the same protocol as in other EuroPrevall centres (28).

Sampling strategies

To obtain representative samples of children of the target age, different sampling techniques are needed for various centres from the three countries. In China, primary school attendance is compulsory by law. Therefore, a random sample of children can be recruited through the school system. A complete list of the primary schools was obtained from the Education Departments from Hong Kong, the urban and rural Beijing regions. Schools were individually allocated a number, and by computer randomization, a list of at least 20 schools was generated for recruitment of schoolchildren. Of the schools agreeing to participate in the study, all schoolchildren from primary one to five were invited. A similar strategy was used in the recruitment of subjects in Russia. In India, many of the parents might not be able to read and understand the nature of the study. Recruitment was carried out by house to house visits of households from randomly selected administrative units of the two areas. All children of the target age range from the selected households were recruited for study.

In line with the EuroPrevall methodology (28), the subjects were first screened by a standardized one-page questionnaire to ascertain possible adverse reactions to food. Subsequently, a case-control



Figure 1. Map showing centers taking part in the EuroPrevall-INCO surveys of food allergy.

sample was recruited for completion of a face-to-face questionnaire, skin-prick test, and blood collection for serum specific IgE measurement. Those who reported adverse reactions to food in the screening stage would be considered as cases while those without reported adverse reaction to food were controls. The questionnaire used in the second stage included questions on potential risks and exposures as well as symptoms of other allergic conditions such as asthma, allergic rhinitis, and eczema along with a detailed enquiry of the possible food adverse reactions. The EuroPrevall screening and case-control questionnaires were translated into the local languages and back-translated into English. This involved an independent person who was bilingual to translate the original English EuroPrevall questionnaires into the local languages. Another bilingual person then back translated this into English. The translated questionnaire was then pilot tested in a group of parents, and necessary modifications were made before the survey was carried out in the community.

Skin-prick test and serum IgE analyses

After completion of the detailed case-control questionnaire, all subjects underwent skin-prick test against a panel of food allergens and aero-allergens along with positive and negative controls as described in Table 1. These allergens were selected because pilot studies and clinical experience suggested that they were the most common reported food allergens in regions of the participating centres. Standardized allergen extracts and control solutions were obtained from ALK-Abelló (Madrid, Spain). A drop of each allergen extract was placed on the skin of the volar side of the forearms and pricked through using ALK lancets (Horsholm, Denmark). After 15 min, the weal reaction was measured as the

mean of the longest diameter and the length of the perpendicular line through its middle. All serum samples were sent to a central laboratory for measurement of serum IgE using an ImmunoCAP 250 system (Phadia, Uppsala, Sweden). As in other EuroPrevall centres, serum IgE to twenty-five foods were determined (Box 1). The sera are first tested on five mixtures (two commercially available and three custom-made). Samples testing positive (≥ 0.35 kU_A/l) to a mix were tested on the individual foods of that mix. Additional serum specific IgE measurements were made for foods of local importance and these include mango and crab in China, egg plant and orange in India. Serum specific IgE against the common environmental allergens including house dust mite, cat, birch, grass, mugwort, *Parietaria* pollen, and total IgE was also measured.

Foodborne and orofecal infections

Sera were tested for the presence of common orofecal infections. Total antibodies against hepatitis A and IgG antibodies to salmonella and *Toxoplasma gondii* were measured. Stool samples were collected and tested for the presence of the trematode *Opisthorchis felinus* in rural and urban populations of Tomsk, Russia (29) and for the presence of *Ascaris lumbricoides*, *Strongyloides stercoralis* and hookworms in China and India. The relationship of these foodborne and orofecal infections and the manifestations of food allergies was determined.

mRNA sampling and analyses

EDTA blood aliquoted into RNA buffer (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) is kept frozen at -80°C until

Table 1. Allergen extracts for skin-prick test in the three participating countries

	China	India	Russia
Dustmite mix	X	X	X
Cockroach	X	X	X
Peanut	X	X	X
Cow's milk	X	X	X
Hen's egg	X	X	X
Soy	X	X	X
Fish	X		X
Shrimp	X		
Mussel	X		
Hazelnut	X		X
Walnut	X		
Melon	X	X	X
Cat	X	X	X
Wheat	X	X	X
Lentils		X	
Chickpea		X	
Egg plant		X	
Banana		X	
Sunflower		X	X
Tomato	X	X	X
Jackfruit		X	
Birch pollen			X
Carrot			X
Beef	X		
Orange	X	X	
Apple	X	X	X
Grass mix	X	X	X
Mugwort	X	X	X
Ragweed		X	X
Chenopodium		X	X
Date palm profilin	X	X	X
Crab	X		
Mango	X		
Peach	X		

analysis. The samples were used to measure by quantitative PCR the expression of genes that were involved in pathogen recognition (30, 31), and in amino acid starvation response (32) to indicate how microbial exposure and nutritional status may be related to allergic outcomes.

Sample size calculation

The sample sizes were calculated based on pilot studies in children from China and estimation of the prevalence of food allergies in urban and rural centres of the participating countries (12, 14). The prevalence rates of IgE mediated food allergy in primary school children were estimated to be 4% in urban areas of the participating countries and 2% in rural areas. We wanted the case-control study to have 90% power to detect an odds ratio of 2 given that 20% of the population

was exposed to the risk factor. Given equal numbers of cases and controls, we estimated the need for 230 cases and 230 controls, and set a target of 240 of each. Making the pessimistic assumption that the prevalence was no greater in the urban than the rural area (i.e. 2%), we would need to sample 12 000 people to identify 240 cases, implying 6000 from urban and 6000 from rural centres. A sample of 1527 in each location would have given 90% power to detect a difference between 2% and 4%, so we regarded this sample size as adequate to describe the variation in prevalence between centres.

Administration of questionnaires

The EuroPrevall questionnaires were developed based on relevant pre-existing questionnaires (16), and the questions inquired about possible adverse reactions to specific food and obtained information related to possible risk factor for food allergies. A total of eight other European centres also used the same methodology and questionnaire to study random sample of primary schoolchildren across different climatic areas in Europe (28). The questionnaires were translated into the local languages and pilot tested as described above. For China and Russia, the screening questionnaires were delivered to the schools for distribution to the parents or legal guardians to complete. In India, the screening questionnaire was filled out during face to face interview with the parents or guardians. The clinical questionnaire was administered by trained interviewers according to the standardized Europrevall protocol (28). Informed consents were obtained from the parents or legal guardians prior to the study.

The progress of the study

At the time of writing, all centres had completed the screening phase of the study and data cleaning has been completed. A total of more than 37 000 children have been screened (China: 16 866; India: 7488; Russia: 12 813). The response rates for the screening phase from the different centres have ranged from 83% to 95%. The data collection for the case-control part is expected to be completed by August, 2009. The serum samples are being analysed for IgE by the same central laboratory as in all other Europrevall centres and direct comparison between our centres and the European centres will be possible.

Conclusion

The EuroPrevall-INCO surveys will be the first comparative study of food allergies using standardized methodology in some of the most populous countries in the world. The three participating countries together represent about 40% of the world population. Both rural and urban children were recruited for study such that the possible protection of the rural environment against the development of food allergies can be determined. Subjective symptoms along with objective markers of IgE mediated sensitization were collected. Serum specific IgE was measured by standardized methodology to ensure comparability of the data. We will also be able to compare with other European countries with data collected by the same standardized methodology. The

Box 1. Foods tested in CAP analysis

Commercial mixes

CAP mix 1 (commercial, fx5): hen's egg, cow's milk, soy, peanut, wheat, fish

CAP mix 2 (commercial, fx6): sesame seed, wheat, buckwheat, corn, rice

EuroPrevall custom mixes

CAP mix 3 (custom made): hazelnut, tomato, walnut, carrot, celery

CAP mix 4 (custom made): shrimp, poppy seed, lentil, mustard, sunflower

CAP mix 5 (custom made): apple, kiwi, melon, banana, peach

results of the study will improve our understanding of this common condition and may facilitate the development of preventive strategies for food allergies.

Acknowledgments

This work was funded by the EU through EuroPrevall (FP6-FOOD-CT-2005-514000) and HK Research Grant Council CUHK

4512/06M. The authors wish to thank all the subjects and their parents in taking part in the EuroPrevall-INCO surveys. We also like to acknowledge the generous supply of food allergen extracts for skin-prick test by ALK-Abelló (Madrid, Spain). We also thank Lucia Jimeno and Domingo Barber of ALK-Abelló (Madrid, Spain) for preparation of the allergen extracts. We also thank Jonas Lidholm, Asa Marknell DeWitt, Caroline Pettersson, and Hakan Molander (Phadia, Uppsala) for their development of the custom food mix IgE tests.

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