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# International Variations in Bronchial Responsiveness in Children: Findings From ISAAC Phase Two

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**Summary.** Rationale: Bronchial responsiveness is an objectively measurable trait related to asthma. Its prevalence and association with asthma symptoms among children in many countries are unknown. Objectives: To investigate international variations in bronchial responsiveness (BR) and their associations with asthma symptoms and atopic sensitization. Methods: Bronchial challenge tests were conducted in 6,826 schoolchildren (aged 8–12 years) in 16 countries using hypertonic (4.5%) saline. $FEV_1$ was measured at baseline and after inhalation for 0.5, 1, 2, 4, and 8 min. BR was analyzed both as a dichotomous (bronchial hyperreactivity, BHR, at least 15% decline in $FEV_1$) and as a continuous variable (time–response slope, BR slope, individual decline in $FEV_1$ per log(min)). Results: Prevalence of wheeze last year ranged from 4.4% in Tirana (Albania) to 21.9% in Hawkes Bay (New Zealand) and of BHR from 2.1% in Tirana to 48% in Mumbai (India). The geometric mean BR slope varied between 3.4%/log(min) in Tirana and 12.8%/log(min) in Mumbai and Rome (Italy). At the individual level, BHR was positively associated with wheeze during the past 12 months both in affluent countries (OR = 3.6; 95% CI: 2.7–5.0) and non-affluent countries (OR = 3.0; 1.6–5.5). This association was more pronounced in atopic children. There was a correlation ($\rho = 0.64$, $P = 0.002$) between center-specific mean BR slope and wheeze prevalence in atopic, but not in non-atopic children. Conclusions: BR to saline in children varied considerably between countries. High rates of BR were not confined to affluent countries nor to centers with high prevalences of asthma symptoms. The association between wheeze and BHR at

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the individual level differed across centers and this heterogeneity can be largely explained by effect modification by atopy. **Pediatr Pulmonol. 2010; 45:796–806.** 

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## INTRODUCTION

Many epidemiological studies rely on questionnaires to assess the prevalence of asthma. In an international setting differences in language, culture, and asthma management pose problems in interpreting variations in prevalence between populations.[1–3] In addition to questionnaires, some studies supplement their asthma definition by more objective procedures including bronchial challenge tests.[4] This approach has been used in international comparisons, both among adults[5] and children.[1,6]

Several agents have been applied in bronchial challenge testing including pharmacological stimuli such as histamine and methacholine, and non-pharmacological stimuli, such as cold air, exercise, and saline.[7,8] Hypertonic (4.5%) saline indirectly stimulates inflammatory and neural cells which in turn interact with effector cells (airway smooth muscle, bronchial endothelium, and mucus-producing cells).[7,8] Bronchial responsiveness (BR) to saline stimulation is associated with clinical manifestations of asthma and allergy.[9–11]

Worldwide variations in BR among children and their correlation with questionnaire-based prevalence of asthma have not been widely analyzed. Therefore, in the International Study of Asthma and Allergies in Childhood (ISAAC) Phase Two, bronchial challenge tests with hypertonic saline were performed according to a standardized protocol[12] on children both in affluent and non-affluent countries. In this article, we describe the worldwide variation in this measure of BR and evaluate it as a marker for asthma symptoms with and without atopic sensitization.

## MATERIALS AND METHODS

### Study Population and Field Work

The rationale and methods of the ISAAC Phase Two are described in detail elsewhere.[12,13] Briefly, the study was performed in 35 centers from 22 countries and bronchial challenge with hypertonic saline was conducted in 22 centers from 16 countries. Random samples of at least 1,000 children from $\geq$10 schools within a defined area were drawn. Study modules included questionnaires, bronchial challenge, and skin prick tests. Because hypertonic saline challenge is time consuming it was optional to the centers whether they offered bronchial challenge to all children, a random subsample or a stratified random subsample of a minimum of 100 ''wheezers'' and 100 ''non-wheezers.'' Based on previous results, the latter was estimated to permit detection of prevalence differences in bronchial hyperreactivity (BHR) of 20% versus

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40% between two centers with 80% power at a significance level of 5%.[14] The study protocol was approved by local ethics committees. Informed consent was obtained by at least one parent of each participating child. Participating centers and the applied sampling schemes are shown in Table 1. Children aged 8–12 years were included in the analysis. The fieldwork of bronchial challenge took place between February 1996 and December 2002. Participation rates in the challenge module varied from 32.5% to 100% (median: 76.9%).

## Questionnaires

Standardized, self-administered questionnaires were given to the parents enquiring about the occurrence and severity of asthma symptoms.[13] In countries where literacy was a problem (India, Ghana) a standardized interview replaced the self-administered questionnaire. The question ''Has your child had wheezing or whistling in the chest in the last 12 months?'' determined the stratum for the stratified random sampling and is presented as the main measure of asthma symptoms in our analyses.

**TABLE 1— Data on Fieldwork and Participation by Study Center**

| Country | Study period questionnaire | Characteristic of study area | Questionnaire | Skin prick test | Bronchial challenge | Gross national income per capita (US$) |
|---|---|---|---|---|---|---|
| | | | n[1] (%)[2] | n[1] (%)[2] | n[1] (%)[2] | |
| Albania | | | | | | |
| Tirana | 2–4/1999 | Urban | 1,052 (94.9) | 929 (84.0) | 206 (ND)[3] | 970 |
| Estonia | | | | | | |
| Tallinn | 12/1996–2/1997 | Urban | 971 (83.9) | 642 (55.5) | 244 (71.8)[3] | 3,540 |
| Georgia | | | | | | |
| Tbilisi | 3/2001–6/2002 | Urban | 1,012 (87.7) | 173 (86.5)[3] | 172 (86.0)[3] | 680 |
| Germany | | | | | | |
| Dresden | 9/1995–6/1996 | Urban | 3,023 (82.8) | 2,259 (61.6) | 701 (62.8) | 25,740 |
| Munich | 9/1995–12/1996 | Urban | 3,301 (87.5) | 2,317 (60.6) | 901 (66.0) | |
| Ghana | | | | | | |
| Kintampo | 2–7/2000 | Rural | 1,354 (ND) | 1,322 (ND) | 251 (ND)[3] | 380 |
| Greece | | | | | | |
| Athens | 10/2000–2/2001 | Urban | 985 (85.3) | 985 (85.3) | 195 (90.3)[3] | 11,700 |
| Thessaloniki | 9–11/2001 | Urban | 1,018 (63.0) | 1,018 (63.0) | 212 (88.3)[3] | |
| India | | | | | | |
| Mumbai | 2000–2001 | Urban | 1,658 (ND) | 1,556 (ND) | 121 (ND)[3] | 450 |
| Italy | | | | | | |
| Rome | 10/2000–4/2001 | Urban | 1,354 (83.5) | 1,307 (62.3) | 123 (38.0)[3] | 20,170 |
| Latvia | | | | | | |
| Riga | 5–11/1999 | Urban | 908 (87.4) | 295 (30.8) | 159 (32.5) | 2,570 |
| Netherlands | | | | | | |
| Utrecht | 4/1997–7/1998 | Urban | 3,541 (64.7) | 1,286 (43.3) | 1,103 (43.7) | 25,270 |
| New Zealand | | | | | | |
| Hawkes Bay | 2–6/2000 | Urban/rural | 1,320 (84.3) | 1,288 (82.2) | 229 (72.0)[3] | 13,480 |
| Norway | | | | | | |
| Tromsø | 3–6/2000 | Urban/rural | 3,669 (81.3) | 722 (60.2) | 185 (92.5)[3] | 35,660 |
| Spain | | | | | | |
| Almeria | 3/2000–6/2001 | Urban | 1,126 (49.9) | 1,075 (47.7) | 210 (86.8)[3] | 14,790 |
| Cartagena | 3/2000–3/2001 | Urban | 1,429 (54.6) | 1,030 (39.6) | 160 (93.1)[3] | |
| Madrid | 2/2001–4/2002 | Urban | 981 (35.8) | 653 (23.9) | 425 (100.0)[3] | |
| Valencia | 12/2000–12/2001 | Urban | 1,362 (40.4) | 1,023 (30.5) | 195 (99.0)[3] | |
| Sweden | | | | | | |
| Linköping | 1–4/1997 | Urban | 907 (81.7) | 857 (77.0) | 184 (65.9)[3] | 28,540 |
| Östersund | 1–4/1997 | Urban/rural | 1,195 (86.0) | 991 (71.4) | 279 (76.2)[3] | |
| Turkey | | | | | | |
| Ankara | 10/1999–4/2000 | Urban | 2,976 (87.6) | 2,747 (81.0) | 346 (96.9)[3] | 2,800 |
| United Kingdom | | | | | | |
| West Sussex | 10/1998–07/1999 | Urban/rural | 1,056 (78.6) | 898 (66.7) | 225 (99.1)[3] | 24,070 |
| Total | | | 36,198 | 25,373 | 6,826 | |

n, Number of participants; %, participation in percent; ND, no denominator to assess response rate.
[1]Number of children aged 8–12 years.
[2]Participation rate refers to those who were invited (either full or subsamples).
[3]Stratified disproportional subsample, otherwise: random subsample or full sample.

### Bronchial Challenge

Participating children were asked to withhold bronchodilator medications before the challenge. Regular use of inhaled steroids was recorded, but not withheld. Spirometry was performed according the ATS criteria.[15] At least two spirograms were recorded, and the higher of two reproducible measurements (with <5% variation) of forced expiratory volume in 1 sec ($FEV_1$) was recorded as baseline $FEV_1$. In children with a baseline $FEV_1$ of <75% of the predicted value (n = 103), no bronchial challenge was performed and an inhaled bronchodilator was administered.

BR was assessed by changes in $FEV_1$ during inhalation of nebulized saline from a DeVilbiss UltraNeb 2000 ultrasonic nebulizer.[12,13,16] The children inhaled hyperosmolar (4.5%) saline for periods of increasing duration: 0.5, 1, 2, 4, and 8 min. $FEV_1$ was measured 1 min after each inhalation period and the next challenge period followed after further 3 min. If the $FEV_1$ decreased by 10–15% from the baseline value, the exposure time was repeated. If, after two repetitions, the $FEV_1$ remained 10–15% below the baseline value, the duration of the inhalation period was doubled again according to the protocol. The bronchial challenge was stopped if either the $FEV_1$ had decreased by ≥15% from baseline or the total inhalation period of 15.5 min had been reached. The saline canister and tubing were weighed before and after the challenge in order to measure the total aerosol dose delivered. Study center representatives were trained in one location to assure a standardized performance of the bronchial challenge according to protocol. BR was assessed in two different ways[16]: (1) children with a decline in $FEV_1$ of at least 15% from baseline value or who had an increase of 25% in $FEV_1$ after bronchodilator inhalation (n = 29) were classified to be positive regarding BHR. (2) The time–response slope (BR slope) was calculated for each child by linear regression of percentage decline in $FEV_1$ against log inhalation time.

### Skin Prick Test Reactivity

Skin prick tests were performed according to a detailed standardized protocol[17] with extracts of six common aeroallergens (*Dermatophagoides pteronyssinus*, *D. farinae*, cat dander, *Alternaria tenuis*, mixed tree pollen, and mixed grass pollen) produced by ALK (Hørsholm, Denmark). Additional allergens of local relevance were tested in 18 centers and include: olive pollen, *Parietaria officinalis*, cockroach, dog, mixed moulds, horse, mixed weeds, *Cladosporium*, bird epithelium, and Turkish tree mix.[18] Atopic children with a positive skin reaction were defined as having a wheal size of 3 mm or greater, after subtraction of the negative control.

### Gross National Income

To allow for the difference between environments with "Western" life style and more traditional or rural life style, centers were classified as affluent and non-affluent as in other ISAAC Phase Two publications.[17] Classification was based on the gross national income (GNI) per capita, converted into U.S. dollars, using the World Bank Atlas method.[19] The chosen threshold for affluent was a GNI of ≥$9,200 per capita, according to the World Bank's definition of "high income group".

### Statistical Analyses

In order to obtain a satisfactory approximation to a normal distribution 10 outliers with extreme increase or decrease after the first 0.5 min of stimulation were excluded from the BR slope analysis and the remaining values were transformed by the formula $\log_{10}(\text{slope}* - 1 + 20)$. In the tables, geometric mean values of BR slope were re-transformed to the original scale (%$FEV_1$ decrease per log(min)).

Prevalence rates were calculated for each center by dividing the number of positive responses by the total number of valid responses. Correlations between prevalence rates and averages at the center level were assessed by Spearman's rank correlation coefficient ρ. For the association between an individual's wheeze status and BHR, odds ratios (OR) were determined by logistic regression. In centers with stratified subsamples, means, prevalences, and OR were weighted to account for the sampling scheme using the SURVEY procedures in SAS.[20,21] This method provides unbiased parameter estimates (extrapolated to the population from which the stratified subsamples were drawn) while preserving appropriate standard errors for the weighted estimates. Results from each center were combined by random-effects meta-analysis (DerSimonian–Laird) to allow for both between-center and within-center (sampling) variation.[22] Heterogeneity was assessed by Cochran's Q-test and quantified by the variance component between study centers ($I^2$).[23] All computations were performed using SAS® 9.2 (SAS Institute, Inc., Cary, NC).

## RESULTS

Data from 6,826 children with questionnaire data and bronchial challenge were available from 22 study centers in 16 countries from Europe, Africa, Asia, and Australasia (Table 1). At the time of bronchial challenge, the children were on average 11.1 years old (ranging in the centers from 9.8 to 12.9 years), had a mean body height of 145.5 cm (132–158 cm) and a mean body weight of 40.0 kg (27–49 kg). The proportion of boys was 49.9%, varying from 36% to 60%.

**TABLE 2— Prevalence of Asthma-Related Symptoms, Skin Prick Test Reactivity, and Bronchial Hyperreactivity (BHR[1]) as well as Means of the BR Slope (% $FEV_1$ per Log(Min))**

| | | | BHR | | | Slope of $FEV_1$ decrease | | |
|---|---|---|---|---|---|---|---|---|
| | Wheeze past year | Positive skin prick test[2] | All children | Atopic[3] children | Non-atopic[4] children | All children | Atopic[3] children | Non-atopic[4] children |
| Country | Prevalence rates in %* | | | | | Geometric means* | | |
| Albania | 4.4 | 15.0[5] | 2.1 | 2.4 | 1.8 | 3.4 | 3.9 | 3.2 |
| Tirana | 3.1–5.6 | 12.7–17.3 | 0.3–3.9 | 0.8–4.0 | 0–4.0 | 2.8–3.8 | 2.2–5.7 | 2.5–3.9 |
| Estonia | 8.4 | 14.6[5] | 3.5 | 2.5 | 4.5 | 3.6 | 4.3 | 3.5 |
| Tallinn | 6.7–10.2 | 11.9–17.4 | 1.2–5.8 | –5.2 | 1.0–8.0 | 3.2–4.0 | 3.0–5.7 | 2.9–4.1 |
| Georgia | 9.2 | 33.0[5] | 29.4 | 36.8 | 25.5 | 7.6 | 7.6 | 7.4 |
| Tbilisi | 7.4–11.1 | 25.1–40.9 | 21.9–37.0 | 22.5–51.1 | 16.6–34.5 | 5.2–10.0 | 2.8–13.5 | 5.1–10.0 |
| Germany | 7.9 | 25.7 | 10.5 | 21.5 | 6.5 | 5.0 | 6.9 | 4.3 |
| Dresden | 6.9–8.8 | 23.9–27.5 | 8.2–12.9 | 15.0–28.0 | 4.2–8.9 | 4.4–5.4 | 5.5–8.5 | 3.8–4.7 |
| Munich | 8.3 | 22.3 | 17.9 | 27.0 | 15.0 | 6.8 | 9.5 | 6.1 |
| | 7.3–9.2 | 20.6–24.0 | 15.1–20.6 | 19.8–34.3 | 12.0–18.0 | 6.4–7.2 | 8.0–11.0 | 5.7–6.6 |
| Ghana | 6.4 | 1.7 | 30.4 | None | 30.4 | 9.8 | None | 9.7 |
| Kintampo | 5.1–7.7 | 1.0–2.4 | 23.6–37.3 | Examined | 23.6–37.3 | 8.6–10.8 | Examined | 8.6–10.9 |
| Greece | 5.6 | 14.4[5] | 13.6 | 27.7 | 7.8 | 4.0 | 6.4 | 3.2 |
| Athens | 4.2–7.1 | 12.2–16.6 | 8.3–18.8 | 14.6–40.9 | 3.0–12.7 | 2.8–5.4 | 4.2–8.7 | 1.8–4.7 |
| Thessaloniki | 8.4 | 26.8[5] | 39.9 | 34.8 | 41.6 | 9.4 | 8.1 | 9.7 |
| | 6.7–10.1 | 24.1–29.5 | 32.2–47.7 | 20.7–48.8 | 32.3–51.0 | 7.6–11.2 | 5.5–11.0 | 7.6–12.0 |
| India | 6.1 | 6.4 | 47.8 | 29.9 | 47.4 | 12.8 | 5.9 | 13.0 |
| Mumbai | 4.9–7.3 | 5.2–7.6 | 36.1–59.5 | –69.3 | 34.7–60.2 | 9.2–16.8 | 0.7–12.5 | 9.0–17.6 |
| Italy | 7.9 | 28.9[5] | 33.1[6] | 45.5[6] | 24.8[6] | 12.8 | 19.1 | 9.3 |
| Rome | 6.5–9.4 | 26.5–31.4 | 23.8–42.4 | 27.9–63.1 | 13.9–35.7 | 9.8–16.2 | 11.8–28.1 | 6.5–12.4 |
| Latvia | 6.9 | 19.3 | 13.5[6] | 20.8[6] | 10.3[6] | 6.2 | 10.0 | 5.4 |
| Riga | 5.3–8.6 | 14.8–23.9 | 8.0–18.9 | 3.3–38.4 | 4.7–16.0 | 4.8–7.6 | 4.5–16.7 | 4.1–6.8 |
| Netherlands | 8.7 | 30.9[5] | 19.8[6] | 33.9[6] | 12.2[6] | 7.4 | 10.8 | 6.0 |
| Utrecht | 7.8–9.6 | 28.4–33.5 | 17.4–22.3 | 28.0–39.7 | 9.5–14.8 | 7.0–8.0 | 9.3–12.4 | 5.5–6.5 |
| New Zealand | 21.9 | 34.5 | 23.9 | 34.4 | 19.4 | 7.6 | 11.6 | 6.1 |
| Hawkes Bay | 19.7–24.1 | 31.9–37.2 | 17.9–29.8 | 17.3–51.5 | 12.8–26.0 | 6.2–8.8 | 6.5–17.8 | 5.0–7.2 |
| Norway | 14.0 | 32.7 | 42.7 | 34.3 | 39.9 | 10.8 | 8.9 | 9.6 |
| Tromsø | 12.9–15.2 | 29.3–36.1 | 31.0–54.3 | 10.5–58.1 | 22.8–57.1 | 9.0–12.8 | 5.3–12.9 | 7.0–12.4 |
| Spain | 15.5[6] | 43.0 | 23.7 | 38.3 | 22.9 | 8.4 | 11.2 | 6.4 |
| Almeria | 12.5–16.7 | 40.0–45.9 | 16.5–30.9 | 25.6–51.0 | 13.5–32.2 | 6.4–10.4 | 8.2–14.6 | 4.1–8.9 |
| Cartagena | 11.9[6] | 23.8[5] | 29.7 | 32.7 | 20.1 | 6.8 | 8.8 | 6.2 |
| | 9.3–12.6 | 21.2–26.4 | 22.2–37.1 | 11.6–53.9 | 11.5–28.7 | 5.6–8.2 | 4.7–13.6 | 4.8–7.7 |
| Madrid | 11.6[6] | 34.5[5] | 8.9 | 10.7 | 7.0 | 4.2 | 4.9 | 3.5 |
| | 9.3–13.4 | 30.8–38.1 | 6.4–11.5 | 6.3–15.1 | 3.9–10.1 | 3.6–4.8 | 3.9–6.0 | 2.7–4.2 |
| Valencia | 9.1[6] | 14.3[5] | 24.4 | 41.2 | 19.1 | 8.2 | 11.5 | 7.3 |
| | 8.2–11.4 | 12.1–16.4 | 17.3–31.5 | 18.3–64.1 | 11.7–26.4 | 6.2–10.4 | 6.8–16.9 | 5.1–9.7 |
| Sweden | 7.9 | 19.8[5] | 18.7 | 26.3 | 16.0 | 6.4 | 7.1 | 6.0 |
| Linköping | 6.2–9.7 | 17.2–22.5 | 12.3–25.1 | 9.3–43.3 | 8.9–23.1 | 5.6–7.4 | 4.2–10.3 | 5.1–7.0 |
| Östersund | 10.2 | 26.5[5] | 33.5 | 35.1 | 30.1 | 9.0 | 10.3 | 8.1 |
| | 8.5–12.0 | 23.8–29.3 | 26.0–41.0 | 19.0–51.3 | 20.9–39.4 | 7.8–10.2 | 8.1–12.7 | 6.6–9.7 |
| Turkey | 10.9 | 24.6[5] | 22.4 | 26.8 | 21.0 | 6.4 | 6.9 | 6.2 |
| Ankara | 9.8–12.0 | 23.0–26.2 | 16.8–27.9 | 15.4–38.2 | 14.6–27.3 | 5.2–7.8 | 4.2–9.8 | 4.9–7.7 |
| United Kingdom | 16.2 | 17.5 | 41.4 | 47.7 | 38.4 | 9.4 | 13.6 | 8.4 |
| West Sussex | 13.9–18.4 | 15.0–20.0 | 32.9–49.9 | 19.7–75.7 | 29.0–47.8 | 7.4–11.6 | 5.7–23.8 | 6.5–10.6 |

*Calculations weighted for disproportional subsampling and shown with 95% confidence intervals.
[1]BHR is defined as a decline in $FEV_1$ of at least 15%.
[2]Wheal size of ≥3 mm to at least one of the tested aeroallergens.
[3]Prevalence or slope calculated only in children with a positive skin prick test reactivity (≥3 mm wheal size).
[4]Prevalence or slope calculated only in children with a negative skin prick test reactivity (<3 mm wheal size).
[5]Local allergens were tested in addition to standard set of six common allergens.
[6]The reported frequencies should not be interpreted as prevalence estimates because participation was <60%.

The center-specific prevalences of wheeze, skin prick test reactivity, and BHR, as well as the geometric mean of the BR slope, are listed in Table 2. There was substantial variation in the prevalence of each outcome across the study centers. The prevalence of wheeze in the past year varied from 4.4% (Tirana, Albania) to 21.9% (Hawkes Bay, New Zealand) and of a positive skin prick test from 1.7% (Kintampo, Ghana) up to 43.0% (Almeria, Spain).

There was also a more than 20-fold variation in the prevalence of BHR (2.1% in Tirana, Albania to 47.8% in Mumbai, India). The slope of the individual $FEV_1$ decrease varied from 3.4%/log(min) (in Tirana, Albania) to 12.8%/log(min) (in Rome, Italy and Mumbai, India) with a combined geometric mean of 7.5%/log(min) (Fig. 1). The rank correlation between the center means of the two measures of BR was high ($\rho = 0.93$). Both affluent and non-affluent countries were found within the lower, middle, and upper thirds of the distribution of geometric mean BR slope. The within country variability could be assessed in Spain (four centers), Germany, Greece, and Sweden (each two centers). In these countries (with the exception of Germany) there were centers with significant differences in BR (i.e., geometric mean BR slope above and below the international median and non-overlapping confidence intervals).

The association between BHR and wheeze at the individual level was explored within study centers. Results are presented in Figure 2. There was a positive association of similar magnitude in affluent and non-affluent countries (OR = 3.63 [95% CI: 2.70–4.88] and OR = 2.95 [1.61–5.40], respectively). All associations between BHR and wheeze within centers were positive and most of them were statistically significant, but the strength of the association varied significantly between centers with $I^2$ of 65% for affluent countries and 72% for non-affluent countries.

To elucidate this between-center heterogeneity, effect modification by atopy was investigated. The association between BHR and wheeze was stronger in atopic children than in non-atopic children (Fig. 3), to a similar degree in affluent and non-affluent countries. The heterogeneity between study centers of the BHR–wheeze association was substantially reduced after stratification into atopic and non-atopic children, with $I^2$ values ranging from 20% to 32% (Fig. 3).

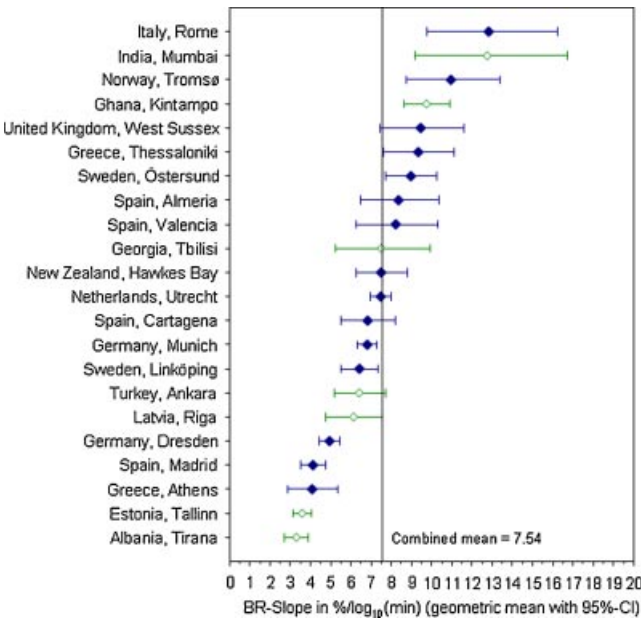


**Fig. 1. Variation in BR slope (decrease of individual $FEV_1$) between study centers (dark, affluent countries; white, non-affluent countries).**

Although BHR was associated with wheeze within centers in both affluent and non-affluent countries, the overall rank correlation at center level between prevalence of wheeze and BR expressed as geometric mean BR slope was weak ($\rho = 0.23$, Fig. 4a). A similar lack of correlation was found between the prevalence of wheeze and the prevalence of BHR across centers ($\rho = 0.31$, $P = 0.16$). After stratification for atopy, there was a significant rank correlation in atopics between the wheeze prevalence and the center geometric mean BR slope of $\rho = 0.64$ ($P = 0.002$), which was consistent in both affluent and non-affluent countries (Fig. 4b). In non-atopics, the equivalent correlation was weaker and non-significant ($\rho = 0.21$, $P = 0.35$), reflecting only a moderate positive correlation at the center level of $\rho = 0.48$ ($P = 0.29$) between the geometric mean BR slope in atopic children and the geometric mean BR slope in non-atopic children (Fig. 5). The few centers with higher BR slope geometric means in non-atopic children (Mumbai, India; Tromsø, Norway; Thessaloniki, Greece) were characterized by a generally high BHR prevalence, a high rate of non-atopic BHR, and a negative association between BHR and skin prick test reactivity at an individual level (data not shown). However, due to low sample sizes within each center the confidence intervals of the center-specific geometric means of the BR slope in atopic and non-atopic children mostly overlap (Table 2).

## DISCUSSION

There were large variations in the prevalence of BR between and within populations and there was a significant association between BR and asthma symptoms at the individual level. However, there was only a modest correlation between BR as determined by challenge with hypertonic saline and wheeze at the center level.

One problem could be misclassification of BR and/or the questionnaire-based reports of asthma symptoms. Bronchial testing reflects the point prevalence of BR at the time of testing, which may be at a time when asthmatic children are free of symptoms and other authors have found that a single test for BR at an arbitrary time point may not be representative for the child's disposition of BR.[24] Comparing summer versus winter time of BR-testing assuming differences in exposure to seasonal inhalant allergens did not reveal distinctions in BHR rates (data not shown). In contrast, the ISAAC questionnaire data assessed period prevalence over 12 months to exclude seasonal and diurnal variations.[25] The questionnaires were

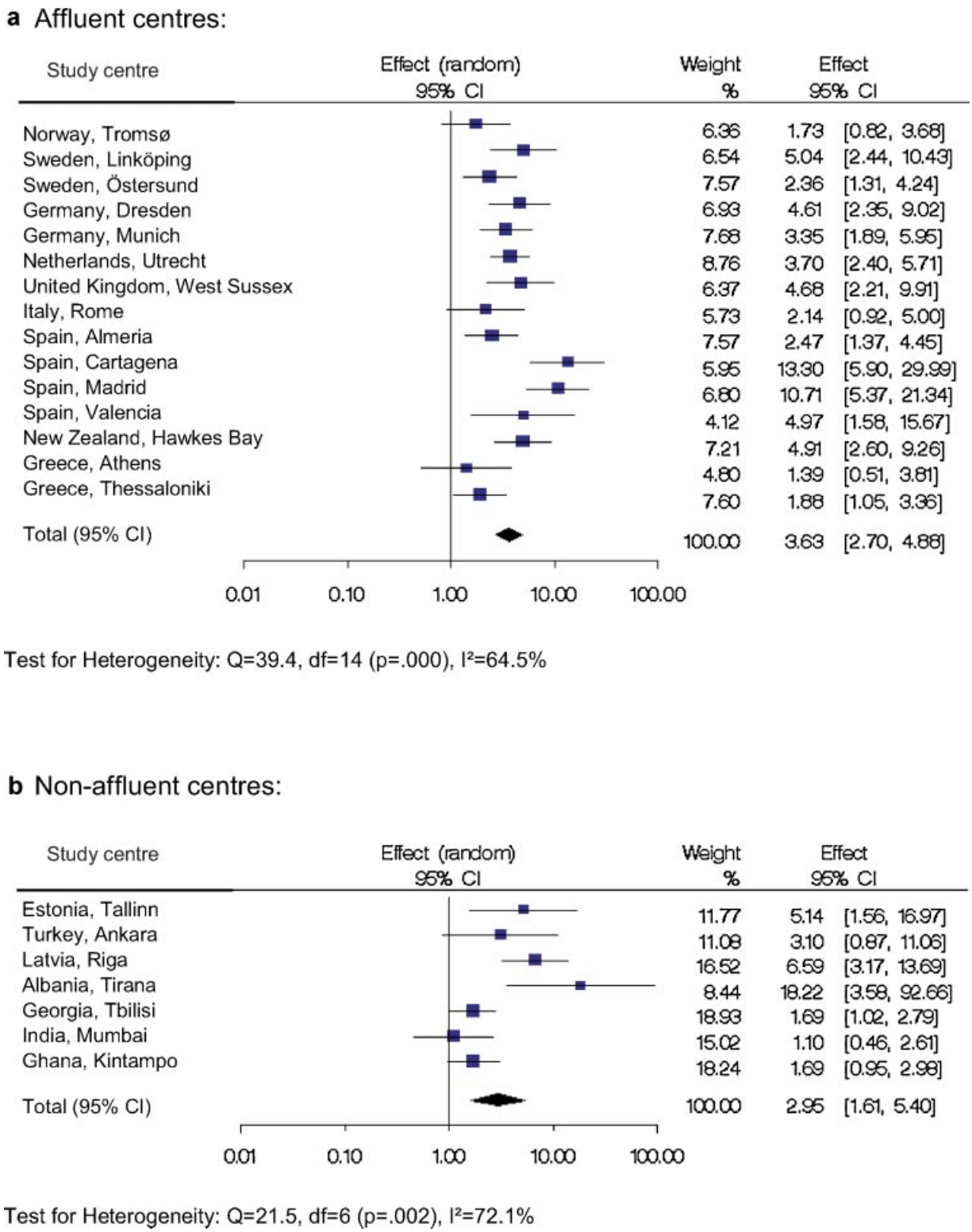


**Fig. 2. Forest plot of the association of wheeze (past year) and bronchial hyperreactivity (defined as a decrease in $FEV_1$ of at least 15%), for (a) affluent and (b) non-affluent countries. Centers are presented in descending order of national per capita GNI.**

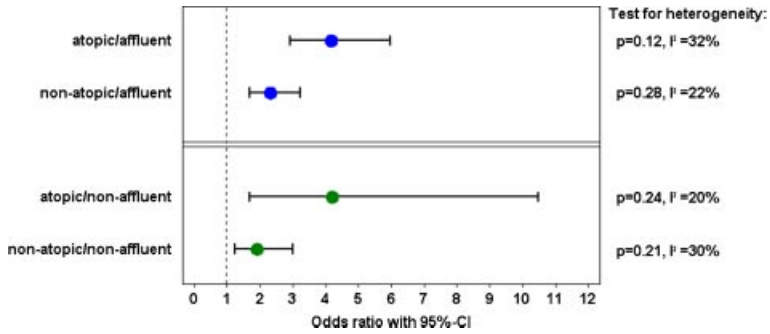


**Fig. 3. Forest plot of the combined association of wheeze (past year) and bronchial hyperreactivity (defined as a decrease in $FEV_1$ of at least 15%), stratified by atopy and affluence.**

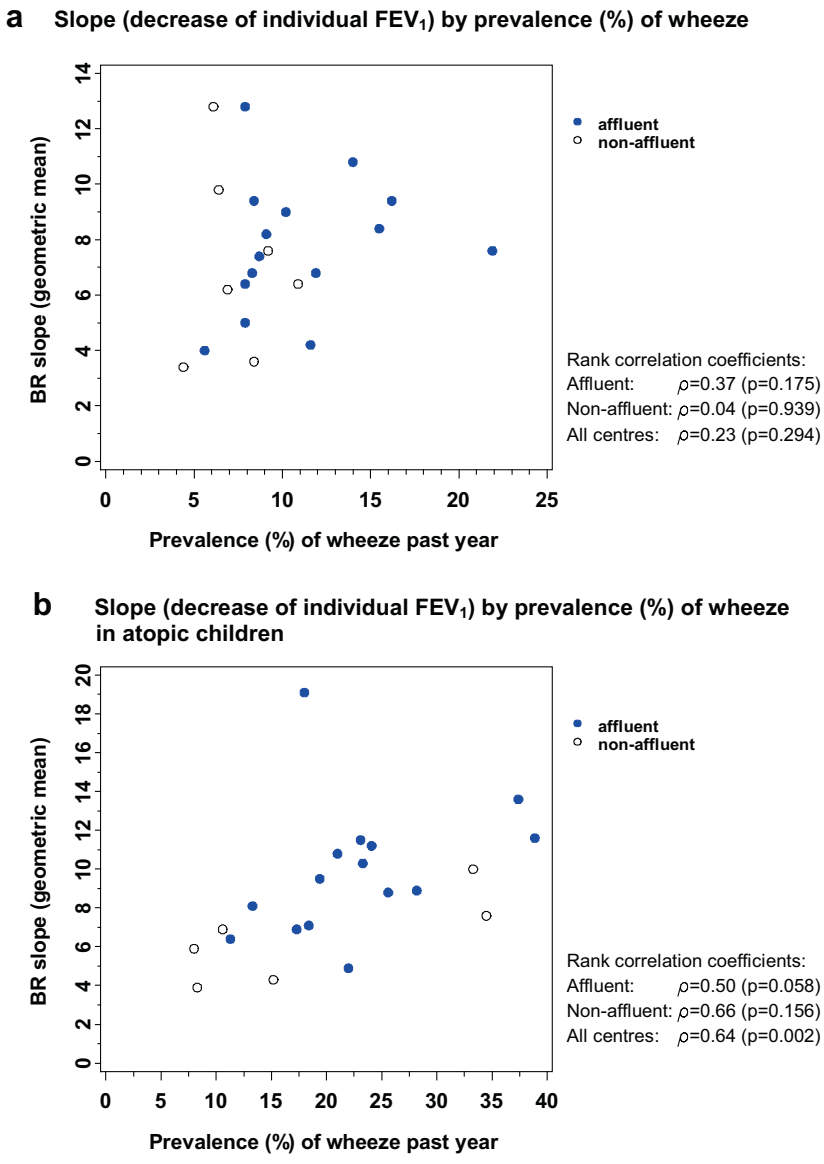


**Fig. 4. Correlations at the center level of mean BR slope versus the prevalence of wheeze in (a) all children and (b) atopic children (with positive skin prick test). BR slope measured as % decrease of individual $FEV_1$ per log(min) inhalation time.**

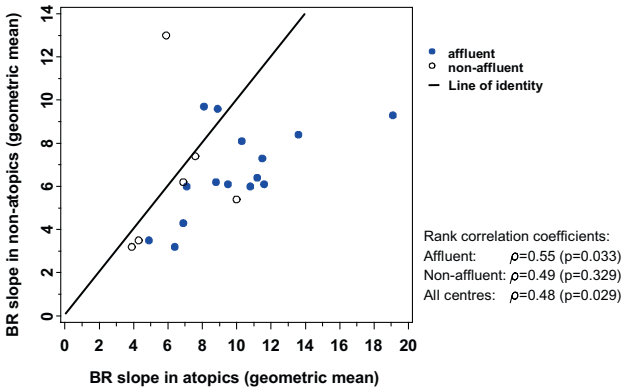


**Fig. 5. Correlation at center level of mean BR slope in atopic children (with positive skin prick test) versus mean BR slope in non-atopic children. BR slope measured as % decrease of individual $FEV_1$ per log(min) inhalation time.**

completed by the parents on average half a year prior than the bronchial challenge test. However, this would tend to affect the association between BR and wheeze more on the individual level than on a population level.

Our questionnaire focused on asthma symptoms rather than on asthma diagnosis since in an international context, the reporting and labeling of diagnoses such as asthma may depend substantially on the local habits.[2] Questions regarding wheeze are widely used in epidemiological studies and have good validity at the individual level as compared to a physician's clinical examination or video questionnaire.[26–28]

Saline was chosen as stimulus in the Phase Two of ISAAC due to its safety, high acceptance by the parents, and availability in centers with diverse economic and environmental conditions.[29] Although there was only a moderate correlation between the nebulized amount of saline and the time of inhalation, the time-based slope showed a good ability to differentiate between asthmatic and non-asthmatic children.[16] The international variability in saline-induced BR was also seen for exercise-induced bronchial reactivity, which was performed additionally in two countries representing the extremes of the worldwide distribution of the frequency of asthma symptoms in ISAAC Phase One and Phase Two, that is, Albania with extreme low prevalence rates and UK with consistently high rates. A reduction in the peak expiratory flow rate of at least 15% after exercise provocation was found about seven times more often in UK than in Albania.[30]

As compared to methacholine challenges, hypertonic saline has a lower sensitivity to detect asthma but is more closely related to allergic asthmatic airway inflammation in adults.[31] This may explain the stronger association between BHR and wheeze on an individual level in atopic children both in affluent and non-affluent countries. Heterogeneity between study centers decreased considerably when atopic and non-atopic children were analyzed separately. Thus, there was a stronger correlation among atopic children at the center level between mean BR slope and prevalence of wheeze. Effect modification by atopy therefore appears to be an important facet in explaining the association between BR to saline and asthma symptoms, both at the individual and population levels. This measure of BR may therefore be indicating a specific atopic asthma phenotype.

Potentially, centers with low or undefined participation rates may not be representative of the population studied. We checked for potential selection bias by comparing the distributions of sex, asthma, eczema, rhinitis, older siblings, and parental atopy and found no evidence that the children participating in the bronchial challenge would not be representative of all eligible children. A sensitivity analysis excluding centers with participation rates below 60% did not alter the conclusions above. The population-based survey in each center within ISAAC Phase Two provided a known sampling frame for cost efficient nested case–control studies enabling us to determine objective markers of disease in informative subgroups across a broad range of countries. Due to the random sampling procedure all subsamples can be considered representative for the underlying population. The applied weights in the analyses of stratified subsamples do not only account for the sampling frame but also for the non-response of the respective item.

Some of the study centers showed a similar ranking for wheeze and BR, for example, low prevalence for both in Tirana, Albania and high prevalence in West Sussex, UK. Some of the centers with high levels of BR, for example, Mumbai in India, and Kintampo in Ghana, showed low prevalence rates for wheeze and positive skin prick tests. The high prevalence of BHR in the study centers in Ghana and India confirms previous reports of high prevalence rates of BHR among children in less affluent areas in Estonia[32] and Western Australia.[33] A study in Indian children suggested that exposure to cooking smoke from solid biomass fuel is significantly associated with a decline in lung function and a higher prevalence of doctor-diagnosed asthma and of other respiratory diseases.[34] Due to the low prevalence of smoking in India in general (16%), and especially amongst women, a contribution of environmental tobacco smoke to the high prevalence of BHR in India seems unlikely.[35] da Silva et al.[36] referred to non-atopic asthma as the predominant phenotype in non-affluent parts of Latin America and attributed this to a high prevalence of infection with helminths. Non-atopic BHR in children often occurs transiently as a reaction to upper respiratory infections, which may be more common in less affluent communities.[37] Overall, it is likely that there is a complex interaction of BR and atopy in causing wheeze in differing environments with different environmental exposures and genetic backgrounds.

In the European Community Respiratory Health Survey (ECRHS) BR was measured in 13,161 adults (aged 20–44 years) in 35 study centers and reported also a wide variation in the prevalence of BHR (defined as a $PD_{20} \leq 1$ mg methacholine) ranging from 3.4% in Galdakao, Spain up to 27.8% in Hawkes-Bay, New Zealand.[38] Although prevalence rates from ECRHS cannot be directly compared to our results, the ranking of comparable centers in same countries partly agreed for some countries, for example, moderate prevalence rates in the Netherlands, high rates in the United Kingdom, and a broad range in Spanish centers. However, in contrast to our findings Norway showed low prevalence rates and New Zealand and Germany high rates of BR in ECRHS. In adults from Estonia (Tartu) a moderate BHR prevalence was measured[39] but Estonian children from Tallinn showed a very low prevalence in ISAAC Phase Two. Norrman et al.[40] discussed the lack of within country variability in Swedish adults, whereas in this analysis children from Östersund and Linköping showed statistically significant differences in the geometric mean BR slope.

Among non-European centers, Mumbai had the highest BHR prevalence in ISAAC Phase Two. In adults, the rate of ''positive'' challenge at $PD_{20} \leq 2$ mg methacholine in Mumbai was only 14%[35] as compared to a median value of 13.0% in the remaining ECRHS centers where BHR was defined (more exclusively) as $PD_{20} \leq 1$ mg.[38] In contrast, for young adults in Brazil, a high BHR prevalence of 31.3% was reported while 10-year-old children in Brazil showed a moderate BHR rate of 16.0% according to ISAAC Phase Two criteria.[36,41]

Differences between our findings and the published international results on adults must be interpreted with caution and may arise from various sources. Firstly, the comparison of prevalence rates in school children and adults may be affected by cohort differences, in that the participants in ECRHS were born several decades earlier than the ISAAC children, and therefore lower prevalence rates might be expected.[42–44] Secondly, some phenotypes of childhood BHR like transient BHR or non-atopic BHR may disappear during adolescence as there is commonly remission of wheeze at this time.[45] Thirdly, the choice of the stimulus in the BR tests may influence the results if, as suggested,[31] response to saline provocation is more specifically related to allergic airway inflammation and there are marked differences in the balance of atopic and non-atopic asthma across the centers being compared. Finally, comparisons may be confounded by the choice of the participating study center against the background of the variability in the level of BR between centers within countries.

In conclusion, this is the first large study to assess BR to hypertonic saline measured by a standardized protocol in combination with questionnaire reports of asthma symptoms and skin prick test reactivity. Association was investigated in children at both an individual level and population level in diverse settings. There was considerable variation in the level of BR between different countries and also between centers within countries. Although there was a clear association of BR with asthma symptoms within individuals, the between-center variability of wheeze prevalence cannot be explained by the level of BR in the population. However, among atopic children, where the association of BR and wheeze was stronger at the individual level, the centers with higher levels of BR also tended to have higher prevalences of asthma symptoms.

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