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International variations in bronchial responsiveness in children: Findings from ISAAC Phase Two

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Keywords:	bronchial responsiveness, bronchial challenge, hypertonic saline, asthma, children, atopy, ISAAC Phase Two

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For Peer Review

International variations in bronchial responsiveness in children:**Findings from ISAAC Phase Two**

Gisela Büchele, MPH¹, Jon Genuneit¹, Gudrun Weinmayr, Ph.D.¹, Bengt Björkstén Ph.D.², Ulrike Gehring, Ph.D.³, Erika von Mutius, MD⁴, Alfred Priftanji, FRCP⁵, Renato T. Stein, Ph.D.⁶, Emmanuel O. Addo-Yobo, MD⁷, Kostas N. Priftis, Ph.D.⁸, Jayant R. Shah, MD⁹, Francesco Forastiere, Ph.D.¹⁰, Vija Svabe, MD¹¹, Julian Crane, MB, BS¹², Wenche Nystad, Ph.D.¹³, Luis García-Marcos, MD¹⁴, Yıldız Saraçlar, MD¹⁵, Nuha El-Sharif, Ph.D.¹⁶, David P. Strachan, MD¹⁷ and the ISAAC Phase Two Study Group

¹ Institute of Epidemiology, University of Ulm, Germany

² Institute of Environmental Medicine, Karolinska Institutet Stockholm, Sweden

³ Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

⁴ Children's Hospital, University of Munich, Germany

⁵ Department of Allergology and Clinical Immunology, University Hospital Center "Mother Teresa", University of Tirana, Albania

⁶ Departamento de Pediatria, Faculdade de Medicina, Pontificia Universidade Catolica, Porto Alegre, Brazil

⁷ Department of Child Health, Komfo Anokye Teaching Hospital, Kumasi, Ghana

⁸ Department of Allergy-Pneumonology, Penteli Children's Hospital, P. Penteli, Greece

⁹ Jaslok Hospital & Research Centre, Mumbai, India

¹⁰ Department of Epidemiology, Rome E Health Authority, Rome, Italy

¹¹ Children's Hospital, Riga, Latvia

¹² Department of Medicine, Wellington School of Medicine and Health Science, New Zealand

¹³ Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway

¹⁴ Pediatric Pulmonology and Allergy Units, Virgen de la Arrixaca University Children's Hospital, University of Murcia, Spain

¹⁵ Hacettepe University, Faculty of Medicine, Pediatric Allergy and Asthma Unit, Ankara, Turkey.

¹⁶ Faculty of Public Health, Alquds University, Jerusalem, Israel

¹⁷ Division of Community Health Sciences, St. George's, University of London, United Kingdom

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Correspondence address: Gisela Büchele
Ulm University
Institute of Epidemiology
Helmholtzstraße 22
89081 Ulm
Germany
Tel: +49 731 5031072
Fax: +49 731 5031069
Email: gisela.buechele@uni-ulm.de

Abbreviated title: BR in children

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For Peer Review

Summary

Rationale: Bronchial responsiveness is an objectively measurable trait related to asthma. Its prevalence and association with asthma symptoms among children in many countries is 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. FEV₁ was measured at baseline and after inhalation for 0.5, 1, 2, 4, and 8 minutes. Bronchial responsiveness was analysed both as a dichotomous (bronchial hyperreactivity, BHR, at least 15% decline in FEV₁) and as a continuous variable (time-response-slope, BR-slope, individual decline in FEV₁ 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 centre-specific mean BR-slope and wheeze prevalence in atopic, but not in non-atopic children.

Conclusions: Bronchial responsiveness to saline in children varied considerably between countries. High rates of BR were not confined to affluent countries nor to centres with high prevalences of asthma symptoms. The association between wheeze and BHR at the individual level differed across centres and this heterogeneity can be largely explained by effect modification by atopy.

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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 paper, 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 II are described in detail elsewhere [12;13]. Briefly, the study was performed in 35 centres from 22 countries and bronchial challenge with hypertonic saline was conducted in 22 centres from 16 countries. Random samples of at least 1000 children from ≥ 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 centres 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 % vs. 40 % between two centres 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 centres 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.

1
2 *Bronchial challenge.* Participating children were asked to withhold
3
4 bronchodilator medications before the challenge. Regular use of inhaled steroids was
5
6 recorded, but not withheld. Spirometry was performed according the ATS criteria [15].
7
8 At least two spiromograms were recorded, and the higher of two reproducible
9
10 measurements (with less than 5 % variation) of forced expiratory volume in one
11
12 second (FEV₁) was recorded as baseline FEV₁. In children with a baseline FEV₁ of
13
14 <75% of the predicted value (n=103), no bronchial challenge was performed and an
15
16 inhaled bronchodilator was administered.

17
18 Bronchial responsiveness was assessed by changes in FEV₁ during inhalation
19
20 of nebulised saline from a DeVilbiss UltraNeb 2000 ultrasonic nebuliser [12;13;16].
21
22 The children inhaled hyperosmolar (4.5 %) saline for periods of increasing duration:
23
24 0.5, 1, 2, 4, and 8 minutes. FEV₁ was measured 1 min after each inhalation period
25
26 and the next challenge period followed after further 3 min. If the FEV₁ decreased by
27
28 10-15 % from the baseline value, the exposure time was repeated. If, after two
29
30 repetitions, the FEV₁ remained 10-15 % below the baseline value, the duration of the
31
32 inhalation period was doubled again according to the protocol. The bronchial
33
34 challenge was stopped if either the FEV₁ had decreased by ≥ 15 % from baseline or
35
36 the total inhalation period of 15.5 min had been reached. The saline canister and
37
38 tubing were weighed before and after the challenge in order to measure the total
39
40 aerosol dose delivered. Study centre representatives were trained in one location to
41
42 assure a standardized performance of the bronchial challenge according to protocol.
43
44 BR was assessed in two different ways [16]: 1) Children with a decline in FEV₁ of at
45
46 least 15 % from baseline value or who had an increase of 25 % in FEV₁ after
47
48 bronchodilator inhalation (n=29) were classified to be positive regarding BHR. 2) The
49
50 time-response-slope (BR-slope) was calculated for each child by linear regression of
51
52 percentage decline in FEV₁ against log inhalation time.

1
2 *Skin prick test reactivity.* Skin prick tests were performed according to a detailed
3
4 standardised protocol [17] with extracts of six common aeroallergens
5
6 (*Dermatophagoides pteronyssinus*, *D.farinae*, cat dander, *Alternaria tenuis*, mixed
7
8 tree pollen and mixed grass pollen) produced by ALK (Hørsholm, Denmark).
9
10 Additional allergens of local relevance were tested in eighteen centres and include:
11
12 olive pollen, *Parietaria officinalis*, cockroach, dog, mixed moulds, horse, mixed
13
14 weeds, *Cladosporium*, bird epithelium, and Turkish tree mix [18]. Atopic children with
15
16 a positive skin reaction were defined as having a wheal size of 3 mm or greater, after
17
18 subtraction of the negative control.

19
20 *Gross National Income.* To allow for the difference between environments with
21
22 “Western” life style and more traditional or rural life style, centres were classified as
23
24 affluent and non-affluent as in other ISAAC Phase Two publications [17].
25
26 Classification was based on the gross national income (GNI) per capita, converted
27
28 into U.S. dollars, using the World Bank Atlas method [19]. The chosen threshold for
29
30 affluent was a GNI of > \$9,200 per capita, according to the World Bank’s definition of
31
32 “high income group”.
33
34

35 *Statistical analyses*

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37 In order to obtain a satisfactory approximation to a normal distribution ten
38
39 outliers with extreme increase or decrease after the first 0.5 minute of stimulation
40
41 were excluded from the BR-slope analysis and the remaining values were
42
43 transformed by the formula $\log_{10}(\text{slope}^* - 1 + 20)$. In the tables, geometric mean values
44
45 of BR-slope were re-transformed to the original scale (%FEV₁ decrease per
46
47 log(min)).
48

49
50 Prevalence rates were calculated for each centre by dividing the number of
51
52 positive responses by the total number of valid responses. Correlations between
53

1
2 prevalence rates and averages at the centre level were assessed by Spearman's
3
4 rank correlation coefficient ρ . For the association between an individual's wheeze
5
6 status and BHR, odds ratios (OR) were determined by logistic regression. In centres
7
8 with stratified sub-samples, means, prevalences, and odds ratios were weighted to
9
10 account for the sampling scheme using the SURVEY-procedures in SAS [20;21].
11
12 This method provides unbiased parameter estimates (extrapolated to the population
13
14 from which the stratified sub-samples were drawn) while preserving appropriate
15
16 standard errors for the weighted estimates. Results from each centre were combined
17
18 by random-effects meta-analysis (DerSimonian-Laird) to allow for both between-
19
20 centre and within-centre (sampling) variation [22]. Heterogeneity was assessed by
21
22 Cochran's Q-test and quantified by the variance component between study centres
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24 (I^2) [23]. All computations were performed using SAS® 9.2. (SAS Institute Inc., Cary,
25
26 NC, USA.).
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Results

Data from 6,826 children with questionnaire data and bronchial challenge were available from 22 study centres 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 centres from 9.8 to 12.9 yr.), had a mean body height of 145.5 cm (132 to 158 cm) and a mean body weight of 40.0 kg (27 to 49 kg). The proportion of boys was 49.9 %, varying from 36 % to 60 %.

The centre-specific prevalences of wheeze, skin prick test reactivity, and bronchial hyperreactivity (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 centres. 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 FEV₁ 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) (figure 1). The rank correlation between the centre means of the two measures of bronchial responsiveness was high ($p=0.93$). 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 (4 centres), Germany, Greece, and Sweden (each 2 centres). In these countries (with the exception of Germany) there were centres with significant differences in BR (i.e. geometric mean BR-slope above and below the international median and non-overlapping confidence intervals).

1
2 The association between BHR and wheeze at the individual level was explored
3
4 within study centres. Results are presented in figure 2. There was a positive
5
6 association of similar magnitude in affluent and non-affluent countries (OR=3.63
7
8 [95 %-CI: 2.70-4.88] and OR=2.95 [1.61-5.40], respectively). All associations
9
10 between BHR and wheeze within centres were positive and most of them were
11
12 statistically significant, but the strength of the association varied significantly between
13
14 centres with I^2 of 65 % for affluent countries and 72 % for non-affluent countries.

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16 To elucidate this between-centre heterogeneity, effect modification by atopy
17
18 was investigated. The association between BHR and wheeze was stronger in atopic
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20 children than in non-atopic children (figure 3), to a similar degree in affluent and non-
21
22 affluent countries. The heterogeneity between study centres of the BHR-wheeze
23
24 association was substantially reduced after stratification into atopic and non-atopic
25
26 children, with I^2 values ranging from 20 % to 32 % (figure 3).

27
28 Although BHR was associated with wheeze within centres in both affluent and
29
30 non-affluent countries, the overall rank correlation at centre level between prevalence
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32 of wheeze and bronchial responsiveness expressed as geometric mean BR-slope
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34 was weak ($\rho=0.23$, figure 4a). A similar lack of correlation was found between the
35
36 prevalence of wheeze and the prevalence of BHR across centres ($\rho=0.31$, $\rho=0.16$).
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38 After stratification for atopy, there was a significant rank correlation in atopics
39
40 between the wheeze prevalence and the centre geometric mean BR-slope of $\rho=0.64$
41
42 ($\rho=0.002$), which was consistent in both affluent and non-affluent countries
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44 (figure 4b). In non-atopics, the equivalent correlation was weaker and non-significant
45
46 ($\rho=0.21$, $\rho=0.35$), reflecting only a moderate positive correlation at the centre level of
47
48 $\rho=0.48$ ($\rho=0.29$) between the geometric mean BR-slope in atopic children and the
49
50 geometric mean BR-slope in non-atopic children (figure 5). The few centres with
51
52 higher BR-slope geometric means in non-atopic children (Mumbai, India; Tromsø,

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2 Norway; Thessaloniki, Greece) were characterised by a generally high BHR
3
4 prevalence, a high rate of non-atopic BHR, and a negative association between BHR
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6 and skin prick test reactivity at an individual level (data not shown). However, due to
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8 low sample sizes within each centre the confidence intervals of the centre-specific
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10 geometric means of the BR-slope in atopic and non-atopic children mostly overlap
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12 (table 2).
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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, but not at the level of study centres. However, there was only a modest correlation between BR as determined by challenge with hypertonic saline and wheeze at the centre 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 vs. 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 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 labelling 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 centres with diverse economic and

1
2 environmental conditions [29]. Although there was only a moderate correlation
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4 between the nebulized amount of saline and the time of inhalation, the time-based
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6 slope showed a good ability to differentiate between asthmatic and non-asthmatic
7
8 children [16]. The international variability in saline-induced BR was also seen for
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10 exercise-induced bronchial reactivity which was performed additionally in two
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12 countries representing the extremes of the worldwide distribution of the frequency of
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14 asthma symptoms in ISAAC Phase One and Phase Two, i.e. Albania with extreme
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16 low prevalence rates and UK with consistently high rates. A reduction in the peak
17
18 expiratory flow rate of at least 15 % after exercise provocation was found about 7
19
20 times more often in UK than in Albania [30].

21
22 As compared to methacholine challenges, hypertonic saline has a lower
23
24 sensitivity to detect asthma but is more closely related to allergic asthmatic airway
25
26 inflammation in adults [31]. This may explain the stronger association between BHR
27
28 and wheeze on an individual level in atopic children both in affluent and non-affluent
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30 countries. Heterogeneity between study centres decreased considerably when atopic
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32 and non-atopic children were analysed separately. Thus, there was a stronger
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34 correlation among atopic children at the centre level between mean BR-slope and
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36 prevalence of wheeze. Effect modification by atopy therefore appears to be an
37
38 important facet in explaining the association between BR to saline and asthma
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40 symptoms, both at the individual and population levels. This measure of BR may
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42 therefore be indicating a specific atopic asthma phenotype.

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44 Potentially, centres with low or undefined participation rates may not be
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46 representative of the population studied. We checked for potential selection bias by
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48 comparing the distributions of sex, asthma, eczema, rhinitis, older siblings, and
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50 parental atopy and found no evidence that the children participating in the bronchial
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52 challenge would not be representative of all eligible children. A sensitivity analysis

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2 excluding centres with participation rates below 60 % did not alter the conclusions
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4 above. The population based survey in each centre within ISAAC Phase II provided a
5 known sampling frame for cost efficient nested case-control-studies enabling us to
6 determine objective markers of disease in informative subgroups across a broad
7 range of countries. Due to the random sampling procedure all subsamples can be
8 considered representative for the underlying population. The applied weights in the
9 analyses of stratified subsamples do not only account for the sampling frame but also
10 for the non-response of the respective item.
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18 Some of the study centres showed a similar ranking for wheeze and BR, e.g.
19 low prevalence for both in Tirana, Albania and high prevalence in West Sussex, UK.
20
21 Some of the centres with high levels of BR, e.g. Mumbai in India, and Kintampo in
22 Ghana, showed low prevalence rates for wheeze and positive skin prick tests. The
23 high prevalence of BHR in the study centres in Ghana and India confirms previous
24 reports of high prevalence rates of BHR among children in less affluent areas in
25 Estonia [32] and Western Australia [33]. A study in Indian children suggested that
26 exposure to cooking smoke from solid biomass fuel is significantly associated with a
27 decline in lung function and a higher prevalence of doctor-diagnosed asthma and of
28 other respiratory diseases [34]. Due to the low prevalence of smoking in India in
29 general (16 %), and especially amongst women, a contribution of environmental
30 tobacco smoke to the high prevalence of BHR in India seems unlikely [35]. Da Silva
31 et al. referred to non-atopic asthma as the predominant phenotype in non-affluent
32 parts of Latin America and attributed this to a high prevalence of infection with
33 helminths [36]. Non-atopic BHR in children often occurs transiently as a reaction to
34 upper respiratory infections which may be more common in less affluent communities
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49 [37]. Overall, it is likely that there is a complex interaction of BR and atopy in causing
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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 yrs) in 35 study centres 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 centres in same countries partly agreed for some countries, e.g. moderate prevalence rates in the Netherlands, high rates in the United Kingdom, and a broad range in Spanish centres. 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. discussed the lack of within country variability in Swedish adults [40], whereas in this analysis children from Östersund and Linköping showed statistically significant differences in the geometric mean BR-slope.

Among non-European centres, 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 centres 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

1
2 cohort differences, in that the participants in ECRHS were born several decades
3
4 earlier than the ISAAC children, and therefore lower prevalence rates might be
5
6 expected [42-44]. Secondly, some phenotypes of childhood BHR like transient BHR
7
8 or non-atopic BHR may disappear during adolescence as there is commonly
9
10 remission of wheeze at this time [45]. Thirdly, the choice of the stimulus in the BR
11
12 tests may influence the results if, as suggested [31], response to saline provocation is
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14 more specifically related to allergic airway inflammation and there are marked
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16 differences in the balance of atopic and non-atopic asthma across the centres being
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18 compared. Finally, comparisons may be confounded by the choice of the participating
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20 study centre against the background of the variability in the level of BR between
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22 centres within countries.
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26 In conclusion, this is the first large study to assess bronchial responsiveness
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28 (BR) to hypertonic saline measured by a standardised protocol in combination with
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30 questionnaire reports of asthma symptoms and skin prick test reactivity. Association
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32 were investigated in children at both an individual level and population level in
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34 diverse settings. There was considerable variation in the level of BR between
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36 different countries and also between centres within countries. Although there was a
37
38 clear association of BR with asthma symptoms within individuals, the between-centre
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40 variability of wheeze prevalence cannot be explained by the level of BR in the
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42 population. However, among atopic children, where the association of BR and
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44 wheeze was stronger at the individual level, the centres with higher levels of BR also
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46 tended to have higher prevalences of asthma symptoms.
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2 Conflicts of Interest Statement:

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4 None of the authors has a financial relationship with a commercial entity that has an
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6 interest in the subject of this manuscript.
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9

10 The ISAAC Phase Two Study group consists of:

11 The ISAAC Phase Two Coordinating and Data Centre: S.K. Weiland † (Director),
12 G. Büchele, C. Dentler, A. Kleiner, P. Rzehak, G. Weinmayr (Institute of
13 Epidemiology, Ulm University, Ulm, Germany).
14
15
16
17

18
19 The principal investigators: A. Priftanji, A. Shkurti, J. Simenati, E. Grabocka, K. Shyti,
20 S. Agolli, A. Gurakuqi (Tirana, Albania); R.T. Stein, M. Urrutia de Pereira,
21 M.H. Jones, P.M. Pitrez (Uruguaiana, Brazil); P.J. Cooper, M. Chico (Pichincha
22 province, Ecuador); Y.Z. Chen (Beijing, China); N.S Zhong (Guangzhou, China);
23 C.K.W. Lai (National Coordinator), G.W.K. Wong (Hong Kong, China); M-A. Riikjärv,
24 T. Annus (Tallinn, Estonia); I. Annesi-Maesano (Créteil, France); M. Gotua,
25 M. Rukhadze, T. Abramidze, I. Kvachadze, L. Karsanidze, M. Kiladze, N. Dolidze
26 (Tbilisi, Georgia); W. Leupold, U. Keil, E. von Mutius, S.K. Weiland † (Dresden,
27 Germany); E. von Mutius, U. Keil, S.K. Weiland † (Munich, Germany); P. Arthur †,
28 E. Addo-Yobo (Kintampo, Ghana); C. Gratziou (National Coordinator), K. Priftis,
29 A. Papadopoulou, C. Katsardis (Athens, Greece); J. Tsanakas, E. Hatziagorou,
30 F. Kirvassilis (Thessaloniki, Greece); M. Clausen (Reykjavik, Iceland); J.R. Shah,
31 R.S. Mathur, R.P. Khubchandani, S. Mantri (Mumbai, India); F. Forastiere,
32 R. Di Domenicantonio, M. De Sario, S. Sammarro, R. Pistelli, M.G. Serra, G. Corbo,
33 C.A. Perucci (Rome, Italy); V. Svabe, D. Sebre, G. Casno, I. Novikova, L. Bagraade
34 (Riga, Latvia); B. Brunekreef, D. Schram, G. Doekes, P.H.N. Jansen-van Vliet,
35 N.A.H. Janssen, F.J.H. Aarts, G. de Meer (Utrecht, the Netherlands); J. Crane,
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

1
2 K. Wickens, D. Barry (Hawkes Bay, New Zealand); W. Nystad, R. Bolle, E. Lund
3
4 (Tromsø, Norway); J. Batlles Garrido, T. Rubi Ruiz, A. Bonillo Perales,
5
6 Y. Gonzalez Jiménez, J. Aguirre Rodriguez, J. Momblan de Cabo,
7
8 A. Losilla Maldonado, M. Daza Torres (Almeria, Spain); L. García-Marcos (National
9
10 Coordinator), A. Martinez Torres, J.J. Guillén Pérez, A. Piñana López,
11
12 S. Castejon Robles (Cartagena, Spain); G. García Hernandez, A. Martinez Gimeno,
13
14 A.L. Moro Rodríguez, C. Luna Paredes, I. Gonzalez Gil (Madrid, Spain);
15
16 M.M. Morales Suarez-Varela, A. Llopis González, A. Escribano Montaner,
17
18 M. Tallon Guerola (Valencia, Spain); L. Bråbäck (National Coordinator), M. Kjellman,
19
20 L. Nilsson, X-M. Mai (Linköping, Sweden); L. Bråbäck, A. Sandin (Östersund,
21
22 Sweden); Y. Saraçlar, S. Kuyucu, A. Tuncer, C. Saçkesen, V. Sumbulöglu, P. Geyik,
23
24 C. Kocabas, (Ankara, Turkey); D.P. Strachan, B. Kaur (West Sussex, UK);
25
26 N. El-Sharif, B. Nemery, F. Barghuthy, S. Abu Huij, M. Qlebo (Ramallah, West Bank).
27

28
29
30 The ISAAC Steering Committee: N. Ait-Khaled (Paris, France); H.R. Anderson and
31
32 D.P. Strachan* (London, UK); C. Flohr* and H. Williams (Nottingham, UK);
33
34 F. Forastiere* (Rome, Italy); I. Asher, P. Ellwood, A. Stewart and E. Mitchell
35
36 (Auckland, New Zealand); J. Crane, N. Pearce and R. Beasley (Wellington, New
37
38 Zealand); B. Björkstén (Stockholm, Sweden); B. Brunekreef* (Utrecht, the
39
40 Netherlands); S. Foliaki (Nuku'alofa, Kingdom of Tonga); L. García-Marcos (Murcia,
41
42 Spain); U. Keil (Münster, Germany) and E. von Mutius* (Munich, Germany);
43
44 S.K. Weiland*†, G. Weinmayr* (Ulm, Germany); C.K.W. Lai and G.W.K. Wong (Hong
45
46 Kong, China); J. Mallol (Santiago, Chile); S. Montefort (Naxxar, Malta); J. Odhiambo
47
48 (Nairobi, Kenya); and C. Robertson (Parkville, Australia).

49 * also members of the ISAAC Phase Two Steering Group, † deceased.
50
51
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For Peer Review

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2 Figure legends:
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6 Figure 1: Variation in BR-slope (decrease of individual FEV₁) between study centres
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8 (dark, affluent countries; white, non-affluent countries).
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11 Figure 2: Forest plot of the association of wheeze (past year) and bronchial
12 hyperreactivity (defined as a decrease in FEV₁ of at least 15%), for (a)
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14 affluent and (b) non-affluent study.
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17 Centres are presented in descending order of national per capita GNI.
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22 Figure 3: Forest plot of the combined association of wheeze (past year) and
23 bronchial hyperreactivity (defined as a decrease in FEV₁ of at least 15%),
24 stratified by atopy and affluence.
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30 Figure 4: Correlations at the centre level of mean BR-slope versus the prevalence of
31 wheeze in (a) all children and (b) atopic children (with positive skin prick
32
33 test).
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35 BR-Slope measured as % decrease of individual FEV₁ per log (minutes) inhalation time.
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40 Figure 5: Correlation at centre level of mean BR-slope in atopic children (with
41 positive skin prick test) versus mean BR-slope in non-atopic children.
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43 BR-Slope measured as % decrease of individual FEV₁ per log (minutes) inhalation time.
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International variations in bronchial responsiveness in children:**Findings from ISAAC Phase Two**

Gisela Büchele, MPH¹, Jon Genuneit¹, Gudrun Weinmayr, Ph.D.¹, Bengt Björkstén Ph.D.², Ulrike Gehring, Ph.D.³, Erika von Mutius, MD⁴, Alfred Priftanji, FRCP⁵, Renato T. Stein, Ph.D.⁶, Emmanuel O. Addo-Yobo, MD⁷, Kostas N. Priftis, Ph.D.⁸, Jayant R. Shah, MD⁹, Francesco Forastiere, Ph.D.¹⁰, Vija Svabe, MD¹¹, Julian Crane, MB, BS¹², Wenche Nystad, Ph.D.¹³, Luis García-Marcos, MD¹⁴, Yıldız Saraçlar, MD¹⁵, Nuha El-Sharif, Ph.D.¹⁶, David P. Strachan, MD¹⁷ and the ISAAC Phase Two Study Group

¹ Institute of Epidemiology, University of Ulm, Germany

² Institute of Environmental Medicine, Karolinska Institutet Stockholm, Sweden

³ Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

⁴ Children's Hospital, University of Munich, Germany

⁵ Department of Allergology and Clinical Immunology, University Hospital Center "Mother Teresa", University of Tirana, Albania

⁶ Departamento de Pediatria, Faculdade de Medicina, Pontificia Universidade Catolica, Porto Alegre, Brazil

⁷ Department of Child Health, Komfo Anokye Teaching Hospital, Kumasi, Ghana

⁸ Department of Allergy-Pneumonology, Penteli Children's Hospital, P. Penteli, Greece

⁹ Jaslok Hospital & Research Centre, Mumbai, India

¹⁰ Department of Epidemiology, Rome E Health Authority, Rome, Italy

¹¹ Children's Hospital, Riga, Latvia

¹² Department of Medicine, Wellington School of Medicine and Health Science, New Zealand

¹³ Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway

¹⁴ Pediatric Pulmonology and Allergy Units, Virgen de la Arrixaca University Children's Hospital, University of Murcia, Spain

¹⁵ Hacettepe University, Faculty of Medicine, Pediatric Allergy and Asthma Unit, Ankara, Turkey.

¹⁶ Faculty of Public Health, Alquds University, Jerusalem, Israel

¹⁷ Division of Community Health Sciences, St. George's, University of London, United Kingdom

1
2
3 Correspondence Gisela Büchele
4 address: Ulm University
5 Institute of Epidemiology
6 Helmholtzstraße 22
7 89081 Ulm
8 Germany
9 Tel: +49 731 5031072
10 Fax: +49 731 5031069
11 Email: gisela.buechele@uni-ulm.de
12
13
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17 Abbreviated title: BR in children
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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 is 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. FEV₁ was measured at baseline and after inhalation for 0.5, 1, 2, 4, and 8 minutes. Bronchial responsiveness was analysed both as a dichotomous (bronchial hyperreactivity, BHR, at least 15% decline in FEV₁) and as a continuous variable (time-response-slope, BR-slope, individual decline in FEV₁ 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 centre-specific mean BR-slope and wheeze prevalence in atopic, but not in non-atopic children.

Conclusions: Bronchial responsiveness to saline in children varied considerably between countries. High rates of BR were not confined to affluent countries nor to centres with high prevalences of asthma symptoms. The association between wheeze and BHR at the individual level differed across centres and this heterogeneity can be largely explained by effect modification by atopy.

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 paper, 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 II are described in detail elsewhere [12;13]. Briefly, the study was performed in 35 centres from 22 countries and bronchial challenge with hypertonic saline was conducted in 22 centres from 16 countries. Random samples of at least 1000 children from ≥ 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 centres 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 % vs. 40 % between two centres 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 centres 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.

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3 *Bronchial challenge.* Participating children were asked to withhold
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5 bronchodilator medications before the challenge. Regular use of inhaled steroids was
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7 recorded, but not withheld. Spirometry was performed according the ATS criteria [15].
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9 At least two spirograms were recorded, and the higher of two reproducible
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11 measurements (with less than 5 % variation) of forced expiratory volume in one
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13 second (FEV₁) was recorded as baseline FEV₁. In children with a baseline FEV₁ of
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15 <75% of the predicted value (n=103), no bronchial challenge was performed and an
16
17 inhaled bronchodilator was administered.
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22 Bronchial responsiveness was assessed by changes in FEV₁ during inhalation
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24 of nebulised saline from a DeVilbiss UltraNeb 2000 ultrasonic nebuliser [12;13;16].
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26 The children inhaled hyperosmolar (4.5 %) saline for periods of increasing duration:
27
28 0.5, 1, 2, 4, and 8 minutes. FEV₁ was measured 1 min after each inhalation period
29
30 and the next challenge period followed after further 3 min. If the FEV₁ decreased by
31
32 10-15 % from the baseline value, the exposure time was repeated. If, after two
33
34 repetitions, the FEV₁ remained 10-15 % below the baseline value, the duration of the
35
36 inhalation period was doubled again according to the protocol. The bronchial
37
38 challenge was stopped if either the FEV₁ had decreased by ≥ 15 % from baseline or
39
40 the total inhalation period of 15.5 min had been reached. The saline canister and
41
42 tubing were weighed before and after the challenge in order to measure the total
43
44 aerosol dose delivered. Study centre representatives were trained in one location to
45
46 assure a standardized performance of the bronchial challenge according to protocol.
47
48 BR was assessed in two different ways [16]: 1) Children with a decline in FEV₁ of at
49
50 least 15 % from baseline value or who had an increase of 25 % in FEV₁ after
51
52 bronchodilator inhalation (n=29) were classified to be positive regarding BHR. 2) The
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54 time-response-slope (BR-slope) was calculated for each child by linear regression of
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56 percentage decline in FEV₁ against log inhalation time.
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Skin prick test reactivity. Skin prick tests were performed according to a detailed standardised 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 eighteen centres 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, centres 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 ten outliers with extreme increase or decrease after the first 0.5 minute 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 (%FEV₁ decrease per log(min)).

Prevalence rates were calculated for each centre by dividing the number of positive responses by the total number of valid responses. Correlations between

1
2
3 prevalence rates and averages at the centre level were assessed by Spearman's
4
5 rank correlation coefficient ρ . For the association between an individual's wheeze
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7 status and BHR, odds ratios (OR) were determined by logistic regression. In centres
8
9 with stratified sub-samples, means, prevalences, and odds ratios were weighted to
10
11 account for the sampling scheme using the SURVEY-procedures in SAS [20;21].
12
13 This method provides unbiased parameter estimates (extrapolated to the population
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15 from which the stratified sub-samples were drawn) while preserving appropriate
16
17 standard errors for the weighted estimates. Results from each centre were combined
18
19 by random-effects meta-analysis (DerSimonian-Laird) to allow for both between-
20
21 centre and within-centre (sampling) variation [22]. Heterogeneity was assessed by
22
23 Cochran's Q-test and quantified by the variance component between study centres
24
25 (I^2) [23]. All computations were performed using SAS® 9.2. (SAS Institute Inc., Cary,
26
27 NC, USA.).
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Results

Data from 6,826 children with questionnaire data and bronchial challenge were available from 22 study centres 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 centres from 9.8 to 12.9 yr.), had a mean body height of 145.5 cm (132 to 158 cm) and a mean body weight of 40.0 kg (27 to 49 kg). The proportion of boys was 49.9 %, varying from 36 % to 60 %.

The centre-specific prevalences of wheeze, skin prick test reactivity, and bronchial hyperreactivity (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 centres. 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 FEV₁ 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) (figure 1). The rank correlation between the centre means of the two measures of bronchial responsiveness 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 (4 centres), Germany, Greece, and Sweden (each 2 centres). In these countries (with the exception of Germany) there were centres with significant differences in BR (i.e. geometric mean BR-slope above and below the international median and non-overlapping confidence intervals).

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2
3 The association between BHR and wheeze at the individual level was explored
4 within study centres. Results are presented in figure 2. There was a positive
5 association of similar magnitude in affluent and non-affluent countries (OR=3.63
6 [95 %-CI: 2.70-4.88] and OR=2.95 [1.61-5.40], respectively). All associations
7 between BHR and wheeze within centres were positive and most of them were
8 statistically significant, but the strength of the association varied significantly between
9 centres with I^2 of 65 % for affluent countries and 72 % for non-affluent countries.

10
11 To elucidate this between-centre heterogeneity, effect modification by atopy
12 was investigated. The association between BHR and wheeze was stronger in atopic
13 children than in non-atopic children (figure 3), to a similar degree in affluent and non-
14 affluent countries. The heterogeneity between study centres of the BHR-wheeze
15 association was substantially reduced after stratification into atopic and non-atopic
16 children, with I^2 values ranging from 20 % to 32 % (figure 3).

17
18 Although BHR was associated with wheeze within centres in both affluent and
19 non-affluent countries, the overall rank correlation at centre level between prevalence
20 of wheeze and bronchial responsiveness expressed as geometric mean BR-slope
21 was weak ($\rho=0.23$, figure 4a). A similar lack of correlation was found between the
22 prevalence of wheeze and the prevalence of BHR across centres ($\rho=0.31$, $p=0.16$).
23 After stratification for atopy, there was a significant rank correlation in atopics
24 between the wheeze prevalence and the centre geometric mean BR-slope of $\rho=0.64$
25 ($p=0.002$), which was consistent in both affluent and non-affluent countries
26 (figure 4b). In non-atopics, the equivalent correlation was weaker and non-significant
27 ($\rho=0.21$, $p=0.35$), reflecting only a moderate positive correlation at the centre level of
28 $\rho=0.48$ ($p=0.29$) between the geometric mean BR-slope in atopic children and the
29 geometric mean BR-slope in non-atopic children (figure 5). The few centres with
30 higher BR-slope geometric means in non-atopic children (Mumbai, India; Tromsø,
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3 Norway; Thessaloniki, Greece) were characterised by a generally high BHR
4 prevalence, a high rate of non-atopic BHR, and a negative association between BHR
5 and skin prick test reactivity at an individual level (data not shown). However, due to
6 low sample sizes within each centre the confidence intervals of the centre-specific
7 geometric means of the BR-slope in atopic and non-atopic children mostly overlap
8 (table 2).
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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, but not at the level of study centres. However, there was only a modest correlation between BR as determined by challenge with hypertonic saline and wheeze at the centre 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 vs. 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 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 labelling 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 centres with diverse economic and

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3 environmental conditions [29]. Although there was only a moderate correlation
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5 between the nebulized amount of saline and the time of inhalation, the time-based
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7 slope showed a good ability to differentiate between asthmatic and non-asthmatic
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9 children [16]. The international variability in saline-induced BR was also seen for
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11 exercise-induced bronchial reactivity which was performed additionally in two
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13 countries representing the extremes of the worldwide distribution of the frequency of
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15 asthma symptoms in ISAAC Phase One and Phase Two, i.e. Albania with extreme
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17 low prevalence rates and UK with consistently high rates. A reduction in the peak
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19 expiratory flow rate of at least 15 % after exercise provocation was found about 7
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21 times more often in UK than in Albania [30].
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27 As compared to methacholine challenges, hypertonic saline has a lower
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29 sensitivity to detect asthma but is more closely related to allergic asthmatic airway
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31 inflammation in adults [31]. This may explain the stronger association between BHR
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33 and wheeze on an individual level in atopic children both in affluent and non-affluent
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35 countries. Heterogeneity between study centres decreased considerably when atopic
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37 and non-atopic children were analysed separately. Thus, there was a stronger
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39 correlation among atopic children at the centre level between mean BR-slope and
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41 prevalence of wheeze. Effect modification by atopy therefore appears to be an
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43 important facet in explaining the association between BR to saline and asthma
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45 symptoms, both at the individual and population levels. This measure of BR may
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47 therefore be indicating a specific atopic asthma phenotype.
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53 Potentially, centres with low or undefined participation rates may not be
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55 representative of the population studied. We checked for potential selection bias by
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57 comparing the distributions of sex, asthma, eczema, rhinitis, older siblings, and
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59 parental atopy and found no evidence that the children participating in the bronchial
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challenge would not be representative of all eligible children. A sensitivity analysis

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3 excluding centres with participation rates below 60 % did not alter the conclusions
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5 above. The population based survey in each centre within ISAAC Phase II provided a
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7 known sampling frame for cost efficient nested case-control-studies enabling us to
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9 determine objective markers of disease in informative subgroups across a broad
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11 range of countries. Due to the random sampling procedure all subsamples can be
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13 considered representative for the underlying population. The applied weights in the
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15 analyses of stratified subsamples do not only account for the sampling frame but also
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17 for the non-response of the respective item.
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22 Some of the study centres showed a similar ranking for wheeze and BR, e.g.
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24 low prevalence for both in Tirana, Albania and high prevalence in West Sussex, UK.
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26 Some of the centres with high levels of BR, e.g. Mumbai in India, and Kintampo in
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28 Ghana, showed low prevalence rates for wheeze and positive skin prick tests. The
29
30 high prevalence of BHR in the study centres in Ghana and India confirms previous
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32 reports of high prevalence rates of BHR among children in less affluent areas in
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34 Estonia [32] and Western Australia [33]. A study in Indian children suggested that
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36 exposure to cooking smoke from solid biomass fuel is significantly associated with a
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38 decline in lung function and a higher prevalence of doctor-diagnosed asthma and of
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40 other respiratory diseases [34]. Due to the low prevalence of smoking in India in
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42 general (16 %), and especially amongst women, a contribution of environmental
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44 tobacco smoke to the high prevalence of BHR in India seems unlikely [35]. Da Silva
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46 et al. referred to non-atopic asthma as the predominant phenotype in non-affluent
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48 parts of Latin America and attributed this to a high prevalence of infection with
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50 helminths [36]. Non-atopic BHR in children often occurs transiently as a reaction to
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52 upper respiratory infections which may be more common in less affluent communities
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54 [37]. Overall, it is likely that there is a complex interaction of BR and atopy in causing
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3 wheeze in differing environments with different environmental exposures and genetic
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5 backgrounds.
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8 In the European Community Respiratory Health Survey (ECRHS) BR was
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10 measured in 13,161 adults (aged 20-44 yrs) in 35 study centres and reported also a
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12 wide variation in the prevalence of BHR (defined as a $PD_{20} \leq 1$ mg methacholine)
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14 ranging from 3.4 % in Galdakao, Spain up to 27.8 % in Hawkes-Bay, New Zealand
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16 [38]. Although prevalence rates from ECRHS cannot be directly compared to our
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18 results, the ranking of comparable centres in same countries partly agreed for some
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20 countries, e.g. moderate prevalence rates in the Netherlands, high rates in the United
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22 Kingdom, and a broad range in Spanish centres. However, in contrast to our findings
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24 Norway showed low prevalence rates and New Zealand and Germany high rates of
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26 BR in ECRHS. In adults from Estonia (Tartu) a moderate BHR prevalence was
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28 measured [39] but Estonian children from Tallinn showed a very low prevalence in
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30 ISAAC Phase Two. Norrman et al. discussed the lack of within country variability in
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32 Swedish adults [40], whereas in this analysis children from Östersund and Linköping
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34 showed statistically significant differences in the geometric mean BR-slope.
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41 Among non-European centres, Mumbai had the highest BHR prevalence in
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43 ISAAC Phase Two. In adults, the rate of “positive” challenge at $PD_{20} \leq 2$ mg
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45 methacholine in Mumbai was only 14 % [35] as compared to a median value of
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47 13.0 % in the remaining ECRHS centres where BHR was defined (more exclusively)
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49 as $PD_{20} \leq 1$ mg [38]. In contrast, for young adults in Brazil, a high BHR prevalence of
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51 31.3 % was reported while 10 year old children in Brazil showed a moderate BHR
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53 rate of 16.0 % according to ISAAC Phase Two criteria [36;41].
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58 Differences between our findings and the published international results on
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60 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

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3 cohort differences, in that the participants in ECRHS were born several decades
4 earlier than the ISAAC children, and therefore lower prevalence rates might be
5 expected [42-44]. Secondly, some phenotypes of childhood BHR like transient BHR
6 or non-atopic BHR may disappear during adolescence as there is commonly
7 remission of wheeze at this time [45]. Thirdly, the choice of the stimulus in the BR
8 tests may influence the results if, as suggested [31], response to saline provocation is
9 more specifically related to allergic airway inflammation and there are marked
10 differences in the balance of atopic and non-atopic asthma across the centres being
11 compared. Finally, comparisons may be confounded by the choice of the participating
12 study centre against the background of the variability in the level of BR between
13 centres within countries.
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32 In conclusion, this is the first large study to assess bronchial responsiveness
33 (BR) to hypertonic saline measured by a standardised protocol in combination with
34 questionnaire reports of asthma symptoms and skin prick test reactivity. Association
35 were investigated in children at both an individual level and population level in
36 diverse settings. There was considerable variation in the level of BR between
37 different countries and also between centres within countries. Although there was a
38 clear association of BR with asthma symptoms within individuals, the between-centre
39 variability of wheeze prevalence cannot be explained by the level of BR in the
40 population. However, among atopic children, where the association of BR and
41 wheeze was stronger at the individual level, the centres with higher levels of BR also
42 tended to have higher prevalences of asthma symptoms.
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3 Conflicts of Interest Statement:
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5 None of the authors has a financial relationship with a commercial entity that has an
6 interest in the subject of this manuscript.
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8
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10
11
12 The ISAAC Phase Two Study group consists of:

13 The ISAAC Phase Two Coordinating and Data Centre: S.K. Weiland † (Director),
14 G. Büchele, C. Dentler, A. Kleiner, P. Rzehak, G. Weinmayr (Institute of
15 Epidemiology, Ulm University, Ulm, Germany).
16
17
18
19
20
21
22
23

24 The principal investigators: A. Priftanji, A. Shkurti, J. Simenati, E. Grabocka, K. Shyti,
25 S. Agolli, A. Gurakuqi (Tirana, Albania); R.T. Stein, M. Urrutia de Pereira,
26 M.H. Jones, P.M. Pitrez (Uruguaiana, Brazil); P.J. Cooper, M. Chico (Pichincha
27 province, Ecuador); Y.Z. Chen (Beijing, China); N.S Zhong (Guangzhou, China);
28 C.K.W. Lai (National Coordinator), G.W.K. Wong (Hong Kong, China); M-A. Riiikjäv,
29 T. Annus (Tallinn, Estonia); I. Annesi-Maesano (Créteil, France); M. Gotua,
30 M. Rukhadze, T. Abramidze, I. Kvachadze, L. Karsanidze, M. Kiladze, N. Dolidze
31 (Tbilisi, Georgia); W. Leupold, U. Keil, E. von Mutius, S.K. Weiland † (Dresden,
32 Germany); E. von Mutius, U. Keil, S.K. Weiland † (Munich, Germany); P. Arthur †,
33 E. Addo-Yobo (Kintampo, Ghana); C. Gratziou (National Coordinator), K. Priftis,
34 A. Papadopoulou, C. Katsardis (Athens, Greece); J. Tsanakas, E. Hatziagorou,
35 F. Kirvassilis (Thessaloniki, Greece); M. Clausen (Reykjavik, Iceland); J.R. Shah,
36 R.S. Mathur, R.P. Khubchandani, S. Mantri (Mumbai, India); F. Forastiere,
37 R. Di Domenicantonio, M. De Sario, S. Sammarro, R. Pistelli, M.G. Serra, G. Corbo,
38 C.A. Perucci (Rome, Italy); V. Svabe, D. Sebre, G. Casno, I. Novikova, L. Bagrade
39 (Riga, Latvia); B. Brunekreef, D. Schram, G. Doekes, P.H.N. Jansen-van Vliet,
40 N.A.H. Janssen, F.J.H. Aarts, G. de Meer (Utrecht, the Netherlands); J. Crane,
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 K. Wickens, D. Barry (Hawkes Bay, New Zealand); W. Nystad, R. Bolle, E. Lund
4
5 (Tromsø, Norway); J. Batlles Garrido, T. Rubi Ruiz, A. Bonillo Perales,
6
7 Y. Gonzalez Jiménez, J. Aguirre Rodriguez, J. Momblan de Cabo,
8
9 A. Losilla Maldonado, M. Daza Torres (Almeria, Spain); L. García-Marcos (National
10
11 Coordinator), A. Martinez Torres, J.J. Guillén Pérez, A. Piñana López,
12
13 S. Castejon Robles (Cartagena, Spain); G. García Hernandez, A. Martinez Gimeno,
14
15 A.L. Moro Rodríguez, C. Luna Paredes, I. Gonzalez Gil (Madrid, Spain);
16
17 M.M. Morales Suarez-Varela, A. Llopis González, A. Escribano Montaner,
18
19 M. Tallon Guerola (Valencia, Spain); L. Bråbäck (National Coordinator), M. Kjellman,
20
21 L. Nilsson, X-M. Mai (Linköping, Sweden); L. Bråbäck, A. Sandin (Östersund,
22
23 Sweden); Y. Saraçlar, S. Kuyucu, A. Tuncer, C. Saçkesen, V. Sumbulöglu, P. Geyik,
24
25 C. Kocabas, (Ankara, Turkey); D.P. Strachan, B. Kaur (West Sussex, UK);
26
27 N. El-Sharif, B. Nemery, F. Barghuthy, S. Abu Huij, M. Qlebo (Ramallah, West Bank).
28
29
30
31
32
33

34
35
36 The ISAAC Steering Committee: N. Ait-Khaled (Paris, France); H.R. Anderson and
37
38 D.P. Strachan* (London, UK); C. Flohr* and H. Williams (Nottingham, UK);
39
40 F. Forastiere* (Rome, Italy); I. Asher, P. Ellwood, A. Stewart and E. Mitchell
41
42 (Auckland, New Zealand); J. Crane, N. Pearce and R. Beasley (Wellington, New
43
44 Zealand); B. Björkstén (Stockholm, Sweden); B. Brunekreef* (Utrecht, the
45
46 Netherlands); S. Foliaki (Nuku'alofa, Kingdom of Tonga); L. García-Marcos (Murcia,
47
48 Spain); U. Keil (Münster, Germany) and E. von Mutius* (Munich, Germany);
49
50 S.K. Weiland*†, G. Weinmayr* (Ulm, Germany); C.K.W. Lai and G.W.K. Wong (Hong
51
52 Kong, China); J. Mallol (Santiago, Chile); S. Montefort (Naxxar, Malta); J. Odhiambo
53
54 (Nairobi, Kenya); and C. Robertson (Parkville, Australia).
55
56
57
58

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60

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For Peer Review

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3 Figure legends:
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8 Figure 1: Variation in BR-slope (decrease of individual FEV₁) between study centres
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10 (dark, affluent countries; white, non-affluent countries).
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15 Figure 2: Forest plot of the association of wheeze (past year) and bronchial
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17 hyperreactivity (defined as a decrease in FEV₁ of at least 15%), for (a)
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19 affluent and (b) non-affluent study.
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22 Centres are presented in descending order of national per capita GNI.
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27 Figure 3: Forest plot of the combined association of wheeze (past year) and
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29 bronchial hyperreactivity (defined as a decrease in FEV₁ of at least 15%),
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31 stratified by atopy and affluence.
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37 Figure 4: Correlations at the centre level of mean BR-slope versus the prevalence of
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39 wheeze in (a) all children and (b) atopic children (with positive skin prick
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41 test).
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44 BR-Slope measured as % decrease of individual FEV₁ per log (minutes) inhalation time.
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49 Figure 5: Correlation at centre level of mean BR-slope in atopic children (with
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51 positive skin prick test) versus mean BR-slope in non-atopic children.
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54 BR-Slope measured as % decrease of individual FEV₁ per log (minutes) inhalation time.
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Table 1 – Data on field work and participation by study centre

Country	Study period questionnaire	Characteristic of study area	Questionnaire	Skin Prick Test	Bronchial challenge	Gross national income per capita (US\$)
			n ^a (%) ^b	n ^a (%) ^b	n ^a (%) ^b	
<i>Albania</i>						
Tirana	2-4/1999	Urban	1,052 (94.9)	9,29 (84.0)	206 (ND) ^c	970
<i>Estonia</i>						
Tallinn	12/1996-2/1997	Urban	971 (83.9)	642 (55.5)	244 (71.8) ^c	3540
<i>Georgia</i>						
Tbilisi	3/2001-6/2002	Urban	1,012 (87.7)	173 (86.5) ^c	172 (86.0) ^c	680
<i>Germany</i>						
Dresden	9/1995-6/1996	Urban	3,023 (82.8)	2,259 (61.6)	701 (62.8)	25740
Munich	9/1995-12/1996	Urban	3,301 (87.5)	2,317 (60.6)	901 (66.0)	
<i>Ghana</i>						
Kintampo	02-07/2000	Rural	1,354 (ND)	1,322 (ND)	251 (ND) ^c	380
<i>Greece</i>						
Athens	10/2000-2/2001	Urban	985 (85.3)	985 (85.3)	195 (90.3) ^c	11700
Thessaloniki	9-11/2001	Urban	1,018 (63.0)	1,018 (63.0)	212 (88.3) ^c	
<i>India</i>						
Mumbai	2000-2001	Urban	1,658 (ND)	1,556 (ND)	121 (ND) ^c	450
<i>Italy</i>						
Rome	10/2000-4/2001	Urban	1,354 (83.5)	1,307 (62.3)	123 (38.0) ^c	20170
<i>Latvia</i>						
Riga	5-11/1999	Urban	908 (87.4)	295 (30.8)	159 (32.5)	2570
<i>Netherlands</i>						
Utrecht	4/1997-7/1998	Urban	3,541 (64.7)	1,286 (43.3)	1103 (43.7)	25270
<i>New Zealand</i>						
Hawkes Bay	2-6/2000	Urban/rural	1,320 (84.3)	1,288 (82.2)	229 (72.0) ^c	13480
<i>Norway</i>						
Tromsø	3-6/2000	Urban/rural	3,669 (81.3)	722 (60.2)	185 (92.5) ^c	35660
<i>Spain</i>						
Almeria	3/2000-6/2001	Urban	1,126 (49.9)	1,075 (47.7)	210 (86.8) ^c	
Cartagena	3/2000-3/2001	Urban	1,429 (54.6)	1,030 (39.6)	160 (93.1) ^c	14790
Madrid	2/2001-4/2002	Urban	981 (35.8)	653 (23.9)	425 (100.0) ^c	
Valencia	12/2000-12/2001	Urban	1,362 (40.4)	1,023 (30.5)	195 (99.0) ^c	
<i>Sweden</i>						
Linköping	1-4/1997	Urban	907 (81.7)	857 (77.0)	184 (65.9) ^c	28540

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3	Östersund	1-4/1997	Urban/rural	1,195 (86.0)	991 (71.4)	279 (76.2) ^c	
4							
5	<i>Turkey</i>						
6	Ankara	10/1999-4/2000	Urban	2,976 (87.6)	2,747 (81.0)	346 (96.9) ^c	2800
7							
8	<i>United Kingdom</i>						
9	West Sussex	10/1998-07/1999	Urban/rural	1,056 (78.6)	898 (66.7)	225 (99.1) ^c	24070
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11	Total			36,198	25,373	6,826	
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n = number of participants; % = participation in percent; ND = no denominator to assess response rate;

^a number of children aged 8-12 years;

^b participation rate refers to those who were invited (either full or subsamples);

^c stratified disproportional subsample, otherwise: random subsample or full sample

Table 2 - Prevalence of asthma-related symptoms, skin prick test reactivity and bronchial hyperreactivity (BHR^a) as well as means of the BR-slope (%FEV₁ per log(min))

Country	Wheeze past year	Positive skin prick test ^b	----- BHR -----			---- Slope of FEV ₁ decrease ----		
			All children	Atopic ^c children	Non-atopic ^d children	All children	Atopic ^c children	Non-atopic ^d children
----- prevalence rates in % ^e -----			----- geometric means ^c -----					
<i>Albania</i>	4.4	15.0 ^e	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
<i>Estonia</i>	8.4	14.6 ^e	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	-0.1-5.2	1.0-8.0	3.2-4.0	3.0-5.7	2.9-4.1
<i>Georgia</i>	9.2	33.0 ^e	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
<i>Germany</i>	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
<i>Ghana</i>	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
<i>Greece</i>	5.6	14.4 ^e	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 ^e	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
<i>India</i>	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	-9.5-69.3	34.7-60.2	9.2-16.8	0.7-12.5	9.0-17.6
<i>Italy</i>	7.9	28.9 ^e	33.1 ^f	45.5 ^f	24.8 ^f	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
<i>Latvia</i>	6.9	19.3	13.5 ^f	20.8 ^f	10.3 ^f	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
<i>Netherlands</i>	8.7	30.9 ^e	19.8 ^f	33.9 ^f	12.2 ^f	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
<i>New Zealand</i>	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
<i>Norway</i>	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
<i>Spain</i>	15.5 ^f	43.0	23.7	38.3	22.9	8.4	11.2	6.4

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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 ^f	23.8 ^e	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 ^f	34.5 ^e	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 ^f	14.3 ^e	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 ^e	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
	10.2	26.5 ^e	33.5	35.1	30.1	9.0	10.3	8.1
Östersund	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
	10.9	24.6 ^e	22.4	26.8	21.0	6.4	6.9	6.2
Turkey	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

^a BHR is defined as a decline in FEV₁ of at least 15%;

^b wheal size of ≥ 3 mm to at least one of the tested aeroallergens;

^c prevalence or slope calculated only in children with a positive skin prick test reactivity (≥ 3 mm wheal size);

^d prevalence or slope calculated only in children with a negative skin prick test reactivity (<3 mm wheal size);

* calculations weighted for disproportional subsampling and shown with 95%-confidence intervals;

^e local allergens were tested in addition to standard set of six common allergens;

^f the reported frequencies should not be interpreted as prevalence estimates because participation was <60%;

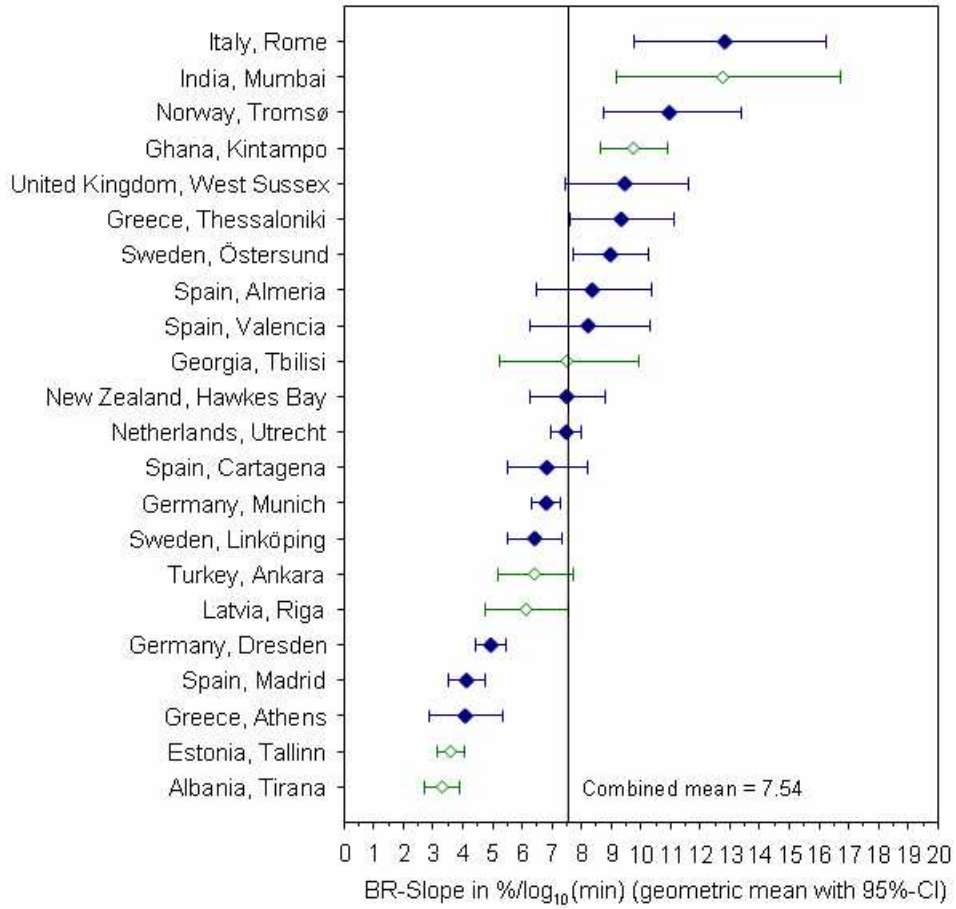
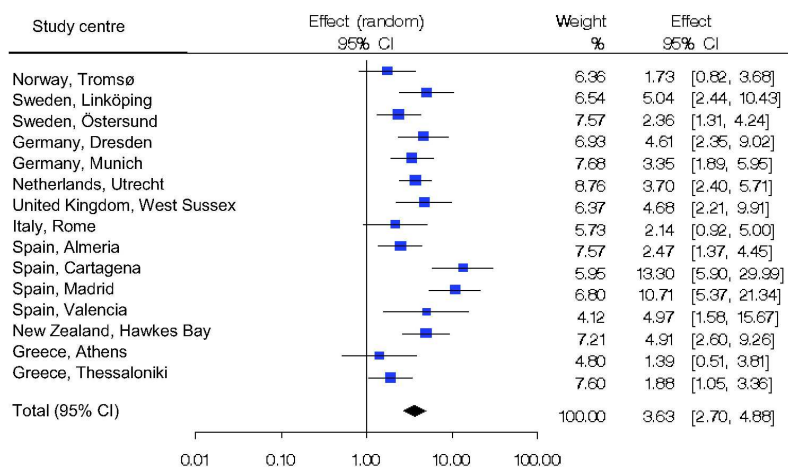


Figure 1: Variation in BR-slope (decrease of individual FEV₁) between study centres (dark, affluent countries; white, non-affluent countries).

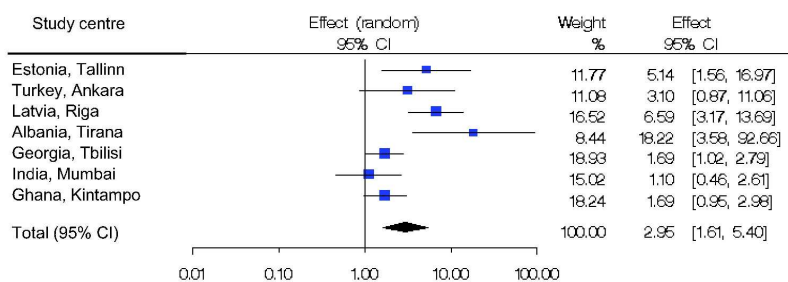
165x163mm (96 x 96 DPI)

a) Affluent centres:



Test for Heterogeneity: $Q=39.4$, $df=14$ ($p=.000$), $I^2=64.5\%$

b) Non-affluent centres:



Test for Heterogeneity: $Q=21.5$, $df=6$ ($p=.002$), $I^2=72.1\%$

Figure 2: Forest plot of the association of wheeze (past year) and bronchial hyperreactivity (defined as a decrease in FEV₁ of at least 15%), for (a) affluent and (b) non-affluent study.

Centres are presented in descending order of national per capita GNI.
159x169mm (600 x 600 DPI)

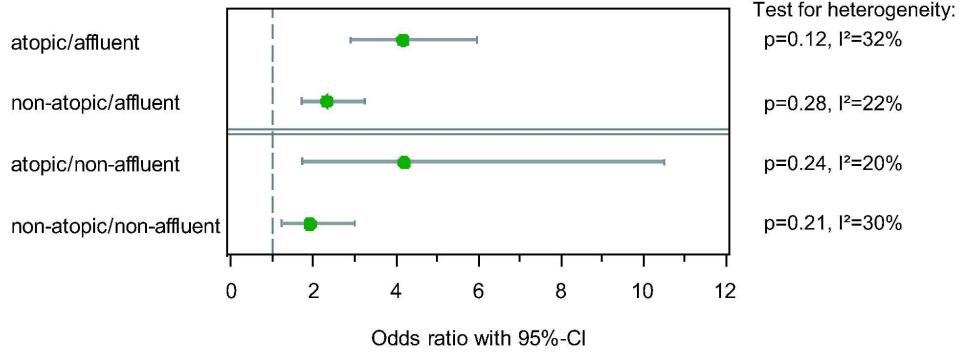
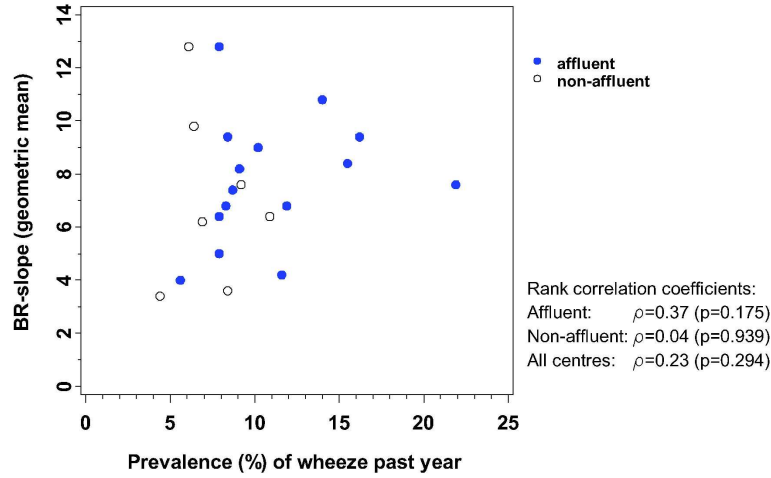


Figure 3: Forest plot of the combined association of wheeze (past year) and bronchial hyperreactivity (defined as a decrease in FEV₁ of at least 15%), stratified by atopy and affluence. 173x75mm (600 x 600 DPI)

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a) Slope (decrease of individual FEV₁) by prevalence (%) of wheeze



b) Slope (decrease of individual FEV₁) by prevalence (%) of wheeze in atopic children

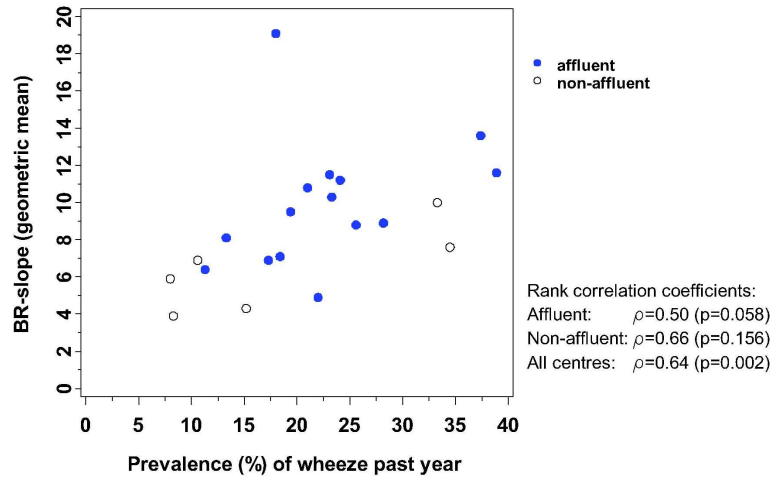


Figure 4: Correlations at the centre level of mean BR-slope versus the prevalence of wheeze in (a) all children and (b) atopic children (with positive skin prick test). BR-Slope measured as % decrease of individual FEV₁ per log (minutes) inhalation time.

159x233mm (600 x 600 DPI)

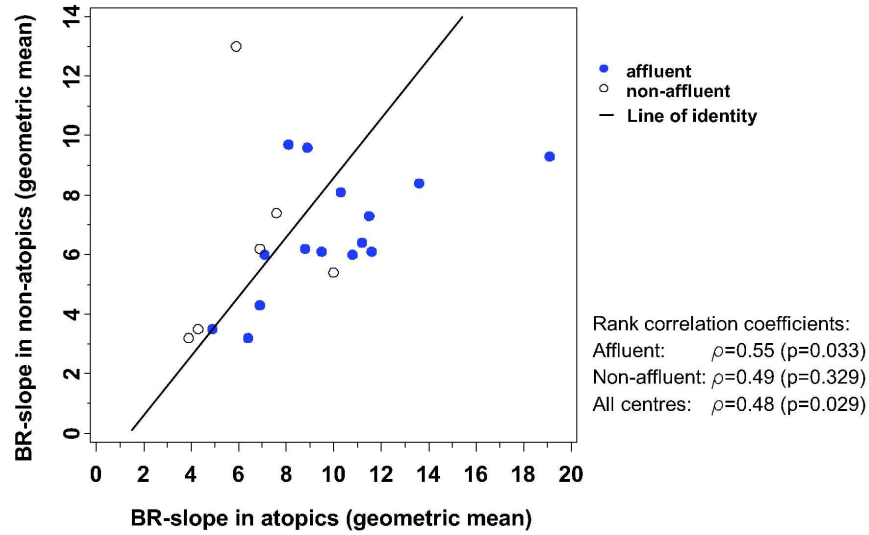


Figure 5: Correlation at centre level of mean BR-slope in atopic children (with positive skin prick test) versus mean BR-slope in non-atopic children.
 BR-Slope measured as % decrease of individual FEV₁ per log (minutes) inhalation time.

172x113mm (600 x 600 DPI)

International variations in bronchial responsiveness in children:**Findings from ISAAC Phase Two**

Gisela Büchele, MPH¹, Jon Genuneit¹, Gudrun Weinmayr, Ph.D.¹, Bengt Björkstén Ph.D.², Ulrike Gehring, Ph.D.³, Erika von Mutius, MD⁴, Alfred Priftanji, FRCP⁵, Renato T. Stein, Ph.D.⁶, Emmanuel O. Addo-Yobo, MD⁷, Kostas N. Priftis, Ph.D.⁸, Jayant R. Shah, MD⁹, Francesco Forastiere, Ph.D.¹⁰, Vija Svabe, MD¹¹, Julian Crane, MB, BS¹², Wenche Nystad, Ph.D.¹³, Luis García-Marcos, MD¹⁴, Yıldız Saraçlar, MD¹⁵, Nuha El-Sharif, Ph.D.¹⁶, David P. Strachan, MD¹⁷ and the ISAAC Phase Two Study Group

¹ Institute of Epidemiology, University of Ulm, Germany

² Institute of Environmental Medicine, Karolinska Institutet Stockholm, Sweden

³ Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

⁴ Children's Hospital, University of Munich, Germany

⁵ Department of Allergology and Clinical Immunology, University Hospital Center "Mother Teresa", University of Tirana, Albania

⁶ Departamento de Pediatria, Faculdade de Medicina, Pontificia Universidade Catolica, Porto Alegre, Brazil

⁷ Department of Child Health, Komfo Anokye Teaching Hospital, Kumasi, Ghana

⁸ Department of Allergy-Pneumonology, Penteli Children's Hospital, P. Penteli, Greece

⁹ Jaslok Hospital & Research Centre, Mumbai, India

¹⁰ Department of Epidemiology, Rome E Health Authority, Rome, Italy

¹¹ Children's Hospital, Riga, Latvia

¹² Department of Medicine, Wellington School of Medicine and Health Science, New Zealand

¹³ Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway

¹⁴ Pediatric Pulmonology and Allergy Units, Virgen de la Arrixaca University Children's Hospital, University of Murcia, Spain

¹⁵ Hacettepe University, Faculty of Medicine, Pediatric Allergy and Asthma Unit, Ankara, Turkey.

¹⁶ Faculty of Public Health, Alquds University, Jerusalem, Israel

¹⁷ Division of Community Health sciences, St. George's, University of London, United Kingdom

1
2
3 Correspondence Gisela Büchele
4 address: Ulm University
5 Institute of Epidemiology
6 Helmholtzstraße 22
7 89081 Ulm
8 Germany
9 Tel: +49 731 5031072
10 Fax: +49 731 5031069
11 Email: gisela.buechele@uni-ulm.de
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17 Abbreviated title: BR in children
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20 Keywords: bronchial responsiveness, bronchial challenge, hypertonic
21 saline, asthma, children, atopy, 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 is 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. FEV₁ was measured at baseline and after inhalation for 0.5, 1, 2, 4, and 8 minutes. Bronchial responsiveness was analysed both as a dichotomous (bronchial hyperreactivity, BHR, at least 15% decline in FEV₁) and as a continuous variable (time-response-slope, BR-slope, individual decline in FEV₁ per log(min)).

Results: Prevalence of BHR ranged from 2.1% in Tirana (Albania) 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 centre-specific mean BR-slope and wheeze prevalence in atopic, but not in non-atopic children.

Conclusions: Bronchial responsiveness to saline in children varied considerably between countries. High rates of BR were not confined to affluent countries nor to centres with high prevalences of asthma symptoms. The association between wheeze and BHR at the individual level differed across centres and this heterogeneity can be largely explained by effect modification by atopy.

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 paper, 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 II are described in detail elsewhere [12;13]. Briefly, the study was performed in 35 centres from 22 countries and bronchial challenge with hypertonic saline was conducted in 22 centres from 16 countries. Random samples of at least 1000 children from ≥ 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 centres 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 % vs. 40 % between two centres 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 centres 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.

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3 *Bronchial challenge.* Participating children were asked to withhold
4 bronchodilator medications before the challenge. Regular use of inhaled steroids was
5 recorded, but not withheld. Spirometry was performed according the ATS criteria [15].
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7 At least two spirometry were recorded, and the higher of two reproducible
8 measurements (with less than 5 % variation) of forced expiratory volume in one
9 second (FEV₁) was recorded as baseline FEV₁. In children with a baseline FEV₁ of
10 <75% of the predicted value (n=103), no bronchial challenge was performed and an
11 inhaled bronchodilator was administered.
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22 Bronchial responsiveness was assessed by changes in FEV₁ during inhalation
23 of nebulised saline from a DeVilbiss UltraNeb 2000 ultrasonic nebuliser [12;13;16].
24 The children inhaled hyperosmolar (4.5 %) saline for periods of increasing duration:
25 0.5, 1, 2, 4, and 8 minutes. FEV₁ was measured 1 min after each inhalation period
26 and the next challenge period followed after further 3 min. If the FEV₁ decreased by
27 10-15 % from the baseline value, the exposure time was repeated. If, after two
28 repetitions, the FEV₁ remained 10-15 % below the baseline value, the duration of the
29 inhalation period was doubled again according to the protocol. The bronchial
30 challenge was stopped if either the FEV₁ had decreased by ≥ 15 % from baseline or
31 the total inhalation period of 15.5 min had been reached. The saline canister and
32 tubing were weighed before and after the challenge in order to measure the total
33 aerosol dose delivered. Study centre representatives were trained in one location to
34 assure a standardized performance of the bronchial challenge according to protocol.
35
36 BR was assessed in two different ways [16]: 1) Children with a decline in FEV₁ of at
37 least 15 % from baseline value or who had an increase of 25 % in FEV₁ after
38 bronchodilator inhalation (n=29) were classified to be positive regarding BHR. 2) The
39 time-response-slope (BR-slope) was calculated for each child by linear regression of
40 percentage decline in FEV₁ against log inhalation time.
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3 *Skin prick test reactivity.* Skin prick tests were performed according to a detailed
4 standardised protocol [17] with extracts of six common aeroallergens
5 (*Dermatophagoides pteronyssinus*, *D. farinae*, cat dander, *Alternaria tenuis*, mixed
6 tree pollen and mixed grass pollen) produced by ALK (Hørsholm, Denmark).
7
8 Additional allergens of local relevance were tested in eighteen centres and include:
9 olive pollen, *Parietaria officinalis*, cockroach, dog, mixed moulds, horse, mixed
10 weeds, *Cladosporium*, bird epithelium, and Turkish tree mix [18]. Atopic children with
11 a positive skin reaction were defined as having a wheal size of 3 mm or greater, after
12 subtraction of the negative control.
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15 *Gross National Income.* To allow for the difference between environments with
16 “Western” life style and more traditional or rural life style, centres were classified as
17 affluent and non-affluent as in other ISAAC Phase Two publications [17].
18 Classification was based on the gross national income (GNI) per capita, converted
19 into U.S. dollars, using the World Bank Atlas method [19]. The chosen threshold for
20 affluent was a GNI of > \$9,200 per capita, according to the World Bank’s definition of
21 “high income group”.
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25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 *Statistical analyses*

45 In order to obtain a satisfactory approximation to a normal distribution ten
46 outliers with extreme increase or decrease after the first 0.5 minute of stimulation
47 were excluded from the BR-slope analysis and the remaining values were
48 transformed by the formula $\log_{10}(\text{slope}^* - 1 + 20)$. In the tables, geometric mean values
49 of BR-slope were re-transformed to the original scale (%FEV₁ decrease per
50 log(min)).
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60 Prevalence rates were calculated for each centre by dividing the number of
positive responses by the total number of valid responses. Correlations between

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3 prevalence rates and averages at the centre level were assessed by Spearman's
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5 rank correlation coefficient ρ . For the association between an individual's wheeze
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7 status and BHR, odds ratios (OR) were determined by logistic regression. In centres
8
9 with stratified sub-samples, means, prevalences, and odds ratios were weighted to
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11 account for the sampling scheme using the SURVEY-procedures in SAS [20;21].
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13 This method provides unbiased parameter estimates (extrapolated to the population
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15 from which the stratified sub-samples were drawn) while preserving appropriate
16
17 standard errors for the weighted estimates. Results from each centre were combined
18
19 by random-effects meta-analysis (DerSimonian-Laird) to allow for both between-
20
21 centre and within-centre (sampling) variation [22]. Heterogeneity was assessed by
22
23 Cochran's Q-test and quantified by the variance component between study centres
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25 (I^2) [23]. All computations were performed using SAS® 9.2. (SAS Institute Inc., Cary,
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27 NC, USA.).
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Results

Data from 6,826 children with questionnaire data and bronchial challenge were available from 22 study centres 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 centres from 9.8 to 12.9 yr.), had a mean body height of 145.5 cm (132 to 158 cm) and a mean body weight of 40.0 kg (27 to 49 kg). The proportion of boys was 49.9 %, varying from 36 % to 60 %.

The centre-specific prevalences of wheeze, skin prick test reactivity, and bronchial hyperreactivity (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 centres. 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 FEV₁ 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) (figure 1). The rank correlation between the centre means of the two measures of bronchial responsiveness 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 (4 centres), Germany, Greece, and Sweden (each 2 centres). In these countries (with the exception of Germany) there were centres with significant differences in BR (i.e. geometric mean BR-slope above and below the international median and non-overlapping confidence intervals).

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3 The association between BHR and wheeze at the individual level was explored
4 within study centres. Results are presented in figure 2. There was a positive
5 association of similar magnitude in affluent and non-affluent countries (OR=3.63
6 [95 %-CI: 2.70-4.88] and OR=2.95 [1.61-5.40], respectively). All associations
7 between BHR and wheeze within centres were positive and most of them were
8 statistically significant, but the strength of the association varied significantly between
9 centres with I^2 of 65 % for affluent countries and 72 % for non-affluent countries.

10
11 To elucidate this between-centre heterogeneity, effect modification by atopy
12 was investigated. The association between BHR and wheeze was stronger in atopic
13 children than in non-atopic children (figure 3), to a similar degree in affluent and non-
14 affluent countries. The heterogeneity between study centres of the BHR-wheeze
15 association was substantially reduced after stratification into atopic and non-atopic
16 children, with I^2 values ranging from 20 % to 32 % (figure 3).

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18 Although BHR was associated with wheeze within centres in both affluent and
19 non-affluent countries, the overall rank correlation at centre level between prevalence
20 of wheeze and bronchial responsiveness expressed as geometric mean BR-slope
21 was weak ($\rho=0.23$, figure 4a). A similar lack of correlation was found between the
22 prevalence of wheeze and the prevalence of BHR across centres ($\rho=0.31$, $p=0.16$).
23 After stratification for atopy, there was a significant rank correlation in atopics
24 between the wheeze prevalence and the centre geometric mean BR-slope of $\rho=0.64$
25 ($p=0.002$), which was consistent in both affluent and non-affluent countries
26 (figure 4b). In non-atopics, the equivalent correlation was weaker and non-significant
27 ($\rho=0.21$, $p=0.35$), reflecting only a moderate positive correlation at the centre level of
28 $\rho=0.48$ ($p=0.29$) between the geometric mean BR-slope in atopic children and the
29 geometric mean BR-slope in non-atopic children (figure 5). The few centres with
30 higher BR-slope geometric means in non-atopic children (Mumbai, India; Tromsø,
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3 Norway; Thessaloniki, Greece) were characterised by a generally high BHR
4 prevalence, a high rate of non-atopic BHR, and a negative association between BHR
5 and skin prick test reactivity at an individual level (data not shown). However, due to
6 low sample sizes within each centre the confidence intervals of the centre-specific
7 geometric means of the BR-slope in atopic and non-atopic children mostly overlap
8 (table 2).
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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, but not at the level of study centres. However, there was only a modest correlation between BR as determined by challenge with hypertonic saline and wheeze at the centre 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. In contrast, the ISAAC questionnaire data assessed period prevalence over 12 months to exclude seasonal and diurnal variations [24]. The questionnaires were 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 labelling 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 [25-27].

Saline was chosen as stimulus in the Phase Two of ISAAC due to its safety, high acceptance by the parents and availability in centres with diverse economic and environmental conditions [28]. 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

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3 exercise-induced bronchial reactivity which was performed additionally in two
4 countries representing the extremes of the worldwide distribution of the frequency of
5 asthma symptoms in ISAAC Phase One and Phase Two, i.e. Albania with extreme
6 low prevalence rates and UK with consistently high rates. A reduction in the peak
7 expiratory flow rate of at least 15 % after exercise provocation was found about 7
8 times more often in UK than in Albania [29].
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17 As compared to methacholine challenges, hypertonic saline has a lower
18 sensitivity to detect asthma but is more closely related to allergic asthmatic airway
19 inflammation in adults [30]. This may explain the stronger association between BHR
20 and wheeze on an individual level in atopic children both in affluent and non-affluent
21 countries. Heterogeneity between study centres decreased considerably when atopic
22 and non-atopic children were analysed separately. Thus, there was a stronger
23 correlation among atopic children at the centre level between mean BR-slope and
24 prevalence of wheeze. Effect modification by atopy therefore appears to be an
25 important facet in explaining the association between BR to saline and asthma
26 symptoms, both at the individual and population levels. This measure of BR may
27 therefore be indicating a specific atopic asthma phenotype.
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44 Potentially, centres with low or undefined participation rates may not be
45 representative of the population studied. We checked for potential selection bias by
46 comparing the distributions of sex, asthma, eczema, rhinitis, older siblings, and
47 parental atopy and found no evidence that the children participating in the bronchial
48 challenge would not be representative of all eligible children. A sensitivity analysis
49 excluding centres with participation rates below 60 % did not alter the conclusions
50 above.
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Some of the study centres showed a similar ranking for wheeze and BR, e.g. low prevalence for both in Tirana, Albania and high prevalence in West Sussex, UK.

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3 Some of the centres with high levels of BR, e.g. Mumbai in India, and Kintampo in
4 Ghana, showed low prevalence rates for wheeze and positive skin prick tests. The
5 high prevalence of BHR in the study centres in Ghana and India confirms previous
6 reports of high prevalence rates of BHR among children in less affluent areas in
7 Estonia [31] and Western Australia [32]. A study in Indian children suggested that
8 exposure to cooking smoke from solid biomass fuel is significantly associated with a
9 decline in lung function and a higher prevalence of doctor-diagnosed asthma and of
10 other respiratory diseases [33]. Due to the low prevalence of smoking in India in
11 general (16%), and especially amongst women, a contribution of environmental
12 tobacco smoke to the high prevalence of BHR in India seems unlikely [34]. Da Silva
13 et al. referred to non-atopic asthma as the predominant phenotype in non-affluent
14 parts of Latin America and attributed this to a high prevalence of infection with
15 helminths [35]. Non-atopic BHR in children often occurs transiently as a reaction to
16 upper respiratory infections which may be more common in less affluent communities
17 [36].

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20 In the European Community Respiratory Health Survey (ECRHS) BR was
21 measured in 13,161 adults (aged 20-44 yrs) in 35 study centres and reported also a
22 wide variation in the prevalence of BHR (defined as a $PD_{20} \leq 1$ mg methacholine)
23 ranging from 3.4% in Galdakao, Spain up to 27.8% in Hawkes-Bay, New Zealand
24 [37]. Although prevalence rates from ECRHS cannot be directly compared to our
25 results, the ranking of comparable centres in same countries partly agreed for some
26 countries, e.g. moderate prevalence rates in the Netherlands, high rates in the United
27 Kingdom, and a broad range in Spanish centres. However, in contrast to our findings
28 Norway showed low prevalence rates and New Zealand and Germany high rates of
29 BR in ECRHS. In adults from Estonia (Tartu) a moderate BHR prevalence was
30 measured [38] but Estonian children from Tallinn showed a very low prevalence in
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3 ISAAC Phase Two. Norrman et al. discussed the lack of within country variability in
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5 Swedish adults [39], whereas in this analysis children from Östersund and Linköping
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7 showed statistically significant differences in the geometric mean BR-slope.
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10 Among non-European centres, Mumbai had the highest BHR prevalence in
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12 ISAAC Phase Two. In adults, the rate of “positive” challenge at $PD_{20} \leq 2$ mg
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14 methacholine in Mumbai was only 14 % [34] as compared to a median value of
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16 13.0 % in the remaining ECRHS centres where BHR was defined (more exclusively)
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18 as $PD_{20} \leq 1$ mg [37]. In contrast, for young adults in Brazil, a high BHR prevalence of
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20 31.3 % was reported while 10 year old children in Brazil showed a moderate BHR
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22 rate of 16.0 % according to ISAAC Phase Two criteria [35;40].
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27 Differences between our findings and the published international results on
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29 adults may arise from various sources. Firstly, the comparison of prevalence rates in
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31 school children and adults may be affected by cohort differences, in that the
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33 participants in ECRHS were born several decades earlier than the ISAAC children,
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35 and therefore lower prevalence rates might be expected [41-43]. Secondly, some
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37 phenotypes of childhood BHR like transient BHR or non-atopic BHR may disappear
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39 during adolescence as there is commonly remission of wheeze at this time [44].
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41 Thirdly, the choice of the stimulus in the BR tests may influence the results if, as
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43 suggested [30], response to saline provocation is more specifically related to allergic
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45 airway inflammation and there are marked differences in the balance of atopic and
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47 non-atopic asthma across the centres being compared. Finally, comparisons may be
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49 confounded by the choice of the participating study centre against the background of
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51 the variability in the level of BR between centres within countries.
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In conclusion, this is the first large study to assess bronchial responsiveness (BR) to hypertonic saline measured by a standardised protocol in combination with

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3 questionnaire reports of asthma symptoms and skin prick test reactivity. Association
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5 were investigated in children at both an individual level and population level in
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7 diverse settings. There was considerable variation in the level of BR between
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9 different countries and also between centres within countries. Although there was a
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11 clear association of BR with asthma symptoms within individuals, the between-centre
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13 variability of wheeze prevalence cannot be explained by the level of BR in the
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15 population. However, among atopic children, where the association of BR and
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17 wheeze was stronger at the individual level, the centres with higher levels of BR also
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19 tended to have higher prevalences of asthma symptoms.
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3 Conflicts of Interest Statement:
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5 None of the authors has a financial relationship with a commercial entity that has an
6 interest in the subject of this manuscript.
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12 The ISAAC Phase Two Study group consists of:

13 The ISAAC Phase Two Coordinating and Data Centre: S.K. Weiland † (Director),
14 G. Büchele, C. Dentler, A. Kleiner, P. Rzehak, G. Weinmayr (Institute of
15 Epidemiology, Ulm University, Ulm, Germany).
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17
18
19
20
21
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23

24 The principal investigators: A. Priftanji, A. Shkurti, J. Simenati, E. Grabocka, K. Shyti,
25 S. Agolli, A. Gurakuqi (Tirana, Albania); R.T. Stein, M. Urrutia de Pereira,
26 M.H. Jones, P.M. Pitrez (Uruguaiiana, Brazil); P.J. Cooper, M. Chico (Pichincha
27 province, Ecuador); Y.Z. Chen (Beijing, China); N.S Zhong (Guangzhou, China);
28 C.K.W. Lai (National Coordinator), G.W.K. Wong (Hong Kong, China); M-A. Riiikjäv,
29 T. Annus (Tallinn, Estonia); I. Annesi-Maesano (Créteil, France); M. Gotua,
30 M. Rukhadze, T. Abramidze, I. Kvachadze, L. Karsanidze, M. Kiladze, N. Dolidze
31 (Tbilisi, Georgia); W. Leupold, U. Keil, E. von Mutius, S.K. Weiland † (Dresden,
32 Germany); E. von Mutius, U. Keil, S.K. Weiland † (Munich, Germany); P. Arthur †,
33 E. Addo-Yobo (Kintampo, Ghana); C. Gratziou (National Coordinator), K. Priftis,
34 A. Papadopoulou, C. Katsardis (Athens, Greece); J. Tsanakas, E. Hatziagorou,
35 F. Kirvassilis (Thessaloniki, Greece); M. Clausen (Reykjavik, Iceland); J.R. Shah,
36 R.S. Mathur, R.P. Khubchandani, S. Mantri (Mumbai, India); F. Forastiere,
37 R. Di Domenicantonio, M. De Sario, S. Sammarro, R. Pistelli, M.G. Serra, G. Corbo,
38 C.A. Perucci (Rome, Italy); V. Svabe, D. Sebre, G. Casno, I. Novikova, L. Bagrade
39 (Riga, Latvia); B. Brunekreef, D. Schram, G. Doekes, P.H.N. Jansen-van Vliet,
40 N.A.H. Janssen, F.J.H. Aarts, G. de Meer (Utrecht, the Netherlands); J. Crane,
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3 K. Wickens, D. Barry (Hawkes Bay, New Zealand); W. Nystad, R. Bolle, E. Lund
4 (Tromsø, Norway); J. Batlles Garrido, T. Rubi Ruiz, A. Bonillo Perales,
5
6 Y. Gonzalez Jiménez, J. Aguirre Rodriguez, J. Momblan de Cabo,
7
8
9 A. Losilla Maldonado, M. Daza Torres (Almeria, Spain); L. García-Marcos (National
10
11 Coordinator), A. Martinez Torres, J.J. Guillén Pérez, A. Piñana López,
12
13 S. Castejon Robles (Cartagena, Spain); G. García Hernandez, A. Martinez Gimeno,
14
15 A.L. Moro Rodríguez, C. Luna Paredes, I. Gonzalez Gil (Madrid, Spain);
16
17 M.M. Morales Suarez-Varela, A. Llopis González, A. Escribano Montaner,
18
19 M. Tallon Guerola (Valencia, Spain); L. Bråbäck (National Coordinator), M. Kjellman,
20
21 L. Nilsson, X-M. Mai (Linköping, Sweden); L. Bråbäck, A. Sandin (Östersund,
22
23 Sweden); Y. Saraçlar, S. Kuyucu, A. Tuncer, C. Saçkesen, V. Sumbulöglu, P. Geyik,
24
25 C. Kocabas, (Ankara, Turkey); D.P. Strachan, B. Kaur (West Sussex, UK);
26
27 N. El-Sharif, B. Nemery, F. Barghuthy, S. Abu Huij, M. Qlebo (Ramallah, West Bank).
28
29
30
31
32
33

34
35
36 The ISAAC Steering Committee: N. Ait-Khaled (Paris, France); H.R. Anderson and
37
38 D.P. Strachan* (London, UK); C. Flohr* and H. Williams (Nottingham, UK);
39
40 F. Forastiere* (Rome, Italy); I. Asher, P. Ellwood, A. Stewart and E. Mitchell
41
42 (Auckland, New Zealand); J. Crane, N. Pearce and R. Beasley (Wellington, New
43
44 Zealand); B. Björkstén (Stockholm, Sweden); B. Brunekreef* (Utrecht, the
45
46 Netherlands); S. Foliaki (Nuku'alofa, Kingdom of Tonga); L. García-Marcos (Murcia,
47
48 Spain); U. Keil (Münster, Germany) and E. von Mutius* (Munich, Germany);
49
50 S.K. Weiland*†, G. Weinmayr* (Ulm, Germany); C.K.W. Lai and G.W.K. Wong (Hong
51
52 Kong, China); J. Mallol (Santiago, Chile); S. Montefort (Naxxar, Malta); J. Odhiambo
53
54 (Nairobi, Kenya); and C. Robertson (Parkville, Australia).
55
56
57
58

59
60 * also members of the ISAAC Phase Two Steering Group, † deceased.

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