ORIGINAL ARTICLE EPIDEMIOLOGY AND GENETICS

International variations in associations of allergic markers and diseases in children: ISAAC Phase Two

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To cite this article: Weinmayr G, Genuneit J, Nagel G, Björkstén B, van Hage M, Priftanji A, Cooper P, Rijkjärv M-A, von Mutius E, Tsanakas J, Forastiere F, Doekes G, Garrido JB, Suarez-Varela MM, Bråbäck L, Strachan DP, the ISAAC Phase Two Study Group. International variations in associations of allergic markers and diseases in children: ISAAC Phase Two. *Allergy* 2010; **65**: 766–775.

Keywords
allergen-specific IgE; allergic disease; ISAAC Phase Two; skin prick test.

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Accepted for publication 24 October 2009

DOI:10.1111/j.1398-9995.2009.02283.x

Edited by: Wytske Fokkens

Abstract

Background: Circulating allergen-specific IgE (sIgE) and skin prick tests (SPT) are used to define atopy. Downregulation of local inflammatory responsiveness has been proposed to explain a low prevalence of positive SPTs in less affluent countries. We analysed the association between SPTs, total and allergen-specific IgE and their relationships to allergic symptoms in centres with diverse living conditions.

Methods: Cross-sectional studies of stratified random samples of 8 to 12-year-old children ($n = 7461$) used the standardized methodology of Phase Two of the International Study of Asthma and Allergies in Childhood (ISAAC). Symptoms of asthma, rhinitis and eczema were ascertained by parental questionnaires. Skin examination, hypertonic saline bronchial challenge, six aeroallergen SPTs and measurements of serum total IgE and sIgE were performed.

Results: In nonaffluent countries, a higher proportion of children with positive SPT had no detectable sIgE (range 37–61%) than in affluent countries (0–37%). Total serum IgE was associated with all disease outcomes among children with both positive SPT and sIgE ($P < 0.001$), but only with self-reported eczema in children with negative SPTs and negative sIgE.

Conclusions: The international pattern of discordance between SPT and sIgE results did not support the downregulation hypothesis. Among children with no evidence of sensitization to common aeroallergens, increased total IgE contributes little to the risk of wheeze and rhinitis in the general population but may play a role in eczema.

Allergen-specific circulating IgE (sIgE) and allergen skin prick tests (SPT) are the two most commonly used markers for atopy. Often they are considered to be largely interchangeable measures, but their inter-relationship has rarely been studied in population-based samples. By comparison with circulating IgE antibody determinations, skin prick tests seem to be more closely related to allergic symptoms (1, 2). They are more susceptible to interobserver variations, however, although this can be reduced by properly standardized procedures (3). Studies in adults are inconsistent regarding the role of total IgE as

a determinant of atopic diseases, independent of SPT positivity and circulating sIgE (4–6).

Julge et al. reported a decrease in SPT reactivity in Estonian children from age three to six, while the prevalence of sIgE remained constant (7). They hypothesized a possible downregulation of local inflammatory responsiveness in Estonian children as opposed to Swedish children, where this phenomenon was not observed. It was suggested that this downregulation might explain the lower prevalence of atopic disease in Estonia and other eastern European countries.

In the present article, we investigated the relationship between circulating total IgE, sIgE and SPT in 8 to 12- year-old children participating in the Phase Two of the International Study of Asthma and Allergy in Children (ISAAC). We hypothesized that the association between sIgE and SPT as well as their relationship to clinical symptoms would differ in affluent and nonaffluent centres, i.e. between countries with a high and a low prevalence of allergic diseases.

Methods

Study populations and field work

The study methods of ISAAC Phase Two have been described in detail elsewhere (8, 9). Briefly, random samples of at least ten schools from defined geographical areas were chosen, and children ($n > 1000$ per centre) attending classes with a majority of 9 to 11-year-olds were invited to participate. Standardized self-administered parental questionnaires on symptoms were used (10). In India, the questions were posed by trained interviewers because illiteracy was common.

The ISAAC Phase Two methodology allowed objective measurements to be performed either in the full sample (option A) or in stratified random subsamples of children (option B) (8). Most centres invited all children to participate in the skin prick testing, while blood samples were collected mostly in stratified random subsamples of children with and without reports of wheeze in the past year (e. 100 in each stratum). All centres obtained approval by local ethics committees, and investigators were trained in one location to assure comparable data quality (8).

Skin prick test

The SPTs were carried out according to a detailed protocol (11). Six extracts of common aeroallergens (*Dermatophagoides pteronyssinus*, *D. farinae*, cat dander, *Alternaria tenuis*, mixed tree pollen and mixed grass pollen) produced by ALK (Horsholm Denmark) were used. The centres were encouraged to add allergens of local relevance. Additional allergens were tested in 13 centres and include the following: Olive pollen, *Parietaria officinalis*, cockroach, dog, horse and *Cladosporium herbarum*. A positive skin reaction was defined as a wheal size ≥3 mm, after subtraction of the negative control. For comparison, prevalences were also calculated using allergen wheal diameter ≥ half the diameter of the positive control (the histamine wheal), as the cut-off for defining a positive SPT.

Allergen-specific IgE

Allergen-specific IgE antibodies to a mix of common inhalant allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, birch, timothy, mugwort, cat, dog, horse, *Cladosporium*, olive pollen and *Parietaria*) were measured by Phadiatop® (Phadia AB, Uppsala, Sweden) in one central laboratory (8) except for the two German centres that used the Pharmacia SX1 CAP (Pharmacia, Lund, Sweden) (12). A positive allergen-specific IgE test was defined as ≥0.35 kU_A/l. Cut-off levels of 0.7 and 3.5 kU_A/l were also tested.

Total IgE

The serum concentrations of IgE were determined in the same laboratories as sIgE by ImmunoCAPTM total IgE (Phadia AB, Uppsala, Sweden) according to the manufacturer's instructions. The lower detection limit was 2 kU/l. The German samples were analysed with the Insulite System (DPC Biermann, Bad Nauheim, Germany) (12). A subsample of 400 German probes was also analysed in the central laboratory, and concordance was good for total IgE and sIgE (8).

Disease outcomes

Standardized parental questionnaires including detailed questions on the occurrence and severity of symptoms of asthma, rhinitis and eczema were administered. These were identical to those used in ISAAC Phase One for parents of children aged 6–7 (8, 10). In addition, children were examined for visible signs of flexural eczema (13, 14).

Statistical analysis

Total IgE levels were set to 1.99 kU/l when below the detection limit of 2 kU/l and transformed to a logarithmic scale to approximate normal distribution. Prevalences and odds ratios (ORs) were calculated using the appropriate weighting and variance estimation to account for stratified subsampling (15, 16) with the SURVEY-procedures of SAS (V9.2, SAS Institute, Inc, Cary, NC, USA).

For sIgE and SPT positivity, respectively, the association with total IgE was estimated with linear regression and is expressed as a geometric means ratio, i.e. the ratio of the geometric mean concentration of total IgE in test-positive (e.g. SPT positive) children to that in test-negative children.

When modelling disease outcomes in relation to allergic markers, models were fitted within each centre using proc surveylogistic, and combined estimates of the odds ratio were calculated using random effects meta-analysis (17).

The associations with total IgE were calculated after logarithmic transformation of IgE level. ORs of disease are presented for a tenfold increase in total IgE. Adjustment for sIgE and SPT was performed by incorporating the dichotomized variables (cutpoint 0.35 kU_A/l for sIgE and 3 mm for SPT) into the model or by using mean diameter for the SPT and the RAST-classes of the Phadiatop (Phadiatop classes: 0 = negative; 1 = 0.35–0.69 kU_A/l; 2 = 0.70–3.4 kU_A/l;

Table 1 Number and proportion of children with and without positive phadiatop (sIgE >0.35 kU_A/l) and skin prick test reaction (SPT ≥3 mm) in affluent (GNI per capita per year ≥ $9200 (9)) and nonaffluent countries

Centre	Total (sub sample) *N*	Total (full sample) *N*	sIgE– SPT– *N*	sIgE– SPT– % (weighted)*	sIgE+ SPT– *N*	sIgE+ SPT– % (weighted)	sIgE– SPT+ *N*	sIgE– SPT+ % (weighted)	sIgE+ SPT+ *N*	sIgE+ SPT+ % (weighted)	SPT– among sIgE+ % (weighted)	sIgE– among SPT+ % (weighted)
Germany, Dresden	1990	1990	1214	61.0	255	12.8	43	2.2	478	24.0	34.8	8.3
Germany, Munich	1664	1664	1043	62.7	241	14.5	7	0.4	373	22.4	39.3	1.8
Greece, Athens	188	992	111	59.6	25	13.2	12	6.8	42	20.5	39.2	25.0
Greece, Thessaloniki	206	1017	108	56.6	28	12.6	20	11.1	51	19.7	39.6	36.9
Italy, Rome	90	1722	42	56.9	4	3.6	–	0.0	47	39.5	8.3	0.0
the Netherlands, Utrecht	926	926	602	65.0	33	3.6	44	4.8	247	26.7	11.8	15.1
Norway, Tromsø	96	3476	52	64.3	1	1.5	6	4.5	38	29.7	5.7	12.8
Spain, Almeria	198	1114	81	48.8	13	6.4	5	2.6	101	42.1	13.5	6.2
Spain, Cartagena	168	1424	75	54.8	30	16.3	5	5.0	60	24.0	41.9	19.2
Spain, Madrid	324	967	178	53.4	33	9.6	22	6.5	105	30.5	23.9	17.7
Spain, Valencia	511	511	332	65.0	102	20.0	9	1.8	68	13.3	60.0	11.7
Sweden, Linköping	150	910	90	66.1	15	11.1	3	2.7	42	20.1	37.7	13.2
Sweden, Östersund	232	1193	125	62.0	11	6.2	7	1.8	90	30.0	18.5	5.3
UK, West Sussex	197	1047	102	57.1	43	21.1	2	0.9	50	20.9	52.1	4.2
Albania, Tirana	106	1056	71	71.0	13	10.2	7	8.2	15	10.6	49.6	44.6
Estonia, Tallinn	117	961	88	78.1	12	10.2	6	6.2	11	5.4	71.0	61.0
Georgia, Tbilisi	169	987	97	57.3	17	9.5	21	12.2	37	21.0	31.1	36.7
India, Mumbai	129	1704	67	51.5	42	32.0	11	7.9	10	8.6	78.9	47.9

sIge+: sIgE >0.35 kU_A/l.
sIge–: sIgE ≤0.35 kU_A/l.
SPT+: diameter (minus negative control) >3 mm for at least one tested allergen.
SPT–: diameter (minus negative control) ≤3 mm for all tested allergens.
N, number of children.
*A weighting procedure was applied to account for stratified subsampling (see Methods).

3 = 3.5–17.4 kU_A/l; 4 = 17.5–49.9 kU_A/l; 5 = 50–99 kU_A/l; 6 = ≥100 kU_A/l).

Statistical interaction on a multiplicative scale was tested by adding an interaction term to the logistic model. Additive interaction was tested calculating the relative excess risk using the SAS program by Lundberg et al. (18).

Results

Relation between sIgE and skin prick test results

The number of children within each centre with positive and negative allergen-specific IgE (sIgE) and SPT are shown in Table 1. Among children with positive sIgE, the proportion with a negative SPT ranged from 5.7% in Tromsø, Norway and 8.3% in Rome, Italy to 71% in Tallinn, Estonia and 78.9% in Mumbai, India (Table 1, Fig. 1). Among SPT-positive children, the lowest proportions of children with negative sIgE were found in Rome (0%) and Munich, Germany (1.8%) and the highest in Mumbai (47.9%) and Tallinn (61.0%). Overall, discordant results between sIgE and SPT were more common in the four less affluent countries, but levels of discordance were also high in some of the affluent centres.

Whereas less affluent centres had a higher proportion of SPT negatives among children with sIgE, as previously reported in Estonia (7), the difference between affluent and nonaffluent countries was more pronounced and more consistent for the proportion of SPT-positive children who had no detectable sIgE, regardless of whether only the standard SPT panel of six allergens or all additional local allergens were included in the analysis (Fig. 1). The pattern was similar employing varying cut-off levels for sIgE and SPTs, including the definition of a positive SPT as at least half the diameter of the histamine control (Table S1).

Relationship between sIgE, skin prick test and total IgE

The concentration of total IgE varied between the four combinations of sIgE/SPT defined in Table 1. The greatest differences in the mean concentration were found between children with and without sIgE, with generally only a moderate increase in total IgE in most centres if the children had a positive SPT (Table 2).

Total IgE was more strongly associated with sIgE than with SPT positivity (Fig. 2). Combining all centres, the geometric means ratio for the association of total IgE with sIgE (adjusted for presence/absence of positive SPT) was 3.66 [95%-confidence interval (95%-CI): 3.12;4.30], whereas the geometric means ratio for the association of total IgE with SPT results (adjusted for sIgE, cutpoint 0.35 kU_A/l) was 1.60 (95%-CI: 1.38;1.84). These ratios were of similar magnitude for centres in affluent countries and nonaffluent countries.

Association between sIgE, SPT results and symptoms

Each allergic disease outcome was analysed by a four-way combination of SPT results and sIgE results within each centre. Pooled estimates did not reveal any systematic difference

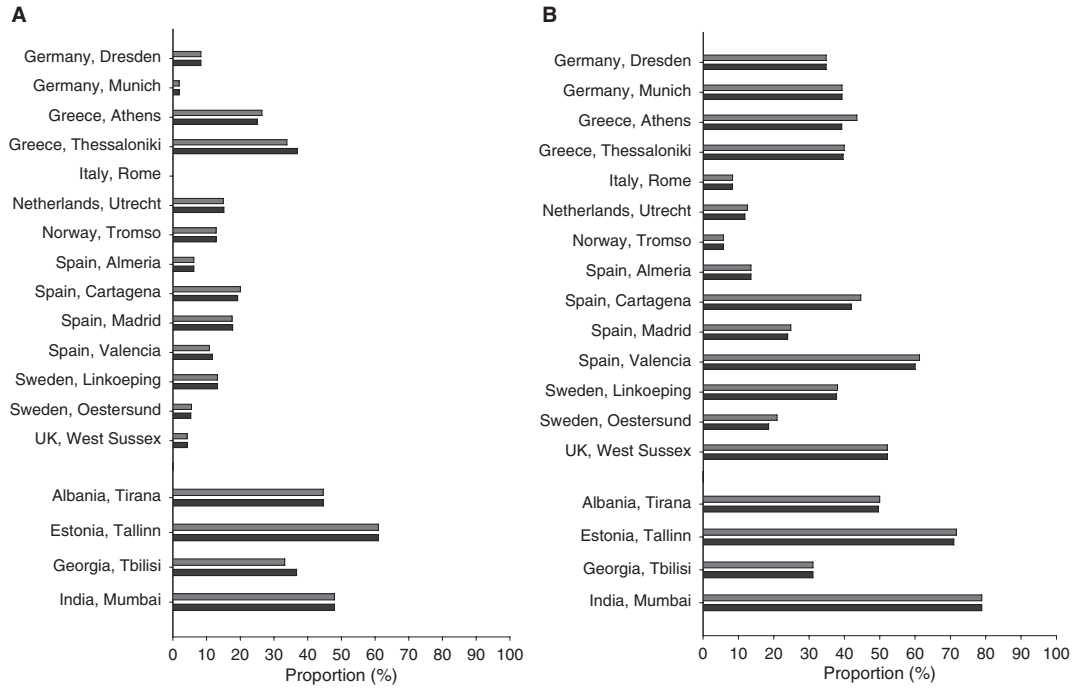

Figure 1 Proportion of children with sIgE ≤0.35 kU_A/l among skin prick test-positive children (A) and proportion of skin prick test-negative children among children with sIgE >0.35 kU_A/l. (B) ▩: local allergens in addition to the standard set were tested in the skin prick test; ■: the standard set of six common aeroallergens was tested (see Methods).

Table 2 Levels of total IgE (geometric mean) in kU/l among children with and without positive phadiatop (sIgE >0.35 kU_A/l) and skin prick test reaction (SPT ≥3 mm)

	Geometric mean of total IgE (weighted§) with 95%-CI				
Centre	All children	sIgE– SPT–	sIgE– SPT+	sIgE+ SPT–	sIgE+ SPT+
Germany, Dresden	71 (67;76)	41 (38;44)	60 (41;88)	130 (110;150)	210 (190;230)
Germany, Munich	64 (59;68)	36 (33;38)	120 (22;670)	110 (96;130)	230 (200;260)
Greece, Athens	75 (60;95)	39 (30;51)	47 (26;83)	220 (150;340)	290 (190;430)
Greece, Thessaloniki	67 (55;83)	45 (35;57)	33 (17;65)	140 (85;220)	210 (130;330)
Italy, Rome	79 (54;120)	33 (22;51)		86 (34;220)	280 (180;430)
the Netherlands, Utrecht	59 (55;63)	39 (34;43)	73 (44;120)	170 (100;270)	210 (180;250)
Norway, Tromsø	44 (33;59)	23 (17;31)	52 (28;97)	31†	134 (69;260)
Spain, Almeria	81 (63;100)	28 (20;38)	35 (6;200)	200 (110;360)	250 (190;320)
Spain, Cartagena	67 (53;84)	34 (25;45)	50*‡ (17;150)	130 (81;210)	150 (89;250)
Spain, Madrid	68 (58;79)	38 (30;46)	43 (24;78)	70 (46;110)	200 (160;240)
Spain, Valencia	55 (49;62)	32 (28;37)	43 (16;110)	130 (100;170)	160 (120;200)
Sweden, Linköping	65 (50;83)	40 (30;53)	227*‡ (9;6010)	120 (51;280)	260 (130;490)
Sweden, Östersund	55 (46;67)	34 (27;42)	19 (6;65)	100 (41;250)	130 (100;180)
UK, West Sussex	67 (53;85)	35 (26;47)	295†	110 (70;180)	230 (160; 340)
Albania, Tirana	120 (93;150)	110 (81;150)	71* (19;260)	160 (65;420)	330 (200;530)
Estonia, Tallinn	57 (45;72)	43 (32;57)	19* (3;100)	160 (86;280)	320 (76;1310)
Georgia, Tbilisi	96 (76;120)	57 (43;75)	54 (26;110)	240 (150;390)	370 (260;520)
India, Mumbai	590 (440;790)	260 (190;370)	260 (120;530)	1910 (1340;2730)	3010 (1730;5240)

*Unweighted because weights could no be calculated.
†No variation because estimate based on one or two children.
‡Estimate based on ≤5 children.
§A weighting procedure was applied to account for stratified subsampling (see Methods).
sIge+: sIgE >0.35 kU_A/l.
sIge–: sIgE ≤0.35 kU_A/l.
SPT+: diameter (minus negative control) ≥3 mm for at least one tested allergen.
SPT–: diameter (minus negative control) <3 mm for all tested allergens.

between affluent and nonaffluent countries, so combined estimates were subsequently calculated including all centres (Table 3).

The association with allergic disease was strongest for children with both a positive sIgE test and one or more positive SPTs. Adjustment for total IgE did not substantially weaken this relationship. Among children with negative SPT, all outcomes were significantly more common if sIgE was positive, whereas for children with a negative sIgE, SPT positivity was significantly associated with wheeze, rhinitis and rhinoconjunctivitis symptoms, but not with BHR, questionnaire reported eczema nor eczema detected by skin examination. Adjustment for total IgE did not greatly alter these effect estimates. Although the risk of all allergic outcomes was highest for children with both SPT and sIgE tests positive, there was no significant multiplicative or additive interaction between these two measures of aeroallergen sensitization, with respect to disease risk.

Association of total IgE with symptoms

Because the pooled estimates did not reveal any significant difference between affluent and nonaffluent countries, all centres were subsequently included in the combined estimates. There was a significant positive association of all outcomes with total IgE, with the crude ORs for a tenfold increase ranging from 1.59 (95%-CI: 1.44–1.76) for eczema detected on skin examination to 3.14 (95%-CI: 2.62–3.77) for rhinoconjunctivitis (Table 4). The strength of the associations decreased, but remained significant after adjusting for SPT results. In contrast, when adjusting for sIgE (Phadiatop class), the association with total IgE remained significant only for wheeze, questionnaire reported and examined eczema. Adjustment for sIgE using the Phadiatop class produced a larger change in effect estimate than the dichotomous variable (cutpoint 0.35 kU_A/l), whereas when adjusting for SPT, the dichotomous variable and the mean diameter of the wheal size gave similar results.

Among the children with both circulating sIgE and positive SPT, the level of total IgE was significantly associated with all six disease outcomes ($P < 0.001$ throughout). After adjustment for Phadiatop class, total IgE was no longer associated with rhinitis [OR per tenfold increase in IgE: 0.92 (95%-CI: 0.68;1.24)] or rhinoconjunctivitis [OR: 0.75 (95%-CI: 0.55;1.04)]. Adjustment for both Phadiatop class and SPT mean wheal diameter did not remove the association of total IgE with wheeze, BHR and eczema (reported or examined) (Table 4).

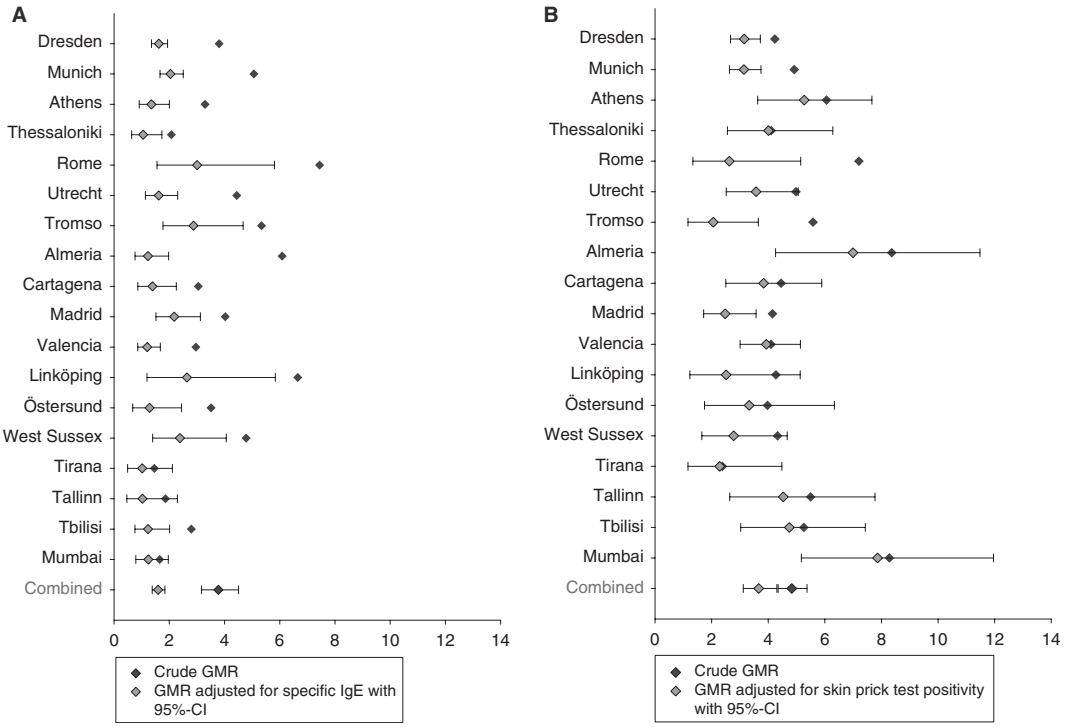

Figure 2 Geometric means ratios (GMR) for the association of total IgE with (A) skin prick test positivity (crude/adjusted for specific IgE+/–) and (B) specific IgE, cutpoint 0.35 kU_A/l (crude/adjusted for skin prick test positivity).

Finally, we investigated associations of total IgE with each disease among individuals with both a negative sIgE and negative SPTs. In this 'nonatopic' group, only the association of total IgE with questionnaire reported eczema was significant, although there was also a nonsignificant increase in prevalence of examined eczema among children with higher IgE. Neither wheeze nor BHR nor rhinitis was associated with total IgE among children with no evidence of aeroallergen sensitization.

Discussion

Relationship between markers of allergy

This large multicentre study observed discordant results of SPTs and allergen-specific IgE levels in children from diverse centres in both affluent and nonaffluent countries, despite standardized field and laboratory protocols.

There are acknowledged limitations of SPTs in epidemiological studies, particularly regarding reproducibility (3, 19). However, these were minimized by development of a standardized protocol using devices that perform most reproducibly under field conditions (20, 21) and by using the same six core allergen extracts in all centres. Staff were carefully trained and tests of reproducibility for each fieldworker were performed.

Some investigators have recommended that SPT reactions are normalized by expressing allergen wheal sizes in relation to histamine wheal diameter (22). Ronchetti et al. found that children from Italy, Libya and Poland differed in histamine skin reactivity and that allergen wheal diameters of 3 mm corresponded to markedly different sIgE concentrations (23). However, this seems not to be important for the pattern observed in our study, as the discordance between SPT and sIgE results remained similar when defining a positive skin prick reaction as a diameter of at least half the histamine control.

The discrepancy between allergen-specific IgE and SPT results is not easily attributed to the slight differences in the allergen panels used in the two tests. *Alternaria* was part of the SPT panel but not included in the Phadiatop. However, few ISAAC Phase Two participants were sensitized to *Alternaria* only *(data not shown)*. Although Phadiatop includes olive and *Parietaria*, these 'Mediterranean' allergens were added to the SPT panel if locally relevant. Some centres also included dog and horse allergens in their skin tests. However, the discordance between SPT and sIgE results was similar with and without inclusion of these additional SPT allergens.

Julge et al. observed in a longitudinal study of children from Estonia and Sweden a high proportion of Estonian children with detectable sIgE but no SPT reaction at the age of 5 (7). This was in contrast to comparable results for sIgE and SPT reactions for Swedish children and for the same Estonian children when they were younger (1–2 years old). The authors hypothesized that there may be 'downregulation' of SPT reactivity following the first years of life that may explain the lower prevalence of atopic disease in Estonia and other less affluent countries.

Table 3 Combined odds ratios with 95%-CI for the association between symptoms and different combinations of sIgE and SPT positivity

		Wheeze in the past year	BHR	Rhinitis symptoms	Rhinoconjunctivitis symptoms	Flexural eczema on examination	Self-reported flexural eczema
Crude	Ref	1.00 (–)	1.00 (–)	1.00 (–)	1.00 (–)	1.00 (–)	1.00 (–)
	sIgE+ SPT–	1.75 (1.39–2.20)	1.49 (1.09–2.04)	1.79 (1.49–2.16)	2.21 (1.53–3.19)	1.87 (1.32–2.64)	1.46 (1.17–1.83)
	sIgE– SPT+	1.68 (1.09–2.58)	1.48 (0.82–2.68)	2.58 (1.85–3.59)	4.20 (2.36–7.46)	1.22 (0.47–3.18)	1.58 (0.95-2.63)
	sIgE+ SPT+	4.09 (3.13–5.33)	2.66 (1.99–3.57)	5.00 (3.76–6.65)	8.81 (5.90–13.13)	2.72 (2.14–3.46)	2.09 (1.77–2.46)
Adjusted for total IgE	Ref	1.00 (–)	1.00 (–)	1.00 (–)	1.00 (–)	1.00 (–)	1.00 (–)
	sIgE+ SPT–	1.63 (1.28–2.08)	1.56 (1.12–2.18)	1.75 (1.43–2.15)	2.06 (1.35–3.15)	1.66 (1.14–2.40)	1.38 (1.08–1.76)
	sIgE– SPT+	1.73 (1.12–2.67)	1.45 (0.80–2.62)	2.51 (1.82–3.45)	3.90 (2.32–6.57)	1.22 (0.43–3.41)	1.56 (0.89–2.74)
	sIgE+ SPT+	3.11 (2.46–3.94)	2.29 (1.73–3.05)	4.27 (3.44–5.31)	6.70 (4.86–9.23)	1.68 (1.19–2.38)	1.67 (1.38–2.02)

sIge+: sIgE >0.35 kU_A/l.
sIge–: sIgE ≤0.35 kU_A/l.
SPT+: diameter (minus negative control) ≥3 mm for at least one tested allergen.
SPT–: diameter (minus negative control) <3 mm for all tested allergens.
BHR: bronchial hyper-responsiveness defined as a fall of >15% in FEV1 after provocation with hyperosmolar saline solution.

Table 4 Combined odds ratios with 95%-CI for a tenfold increase in total IgE [1 unit increase in lg (IgE)]

	Wheeze in the past year	BHR	Rhinitis symptoms	Rhinoconjunctivitis symptoms	Flexural eczema on examination	Self-reported flexural eczema
Crude	2.27 (1.97–2.62)	1.90 (1.59–2.26)	2.05 (1.74–2.41)	3.14 (2.62–3.77)	1.73 (1.19–2.51)	1.59 (1.44–1.76)
Adjusted for skin prick test*	1.73 (1.53–1.96)	1.43 (1.23–1.66)	1.41 (1.27–1.57)	1.99 (1.74–2.26)	1.60 (1.11–2.31)	1.42 (1.26–1.60)
Adjusted for Phadiatop class	1.20 (1.05–1.38)	1.24 (0.97–1.58)	1.02 (0.90–1.15)	1.04 (0.89–1.20)	1.45 (1.08–1.95)	1.28 (1.12–1.46)
Adjusted for skin prick test* and Phadiatop class	1.29 (1.11–1.49)	1.17 (0.96–1.43)	1.03 (0.91–1.15)	1.10 (0.93–1.30)	1.49 (1.07–2.06)	1.30 (1.13–1.50)
Nonatopic individuals (sIgE– SPT–)	1.03 (0.85–1.25)	0.95 (0.76–1.18)	1.09 (0.88–1.34)	1.15 (0.81–1.65)	1.39 (0.86–2.25)	1.29 (1.08–1.53)
Atopic individuals (sIgE+ SPT+)	2.32 (1.76–3.07)	2.39 (1.73–3.31)	2.32 (1.87–2.88)	2.64 (2.11–3.31)	3.12 (1.65–5.92)	1.66 (1.29–2.12)
Atopic individuals (sIgE+ SPT+) adjusted for SPT mean diameter and Phadiatop class	1.78 (1.27–2.50)	2.21 (1.37–3.57)	0.89 (0.65–1.21)	0.73 (0.53–1.02)	1.91 (1.05–3.46)	1.52 (1.05–2.19)

*diameter minus negative control < or ≥3 mm.
sIge+: sIgE >0.35 kU_A/l.
sIge–: sIgE ≤0.35 kU_A/l.
SPT+: diameter (minus negative control) ≥3 mm for at least one tested allergen.
SPT–: diameter (minus negative control) <3 mm for all tested allergens.
BHR: bronchial hyper-responsiveness defined as a fall of >15% in FEV1 after provocation with hyperosmolar saline solution.

In the present study of 8 to 12- year-old children, we could confirm a high proportion of children with positive sIgE but negative SPT in Estonia when compared to Sweden. Overall, the proportion of children with this result was higher in the nonaffluent countries, but high proportions could also be found in two centres in affluent countries. The proportion of sIgE negative subjects among children with positive SPTs varied even more, however, with consistently higher proportions in nonaffluent centres. This would suggest an enhanced SPT reactivity, in contrast to the pattern predicted by Julge et al. (7). Therefore, the overall picture in ISAAC Phase Two does not unequivocally support the hypothesis of 'downregulation' of SPT reactivity in less affluent countries.

Theoretically, the discrepancies between the results of the two markers of aeroallergen sensitization could depend on the fact that Phadiatop measures circulating sIgE, whereas SPT reactions integrate a sequence of events leading to activation of cell-bound sIgE and resulting inflammatory mechanisms. Pierson-Mullany et al. observed a considerable overlap of sIgE concentrations among SPT-positive and SPT-negative persons and suggested that this inconsistency could be because of individual differences in antibody affinities (24). Thus, at a given sIgE concentration, SPT-positive individuals would have higher mean binding affinities. This would predict that SPT positivity would be more strongly associated than levels of circulating sIgE with target organ effects including allergic symptoms. Although there has been no confirmation of this hypothesis by experimental studies, positive SPTs were found to have a closer relationship than did circulating allergen-specific IgE to responses to nasal provocation with purified recombinant allergens in 24 pollen sensitive adults (1).

Associations with allergic diseases

Previous publications of ISAAC Phase Two results have mainly used SPTs alone as an indication of atopy in relation to the risk of wheezing and rhinoconjunctivitis and eczema, both within and between centres (9, 25, 26). In the present study based on a subsample of ISAAC Phase Two participants, we examined the independent effects of SPT positivity, circulating sIgE and total IgE upon disease risk. Both SPT and sIgE were associated (individually and in combination) with wheeze, rhinitis and reported eczema but the associations of SPT positivity with examined eczema were weaker, after allowance for the effect of sIgE.

After adjustment for levels of sIgE, there was no independent effect of total IgE on three of the outcomes (BHR, rhinitis and rhinoconjunctivitis symptoms), whereas a significant association with total IgE persisted for wheeze and eczema. Similarly, in three UK ECRHS centres, adjustment for sIgE removed the effect of total IgE on BHR (5).

In our study, the association of total IgE with all disease outcomes investigated was reduced but still positive and statistically significant when SPT reactivity was included in the model. These findings partially agree with results of the Tucson study that reported an independent effect of total IgE on wheeze/asthma but not on allergic rhinitis (27) after adjustment for SPT reactivity.

In the British part of the ECRHS study, adjustment for SPT reactions had less capacity than sIgE to explain the relationship between total IgE and BHR (5). This was interpreted as incomplete control of confounding factors when employing SPT results (5). Our findings confirm that specific IgE may have a stronger potential for confounding. However, in our data, after adjustment for total IgE, both SPT and sIgE had similar independent effects on the prevalence of BHR (as we observed with wheeze), suggesting multiple biological pathways may be operating.

To elucidate the independent effect of total IgE, we concentrated on the association with diseases among children with no evidence of aeroallergen sensitization. In this group, only eczema showed evidence of association with total IgE levels, and there was no effect of total IgE on wheeze. Among the nonatopic individuals in the Spanish ECRHS centres wheeze in the past year in combination with BHR, but not wheeze only, was significantly associated with total IgE (4). However, we found similar patterns for wheeze and BHR.

In conclusion, the international pattern of discordance between SPT and sIgE results did not support the hypothesis that SPT reactivity is downregulated in less affluent countries. Across diverse study centres, both these allergic markers were associated (individually and in combination) with symptoms of wheeze, rhinitis and eczema in children, independent of total IgE. Among children with no evidence of aeroallergen sensitization, total IgE contributes little to the risk of wheeze and rhinitis in the general population but may play a role in eczema.

Acknowledgement

We wish to thank all children, parents, teachers, field workers and laboratory workers for their enormous contributions to this collaborative study. ALK generously provided reagents for field work in several low income countries without charge.

The agencies that funded the field work are listed elsewhere (8).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Proportion of children with and without positive phadiatop (sIgE >0.35 kUA/l) and skin prick test reaction (SPT $\geq$ half of the histamine control).

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