# Helminth infections, allergic disorders and immune responses: studies in Indonesia

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# Chapter 7

## Clustering of allergic outcomes within families and households in areas endemic for helminth infection

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## Abstract

**Background**: Allergy and helminth infections share key immunological features in terms of T helper (Th) 2 responses. Although in industrialized countries clustering of allergic disorders within families has been reported frequently, such information is lacking from areas where helminth infections are endemic.

**Methods**: A total 466 subjects within 29 families and 112 households participated in this study. Filarial infection, skin test reactivity and IgE to mite as well as total-IgE were measured in all samples. Clustering of the allergy related outcomes due to genetic and due to household factors was tested.

**Results**: Genetic factors contributed significantly to the clustering of total-IgE and allergen specific-IgE, whereas only household factors contributed to the clustering of skin prick test (SPT) positivity.

**Conclusion**: Similar to several studies conducted in western populations, total-IgE and allergen specific-IgE are influenced by genetic factors in a population resident in a helminth endemic area. However, clustering of SPT positivity due to genetic factors was not significant in the current study raising the question whether presence of helminth infections may override genes that are associated with expression of tissue reactivity to allergens in the west.

## Introduction

Like in industrialized countries, the prevalence of allergy in developing regions of the world, such as in Asia, is also increasing. A study conducted in Thailand reported a high prevalence of asthma and other allergic diseases (wheezing, rhinitis and eczema) among children and indicated that symptoms have been increasing four-fold over the last decade [404]. In Malaysia, asthma and related symptoms were common among school children in inner city Kuala Lumpur, where 10% of the children reported to have ever had asthma [181]. However, there is an important difference between urban and rural populations within developing countries with respect to the prevalence of allergic disorders. For example in the West Bank the prevalence of wheeze and asthma was higher in urban than in rural areas [166]; a significantly greater prevalence of allergic symptoms was found in urban compared with rural children in Saudi Arabia [165]; higher prevalence of exercise-induced bronchospasm was reported in urban compared to rural children in India [350]; and several African studies have indicated significant differences in allergic disorders when comparing urban with rural communities [169;170;209;249].

Although there is a strong evidence that genetic factors influence allergic disorders, this alone can not explain the increase that is being observed worldwide. However, the gene by environment interactions could play an important role in changing disease patterns in

continuously evolving environments [405;406]. It is therefore important to investigate how genetic factors modify allergic outcomes in varying environments where the expression of allergic disorders is widely different.

In western populations, genetic factors have been shown to influence allergic disorders. Not only asthma [191] but also related phenotypes such as total-IgE [407;408], allergen specific-IgE [409;410], SPT positivity [409;410] and bronchial hyperresponsiveness [411;412] have been shown to be influenced by several genes. Among environmental factors which may influence allergic disorders, increasing air pollution [174;175], rise in indoor allergen exposure [176], altered diet [177;178] and changes in exposure to microbes [179;180;413] have been studied intensively.

Recently, few studies have indicated that exposure to helminth infections which are often endemic in rural areas of the developing world may influence allergic outcomes, positively and negatively [252;282]. Helminth infections like allergic diseases are characterized by elevated levels of IgE, tissue eosinophilia, mastocytosis, and CD4+ T cells that preferentially secrete the Th2 cytokines [414]. However, chronic helminth infections are also associated with immune suppression, primarily to parasite antigens, but spill over suppression has also been described [241;277]. This suppression may play an important role for any inverse relationship observed between helminth infections and allergic disorders [180].

Th2 responses have been shown to be under genetic control as reviewed by Wills-Karp and Ewart [191]. Several chromosomal regions for instance chromosome 4q, 6, 11q and 13q showed evidence for linkage to asthma, atopy, total IgE, SPT, blood eosinophils and bronchial hiperresponsiveness; chromosomes 2, 6, 9 and 12 showed linkage to asthma, specific-IgE, and total IgE; and chromosomes 1p31, 7q, 11p13, 11q13, 12q24, 13q31, 17q12-21 and 19q13 showed linkage to asthma, bronchial hiperesponsiveness, SPT, total IgE, blood eosinophils, asthma age of onset. Thus specific chromosomal regions appear to be important in susceptibility to different phenotypes of allergic responsiveness.

In helminth infections, genetic factors play an important role in controlling outcome of an infection. A genome scan for human schistosomiasis carried out in Brazil identified chromosome 5q31-q33 as a locus responsible for controlling the intensity of *Schistosoma mansoni* infection [145] and there is also evidence for genetic control of pathology by a region containing the gene for the interferon-gamma receptor 1 subunit in this disease [146]. For nematodes, there is also evidence for genetic control of filarial infection [160;415] and a recent study conducted in Nepal has found a locus controlling *Ascaris lumbricoides* intensity on chromosomes 1 and 13 [147].

To start understanding the relationship between helminths and allergic disorders at the genetic level, it is important to investigate the behaviour of allergic disorders in populations residing in areas where helminths are endemic. As a first step towards this, we have characterized the clustering of allergic phenotypes and using a score statistics model has dissected the contribution of genetic and non-genetic factors to the clustering.

## Material and methods

### The study design

The study population consisted of life-long residents in Salubarana, Karossa district, South Sulawesi, Indonesia, an area where *Brugi malayi* is endemic. The village is located 3 kilometres from the seaside and most of the houses are situated along the main road and spread over a distance of approximately 1.2 kilometres. The major economic activity of the population is subsistence farming, which mainly involves the growing of cacao and corn. In cooperation with the head of the village and the health workers of the local District Health Centre, all inhabitants were informed about the study and invited to participate. Informed consent was obtained from all participants before skin prick testing, clinical and parasitological examination and blood withdrawal in accordance with the guidelines of Indonesian Department of Health and Human Services.

The households were visited and information was obtained about the members of the household; name, age, sex and the relation from each to another. To maximize information of the link among individuals, multi checks were made before constructing pedigrees. Information on children was obtained from parents or from adults who lived with the children whenever both mother and father were not available.

SPT reactivity for aeroallergens was tested with extract of *Dermathophagoides pteronyssinus*, *Dactylis glomerata* grass pollen, cat dander and dog dander preparation (HAL Allergen Laboratories, the Netherlands). Histamine chloride (10mg/ml) was used as the positive control and allergen diluent as the negative control (HAL Allergen Laboratories, The Netherlands). SPT was done on the volar side of lower arm, using separate 25 gauge needles. The results were measured after 15 minutes. Skin prick reactivity was determined to be positive if diameter of erythema >= 10 mm [416-418]. The same investigator performed all the skin prick tests.

Microfilariae (mf) were enumerated by filtration of 1 ml nocturnally collected blood through a Millipore, fixed with methanol, stained with Giemsa's stain and examined microscopically.

Plasma, for immunological studies was stored (immediately after separation) at -20°C for several months and subsequently transported to The Netherlands on dry ice, where it was stored at -70°C until use.

**Parasite antigen**

Adult *B.malayi* worm was purchased from TRS laboratories, Athens, Georgia, USA. Hundred female worms were freeze-dried, ground to powder on ice, dissolved in PBS (phosphate buffer saline) and slowly stirred at 4°C. The protein concentration was determined by the BCA (2,2'-Biquinoline-4,4'-dicarboxylic acid disodium salt hydrate) method before storage at −20°C [303].

**Total-IgE antibodies**

Total-IgE antibodies were measured by an enzyme linked immunosorbent assay (ELISA) in serum samples as described previously [265]. Briefly, Maxisorp plates (Nunc, Roskilde, Denmark) were coated at 4°C with 100 µl rabbit anti-human IgE (Dako, A/S Denmark) (1:1000) in 0.1 M bicarbonate buffer pH 9.6), and blocked with PBS/2%BSA (Bovine Serum Albumin) at room temperature. After washing with PBS 5% and 0.1% Tween-20, 100 µl of sera diluted in Tris 0.1 M pH 7.5 containing 0.05% Tween-20 (1:200) were added in to the wells. As a reference, the WHO standard serum of human IgE (National institute for Biological standards and Control, Hertfordshire, UK) was used, applying a nine fold serial dilution (1:2), starting at concentration of 50 IU/ml. Plates were incubated for one hour at room temperature, and after washing goat anti-human IgE-biotinylated antibody (1:1000) (Vector Burlingame, CA) was added, followed by incubation with streptavidine-alkaline phosphates conjugate (1:3000) (Dako, Glostrup, Denmark) for one hour at room temperature. Finally 100 µl 1 mg/ml p-nitrophenylphosphatase (pNPP) (Boehringer, Mannheim, Germany) in 0.1-ml diethanolamine (DEA) was added and the reaction was stopped after 20 minutes by adding 100 µl 3 M NaOH. Absorbance was measured at 405 nm. One international unit (IU) is 2.4 ng IgE.

**Allergen specific-IgE**

Serum level of mite-, dog-, cat-, grasspollen-specific-IgE were determined by radio allergosorbent test (RAST) as described previously [363]. Briefly, 50 µl serum was incubated overnight with 1.5 mg of Sepharose-coupled allergen in a final volume of 300 µl PBS, 3% BSA, 0.1% Tween-20. After washing away non-bound serum components, radiolabelled sheep antibodies (Sanquin, Amsterdam, Netherlands) directed to human IgE, were added. After overnight incubation and washing, bound radioactivity was measured. The outcomes were expressed as % binding. To convert these values into IU/ml, the results were plotted to a non-linear regression curve of chimaeric monoclonal IgE antibody dilution series against the major house-dust-mite allergen *Der p 2* and Sepharose-coupled mite extracts [304]. The cut off of the assay was 0.3 IU/ml and subjects were considered to have high levels of specific-IgE when the value was more than 1.0 IU/ml.

**ELISA for detection anti-filarial specific IgG4**

The levels of anti-filarial IgG4 were determined in ELISA as described elsewhere [305]. The OD of patient's plasma were converted into arbitrary unit (A.U.) by drawing a standard curve of a donor positive for filarial infections from Central Sulawesi who was given a value of $10^6$A.U./ml. Anti-filarial IgG4 is a valuable diagnostic tool which detects filarial infection not detectable by standard parasitological examination (blood mf). The anti-filarial IgG4 drops after treatment and it might detect not only the presence of adult worm and mf but also the earlier stages of infective larvae [419;420]. The test when using crude filarial antigen extracts has a 100% sensitivity and 95 % specificity [421].

**Statistical analysis**

Standard statistical analyses were performed in SPSS for windows version 10. More advanced analyses were performed in S-PLUS 2000 for windows. A log transformation was used to obtain normally distributed data; specific-IgG4 level, total-IgE and mite-specific IgE plus one were transformed to log 10. To derive a threshold between high and low levels of total-IgE antibodies, the frequency distribution of this antibody was plotted. Based on this distribution, the cut off of total-IgE was set at 1400 IU/ml.

Although the presence of mf is a gold standard to assess infection status in individuals, it requires a minimum threshold to visualize in blood and hence fails to establish low parasitemia and cryptic infections. Since measurement of IgG4 is more sensitive and IgG4 levels were shown to be associated with microfilaremia [419;420], a threshold for the infection status was estimated from the anti-filarial IgG4 levels. Briefly, it was assumed that the bimodal distribution of anti-filarial IgG4 levels is a mixture of two normal distributions, one representing the uninfected and one representing the infected subjects. The means of these two distributions were estimated using the program k-means in S-PLUS for windows version 2000. The value of 4.50 A.U./ml (log10) which was just in between the two means was used as threshold to define infected and uninfected subjects. This threshold was higher than 4.20 A.U./ml (log 10) when using 20 Dutch blood donors plus three standard deviation as a cut-off.

For females and males and for various age strata, the prevalence of mf, IgG4 > 4.5 A.U./ml, mite-SPT, mite-IgE>0.3 IU/ml and mite-IgE>1 IU/ml were estimated and compared using Pearson *chi-square* statistic. For the continuous outcomes anti-filarial IgG4 level, mite-IgE and total-IgE level the mean and standard deviations were computed for the females and males and for the various age strata. The means were compared using ANOVA. Next, a regression model was fitted to assess the relation between age and gender and the outcomes. We verified whether interaction between age and gender existed.

For filarial infected and uninfected subjects, the prevalence of mite-SPT positivity, of mite-IgE>0.3 IU/ml, of mite-IgE>1 IU/ml, of total-IgE>1400 IU/ml and the mean of mite-IgE and total-IgE were estimated and compared by using Pearson *chi-square* statistic for the binary outcomes and ANOVA for the quantitative outcomes. The relation between total-IgE and specific-IgE and skin prick reactivity was studied using logistic regression analysis. Here adjustments for age and gender were made.

Genetic and household clustering of allergy were tested by statistic test of Houwing-Duistermaat *et al.*, [339] using S-PLUS 2000 for windows. This has been applied to test clustering of filarial infection due to genetic and household effects [160;339;422]. Briefly, the genetic and the household correlation structures were based on distances between individuals. The correlation between genetic effects of two individuals equals $2^{-dg}$ in which dg is the genetic distance in the pedigree. Thus parents and offspring have dg=1, pairs of siblings have dg=1, grandparents and grandchildren have dg=2, aunts/uncles and nephews have dg=2, cousins have dg=3, great-grandparents and great-grandchildren have dg=3, great-uncles/aunts and nephews have dg=3, and grand-nephews can have dg=4 or dg=5 depending on the degree of kinship. Individuals who are not related have infinity as distance. In the data the distances between relatives varied from 1 to 5. The distances for the household model were defined by dh, with dh=0 for individuals sharing the same house (correlation 1) and distance infinity for inhabitants of different households (correlation 0). The hypotheses of no correlation due to genetic or household effects were tested versus the alternatives using the test statistic of Houwing-Duistermaat *et al*. [339]. The allergy related parameters were adjusted for age and sex.

## Results

### Study population: infection and allergy

A total of 466 participants from Salubarana, aged 5 – 84 years (mean 28.8 years) participated in this study and the number of females and males in this village was comparable (225 females and 241 males). Filarial infection was determined by conventional blood filtration technique and by specific anti-filarial IgG4 as an indicator of active infection. Of the 466 subjects screened 8.6% were microfilaremic whereas 53.15% were found positive for anti-filarial IgG4. As already described in materials and methods, this test is highly sensitive (100%) with a 95% specificity [421].

In the total population skin prick reactivity was 11.6% and predominantly found against house dust mite allergen (6.0%), the rest of the reactivity was to grass pollen, cat and dog (4.2 %, 1.5% and 2.1%, respectively), all participants showed a positive response to the histamine control.

**Effect of gender and age on infection and allergy**

Tables 1 and 2 show the prevalence of mf, active infection status (detected by antifilarial-IgG4), mite-SPT and the level of mite-IgE (>0.3 IU/ml and >1 IU/ml) as well as the means of anti-filarial IgG4, mite-IgE level and total-IgE levels as a function of gender and age. In the total population it was shown that mf prevalence and anti-filarial IgG4 were higher in males than in females and increased significantly with age. For allergy phenotypes, both the levels and the prevalence of specific mite-IgE (using cut off points 0.3 or 1 IU/ml) as well as total-IgE were higher in males compared to females but no difference was found in mite-SPT reactivity. When age was divided into four group, as shown in table 2, the prevalence of mite-SPT as well as levels of mite-IgE reached a peak in age group 15-29 years and then decreased with increasing age. The levels of total-IgE also decreased with increasing age. From scatter plots of age versus mite-IgE and age versus total-IgE (data not shown), it appeared that the relation between age and the levels was best described by using the natural logarithm of age (lnage). The lnage had also been used when analysing the clustering of filarial infection (35). Therefore in all further analyses we used lnage as covariate. Using regression analysis we found an interaction between age and gender for infection status and mite-IgE, whereas no interaction for mite-SPT and total-IgE was detected.

| | Female(n=225) | Male(n=241) | p |
|---|---|---|---|
| Microfilariae prev., % | 5.8 | 11.2 | 0.037 |
| Filarial infection prev., % | 39.1 | 67.2 | <0.001 |
| Mite-SPT prev., % | 7.1 | 4.1 | 0.24 |
| Mite-IgE >0.3 IU/ml prev., % | 56 | 66.4 | 0.021 |
| Mite-IgE >1.0 IU/ml prev., % | 21.3 | 33.6 | 0.003 |
| Total-IgE > 1400 IU/ml prev., % | 28.2 | 34.8 | 0.001 |
| Anti-filarial IgG4 level, A.U./ml | 4.24(3.40-5.16) | 4.90(4.22-5.77) | <0.001 |
| Mite-IgE level, IU/ml | 0.20(0.05-0.27) | 0.27(0.09-0.38) | <0.001 |
| Total-IgE level, IU/ml | 3.30(2.97-3.72) | 3.53(3.20-3.94) | <0.001 |

**Table 1**. Measurements as a function of gender.
Prevalence of microfilariae, filarial infection (determined by anti-filarial IgG4), skin test reactivity to mite (mite-SPT), IgE to mite (mite-IgE; low level sensitization: >0.3 and high level sensitization: >1.0 IU/ml), high level of total-IgE (>1400 IU/ml) and the levels of anti-filarial IgG4, mite-IgE and total-IgE are indicated for 466 subjects. P-values were measured using Pearson *chi-square* for comparing prevalences and one way ANOVA for comparing anti-filarial IgG4, specific- and total-IgE levels.

**Effect of infection on SPT and IgE antibodies**

When mf presence was used to identify infected subjects, none of the 40 subjects positive for mf were positive for mite-SPT, whereas all 27 mite-SPT positive subjects were amicrofilaremic; this difference fell short of statistical significance (p=0.10). When using anti-filarial IgG4 to assign study subjects into "infected" and "non infected" categories, prevalence

of skin test reactivity to mite was similar in infected and non infected groups whereas infected group showed higher total-IgE and sensitisation to mite (mite-IgE) (Table 3).

| | Age <=14 (n=122) | Age 15-29 (n=138) | Age 30-44 (n=111) | Age >44 (n=95) | p |
|---|---|---|---|---|---|
| Microfilariae prevalence, % | 4.1 | 5.1 | 11.7 | 15.8 | 0.005 |
| Filarial infection prevalence, % | 32 | 55.1 | 59.5 | 72.6 | <0.001 |
| Mite-SPT prevalence, % | 6.6 | 7.2 | 5.4 | 3.2 | 0.59 |
| Mite-IgE >0.3 IU/ml prevalence, % | 58.2 | 65.9 | 62.2 | 57.9 | 0.52 |
| Mite-IgE >1.0 IU/ml prevalence, % | 23 | 35.5 | 29.7 | 20 | 0.035 |
| Total-IgE >1400 IU/ml prevalence, % | 30.8 | 38.3 | 44.1 | 48.1 | 0.32 |
| Anti-filarial IgG4 level, A.U./ml | 4.15(3.31-5.07) | 4.50(3.82-5.42) | 4.80(4.14-5.75) | 5.00(4.46-5.67) | <0.001 |
| Mite-IgE level, IU/ml | 0.22 (0.06-0.28) | 0.27 (0.06-0.40) | 0.24 (0.07-0.37) | 0.19 (0.07-0.26) | 0.046 |
| Total-IgE level, IU/ml | 3.54 (3.18-3.94) | 3.44 (3.09-3.92) | 3.36 (3.05-3.78) | 3.29 (2.94-3.77) | 0.013 |

**Table 2**. Mesurements as a function of age. Prevalence of microfilariae, filarial infection (determined by anti-filarial IgG4), skin test reactivity to mite (mite-SPT), IgE to mite (mite-IgE; low level sensitisation; >0.3 and high sensitization: >1.0 IU/ml), high level of total-IgE (>1400 IU/ml) and the levels of anti-filarial IgG4, mite-IgE and total-IgE are indicated for 466 subjects. P-values were measured using Pearson *chi-square* for comparing prevalences and one way ANOVA for comparing the levels of anti-filarial IgG4, specific- and total-IgE.

| | Non infected (n=216) | Infected (n=250) | p |
|---|---|---|---|
| Mite-SPT prev., % | 6.0(13/216) | 5.6(14/250) | 0.85 |
| Mite-IgE >0.3 IU/ml prev., % | 56.5(122/216) | 65.6(164/250) | 0.04 |
| Mite-IgE >1.0 IU/ml prev., % | 21.8(47/216) | 32.8(82/250) | 0.008 |
| Total-IgE >1400 IU/ml prev., % | 62.5(135/216) | 77.6(194/250) | <0.001 |
| Mite-IgE level, IU/ml | 0.21(0.06-0.29) | 0.25(0.07-0.37) | 0.021 |
| Total-IgE level, IU/ml | 3.33(2.97) | 3.50(3.22-3.88) | 0.001 |

**Table 3**. Measurements as a function of infection status as determined by anti-filarial IgG4. Skin test reactivity to mite (mite-SPT), IgE to mite (mite-IgE; low level sensitization: >0.3 and high sensitization: >1.0 IU/ml), high level of total-IgE (>1400 IU/ml) and the levels of mite-IgE and total-IgE are indicated for 466 subjects. P-values were measured using Pearson *chi-square* for comparing prevalence and one way ANOVA for comparing specific- and total-IgE levels.

### Determinant of skin reactivity

In industrialized countries total-IgE and specific-IgE are identified as risk factors for skin prick reactivity and allergic diseases. Regression analysis was performed to determine the contributions of total-IgE and specific-IgE to the outcome of a positive SPT to mite. Association for these independent variables with skin test reactivity was adjusted for age and gender. Mite-SPT was strongly associated with mite-IgE reactivity and total-IgE (b=3.315, CI=2.63-4.00, p<0.001 and b=1.163, CI=0.92-1.77, p=0.007, respectively).

### Clustering analysis of mite-SPT, mite-IgE and total-IgE

The study comprised of 29 families which ranged in size from one to 214 individuals, the mean number of members per family was 16.1. The 466 inhabitants examined were

spread over 112 households, the households ranged in size from one to 18 individuals and the mean number of members per household was 4.16. Mite-SPT reactivity, mite-IgE and total-IgE were analysed using the test statistics developed by Houwing-Duistermaat [339]. The result showed that both genetic and household factors could explain the clustering of mite-IgE as well as total-IgE responses, whereas only household effects significantly explained the clustering of mite-SPT reactivity (table 4).

| Outcome | Genetic | Household |
|---|---|---|
| Mite-SPT | 0.121 | 0.018 |
| Mite-IgE >0.3 IU/ml prevalence | <0.001 | <0.001 |
| Mite-IgE >1.0 IU/ml prevalence | 0.007 | 0.122 |
| Total-IgE >1400 IU/ml prevalence | <0.001 | <0.00 |
| Mite-IgE level, IU/ml | 0.004 | 0.037 |
| Total-IgE level, IU/ml | <0.001 | <0.001 |

**Table 4**. P-values for testing the null hypothesis of clustering of mite-SPT reactivity, prevalence and the level of mite- and total-IgE due to genetic and household effects. Mite-SPT and total-IgE were adjusted for sex and age, whereas for mite-IgE, sex, age and interaction of sex and age were used as adjustment.

## Discussion

### Skin prick test

The prevalence of skin prick reactivity to aeroallergens in this study population was found to be 11.6%. This number is comparable with the results of studies conducted in developing countries in Africa [168;265;423], but much lower than those reported in the developed countries [424]. Moreover, similar to studies carried out in Africa and in South America [168;248;265;423], a positive SPT and IgE sensitization was predominantly against house dust mite in residents of Salubarana.

### Differences in allergy phenotypes as a function of gender and age

Although we did not find any significant differences in mite-SPT as a function of gender, sensitization to mite (mite-IgE) was significantly higher in males than in females. The higher prevalence of allergies in males, when described by mite-IgE [425;426] or by mite-SPT [427;428] have been described in several studies in Europe. The absence of a gender related difference in mite-SPT has been observed in other studies conducted in developing countries [249;429] and the mechanisms behind this discrepancy in the data generated in Western populations and those in developing countries has not been investigated. The mean of mite-IgE reached a peak in the age group 15-29 years and then decreased in older age groups. The decline is similar to studies carried out in age groups 20-70 years which found a decline in mite-IgE with increasing age [425].

**Effect of infection**

The general expansion of IgE producing B-cells during helminth infections may explain the higher levels of IgE antibodies in the infected subjects. The question of whether any cross reactivity between house dust mite and helminth antigens occurs, as reported in a study before [430] needs to be investigated in our samples. Considering SPT reactivity, none of the mf positive individuals were positive in mite-SPT. Although the results do not reach statistical significance (P=0.10), most probably due to the small number of mf positive subjects, the data suggest that the presence of mf in blood has a protective effect on mite-SPT reactivity. This is in agreement with the findings that schistosomes and intestinal helminths reduce the risk of atopy [241;249], asthma [256] or wheeze [170]. However, when anti-filarial IgG4 was used as an indicator of active infection, no effect of infection on SPT positivity was observed. The discrepancy between the results when using mf positivity versus positivity for anti-filarial IgG4 may be due to the fact that only high infection levels (those with detectable mf) are able to protect from SPT positivity. Indeed anti-filarial IgG4 is believed to detect low level infections or amicrofilaremics who have cryptic or single sex infections [73;307].

**Contribution of mite-IgE and total-IgE to SPT positivity**

As in studies carried out in western populations, the high level of mite-IgE in this study population was associated with a 6-fold increase in the risk of responding to mite extracts in SPT. However this risk factor is relatively low compared with industrialized countries where having specific-IgE generally translates into positivity in SPT [362;431]. In the Netherlands the presence of mite-IgE was associated with a 39-fold increase in the risk of SPT to mite (van der Zee, unpublished data). Several reasons may be considered for this difference. Total-IgE: Some investigators have attributed the negative association between helminth infections and allergic disorders to the presence of high levels of total-IgE in helminth infected subjects; non specific-IgE may be able to saturate Fcε receptors and therefore compete away allergen specific-IgE [432]. However, more recent studies using appropriate statistical methods have found no effect of total-IgE on the risk of skin prick reactivity or wheeze [251;265] supporting our findings here. Specific-IgE: It is possible that the affinity of IgE antibodies to mite is lower in individuals infected with helminths. The lower affinity of mite-IgE would then lead to lower mast cell degranulation [180]. This possibility has yet to be investigated in detail. Immunoregulation: In helminth infected subjects, there is increased regulatory responses characterized by high IL-10 production [241;433] and the presence of regulatory T cells [434]. These suppressory responses may contribute to lower allergic outcomes in the face of elevated IgE antibodies [241].

**Clustering of allergic phenotypes**

From studies carried out in the West, it is clear that genetic and household factors influence predisposition to allergic disorders. In our study carried out in a rural area in a developing country, we have evaluated the influence of family and household factors on mite-SPT, mite-IgE and total-IgE in an area endemic for filariasis. We used a test

statistic that screens data sets for different correlation structures [339] to test whether clustering exist due to genetic or household factors. The genetic model indicates that a phenotype is more similar between genetically related subjects who may belong to different households whereas the household model indicates that the phenotype is clustered within a household due to factors that govern lifestyle within a household.

When the test was applied to allergic responses; namely mite-SPT reactivity, mite-IgE sensitization and total-IgE, it showed different results. When IgE response to mite was considered, both genetic and household factors could significantly explain clustering of this trait. Thus both household factors and genetic factors may contribute to mite-IgE and total-IgE responses in this population. Interestingly when the test is used to examine the aggregation of mite-SPT, only household effect was found to be significant, indicating that household factors affect this outcome. The evidence for a genetic effect on SPT has been observed in some studies in the West [191]. The weak genetic effect on SPT reactivity in our population may result from the ability of helminth infections to modulate skin reactivity to allergens. When using mf positivity as a measure of infection, it was clear that indeed there may be an effect of filarial infection on SPT, however, when anti-filarial IgG4 was used to also detect low level infections or exposure to larval stages of parasites, we did not see any effect of infection on SPT outcomes. These results suggest that high level infections, but not light infections, modulate the SPT. The specificity of anti-filarial IgG4 has been estimated to be 95% using sera from Europeans infected with other nematode or trematode infections [421]. The assay may therefore also indicate the minor presence of some other helminth infections. Considering this, one would interpret the result that low level filarial infections and other helminths such as intestinal nematodes, are less effective in modulating SPT. To substantiate this, studies need to be undertaken in another area where mf prevalence is higher in order to be able to determine with certainty the modulating effect of SPT by mf.

This study has shown that some allergic determinants such as IgE antibodies are under genetic control in populations residing in areas where encounter with pathogen such as helminths. However, not all allergic outcomes are under genetic control. For skin reactivity to house dust mite, which in areas of low pathogen exposure has been reported to be under genetic control, may be also affected by environmental effects. These data raise the question whether frequent exposure to pathogens such as to helminths can override the genetic influences of allergic traits, indicating a situation of gene by environment interaction.

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