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THE EFFECTIVENESS OF MULTIFACETED INTERVENTION PROGRAMS IN THE PRIMARY PREVENTION OF ASTHMA IN HIGH-RISK TODDLERS AND PRESCHOOLS AFTER THIRTY SIX MONTHS OF FOLLOW-UP

By

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ABSTRACT

THE EFFECTIVENESS OF MULTIFACETED INTERVENTION PROGRAMS IN THE PRIMARY PREVENTION OF ASTHMA IN HIGH-RISK TODDLERS AND PRESCHOOLS AFTER THIRTY SIX MONTHS OF FOLLOW-UP

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Design: European (England, Germany, Greece, Lithuania) multi-center prospective single-blind randomized control trial with a follow-up of 36 months

Participants: Toddlers and preschoolers, with at least 1 parent with atopic symptoms and sensitization, who initially were not sensitized to house dust mite allergens.

Interventions: A combination of education and mattress cover.

Main Outcome Measure: Asthma Diagnosis by Physician and wheezing.

Results: We demonstrated that a prevention program in high risk toddlers and preschoolers with a follow-up of 36 months resulted in a modest but significant reduction in the risk of asthma (OR= 0.66, 0.45-0.98) and wheezing (OR= 0.66, 0.45-0.98).

Conclusion: In this analysis we have demonstrated that follow up of 36 months intervention program, focused on mattress cover reduced the incidence of asthma and wheezing in high risk children.

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INTRODUCTION

Asthma is an important health problem in the United States and worldwide (1). It is defined by the US National Heart, Lung and Blood Institute, as a chronic inflammatory disorder of the airways. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, and chest tightness, particularly at night or in the early morning (1). In 1995 the US National Health Interview Survey, reported that approximately 5% (15 million) Americans have asthma, with 5 million under the age of 18 years (2). Both U.S. and international studies assessing changes in the prevalence of asthma symptoms using standardized methods in the same community at different times have reported increased asthma prevalence over the last years.(3) This increase has been observed in a wide range of countries with varying lifestyles (4). Increased prevalence of diagnosed asthma or asthma symptoms in children and adolescents reported in either standardized national or regional surveys ranges between 25% and 75% per decade during the period from 1960 to 1990(2). Consistently higher prevalence rates are reported among children of color as compared to white children, and in urban compared with rural areas (5). Limitations of these studies include lack of objective markers of asthma, and perhaps increased awareness and reporting of asthma by patients and physicians (5).

Asthma morbidity also shows evidence of increase throughout the world. Rate of hospital admissions is considered a reliable measure of asthma morbidity. This increase, as measured by hospital admissions, is most pronounced in young children beginning in the 1960s. Between 1960 and 1980, U.S. hospitalization rates for asthma increased by more than 200% in children and 50% in adults (6). For most cases, diagnostic transfer

does not explain these increases. Increased rates of hospital admission reflect an increase in severity of asthma (2).

During pregnancy there is a relative dominance of Th2-lymphocytes, necessary to prevent rejection of the fetus by the mother. During the first years of life, this Th2-state usually converts to a Th1-dominance, which is the normal state for a non-allergic child. It seems likely that environmental allergens during the first years of life influence the maturation of the T cell system, leading to persisting predominance of Th2 reactivity. Stimulated Th2-cells secrete IL-4, IL-5,IL-10 AND IL-13, which facilitate IgE immune response and eosinophil activation, increasing the risk of becoming sensitized and developing allergic symptoms(7).

Important factors thought to implicate in the rapid increase in IgE-mediated disease in our society are polluted air outdoors, changes in the infant bacterial gut flora, or changes in the panorama of infections during early life (8). It is likely that the causes of sensitization are complex. Our knowledge about etiologic factors must still be regarded as fragmentary (7).

Primary prevention of asthma means preventing the development of asthma in a child at risk or the development of allergy and thus preventing allergic asthma, and secondary prevention to prevent the onset of symptoms in a child with asthma. There is general agreement about the importance of secondary prevention measures in patients with asthma or in those already sensitized to allergens to reduce the burden of disease. Primary prevention is optimal, but it is often unclear what kinds of intervention will be effective (7).

Among indoor allergens, House Dust Mite (HDM) is the most common sensitizer especially in mild climates or and in houses with high humidity. The proportion of sensitized children increases by age during the first two decades of life (9). Because sensitization often precedes allergic airway disease, public health efforts to reduce exposure before sensitization occurs can reduce the burden of disease.

Both prospective and cross-sectional studies have shown a dose- response relationship between the level of exposure to HDM allergens and allergic sensitization. An amount of HDM allergen necessary to sensitize genetically at-risk children is unclear (8, 10).

Mattresses, underbedding, quilts and pillows are significant reservoirs of HDM allergens. Many studies have been conducted to determine the efficacy of encasing bedding items to reduce the levels of HDM allergens. Recent studies have generally found an effective reduction in HDM as well as compliance with the use of the encasing (11).

LITERATURE REVIEW

The following studies will be reviewed (Table 1): The ISLE study (11,12,13), the CAPPS study (14), the SPACE study (the birth cohort part) (15), the CAPS study (16), the PIAMA study (17), and the MAAS study (18). The Clinical outcome measures for those primary prevention studies, intervention vs. control group are presented in Table 2 (19).

Ongoing primary prevention studies attempt to evaluate the effect of different intervention strategies, especially dietary intervention or/and HDM allergens avoidance. These trials have dissimilar end points involving sensitization to different allergens and

airway symptoms such as cough and wheeze. The challenge to interpretation is that the studies used different protocols, and some combined two or more intervention procedures, such as dietary intervention plus avoidance of HDM and pet allergens as well as tobacco smoke.

Outcome definition in studies

Infants at risk of asthma are often also at risk for atopy. "Atopy" has previously been used as a poorly defined term to refer to allergic conditions which tend to cluster in families, including hay fever (allergic rhinitis), asthma, eczema, and other specific and non-specific allergic states (20). More recently, the term atopy has been restricted to conditions that are associated with the production of specific IgE in response to common environmental allergens. Skin prick testing provides a convenient test for atopy in epidemiological studies (21). However, it has been suggested that total serum IgE provides an overall estimate of the allergic component in asthma, and that total serum IgE is associated with asthma independently of specific IgE levels (20).

Infant atopy is usually easy to assess by skin prick test, while determining asthma is more challenging. Lung function tests are routinely used in adults and older children, but are difficult and time-consuming to perform in young children, particularly in those under 4 years of age. Some new techniques have emerged in recent years, for example, body plethysmography and airways resistance using Impulse Oscillometry (IOS). IOS uses quiet breathing maneuvers to detect subtle changes in airways resistance. The minimal requirements for patient cooperation enable better data collection in children and adults who may not be able to fully cooperate with standard pulmonary function measures. However, these methods are often not available for larger epidemiological

studies. As a result, in studies of young children (with the notable exception of the ISLE study), the diagnosis of asthma is mainly based on parental self report of the child's wheezing, cough, other respiratory symptoms, or physician assessment (12). However, a limitation of lung function tests is that one test provides only current status, as contrasted to, information on a history of wheezing and coughing.

Inclusion / Exclusion Criteria

To evaluate the outcomes of primary prevention, almost all studies identify infants with a family history of asthma or allergy/atopy before or shortly after birth. Note, the reviewed studies did not identify genetic susceptibilities, since this would have required determining genetic polymorphisms. However, studies employ different definitions of ''high risk for atopy''. ISLE (11,12,13), the first randomized trial of asthma prevention, used, as inclusion criteria, presence of either one or two parents with positive food hypersensitivity plus elevated cord blood immunoglobulin E (IgE)>0.5 kU/L. (CAPPS) (14) included infants with at least one first degree relative with asthma or two first degree relatives with other IgE mediated diseases. A few studies used inclusion criteria such as (1) self report of atopy history in both parents, and (2) a positive SPT or specific IgE test in a panel of common aero-allergens in one or both parents. Two of the six studies (Table 1) included newborns with non-allergic parents as well as newborn of allergic parents (14, 17).

Prior prevention trials had only few exclusion criteria. These included avoidance of pets for all or part of the study participants (14, 18). No other special exclusive criteria were reported in any of the studies.

Length and Timing of Allergic Avoidance

Most studies started intervention from birth and not before birth. In the Manchester Allergy and Asthma Prevention Study (18) the intervention was started in the 3rd trimester of pregnancy. There is inconsistency in the literature in regards to when is the most beneficial time to start an intervention program in the last trimester of pregnancy compared to starting at birth (18).

In addition, there was no consensus among the prevention studies as to an effective length of intervention and observation period. SPACE (15), with 696 newborns, conducted a 1 year intervention program with assessment at 1 year of age. The Australian study CAPS has reported on the longest intervention period to date with results at 18 months within a study that will follow children up to 36 months (16). Only one study (The Isle of Wight Study) (11, 12, 13) tracked children for 8 years after an intervention in infancy. Positive results after the age of 1-2 years are encouraging, but are insufficient to assess whether long term reduction of house dust mite reduces the risk of allergy or asthma.

HDM Allergens Eradication & Methods

The amount of allergen necessary to sensitize genetically at-risk children is unclear. Prospective and cross-sectional studies have shown a dose response relationship between atopy and the level of exposure to HDM. Thus we would expect to see a lower prevalence of atopy in an intervention group (19). Mattresses, bedding, quilts and pillows have been found to be significant reservoirs of HDM and thus a target for intervention. Many studies have been conducted to determine the efficacy of encasing bedding items to reduce the level of HDM allergens. Recent evidence shows a generally positive effect (as

covers for the baby's bed and for parents' bed, since in some families the newborn spends a significant amount of time in his or her parents' bed. In some trials the prevention program included a request to cover any additional mattress in the child room (12, 14, 18). Other common dust mite allergens eradication methods have included chemical eradication, replacing the carpet in the infant room with vinyl flooring, usage of high filtration vacuum cleaning, usage of hot washable toys, and regular hot washing of bedding (11, 13, 14, 18). There is no agreement among the main randomized trials as to which are the most effective interventions to reduce dust mite allergen levels. The Manchester study (18) used the most stringent intervention by including all known methods. In contrast, the SPACE study (15) used only the physical method (covering the mattress on the infant's bed) and gave recommendation to the parents in the intervention group to use other methods.

Most studies (except SPACE) conducted home visits to assess the allergen levels in a few locations in the child's home, such as bed, bedroom, and living room. There was no agreement across studies as to which areas needed to be checked (11-18).

Blinding

The PIAMA study (17) was the only double blind, placebo-controlled trial evaluating the use of mite-impermeable mattresses and pillow covers. The study encountered a methodological problem when it provided the placebo group with placebo covers. Typically made out of cotton, the placebo cover proved to some extent to impermeable to dust mite.

Most of the studies gave up the option of blinding researchers and patients, since it limited extensively the kind of intervention program they could use. Most other components of the prevention program don't have 'placebo' type options.

Combination of Intervention Procedures

One challenge in evaluating prevention efficacy is the fact that all of the primary prevention studies had different study protocols, often combining two or more intervention strategies. However, even when two studies used the same type of intervention strategies, usually nutrition and indoor allergens, they followed different protocols. For instance, in both the Manchester and Australian studies, a combination of HDM reduction and dietary intervention was introduced. While the HDM avoidance methods were almost identical, the dietary interventions were different. The Manchester study (18) asked mothers to follow a special, restrictive diet in case they were breastfeeding their babies, and not to introduce solid food to babies before 6 months of age. In contrast, the Australian study (CAPS) (16) asked mothers to follow a dietary intervention involving daily use of oil supplement to increase omega-3 intake.

Summary

In summary, Asthma is the most common chronic disease of childhood in developed countries and one of the few treatable conditions that has increased in prevalence and severity over the last 20 years (2). In the last decade, epidemiological studies have contributed important information about the environmental risk factors associated with childhood asthma, and about which modifications of these factors offer the best opportunities for prevention. The most important factors include exposure to

indoor and outdoor allergens or environmental tobacco smoke, the presence of respiratory infections in early life, and dietary factors.

The objectives of this work are to prospectively determine whether HDM indoor allergen avoidance by physical methods and education programs for 36 months in toddlers and preschoolers genetically predisposed to atopy, would reduce the development of asthma and wheezing. The hypothesis is that allergen avoidance (HDM) in toddlers and preschoolers (~2-5 years old), in children genetically predisposed to atopy would reduce the development of asthma and wheezing, after 36 months of follow-up. The hypotheses were tested in a randomized controlled trial of large sample size for children in risk. The intervention that has been assessed is simple and affordable and thus, can be use on a large scale in 'real life' by the public.

METHODS

Population

The data used in this research was assembled through the "Study on the Prevention of Allergy in Children in Europe" (SPACE). This study was a multi-center, population-based, randomized control study of children at high risk of allergy from the countries of Austria, Germany, Greece, Great Britain, and Lithuania. The objective of the SPACE study was to prevent sensitization to house dust mite and food allergens, as well as the development of atopic symptoms during infancy through the use of mite allergen impermeable mattress covers. The SPACE study consisted of three cohorts of participants: schoolchildren, preschool children/toddlers, and newborns. This analysis focuses on preschool children and toddlers from Lithuania, Greece, Great Britain, and Germany.

Prior to initiation of the SPACE study, the local ethical committees at each of the research sites approved the working protocol of the study. During recruitment, informed consent was obtained from the parents of each child prior to the collection of all measurements proposed in the study (Figure 1). Preschool children and toddlers were recruited into the SPACE study based on the atopic history of their parents. Through the recruitment process, the parents were instructed to complete screening questionnaires for symptoms associated with the presence of allergic disease. If a history of bronchial asthma, atopic eczema, allergic rhinitis, or hay fever was reported by either of the parents, skin prick testing or serum IgE measurements were performed on the parents. If one or both of the parents reacted positively to the SPT or positively to at least one allergen out of the panel of five aeroallergens tested (*Dermtophagoides pterosyssinies*, *D. farinae*, birch pollen, grass pollen, and cat dander) or if measurements of allergen-specific IgEs were equal or larger than 1.43 kU/L, then their child was eligible for the study (Figure 1).

Exclusion criteria implemented in this study were children who (1) were not residents of the respective country and (2) were sensitized to mite allergens at the beginning of the study (per the results of the prick test).

Between May, 1997 and May, 1999, 636 Preschool children and toddlers were recruited into the study and followed up through August, 2001. Children were then randomly allocated to the intervention group or to the control group. The allocation to the study groups within each country was based on the day of the visit, according to a block randomization of a 2-week time period.

Exposure Measurement

A standardized questionnaire focusing on allergens exposures was also completed at each of the 12, 18, 24, and 36 month follow-up periods (Figure 1). For each survey period, the questionnaires were developed so that they would obtain exposure information since the previous survey period in order to develop a continuous and complete record of exposure throughout the three years of the study. These questions targeted exposures in regard to the child's living environment, as well as the child's health status. Specific questions dealt with issues of passive smoking, presence of chest infections, and, for the intervention group, questions in regards to their compliance with the intervention measures (e.g. mattress cover used, bed linen and/or soft toys washed, external contact with pets)

Outcome Measurement

Wheezing and asthma was measured at all the survey periods (Figure 1). Wheezing information was based on parental observations; asthma was based on parental records of doctor diagnoses. To ascertain the outcomes of asthma and wheezing separately at each of the follow-ups, questions regarding these conditions were placed into the standardized questionnaire completed by the child's parents. Repeated measurements of these conditions allowed us to track the progression of the diseases and conditions over the three years of the study. Skin prick test were conducted three times during the study period: at the beginning, and after 12 and 24 months of the study (Figure 1).

Statistical analysis

The statistical analysis was conducted through two approaches:

I. Cross sectional analysis of each of the study periods of follow-up. Logistic regression was used to estimate the odds ratios for developing the outcomes of asthma and wheezing in each of the study periods by intervention vs. control arm (GENMOD procedure of SAS without the REPEATED command was used).

II. Generalized estimation equation (GEE) model was applied to test the significance of the repeated outcomes of asthma and wheezing on the intervention vs. the control group (GENMOD procedure). GEE analysis conducted due to the dichotomous nature of the outcome variables (asthma and wheezing), which was repeatedly measured over time to estimate marginal probabilities.

Even though, randomized trial do not require adjustment for confounders, controlling for confounders provide information on whether the randomization was effective. Thus, adjusted odds ratios were estimated taking the following potential confounders into account: country, gender, age, age mother, age father, child's birth weight, smoking in the household, mothers' education, fathers' education, exposure to pet, paternal asthma, maternal asthma, asthma of a sibling, child's birth order, child ever breastfed, child ever had eczema, asthma or wheezing at baseline, and history of pneumonia. In addition, time and the interaction between time and intervention (combined effects) were being tested to evaluate changes over time. The variable 'country' was chosen to appear in all adjusted models since there are differences across the four sites due to different cultures (Table 7).

What is the evidence that the randomization did not work within some countries. If so, this belongs to results and discussion.

In order to reach the most parsimonious models for asthma and wheezing, we chose to use the backward modeling strategy. In backward elimination, a model that contains the treatment variable, a full set of confounders, and combined effects is fitted first. Interaction terms were then excluded if they do not contribute to the explanatory model ($p>0.05$). Then, potential confounders are eliminated from the model following the 10% rule of confounding (22). This rule indicates that if the relative change in the odds ratio of the treatment variable after adjustment for certain variable(s) is greater than 10 percent, then the variable(s) is selected to be included in the model (23).

The GEE models for each outcome produced effect coefficient estimates for each variable in the model, along with their 95% confidence intervals. These estimates were then transformed into odds ratios (along with the 95% confidence intervals of the odds ratio) to express the adjusted effect of each parameter on the outcome specified in the model. All statistical analyses performed using the SAS program (SAS version 8.2).

In order to determine whether losses to follow-up that occurred during the entire study period are explained by the treatment variable and the confounders, a GEE model was developed to determine if any of the predictors used in the models were able to account for the loss to follow-up. A new variable, based on the absence of information regarding the outcomes of asthma and wheezing, coded the loss to follow-up *at each* survey period. At any given survey period, if information on any of the outcomes was missing the loss to follow-up for this participant, at that particular time, was coded as

positive. The losses for each survey period were then combined in a GEE model with loss to follow-up as the outcome.

RESULTS

The study sites recruited and followed up 636 toddlers and preschoolers children. 330 children were randomly allocated to the intervention group and 306 to the control group (Figure 2).

Out of the 636 total toddlers participants, 51.9% (n=330) were assigned to the intervention group and 48.1% (n=306) were assigned to the control group (Table 3). On average 88.8% of the patients in the intervention group and 87.4% of the patients in the control group participated in the study. The attendance proportion of both groups for the four study visits (12, 18, 24, 36 months) ranged from 84%-94%; and there were no significant differences between the intervention and control groups. The great majority of the children had normal birth weight (>=2.5 kg). There were no significant differences between the intervention and control groups. Parents' education was grouped into three levels: Low education: up to high school & vocational school; Medium education: technical school; High education: university. 41% intervention group and 45% control group of the study participants had mothers with medium level of mother's education. Regarding father's education, 39% intervention group and 37% control group had high levels of education. Child's birth order, dichotomized as first born or not first born. 46% of the intervention group and 45% of the controls were first born. Mean age of the child at the beginning of the study was 2.3 years old in the intervention group and 2.1 years old at the control group. There was no significant difference between the intervention and control in mean age by parents (Table 3).

There was also no significant difference in parents or sibling history of asthma between intervention and control, and ranging from 8.8-14% (Table 4). 12.4% of the children in the intervention and 12.8% in the control had asthma on the study entry. No child from Germany entered the study having asthma; the higher percentage of children with asthma history came from children recruited in England. The minority of the children had ever diagnosed with pneumonia prior to the study entry in the intervention and the control group (12.7 and 9.2 respectively). None of the children recruited in England had ever been diagnosed with pneumonia prior to the study entry. A large number of the study participants had experience wheezing prior to the study entry in both arms (37.6% intervention and 43.5% control group). The majority of the study children were breastfed (85.5% intervention group and 82% control group). Approximately one third of the families reported that the child ever had a pet or had pet at the entry of the study. Only 14% of the mothers in the intervention group, and 13.7% in the control reported smoking during pregnancy. However, 40.6% of the families in the intervention group and 41.8% in the control group reported of smoking in the child's home. Compared with the other centers, a low percentage of families from Germany (6.7% and 4.7%) reported smoking at home. On the other hand, the majority of the parents from Greece said that they smoked at home (62.7 and 63.3, Table 4).

In a sensitivity analysis we investigated two extreme scenarios. When assuming that all participants lost to follow up had asthma, the prevalence of developing asthma after 12 months was 20.3% in the control and 14.6% in the intervention group ($p=0.06$, Table 5). The difference showed up in all the centers (Table 7). Following the same

assumption, the prevalence of developing asthma after 18, 24 and 36 months were not significantly difference between the intervention and the control (Table 5).

However, supposing that all participants lost to follow up had no asthma, the prevalence of developing asthma after 24 months was 5.9% in the control and 2.7% in the intervention group (p=0.05, Table 5). The difference did not show up in all the centers equally (Table 7). In Germany, no asthma cases were reported in both groups after 24 months; in England more cases were reported in the intervention group compare to the control (6.9% and 5.9% respectively, p=0.83, Table 7). Following the same assumption, the prevalence of developing asthma after 12, 18, and 36 months were not significantly difference between the intervention and the control group (Table 5).

In the case that all participants lost to follow up had wheezing, the prevalence of developing wheezing after 12, 18 and 24 months was significantly different (p=0.04, 0.008, 0.05 respectively, Table 6) between the intervention and the control, but not in the last followed up visit (p=0.51, Table 6). A higher prevalence of 37.2% occurred after 12 months in the control, compared to 29.4% in the intervention group (p=0.04, Table 6). The pattern of having fewer cases in the intervention group versus in the control group was not detected in all centers (Table 7).

When assuming that all participants lost to follow up had no wheezing, the prevalence of developing wheezing after 18 months (17% and 10.9%, respectively, Table 6) and 24 months (16.7% and 10%, respectively, Table 6) was significantly different among control and intervention. The difference did not showed up in all centers (Table 7). The prevalence of wheezing after 36 months was lowest and there was not significant difference between the intervention and the control (p=0.22, Table 6).

Figures 3 and 4 demonstrate the number of events (wheezing or asthma respectively) for each time period for the control and the intervention groups. The number of asthma occurrences ranged between n=14 and n=21 for the control group and between n=9 and n=18 for the intervention arm (Figure 3a). New events reported in each survey ranged between n=4 and n=21 for the control group and between n=6 and n=18 for the intervention group (Figure 3b). The number of children with wheezing were higher and ranged between n=33 and n=74 for the control group and between n=26 and n=67 for the intervention group (Figure 4a). For new events, occurring since the last survey, the number was between n=6 and n=74 for the control arm and between n=8 and n=67 for the intervention arm (Figure 4b).

Odds ratios of the crude model (not adjusted for any confounder) for the presence of each of the two outcomes asthma diagnosis by physician and wheezing separately for each time periods (12, 18, 24, 36 months) are presented in figures 5 and 6. The control group had a higher risk of having asthma compared to the intervention group at 24 months (OR=0.66, P=0.05) and 36 months (OR=0.64, P=0.02) of follow-up (Figure 5). As for wheezing, at 18 months (OR=0.68, P=0.008), 24 months (OR=0.64, P=0.0004), and 36 months (OR=0.65, P=0.0002) the control group was in a significantly risk of wheezing compared to the intervention group (Figure 6).

To address the research question a GEE model was used to determine whether the intervention program was effective over the study follow up of 36 months. Odds ratios, 95% confidence interval, and p-value for each of the two outcomes asthma (diagnosis by physician) and wheezing are given in Tables 8 and 9.

To investigate the effect of intervention, four models were developed for each outcome:

I. An un-adjusted model,

II. A model controlling for country, chest infection, and asthma or wheezing at baseline respectively for each outcome.

III. A model controlling for the variables mentioned in for model II as well as for the variable time variable.

IV. A model controlling for the variables mentioned in for model III as well as for the interaction between intervention arm and time.

Going backward from Model IV to I, we found that the combined effect, supposing that being in the intervention group is related to a different time trend of wheezing and asthma, was not significant. This left us with Model III: time didn't have effect on the association between intervention and occurrence of asthma nor wheezing. Thus, model II was found to be the most parsimonious model for asthma and wheezing. We were able to eliminate all confounders except three (child ever asthma or wheezing, child ever pneumonia, and country) from the initial model (Tables 8a, 8b, 9a and 9b). In the repeated measurement analyses across models I, II, III we found that children in the control arm had a significantly higher risk for having wheezing ($p=0.01$, Table 8a) than the children in the intervention arm. Also for asthma, children in the control group had a significantly higher risk than the intervention group after a follow-up of 36 months ($p=0.02$, Table 9a).

In addition to the explanatory models for asthma and wheezing, loss-to- follow-up was also analyzed with a repeated measurement model to investigate whether any of the

predictors used in the models where able to significantly predict the non-participation (Table 10). Being in the intervention arm was not related to loss-to-follow-up. However, there were differences for the different centers with lowest loss in Greece ($p=0.01$) and highest in Lithuania ($p<0.0001$).

DISCUSSION

In this data, we demonstrated that a prevention program in high risk toddlers and preschoolers with a follow-up of 36 months resulted in a modest but significant reduction in the risk of asthma (OR= 0.66, 0.45-0.98, Table 8a) and wheezing (OR= 0.66, 0.45-0.98, Table 9a). This is the first report, to our knowledge that house dust mite avoidance seems to be effective in toddlers and preschoolers after long follow up of 36 months with intervention compliance of 75% to 48% at 36 months with Mattress cover usage (Table 11).

The study included only toddlers and preschoolers at high risk of atopy to increase efficacy. The selection criteria of high risk children in this data followed the criteria used in previous studies (11-19). It was required that 1 parent had a history of asthma, atopic eczema, or hay fever, and at least 1 parent had a positive SPT or specific IGE results to one of the common allergens tested. The study was not originally designed to evaluate the outcomes of asthma and wheezing but to evaluate house dust mite sensitization among the two groups intervention versus control. Therefore, at baseline children were not excluded if they had an asthma diagnosis by physician or/and wheezing, only if their IgE test or skin prick test was positive. 12.8% in the control group and 12.4% in the intervention group had asthma at the beginning of the study and 43.5% in the control group and 37.6% of the intervention group had wheezing (Table 2). Having

sub-group of children that have asthma at baseline complicates the analysis and shifts the nature of the study from primary prevention only to a combination of primary and secondary analysis.

Risk factors and primary prevention for asthma have been studied extensively in infants within their first year of life but there is little information about younger children (toddlers and preschoolers). The mean age of the children who participated in this study ranged between 2.3 years at enrollment to 5.3 in the end of the study. The advantage of studying toddlers and preschool age children is that information about potential risk factors and their effect is collected closer to the time of disease inception. However, misclassification of asthma or wheezing may introduce an information bias, which however is likely to be non-differential, i.e. comparable for the intervention and control group.

There is no 'gold standard' for asthma diagnosis, and especially not in early childhood. For this analysis, we investigated whether a physician ever told the parents that their child had asthma and 'wheezing not associated with cold' rather then just cough and wheezing. However, doctor-diagnosed asthma was found not to be an ideal measure, but as reliable as a combination of clinical diagnosis combined with bronchial hyper-responsiveness (BHR) test (23).

Other studies have used a variety of measures to eradicate house dust mites, including regular or high filtration vacuum cleaning, air filtration, ionizers, and plastic or semi permeable mattress covers. In the case of covering of mattress, a significant reduction of the allergen load was reported in the literature for different age groups in varies countries. In this study the primary eradication method was mattress covers with a

combination of education booklets including other recommendations (e.g., hot washing of bedding). Assessment of allergen load was not done in this study. The goal was to keep the intervention simple, as least disturbing, and as inexpensive as possible to be able to apply the program in case it showed sufficient efficacy in real life.

Some variables showed differences in the distribution between control and intervention arm within specific countries (e.g., cigarette smoking during pregnancy, Table 3-4) and others between countries (e.g., child ever asthma, child ever pneumonia, Table 2). However, all confounders except three (child ever had asthma or wheezing, child ever had pneumonia, and country) could be removed from the explanatory model. This reassured us that random allocation into control and intervention arm was effective. On the other hand, the variable country could not be removed from the final model due to center and potential cultural differences.

A limitation is that children were not excluded from the study or the analysis if they had asthma or/and wheezing at enrollment since it was not the study goal to assess asthma but HDM sensitization. Therefore, it was necessary to control for asthma or wheezing at baseline. The only other predictor, which confounded the association between intervention and both outcomes (wheezing and asthma), was the variable 'child ever pneumonia', ascertained in the initial survey (Table 2). There was no important initial difference in prevalence of children having pneumonia at baseline between the two groups (Table 4). It is unlikely that all asthma and wheezing cases were due to pneumonia history, as only 12.7% of the children in the intervention and 9.2% of the control reported pneumonia history. It would be ideal to control for the upper respiratory illness history of the children since the literature indicated strong association between

asthma and upper respiratory illness. Unfortunately we do not have such information in our data and therefore we were not able to adjust for this potential confounder. However, there is not reason to assume that any unmeasured respiratory disease that occurred after randomization occurred in higher incidence in the two arms.

The compliance for the prevention methods among the intervention group was good for such a long intervention program. However, the compliance faded over time for the major intervention method 'always mattress cover use' ranging between 65.5% at 12 months to 48.2% at 36 months, others prevention methods were fairly stable used during the entire study period (Table 11). When testing the effect of time we found significant effect in regards to the wheezing outcome (OR=0.8, $p<0.0001$, Table 8b) but showed a non significant effect in regards to the outcome of asthma (OR=0.7, $p=0.09$, Table 9b). In both cases, time had no significant effect on the prevention program. The interaction between time and intervention group was also not significant for both study outcomes. In this case we can conclude that there was not different time effect during follow-up in the intervention and the control arm. In addition, the variable time did not confound the associations between intention-to-treat and the asthma or wheezing and thus was excluded from the final model.

The study design used for this research has strength in that repeated measurements were taken over three years of toddlers and preschoolers. A total of five outcome measurements (baseline and four visits) for each participant were incorporated into a repeated measurement (generalized estimation equation, GEE) model enabling within subject associations to be accurately accounted. In addition, repeated observations increased the statistical power of the trial.

Number needed to treat (NNT) analysis for the study data showed that in order to avoid one case of asthma in high risk children we need to provide mattress covers to 52 children and 36 mattress covers in order to avoid one case of wheezing. Considering the low price of this eradication tool, health policy maker may consider recommending its use.

Loss to follow-up does not seems to be a problem for this study 77.6% of the intervention participants and 74.6% of the control participants came to all the four follow up visits and only 1.8% in the intervention group and 1.6% of the control group didn't come to any of the visits except the baseline visit. The attendance percentage to the periodically visits (12, 18, 24, and 36 months) ranged between 91.5% to 84.3% for the control group and 94.2% to 84% for the intervention group. Loss-to-follow up was not explained by being in the intervention arm or by the control arm, with the exception of two centers. Thus loss-to-follow-up was deemed not to bias the results (Table 10).

One limitation of this study is that it was able to follow-up the participants until preschool age. It has been known that asthma and wheezing may take more years to develop (24). Further, it has also been noted that even if asthma and wheezing occurs in the first years of life this does not necessarily mean that these disorders will persist later in life. However, most studies did not follow the children for more then 2 years. Only one study (The Isle of Wight Study) (11, 12,13) followed the children till their school years (8 years) and showed significant long term effect of the intervention program.

In order to be compliant with general recommendations, all parents received booklet about asthma prevention methods. Only the intervention group received a mattress cover. Thus, we can not exclude that some families in the control group

practiced some of the intervention measures, possibly because of increased general awareness of health in general and asthma in particular. In addition, we analyzed symptoms that partially depend on parental report. However, it is extremely difficult to conduct a double blind trial in this case since the 'placebo' methods might prevent exposure to allergens. For this reason most studies gave up blindness. Nevertheless, the HDM avoidance seems to be significantly effective in toddlers and preschoolers after long follow up of 36 months.

CONCLUSION

There is a worldwide concern about the increasing burden of asthma over the last decades, in terms of increasing prevalence, morbidity, and economic costs. The overall pattern of an increasing burden from asthma is broadly consistent between countries with different lifestyles and medical practices. Also of concern is the incomplete understanding of the underlying risk factors which may be responsible for the increasing of the trend. Therefore, the emphasis should be on the effort to study primary and secondary prevention approaches that might help enhance our understanding the risk factors' role and reduce morbidity rates.

In this analysis we have demonstrated that follow up of 36 months intervention program, focused on mattress cover reduced the incidence of asthma and wheezing in high risk children. By focusing on these high risk children between the age of 2.3 and 5 years old, we might have limited our ability to generalize. Nevertheless, our findings provide evidence that reducing exposure to house dust mite allergen reduces the risk of developing asthma and wheezing in toddlers and preschoolers.

This research has a potential implication for policy makers, namely to consider mattress covers in primary prevention of asthma. We found that reduction in exposure of house dust mites through simple and inexpensive methods such as mattress cover combined with fairly simple recommendation to families seem to be effective as prophylactic treatment for asthma and wheezing for high risk children. Further research is needed for establishing recommendations concerning the entire population.

In light of recent findings, future studies should focus on four main areas. First, evaluate an affordable and simple primary prevention program for asthma and allergy. Second, test the hypotheses in a randomized controlled trial with large samples of children who do not have asthma at baseline. Third, examine more than one intervention through the use of a factorial design. And fourth, explore the long-term effects of primary prevention program in school age children with extended follow-up, since asthma diagnoses at a later age is more reliable then in early childhood.

TABELS & FIGURES

Table 1: Primary Prevention Studies to Implement an Intervention Design to Reduce Exposure to Inhalant Dust Mite Allergens

Study	Location	Age	Study design	Type of intervention
ISLE of WIGHT study	England N=120	Newborn- 8 years	Randomized controlled study, Two groups (control and intervention) Length of intervention: 9 months Length of follow-up: 8 years	Combined of food and dust mite allergens avoidance
CAPPS Canadian Primary Prevention Study	Canada N=497	Antenatally-1 years	Randomized controlled study, Two groups (control and intervention) Length of intervention: 1 year Length of follow-up: 1 years	Combined of food and dust mite allergens avoidance
SPACE The study on the prevention of allergy in children in Europe (Newborn part)	Europe: Austria, Germany, UK N=696	Newborn- 1 years	Multi-center randomized controlled study, Two groups (control and intervention) Length of intervention: 1 year Length of follow-up: 1 years	Combined of food and dust mite allergens avoidance
CAPS The childhood asthma prevention study	Sydney, Australia N=616	Newborn- 18 months	Multi-center parallel group randomized controlled study, Four groups (control and 3typs of interventions groups) Length of intervention: 18 year Length of follow-up: 18 years	Factorial design, Combined and non combined of dust mite avoidance and dietary intervention
PIAMA The prevention and incidence of asthma and mite allergy study	Netherland N=810	Newborn- 2 years	Randomized intervention controlled study within birth cohort Two groups (control and intervention) Length of intervention: 2year Length of follow-up: 2 years	Dust mite allergens avoidance
MAAS Asthma and allergy study	England N=191	Newborn- 1 years	Nested randomized intervention controlled study within whole population birth cohort Two groups (control and intervention) Length of intervention: 1year Length of follow-up: 1 years	Stringent intervention of combined food and dust mite allergens avoidance

Table 2: Clinical Outcome Measures in Primary Prevention Studies, Intervention (I) Group vs. Control (C) Group

	MAAS N=191	PIAMA N=810	CAPS N=616	SPACE N=696	CaPPS N=497	ISLE N=120
Sensitization to mite	2% I vs. 1% C NS	0.7% I vs. 0.8% C, NS	~10% All groups	1.9% I vs. 5% C P<0.03	?	11% I vs. 31% C P 0.01
Atopy	17% I vs. 14%C, NS	14.4% I vs. 14%C, NS	18-21%, p=0.96	6% I vs. 10.7%C, NS p<0.03	4.6% I vs. 4.4%C, p=0.4	20% I vs. 46.8%C, p=0.003
Wheeze	Lower severe wheeze with SOB or after exertion Lower medication for wheeze p<0.05	No difference	Lower in dietary intervention group only (p=0.02)	Wheeze bronchitis 18.1% i vs. 17.8%C, NS	-	13.8% I vs. 27.4%C, p=0.08
Cough	No difference	Night cough without cold	No difference	-	-	13.8% I vs. 32.3%C, p=0.02
Asthma	Too young	Too young	No difference but less oral steroid use in mite avoidance group (p=0.04)	Too young	15.1% I vs. 20.2%C, (p=0.04 for OR)	9.6% I vs. 15.5%C, p=0.4, (wheeze+ BHR)

Table 3a: Study Population Characteristics (N=636)

		Germany		Greece		Lithuania		England		Total	
		Interv	Control	Interv	Control	Interv	Control	Interv	Control	Interv	Control
		n=53	n=38	n=151	n=152	n=68	n=65	n=58	n=51	n=330	n=306
	%	58.2	41.8	49.8	50.2	51.1	48.9	53.2	46.8	51.9	48.11
Characteristics											
Children who came to visit 1	Yes %	100	97.4	94	89.5	69.1	67.7	100	96.1	90.9	86.9
Children who came to visit 2	Yes %	98.1	89.5	91.4	96.1	92.7	80	100	94.1	94.2	91.5
Children who came to visit 3	Yes %	90.6	94.7	78.8	82.9	78	72.3	98.3	96.1	84	84.3
Children who came to visit 4	Yes %	88.7	94.7	82.1	88.2	83.8	78.5	96.6	88.2	86.1	86.9
Education*: Mother	Low %	20.8	13.2	11.9	13.8	16.2	24.6	67.2	64.7	23.9	24.5
	Medium %	54.7	57.9	39.7	51.3	44.1	38.5	27.6	25.5	40.9	45.4
	High %	24.5	29	48.3	34.9	39.7	36.9	3.4	7.8	34.8	30.1
Father	Low %	45.3	42.1	15.2	24.3	22.1	29.2	67.2	56.8	30.6	33
	Medium %	18.9	10.5	33.1	32.2	38.2	30.7	15.5	29.4	28.7	28.8
	High %	35.8	47.4	51	43.4	36.8	38.5	13.8	9.8	39.1	37.2

*Low education: up to high school & vocational school; Medium education: technical school; High education: university.

Table 3b: Study Population Characteristics (N=636)

		Germany		Greece		Lithuania		England		Total	
		Interv	Control	Interv	Control	Interv	Control	Interv	Control	Interv	Control
		n=53	n=38	n=151	n=152	n=68	n=65	n=58	n=51	n=330	n=306
Child birth order (first child/ yes)	Yes %	56.6	63.2	53.3	42.1	48.5	47.7	19	37.3	46.4	45.1
Child birth weight	< 2.5 Kg	90.6	97.4	93.4	94.1	95.6	97	93.1	96.1	93.3	95.4
Gender (Male/yes)	Yes %	39.6	63.2	57.6	47.4	60.3	61.5	53.5	58.8	54.4	54.3
Mean age of the child		1.8	2.1	2.7	2.3	2.5	2.3	1.4	1.2	2.3	2.1
Mean age of mother at child's birth		31.7	31.9	33.3	32.8	28.8	28.9	31.8	30.2	31.4	31.9
Mean age of father at child's birth		34.8	35.1	36.9	36.3	30.7	30.3	35.9	33.7	35.1	34.5

Table 4: Study Population Characteristics (N=636), family & child medical history

		Germany		Greece		Lithuania		England		Total	
		Interv	Control	Interv	Control	Interv	Control	Interv	Control	Interv	Control
		(n=53)	(n=38)	(n=151)	(n=152)	(n=68)	(n=65)	(n=58)	(n=51)	(n=330)	(n=306)
%		58.2	41.8	49.8	50.2	51.1	48.9	53.2	46.8	51.9	48.11
Family Medical History:											
Asthma father	Yes %	15.1	15.8	3.3	4.6	8.8	3.1	20.7	27.5	9.4	9.5
Asthma mother	Yes %	20.8	13.2	9.3	7.9	4.4	3.1	31	41.2	14	13.1
Asthma sibling	Yes %	3.8	7.9	6.6	7.9	4.4	0	24.1	39.2	8.8	11.4
Child Medical History:											
Child ever asthma	Yes %	0	0	17.9	15.8	2.9	7.7	20.7	19.6	12.4	12.8
Child ever wheezing	Yes %	28.3	31.6	37.1	50.7	38.2	33.9	46.6	43.1	37.6	43.5
Child ever had eczema	Yes %	17	34.2	14.6	19.7	16.2	16.9	43.1	56.9	20.3	27.1
Child ever diagnosed Pneumonia	Yes %	15.8	9.4	4.6	8.6	23.1	35.3	0	0	12.7	9.2
Potential Risk Factor History:											
Cigarettes during pregnancy	Yes %	28.3	13.2	17.9	16.5	0	3.1	8.6	19.6	14.2	13.7
Cigarettes in the home	Yes %	6.7	4.7	62.7	63.3	17.9	18.8	12.7	13.3	40.6	41.8
Child ever has pet	Yes %	18.9	26.3	21.9	19.7	35.3	33.9	65.5	54.9	31.8	29.4
Child has pet now	Yes %	22.2	11	24.4	43.9	13.3	9.8	40	35.4	27.3	26.8
Child ever breast feed	Yes %	86.8	94.7	82.1	79.6	94.1	89.2	82.8	70.6	85.5	82

Table 5: Sensitivity Analysis for Asthma Diagnosis by Physician Prevalence for the Total Study Population, Intervention vs. Control group

Assuming all participants lost to follow-up **have** Asthma Diagnosis by Physician			
Time (Months)	**Control %**	**Intervention %**	**p-value**
12M	20.3	14.6	0.06
18M	13.4	10.6	0.32
24M	22.2	19.4	0.43
36M	18.6	17.2	0.67
Assuming all participants lost to follow-up **have N0** Asthma Diagnosis by Physician			
12M	6.9	5.4	0.51
18M	4.9	3.9	0.56
24M	5.9	2.7	0.05
36M	4.6	2.7	0.28

Table 6: Sensitivity Analysis for Wheezing Prevalence for the Total Study Population, Intervention vs. Control Group

Assuming all participants lost to follow-up **have** Wheezing			
Time (Months)	**Control %**	**Intervention %**	**p-value**
12M	37.2	29.4	0.04
18M	25.5	16.7	0.008
24M	33.0	26.1	0.05
36M	24.8	22.4	0.51
Assuming all participants lost to follow-up **have N0** Wheezing			
12M	24.2	20.3	0.25
18M	17.0	10.9	0.03
24M	16.7	10.0	0.01
36M	10.8	7.9	0.22

Table 7: Wheezing and Asthma Prevalence by Countries

		Germany			Greece			Lithuania			England		
Outcome	Visit	Control N=38	Interv N=53	*p*-value	Control N=152	Interv N=151	*p*-value	Control N=65	Interv N=68	*p*-value	Control N=51	Interv N=58	*p*-value
Wheezing Assuming all participants lost to follow-up **have N0** symptoms	12	13.2	15.1	0.79	27	23.2	0.45	21.5	16.2	0.43	27.5	22.4	0.54
	18	13.2	7.6	0.38	11.8	6.6	0.12	33.9	16.2	0.02	13.7	19.0	0.46
	24	7.9	7.6	0.95	13.8	9.3	0.22	26.2	7.3	0.004	19.6	17.2	0.75
	36	7.9	9.4	0.80	7.9	4.6	0.24	18.5	11.8	0.28	11.8	10.3	0.81
Wheezing Assuming all participants lost to follow-up **have** symptoms	12	15.8	15.1	0.93	37.5	29.1	0.12	53.9	47.1	0.43	31.4	22.4	0.29
	18	23.7	9.4	0.06	15.8	15.2	0.89	53.9	23.5	0.0003	19.6	19.0	0.93
	24	15.8	17.0	0.88	30.9	30.5	0.93	53.9	29.4	0.004	25.5	19.0	0.41
	36	13.2	20.8	0.35	19.7	22.5	0.55	44.6	30.9	0.10	23.5	13.8	0.19
Asthma Assuming all participants lost to follow-up **have N0** symptoms	12	0	0	-	8.6	6.6	0.53	4.6	2.9	0.61	9.8	10.3	0.93
	18	5.3	0	0.09	2.0	1.3	0.66	7.7	7.4	0.94	9.8	10.3	0.93
	24	0	0	-	7.2	2.7	0.07	6.2	1.5	0.16	5.9	6.9	0.83
	36	5.3	0	0.09	2.6	3.3	0.73	6.2	0	0.04	7.8	6.9	0.85
Asthma Assuming all participants lost to follow-up **have** symptoms	12	2.6	0	0.42	19.1	12.6	0.12	38.5	33.8	0.58	13.7	10.3	0.59
	18	15.8	1.9	0.01	5.9	9.9	0.20	27.7	14.7	0.07	15.7	15.5	0.98
	24	5.3	11.3	0.31	24.3	23.8	0.92	33.9	23.5	0.19	13.7	10.3	0.59
	36	10.5	11.3	0.90	14.5	21.2	0.13	32.3	19.1	0.08	19.6	10.3	0.17

Table 8a: Associations of Intervention Controlling for Confounding on Wheezing using Repeated Measurement Models for a Follow-up of 36 Months

Parameter	Model I*				Model II**			
	OR	95% CI-Low	95% CI-high	p-value	OR	95% CI-Low	95% CI-high	p-value
Intervention group	0.66	0.48	0.91	0.01	0.66	0.48	0.91	0.01
Wheezing at baseline					5.18	3.61	7.43	<.0001
Chest infection at baseline					1.56	0.92	2.64	0.09
Country: Germany					0.63	0.34	1.18	0.15
Country: Greece					0.70	0.92	1.1	0.12
Country: Lithuania					1.59	0.91	2.76	0.1
Country: England (Ref.)					-	-	-	-
Time								
Group*time								

* Unadjusted model

** Adjusted model for wheezing and chest infection at baseline and country

*** Adjusted model for wheezing and chest infection at baseline country, and time

**** Adjusted model for wheezing and chest infection at baseline country, time, and Group*time

Table 8b: Associations of Intervention Controlling for Confounding on Wheezing using Repeated Measurement Models for a Follow-up of 36 Months

Parameter	Model III***				Model IV****			
	OR	95% CI-Low	95% CI-high	p-value	OR	95% CI-Low	95% CI-high	p-value
Intervention group	0.66	0.48	0.92	0.01	0.83	0.48	1.43	0.5
Wheezing at baseline	1.69	1.33	2.06	<.0001	5.43	3.76	7.83	<.0001
Chest infection at baseline	1.6	0.93	2.74	0.09	1.61	0.94	2.76	0.08
Country: Germany	0.61	0.32	1.16	0.13	0.61	0.32	1.15	0.13
Country: Greece	0.71	0.45	1.12	0.14	0.71	0.45	1.12	0.14
Country: Lithuania	1.63	0.93	2.87	0.09	1.63	0.92	2.87	0.09
Country: England (Ref.)	-	-	-	-	-	-	-	-
Time	0.70	0.63	0.77	<.0001	0.73	0.64	0.83	<.0001
Group*time					0.9	0.73	1.11	0.32

* Unadjusted model
** Adjusted model for wheezing and chest infection at baseline and country
*** Adjusted model for wheezing and chest infection at baseline country, and time
**** Adjusted model for wheezing and chest infection at baseline country, time, and Group*time

Table 9a: Associations of Intervention Controlling for Confounding on Asthma Diagnosis by Physician, Using Repeated Measurement Models for a Follow-up of 36 Months

Parameter	Model I*				Model II**			
	OR	95% CI-Low	95% CI-high	p-value	OR	95% CI-Low	95% CI-high	p-value
Intervention group	0.65	0.41	1.04	0.08	0.58	0.36	0.91	0.02
Asthma at baseline					5.15	3.13	8.46	<.0001
Chest infection at baseline					2.27	1.27	4.06	0.006
Country: Germany					0.16	0.04	0.59	0.006
Country: Greece					0.47	0.26	0.84	0.01
Country: Lithuania					0.65	0.33	1.28	0.21
Country: England (Ref.)					-	-	-	-
Time								
Group*time								

* Unadjusted model

** Adjusted model for asthma and chest infection at baseline and country

*** Adjusted model for asthma and chest infection at baseline country, and time

**** Adjusted model for asthma and chest infection at baseline country, time, and Group*time

Table 9b: Associations of Intervention Controlling for Confounding on Asthma Diagnosis by Physician, Using Repeated Measurement Models for a Follow-up of 36 Months

Parameter	Model III***				Model IV****			
	OR	95% CI-Low	95% CI-high	p-value	OR	95% CI-Low	95% CI-high	p-value
Intervention group	0.58	0.38	0.91	0.02	0.87	0.38	1.98	0.75
Asthma at baseline	5.09	3.1	8.4	<.0001	5.10	3.1	8.4	<.0001
Chest infection at baseline	2.30	1.3	4.2	0.005	2.32	1.29	4.19	0.005
Country: Germany	0.15	0.04	0.56	0.005	0.15	0.04	0.57	0.005
Country: Greece	0.47	0.26	0.84	0.01	0.46	0.26	0.82	0.009
Country: Lithuania	0.66	0.34	1.30	0.22	0.65	0.33	1.28	0.22
Country: England (Ref.)	-	-	-	-	-	-	-	-
Time	0.87	0.74	1.02	0.09	0.94	0.75	1.16	0.55
Group*time					0.83	0.59	1.14	0.25

* Unadjusted model

** Adjusted model for asthma and chest infection at baseline and country

*** Adjusted model for asthma and chest infection at baseline country, and time

**** Adjusted model for asthma and chest infection at baseline country, time, and Group*time

Table 10: Lost to Follow up vs. Others Predictors Used in the Analysis

Outcome	Variable	OR	95% CI- Low	95% CI- high	p-value
Asthma diagnosis by physician	Intervention group	0.8	0.57	1.19	0.3
	Asthma at baseline	1.2	0.64	2.18	0.6
	Chest infection at baseline	1.6	0.92	2.63	0.1
	Country: Germany	1.08	0.47	2.45	0.86
	Country: Greece	2.30	1.21	4.36	0.01
	Country: Lithuania	5.65	2.94	10.86	>0.0001
	Country: England (Ref.)	.	.	.	.
	Time	1.1	0.96	1.18	0.3
Wheezing	Intervention group	0.8	0.55	1.17	0.3
	wheezing at baseline	1.2	-0.18	0.9	0.4
	Chest infection at baseline	1.4	0.84	2.46	0.2
	Country: Germany	1.4	0.54	3.47	0.5
	Country: Greece	2.9	1.37	6.3	0.005
	Country: Lithuania	7.4	3.4	16.2	>0.0001
	Country: England (Ref.)	.	.	.	.
	Time	1.02	0.89	1.16	0.8

Table 11: Intervention Measures in Children of the Prophylactic Group at 12,18,24,36 Month Observation Periods (n=330)

Time	12 months	18 months	24 months	36 months
Mattress cover use (%)				
Most of the time	15.8	10	13	23.9
Sometime	6.1	3.9	5.8	4.2
Always	63.3	76.7	58.8	46.4
Never	2.7	2.4	3.0	10
Bed linen washed (%)				
Never	0.3	0.3	0.9	0.3
>4-week intervals	2.4	1.5	3.0	2.4
2-4 week intervals	15.2	19.4	16.1	16.7
<2-week intervals	22.7	25.5	23.9	21.8
Every week	49.1	46.7	38.8	43.6
Soft toys washed (%)				
Yes	43.6	50.9	39	33.3
External contact with pets (%)				
Yes	29.7	37.6	28.5	30
Carpet (%)				
Never carpet	5.8	9.4	7.3	11.5
Carpet in child room	70.9	75.8	60.3	62.7
Carpet removed	11.8	9.0	15.8	4.9
Smoking in the household (%)				
Yes	33.3	34.9	30.9	32.7

Figure1: Schematic of Flow of Study

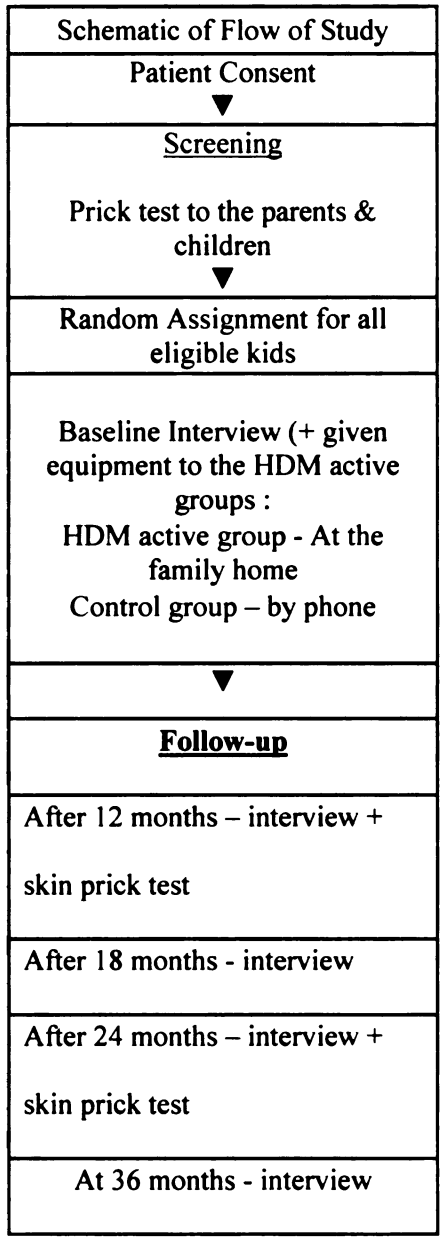


Figure 2: Profile of the Study of Asthma Primary Prevention for High Risk Toddlers and Preschoolers

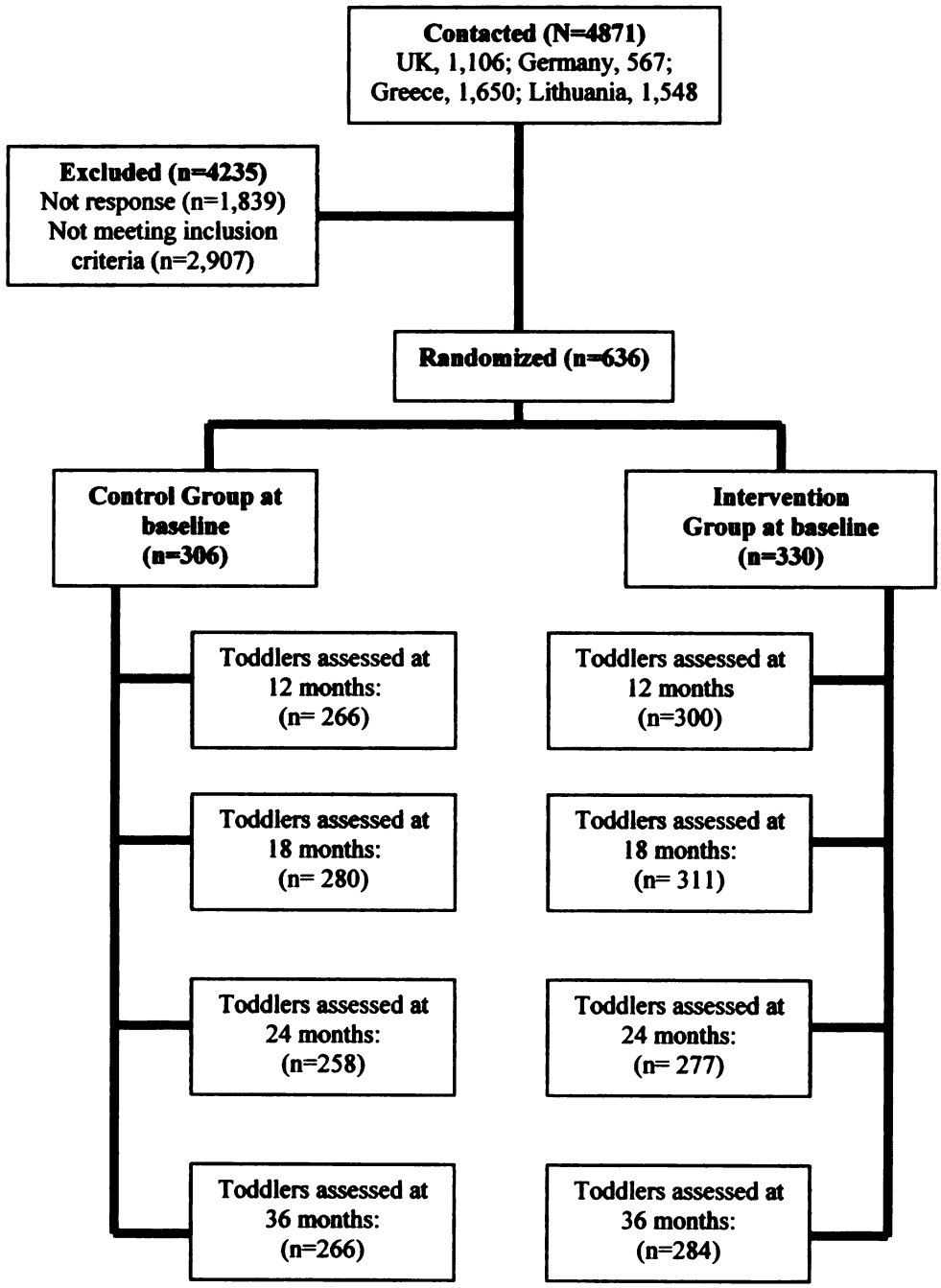


Figure 3a: Total Number of Asthma Events for Each Time Period, Control (n=306) vs. Intervention (n=330)

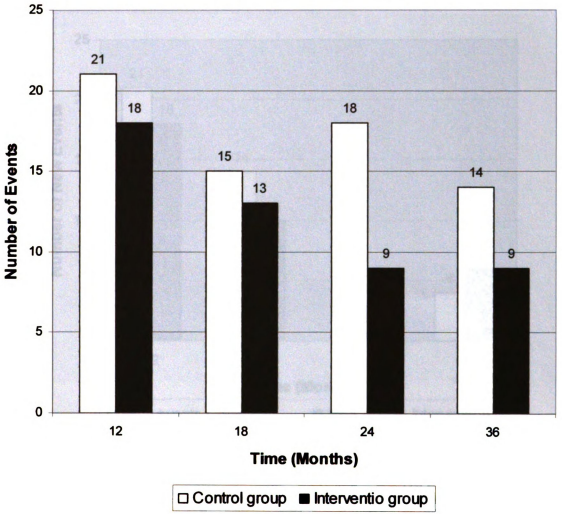


Figure 3b: Number of New Asthma Events for Each Time Period, Control (n=306) vs. Intervention (n=330)

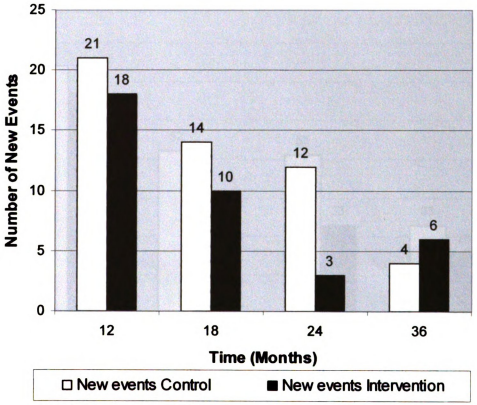


Figure 4a: Total Number of Wheezing Events for Each Time Period, Control (306) vs. Intervention (n=330)

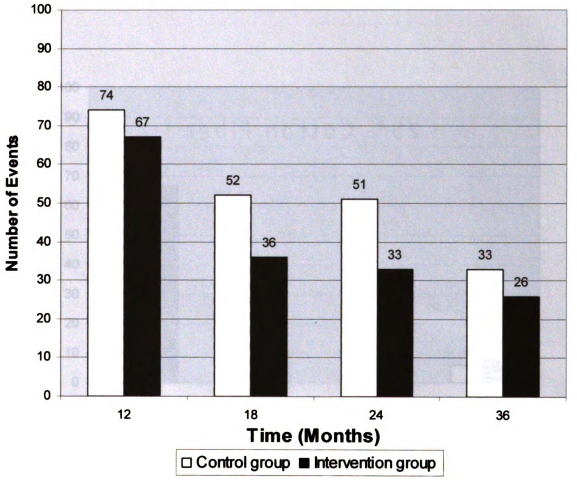


Figure 4b: Number of New Wheezing Events for Each Time Period, Control (n=306) vs. Intervention (n=330)

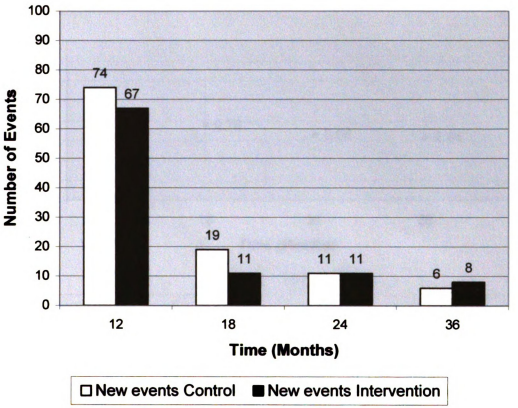


Figure 5: Asthma diagnosis by physician OR calculation for each time period (Unadjusted Model)

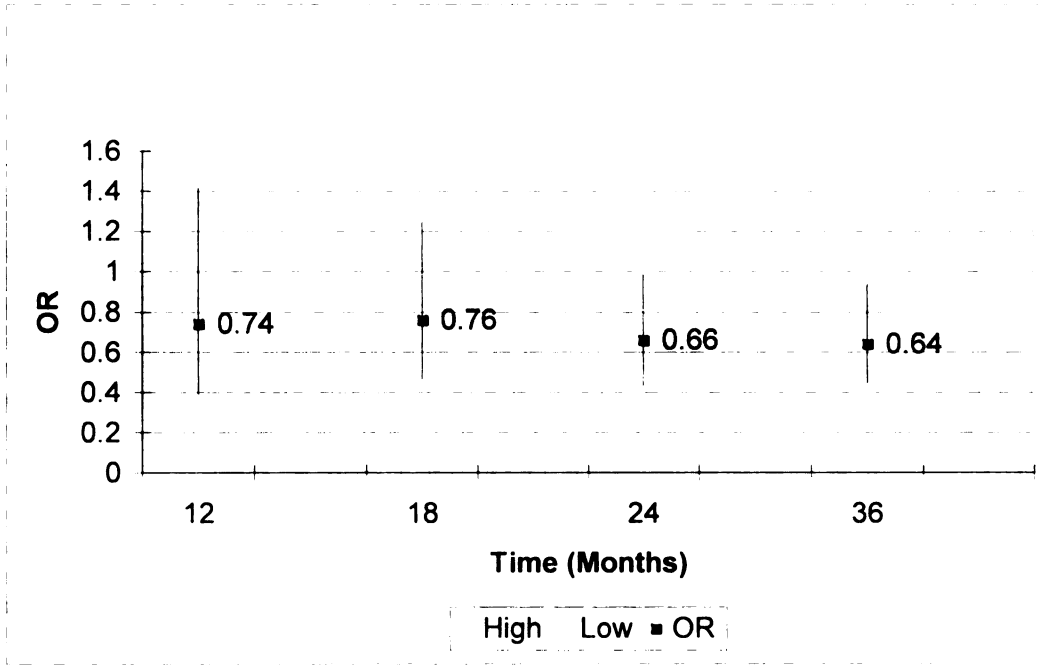
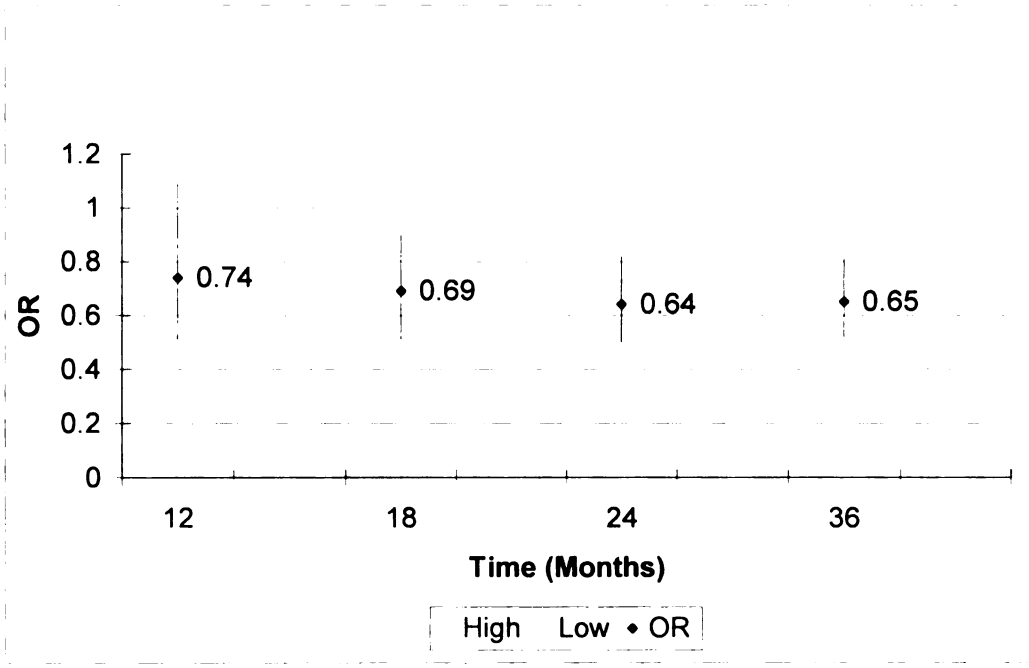


Figure 6: Wheezing OR calculation for each time period (Unadjusted Model)



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