

Confounding and effect modification in studies of diet and childhood asthma and allergies

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Abstract

Objective: To propose a comprehensive set of confounders and effect modifiers that should be considered in epidemiologic investigations.

Methods: Two reviewers independently critiqued studies included in a recent systematic review and extracted data on the confounders and effect modifiers that were considered and the approaches used to justify inclusion.

Results: Of the 62 studies reviewed, 20 were cohort, 16 case-control, 25 cross-sectional studies, and one ecologic study. All cohort, cross-sectional, and ecologic studies had some adjustment for confounding or consideration of effect modification, but this was only the case for 7/16 (44%) case-control studies. Of the 53 studies that considered confounding or effect modification, 39/53 (74%) gave no justification for the inclusion of the variables considered. Studies that justified the inclusion of the variables did so based on empirical evidence ($n = 10$), conceptual justification ($n = 7$), or a combination of the two ($n = 3$). Confounding was handled mainly by using regression modeling, but some case-control studies utilized matching and ANOVA. Ten studies handled effect modification by stratification, eight tested for interaction, and five used both strategies.

Conclusions: We have found substantial shortcomings in the handling of confounding and effect modification in studies of diet and development of childhood asthma/allergies. Selection of variables should be based on conceptual considerations and empirical evidence. Using this approach, we have proposed a comprehensive set of confounders and effect modifiers that need to be considered in future studies.

Background

There is a substantial international interest in the etiologic role of nutrients and other dietary factors in the development of asthma and atopic disease. Although observational studies have reported associations between nutrients and asthma and atopic outcomes, there are concerns that these associations may be a consequence of confounding by complex social, behavioral, and lifestyle factors acting across the life course (1). The scientific and resource consequence of confounding in observational studies of nutrition and disease has been highlighted by the failure of many well-conducted randomized controlled trials (RCTs) to show beneficial effects of nutrient supplementation despite numerous reports of beneficial

associations between nutrients and cardiovascular disease, cancer, and all-cause mortality. To achieve the validity in the evidence provided by observational epidemiologic studies, extra effort therefore needs to be dedicated to the identification, measurement, and adjustment of potential confounding variables (2–5). It is for this reason that the Strengthening The Reporting of Observational Studies in Epidemiology (STROBE) guidelines highlight the importance of considerations of confounding in observational studies (6).

Our recent systematic review found a large body of relevant epidemiologic evidence relating early life and childhood dietary exposures to atopic and asthma outcomes, but much of this was judged to be of poor methodological quality on the grounds of, among other things, the potential

for confounding bias (7). In order to catalyze improvements in the methodological quality and reporting of future epidemiologic investigations, we undertook a further critique of the 62 international studies (8–69) in our dataset to identify and propose a comprehensive set of confounders and effect modifiers that should be considered and suggest ways in which these may be accounted for.

Confounding can be defined as ‘the distortion of a measure of the effect of an exposure on an outcome due to the association of the exposure with other factors that influence the occurrence of the outcome.’ (70) Confounders are variables used to ameliorate the effect of confounding by suitable adjustment (70). The selection of confounders is guided by the knowledge of the subject matter under investigation (70, 71), and while associated with both outcome and exposure, they are not on the causal pathway between exposure and outcome (71). Confounding is addressed by appropriate adjustment in the design of studies and, more commonly, statistical analysis and a variety of design-related strategies, which include restriction, matching, stratification, and regression modeling (71).

Effect modification can be defined as the ‘variation in the selected effect measure for the factor under study across levels of another factor’ (70, 71). Although not a bias, appropriate consideration of effect modification helps to uncover the details of the property of the effect under study (72). In practice, effect modification is assessed by (i) including the product of the exposure and the effect modifier(s) in a regression model and computing statistical significance and (ii) stratifying the effect of the exposure on the outcome by the effect modifier(s) (71–73).

Methods

Details of the methodology for the identification, selection, and inclusion of the studies have been previously reported (7). In summary, the included studies (8–69) were of children aged ≤ 16 years and included mothers during pregnancy. The exposures of interest were food consumption, nutrient intake, and nutrient biomarkers during pregnancy, infancy, and childhood. In addition, we included studies investigating both nutrient deficiency and the role of increased nutrient consumption, whether through diet or supplementation. The primary outcomes of interest were asthma and atopic disorders as assessed by any objective measure, that is, the incidence of physician-confirmed asthma/atopic disease, lung function, and atopic sensitization. Secondary outcomes of interest were measures of disease severity, health economic data, and data on adverse effects.

These studies were reappraised by two independent reviewers (UN and BIN) to extract detailed information on the confounders and effect modifiers included in the studies; examine the approaches and justifications employed in the selection of the confounders and effect modifiers; appraise the analysis strategies utilized to handle the confounders and effect modifiers. A customized data extraction form was used to record the extracted set of information. After the independent review of studies, any disagreements were resolved

through discussion between the two reviewers. A third reviewer arbitrated if discussion could not be reached. BIN and GD did not participate in the discussion of the studies in which they were co-authors.

The information extracted from the studies was transferred to a Microsoft Excel spreadsheet, and basic descriptive statistics of study characteristics were computed and stratified by study design.

Results

The main features of the included studies are presented in Table 1, arranged in alphabetical order of authorship. Table 2 contains the summary statistics relating to issues of confounders and effect modifiers, extracted from the information in Table 1.

Time-line of studies

The earliest of these studies were conducted in 1990 [one cross-sectional study (24) and one case-control study (59)]. Subsequent studies were conducted in 1994 [two case-control studies (56, 62)]. One cross-sectional study was carried out in 1997 (19), and after that, the remaining studies were conducted between 2000 and 2010, with the first cohort studies emerging in 2004 (39, 46, 61)(Table 1).

Selection of confounders and effect modifiers

Of the 62 studies reviewed, 20 utilized cohort design; 25 employed cross-sectional design; 16 were case-control studies; and one study was of ecologic design (Table 2). Nine of the studies (26, 36, 37, 40, 41, 49, 53, 62, 64), which were all case-control studies, did not take into account confounding or effect modification in the analysis. Of the studies that considered either confounding or effect modification ($n = 53$), 40 considered only confounding (9, 10, 15–25, 27, 30, 31, 34, 35, 38, 39, 42–45, 47, 48, 50–52, 54–56, 58, 61, 63, 65–69), while 13 considered both confounding and effect modification (8, 11–14, 28, 29, 32, 33, 46, 57–59). Thirty-nine of these studies did not have any justification for the selection of confounders or effect modifiers included in the studies: 15 (38%), five (13%), and 19 (49%) of these studies were cohort, case-control, and cross-sectional studies, respectively (Table 2). Of the 14 studies (five cohort, two case-control, six cross-sectional, and one ecological) that justified the selection of the confounders or effect modifiers, seven employed a conceptual approach (i.e., based on knowledge of the subject matter or previous evidence in the literature), 10 employed an empirical approach (usually based on statistical tests), while only three employed both conceptual and empirical approaches (Table 2).

Although the number of confounders considered in the studies varied considerably, the most common were age, sex, familial or parental history of atopic disease, parental or household socioeconomic status (education, occupation, income), smoking (maternal or household), siblings (or parity or birth order). Some studies also considered birth weight,

Table 1 Treatment of confounders and effect modifiers in studies investigating the relationship of diet to asthma and allergic diseases

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Antova et al., Multicenter (7)	Cross-sectional study of 7- to 11-year-old children; <i>n</i> = 20271	Intakes of fish, fruits, vegetables	Cough and wheeze assessed by symptom-based questionnaire	C: age, sex, area, pets at home, indoor moisture at home, use of gas oven for heating, additional gas heating, smokers in household, maternal education, paternal occupation, parental allergy, questionnaire respondent, overcrowding at home EM: country of study	No justification given	C: Multiple regression EM: Interaction and stratification
Arora et al., India (8)	Case-control study of 2- to 12-year-olds Cases: <i>n</i> = 19 Controls: <i>n</i> = 25	Serum levels of vitamin A	Asthma based on documented clinical history	C: age, sex EM: None	No justification given	C: Matching: cases and controls matched by age and sex C: Multiple regression
Awasthi et al., India (9)	Cross-sectional study of 6- to 7- and 13- to 14-year-olds; <i>n</i> = 6000	Fruits and vegetables	ISAAC-based wheeze and asthma	C: traffic pollution, parental smoking, cooking fuels, proximity to pets, breastfeeding, TV watching, exercise, antibiotic and paracetamol use in year 1, and parental education EM: None	No justification given	C: Multiple regression
Battle et al., Mexico (10)	Cross-sectional study of 6- to 7-year-olds; <i>n</i> = 1476	Mediterranean diet	ISAAC-based asthma, wheeze, allergic rhinitis	C: sex, siblings, birth weight, BMI, breastfeeding, parental education, child's exercise, smoking exposure at home, parental allergic history, animals at home during yr 1, living in ranch during pregnancy, premature birth, respiratory infections during early life, silhouette, type of combustible used for cooking at home, sleeping alone at home, and mold dampness at home EM: gender, smoking, maternal education, maternal asthma and allergic rhinitis	Conceptual: based on previous literature. Empirical: based on statistical test in relation to the exposure and outcome	C: Multiple regression EM: Stratification
Bäck et al., Sweden (11)	Cohort study of 6-year-old children; <i>n</i> = 123	Vitamin D ₃ intake	ISAAC-based asthma, atopic dermatitis, and allergic rhinitis	C: Parental atopy, family allergic history EM: Family allergic history	Conceptual: family allergic history as an important risk factor for allergy	C: Multiple regression EM: Interaction and stratification
Burney et al., Multicenter (12)	Case-control study of 20- to 45-year-olds; Cases: <i>n</i> = 569 Controls: <i>n</i> = 576	Plasma selenium	Self-reported asthma	C: gender, age, smoking, SES, supplement use, paracetamol use, and BMI EM: gender, atopy, and smoking	No justification given	C: Multiple regression EM: Interaction and stratification

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Camargo et al., the United States (13)	Cohort study of 3-year-olds; $n = 1194$	Vitamin D intake	Questionnaire-based wheeze and eczema	C: sex, birth weight, income, maternal age, maternal pre-pregnancy BMI, passive smoking exposures, breastfeeding, siblings <12 years, maternal asthma/eczema, paternal asthma/eczema, maternal intake of supplements, calcium, and retinol EM: pre-pregnancy BMI, maternal intake of calcium and retinol, parental asthma, white maternal race, and season of last menstrual period	No justification given	C: Multiple regression EM: Interaction and stratification
Castro-Rodriguez et al., Spain (14)	Cross-sectional study of preschoolers of 4.08 mean age; $n = 1784$	Mediterranean diet	ISAAC-based wheeze	C: age, birth weight, livestock during pregnancy, cesarean section, antibiotic during year 1, acetaminophen intake during year 1, rhinoconjunctivitis, dermatitis, paternal asthma, maternal asthma, maternal age, maternal education, paternal smoking, maternal smoking, physical activity, cats at home in the past 12 months EM: None	Empirical: based on statistical test ($P < 0.05$) in bivariate association between the covariates and the outcome	Multiple regression
Chatzi et al., Greece (15)	Cross-sectional study of 7- to 18-year-olds; $n = 690$	Fruits, vegetables, and Mediterranean diet	ISAAC-based wheeze and allergic rhinitis: skin prick test (SPT) for atopy	C: age, sex, BMI, parental asthma, number of siblings EM: None	No justification given	Multiple regression
Chatzi et al., Spain (16)	Cross-sectional study of 6.5-year-olds; $n = 460$	Fish, fruits, vegetables	Questionnaire-based wheeze; SPT	C: gender, parental asthma, parental atopy, maternal smoking, BMI at 6.5 years, parental education and social class, breastfeeding, fish intake during pregnancy, number of siblings EM: None	Empirical: based on statistical test ($P < 0.20$) in bivariate association between the covariates and the outcome	Multiple regression
Chatzi et al., Spain (17)	Cohort study of 6.5-year-olds; $n = 460$	Mediterranean diet	Questionnaire-based wheeze; SPT	C: gender, maternal age, parental education and social class, maternal smoking during pregnancy, supplement use during pregnancy, parental asthma, breastfeeding, lower tract infection at year 1, birth weight, gestation, birth order, number of siblings, BMI at year 6.5 EM: None	Empirical: based on statistical test ($P < 0.20$) in bivariate association between the covariates and the outcome	Multiple regression

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Cook et al., UK (18)	Cross-sectional study of 8- to 11-year-olds; $n = 2650$	Fruits and vitamin C in plasma	Questionnaire-based wheeze; lung function (FEV ₁)	C: age, sex, height, town of study, instrument, observer, room temperature, obesity index, social class, salivary concentration of cotinine, and birth weight EM: None	Conceptual: based on previous literature (only for birth weight and not other confounders)	Multiple regression
Devereux et al., UK (19)	Cohort study of 5-year-olds; $n = 1861$	Plasma vitamin E and zinc	ISAAC-based asthma, wheeze, eczema, and hayfever; SPT	C: maternal age, maternal atopy, maternal smoking, maternal vitamin intake, maternal vitamin E or zinc intake, paternal social class, maternal age at leaving full-time education, deprivation index, birth weight, birth head circumference, crown-heel length, sex, birth order, breastfeeding, and use of antibiotics in year 1 EM: None	No justification given	Multiple regression
Devereux et al., UK (20)	Cohort study of 5-year-olds; $n = 1924$	Plasma selenium	ISAAC-based wheeze and asthma	C: gender, maternal atopy, maternal smoking, maternal age, parity, paternal social class, maternal age at full-time education, birth weight, crown-heel length, head circumference, maternal plasma ascorbate, α -tocopherol, zinc, antibiotics during infancy, breastfeeding, and deprivation index EM: None	Conceptual: based on the previous literature. Empirical: based on statistical test ($P < 0.25$) in bivariate association between the covariates and the outcome	Multiple regression
Devereux et al., UK (21)	Cohort study of 5-year-olds; $n = 1212$	Vitamin D intake	ISAAC-based wheeze and asthma; SPT; bronchodilator response, spirometry, and exhaled nitric oxide	C: maternal atopy, maternal age, maternal smoking, maternal age at leaving full-time education, paternal social class, deprivation index, breastfeeding, sex, use of antibiotics in year 1, birth weight, birth order, season of last menstrual period, maternal intakes of vitamin E, zinc, and calcium EM: None C: GDP of countries EM: None	No justification given	Multiple regression
Ellwood et al., Multicenter (22)	Ecological study of 53 countries	Per capita food intake	ISAAC-based asthma allergic rhinoconjunctivitis, and atopic eczema		Conceptual: based on the previous literature – GDP correlated with allergic symptoms	Mixed models regression

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
El-Kholy et al., Egypt (23)	Case-control study of 2- to 12-year-olds Cases: 40 Controls: 20	Serum and hair zinc and copper	Hospital-based bronchial asthma and atopic dermatitis	C: age, sex, socioeconomic status EM: None	No justification given	Matching: cases and controls matched by age, sex, and socioeconomic status
Erkkola et al., Finland (24)	Cohort study of 5-year-olds; <i>n</i> = 1669	Vitamin D intake	ISAAC-based asthma, allergic rhinitis, and atopic eczema	C: sex, place of birth, gestation, maternal age, maternal education, maternal smoking during pregnancy, siblings, parental asthma and allergic rhinitis, eczema by 6 months, pets at home by 1 year, fruits, vegetables, vitamin C, vitamin E, selenium, zinc EM: None C: None EM: None	No justification given	Multiple regression
Ermis et al., Turkey (25)	Case-control study of children mean age of 7.6 years; Cases: <i>n</i> = 41 Controls: <i>n</i> = 30	Serum zinc, copper, and magnesium levels	Reported bronchial asthma	C: None EM: None	NA	NA
Farchi et al., Italy (26)	Cross-sectional of 6- to 7-year-olds; <i>n</i> = 4104	Vegetables, fruits, fat products, dairy products	ISAAC-based wheeze, shortness of breath, and allergic rhinitis	C: sex, study area, paternal education, household crowding, maternal smoking, paternal smoking, dampness or mold in child's room, and parental asthma EM: None	No justification given	Multiple regression
Fitzsimon et al., Ireland (27)	Cohort study of 3-year-olds; <i>n</i> = 631	Vegetables, fruits, oily fish	Questionnaire-based asthma	C: maternal age, maternal BMI, maternal smoking, exposure to smoke at home, mold or dampness in the home, pollution problems in local area, maternal university education, GMS status, income, parity, birth weight, gender, breastfeeding EM: hospital of birth	No justification given	C: Multiple regression EM: Stratification
Forastiere et al., Italy (28)	Cross-sectional study of 6- to 7-year-olds; <i>n</i> = 4104	Citrus and kiwi fruits	ISAAC-based wheeze, shortness of breath, and allergic rhinitis	C: sex, study area, paternal education, household crowding, maternal smoking, paternal smoking, dampness or mold in the room, parental asthma EM: sex area of residence, paternal education, paternal smoking, parental asthma, life history of asthma	No justification given	C: Multiple regression EM: Interaction

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Gale et al., UK (29)	Cohort study up to the age of 9 years; <i>n</i> = 466	25-(OH)-vitamin concentration	Questionnaire-based eczema and asthma	C: maternal education, season of birth EM: None	No justification given	Multiple regression
Garcia et al., Columbia (30)	Cross-sectional study of 6- to 7- and 13- to 14-year-olds; <i>n</i> = 7085	Food consumption	ISAAC-based wheeze and asthma	C: age, sex, contact with animals, cesarean section, breastfeeding, presence of cat/dog at home in year 1, exercise, TV watching, maternal smoking during year 1, maternal education, frequency of traffic around the house, type of cooking fuel, family size, use of acetaminophen and antibiotics during year 1 EM: None	No justification given	Multiple regression
Garcia-Marcos et al., Spain (31)	Cross-sectional study of 6- to 7-year-olds; <i>n</i> = 20106	Mediterranean diet	ISAAC-based asthma and rhinoconjunctivitis	C: sex, obesity, maternal smoking, siblings, exercise EM: sex	No justification given	C: Multiple regression EM: Stratification
Gilliland et al., the United States (32)	Cross-sectional study of 11- to 19-year-olds; <i>n</i> = 2566	Antioxidant vitamins, fruits, and vegetables	Lung functions	C: parental education, household income, BMI, age, sex, insurance status, personal smoking, environmental smoke exposure, respiratory illness EM: sex, asthma status	Empirical: based on causing a% change in estimate ($\geq 10\%$) of the association between exposure and outcome No justification given	C: Multiple regression EM: Interaction and stratification Multiple regression
Harik-Khan et al., the United States (33)	Cross-sectional study of 6- to 17-year-olds; <i>n</i> = 4093	Vitamins A, C, and E and carotenoids	Questionnaire-based asthma	C: age, sex, household size, BMI, household head educational status, household head sex, household head employment, parental allergic history, race, smoker in household EM: None	Conceptual: based on the previous literature. Empirical: based on the statistical test in relation to the outcome NA	Multiple regression
Hijazi et al., Saudi Arabia (34)	Case-control study of 12-year-olds Cases: <i>n</i> = 114 Controls: <i>n</i> = 202	Foods and nutrients	ISAAC-based asthma	C: place of residence, family allergic history, SPT positivity, maternal education, sex, social class EM: None		
Hozvasz et al., Poland (35)	Case-control study of 1- to 9-year-olds Cases: 25 Controls: 18	Serum α -tocopherol	Atopic dermatitis	C: None EM: None		NA

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Hozyasız et al., Poland (36)	Case-control study of 1- to 9-year-olds Cases: 19 Controls: 18	Plasma retinol	Atopic dermatitis	C: None EM: None	NA	NA
Huang et al., Taiwan (37)	Cross-sectional study of 13- to 17-year-olds; $n = 1166$	Nutrients	Questionnaire-based asthma and allergic rhinitis	C: sex and urbanization EM: None	No justification given	Multiple regression
Hypönen et al., Finland (38)	Cohort of 7648 adults	Vitamin D supplementation	Questionnaire-based asthma and allergic rhinitis; SPT	C: family allergic history and asthma, social class, maternal attitude to health education, maternal frame of mind during delivery, pregnancy wanted by mother, sex, birth order, maternal age, maternal education EM: None	No justification given	Multiple regression
Kalayci et al., Turkey (39)	Case-control study of 13- to 15-year-olds Cases: 14 Controls: 12	Serum α -tocopherol, beta carotene, and ascorbic acid	Hospital-based asthma	C: None EM: None	NA	NA
Kocabaş et al., Turkey (40)	Case-control study of 0- to 3-year-olds Cases: 61 Controls: 61	Serum selenium	Questionnaire-based wheeze	C: None EM: None	NA	NA
Lewis et al., UK (41)	Cross-sectional study of 4- to 6-year-olds; $n = 1162$	Fruits	ISAAC-based wheeze and asthma	C: age, sex, area, smokers at home, distance from roads, Townsend index EM: None	No justification given	Multiple regression
Litonjua et al., the United States (42)	Cohort study of 2-year-olds; $n = 1290$	Antioxidant nutrients and minerals	Questionnaire-based wheeze and eczema	C: birth weight, sex, maternal age, maternal pre-pregnancy BMI, breastfeeding, siblings ≤ 12 years, postnatal passive smoking exposure, family income, parental asthma, parental eczema, maternal intake of fruits and vegetables, and child multivitamin intake in year 1 EM: None	Empirical: based on statistical test ($P < 0.05$) in relation to the outcome and % of change (8%) in the association between the exposure and outcome	Multiple regression

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Martindale et al., UK (43)	Cohort study of 2-year-olds; n = 1924	Antioxidants	ISAAC-based wheeze and eczema	C: sex, maternal age, paternal social class, maternal atopy, maternal smoking, siblings, antibiotic use, vitamin E or C EM: None	No justification given	Multiple regression
McKeever et al., the United States (44)	Cross-sectional study of 6- to 16- (n = 4428) and 17- to 59-year-olds (n = 5858)	Markers of antioxidants, lipid, and minerals	SPT	C: age, sex, smoking, BMI, poverty index, race, survey design EM: None	No justification given	Multiple regression
Milner et al., the United States (45)	Cohort study of 3-year-olds; n > 8000	Multivitamin supplementation	Questionnaire-based asthma and food allergies	C: education, race, sex, breastfeeding, degree of prematurity, household smoking, household income, enrollment in day care EM: race, breastfeeding C: maternal age, gestation, residential municipality, family income, parental education, parental asthma, allergic rhinitis, and atopic eczema, maternal diet during past 1 month, season of data collection, maternal smoking during pregnancy, older siblings, sex, birth weight, household smoking, breastfeeding, age EM: None	Empirical: based on statistical test ($P < 0.05$) in relation to the outcome No justification given	C: Multiple regression EM: Stratification Multiple regression
Miyake et al., Japan (46)	Cohort study of 16- to 24-month-olds; n = 763	Vegetables, fruits, and antioxidants	ISAAC-based wheeze and eczema	C: maternal age, gestation, residential municipality, family income, parental education, parental asthma, allergic rhinitis, and atopic eczema, maternal diet during past 1 month, season of data collection, maternal smoking during pregnancy, older siblings, sex, birth weight, household smoking, breastfeeding, age EM: None	No justification given	Multiple regression
Miyake et al., Japan (47)	Cohort study of 16- to 24-month-olds; n = 763	Dairy products, calcium, and vitamin D	ISAAC-based wheeze and eczema	C: maternal age, gestation, residential municipality, family income, parental education, parental asthma, allergic rhinitis, and atopic eczema, maternal diet during past 1 month, season of data collection, maternal smoking during pregnancy, older siblings, sex, birth weight, household smoking, breastfeeding, age EM: None C: None EM: None	No justification given	Multiple regression
Mizuno et al., Japan (48)	Case-control study Cases: n = 26 (mean age 5.5 years) Controls: n = 25 (mean age 6.4 years)	Serum vitamin A	Asthma	C: None EM: None	NA	NA

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Murray et al., UK (49)	Nested case-control study of 3- to 5-year-olds Cases: $n = 37$ Controls: $n = 37$	Multivitamins, vitamins, minerals, and fatty acids	Wheeze	C: total energy, total fat (matching factors), sex, month of birth, pet ownership, indoor allergen exposure, parental allergic sensitization EM: None	No justification given	ANOVA
Niä et al., Norway (50)	Follow-up study of 6- to 16-year-olds; $n = 4585$	Fruits, vegetables, vitamin supplementation, cod liver oil	Questionnaire-based asthma and wheeze; SPT	C: age, sex, area, parental education, parental atopy, exposure to dog and cat, maternal smoking during pregnancy and at child's 0–11 months EM: None	No justification given	Multiple regression
Okoko et al., UK (51)	Cross-sectional study of 5- to 10-year-olds; $n = 2640$	Fruits	ISAAC-based asthma and wheeze	C: sex, age, paracetamol ibuprofen exposure, vitamin use, living in the farm, mold or mildew in bedroom, in living areas, and in hallways, financial source for home repairs, exposure to passive smoking, ethnicity, birth weight, breastfeeding, number of parents living with child, siblings, parental education, EM: None C: None EM: None	No justification given	Multiple regression using propensity scores of the selected covariates
Omata et al., Japan (52)	Case-control study of 3- to 15-year-olds Cases: $n = 27$ Controls: $n = 25$	Selenium concentration in urine	Reported atopic dermatitis		NA	NA
Pastorino et al., Brazil (53)	Case-control study of 13- to 14-year-olds; $n = 141$ Controls: $n = 387$	Fresh fruits and cooked vegetables	ISAAC-based asthma	C: Pertussis disease, siblings, cesarean section, rhinoconjunctivitis, eczema, allergic mother, SPT positivity, rhinitis, prematurity EM: None	Empirical: based on statistical test ($P < 0.20$) in relation to the outcome	Multiple regression
Pesonen et al., Finland (54)	Cohort study of 20-year-olds; $n = 200$	Plasma retinol concentration	Atopic dermatitis, allergic rhinoconjunctivitis, wheeze, SPT, IgE	C: sex, breastfeeding, family allergic history, smoking in household during year 1, personal smoking at 20 years, maternal education, maternal age, sibship, day care during year 1 EM: None C: age, sex EM: None	No justification given	Multiple regression
Powell et al., UK (55)	Case-control study of 18-month- to 16-year-olds Cases: 37 Controls: 35	Plasma tocopherol, retinol, vitamin C, and selenium	Clinical-documented asthma		No justification given	Simple regression analysis

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Romieu et al., the United States (56)	Cross-sectional study of 2- to 16-year-olds; <i>n</i> = 7904	Vitamin C intake	Questionnaire-based asthma and wheeze	C: BMI, sex, race, parental asthma, parental hay fever, passive smoking, poverty level, presence of atopy, TV watching EM: age	No justification given	C: Multiple regression EM: Stratification
Rubin et al., the United States (57)	Cross-sectional study of 4- to 16-year-olds; <i>n</i> = 7505	Serum vitamin E, β -carotene, vitamin C, and selenium	Questionnaire-based asthma	C: BMI, education level of household head, household crowding, urban residence, passive and active smoke exposure, parental asthma/hay fever, child history of hay fever, avoidance of pets because of allergy EM: cigarette smoke exposure	No justification given	C: Multiple regression EM: Interaction
Swartz & Weiss, the United States (58)	Cross-sectional study of ≥ 30 -year-olds; <i>n</i> = 9074	Serum vitamin C, dietary vitamin D, fish intake, dietary sodium/potassium, serum zinc/copper	Questionnaire-based bronchitis and wheeze	C: cigarette smoking, age, race, triceps skin fold, sex, family income, residence inside city center, head of household education, region EM: age, race, sex, smoking	Conceptual: based on the previous literature.	C: Multiple regression EM: Interaction
Shaheen et al., UK (59)	Cohort study of 7.5-year-olds; 14062	Dietary patterns	Questionnaire-based eczema, wheeze, hay fever, asthma; lung functions, and SPT	C: maternal smoking, infections, maternal antibiotic and paracetamol use, maternal education, housing tenure, financial difficulties, pre-pregnancy BMI, ethnicity, parity, maternal asthma, eczema, and rhinoconjunctivitis, maternal migraine, sex, gestation at birth, breastfeeding during first 6 months, day care by 8 months, multiple pregnancy, pets in infancy, damp/condensation/mold, child exposure to smoking, season of birth, season of FFQ, birth weight, head circumference, birth length, siblings, and BMI EM: None	No justification given	Multiple regression using propensity scores of the selected covariates
Shaheen et al., UK (60)	Cohort study of 30- to 42-month-olds; <i>n</i> = 2973	Umbilical cord trace elements and minerals	Questionnaire-based wheeze and eczema	C: sex, maternal age, parity, gestation, maternal smoking during pregnancy, maternal education, maternal housing tenure, maternal ethnicity, birth weight, maternal atopy, head circumference, crown-heel length, maternal BMI, breastfeeding during 6 months, day care by 6 months, analgesic use, and infections in late pregnancy EM: None	No justification given	Multiple regression

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Shaw et al., New Zealand (61)	Nested case-control study of 8- to 13-year-olds Cases: 26 Controls: 61	Serum selenium	Questionnaire-based wheeze	C: None EM: None	NA	NA
Tabak et al., The Netherlands (62)	Cross-sectional study of 7- to 13-year-olds; $n = 598$	Fruits, vegetables, dairy products, whole grain products, fish	ISAAC-based asthma and wheeze; BHR and SPT	C: maternal education, foreign descent, age, sex, siblings, asthma in siblings, asthma in parents, maternal smoking, breastfeeding, passive smoking, pets at home, BMI EM: None	No justification given	Multiple regression
Tahan & Karakucuk, Turkey (63)	Case-control study of 1- to 3-year-olds; Cases: $n = 34$ Controls: $n = 14$	Zinc (in hair) deficiency	Questionnaire-based wheeze	C: None EM: None	NA	NA
Tamay et al., Turkey (64)	Cross-sectional study of 6- to 12-year-olds; $n = 2387$	Foods	ISAAC-based allergic rhinitis	C: sex, family atopic history, food allergy, eczema, respiratory tract infection, frequent sinusitis, tonsillectomy, adenoidectomy, antibiotics and paracetamol use in year 1, maternal smoking, cat and dog at home at year 1, dampness at home, heating system, born in Istanbul, time lived in Istanbul, trucks passed on weekdays, perinatal redness EM: None	No justification given	Multiple regression
Tsai et al., Taiwan (65)	Cross-sectional study of 11- to 12-year-olds; $n = 2290$	Foods	Questionnaire-based asthma	C: residential districts, sex, doctor-diagnosed allergies EM: None	No justification given	Multiple regression
Willers et al., The Netherlands (66)	Cohort study of 8-year-olds; $n = 2632$	Foods	ISAAC-based asthma and wheeze	C: sex, maternal education, parental allergy, maternal smoking during pregnancy, smoking in the home at 8 years, breastfeeding, older siblings, birth weight, maternal overweight at yr 1, maternal supplement use during pregnancy, region, study arm EM: None	No justification given	Multiple regression

Table 1 (continued)

Reference, country	Study design and population	Exposure	Outcomes	Confounders (C) and effect modifiers (EM) considered	Justification/approaches to the selection of C and EM	Approaches used to deal C and EM
Willers et al., UK (67)	Cohort study of 5-year-olds; <i>n</i> = 1924	Foods	ISAAC-based asthma, wheeze, eczema, hay fever	C: maternal age, paternal social class, maternal age at leaving full-time education, maternal smoking during pregnancy, maternal asthma and atopy, birth weight, sex, siblings, breastfeeding, smoking in the child's home at 5 years EM: None	No justification given	Multiple regression
Wong et al., China (68)	Cross-sectional study of 10-year-olds; <i>n</i> = 10902	Fruits and vegetables	Questionnaire-based asthma	C: propensity scores of several unspecified covariates and sex EM: None	No justification given	Multiple regression using propensity scores of the selected covariates

BMI, body mass index; C, confounders; EM, effect modifiers; FFQ, Food Frequency Questionnaire; GDP, gross domestic product; ISAAC, The International Study of Asthma and Allergies in Childhood; RCTs, randomized controlled trials; SPT, skin prick test.

length, gestational age, use of antibiotics, and paracetamol. The most common effect modifiers considered included sex, smoking, history of atopy, place of residence, and race (data not shown).

None of the studies employed restriction for controlling confounding in the design stage of the study. In the handling of confounding in the statistical analysis, regression modeling was the most common approach utilized, this being employed by all the cohort (12, 14, 18, 20–22, 25, 30, 39, 43, 44, 46–48, 55, 60, 61, 67) and cross-sectional (10, 11, 15–19, 27, 29, 31–33, 38, 42, 45, 51, 52, 57–59, 63, 65, 66, 69) studies and four of the case-control studies (13, 35, 54, 56); two (9, 24) case-control studies employed matching and one (50) employed ANOVA. Of the studies that considered effect modification (*n* = 13), 10 employed stratification; eight tested for interaction, while five used both stratification and interaction (Table 2).

Summary of the comprehensive set of confounders and effect modifiers that should be considered

Based primarily on a careful examination of the factors that were considered in the appraised studies and evidence from other studies (62, 74–88), we propose in Table 3 a comprehensive set of confounders and effect modifiers that should be considered in future epidemiologic studies investigating the role of diet in the development of childhood asthma and atopic disease. These factors can be grouped mainly into maternal characteristics; birth measurements; socioeconomic characteristics; and environmental exposures. These potential confounders have been grouped into primary and secondary confounders. For the primary confounders, we suggest that these are factors that must always be considered based on conceptual justification, while the secondary confounders are factors that need to be confirmed by performing appropriate statistical tests.

Discussion

The evidence from this comprehensive review of the international evidence indicates a general inadequacy in the handling of confounding and effect modification in observational studies investigating the potential effects of diet during pregnancy, infancy, and childhood on the development of childhood atopic disease and asthma. A majority of the case-control studies did not adjust for confounding or consider effect modification, while all the cohort, cross-sectional, and ecologic studies did. Of the studies that considered confounding or effect modification, most gave no justification for including the selected variables; this was the case irrespective of the study design employed. Empirical justification (usually through statistical tests), compared to conceptual justification (based on knowledge of the subject matter or evidence in previous literature), was the major criteria used in selecting the confounders and effect modifiers among the studies that provided justifications for the variables included. All cohort, cross-sectional, and ecologic studies applied regression modeling for confounder adjustment, while the case-control

Table 2 Summary statistics on confounding and effect modification, extracted from Table 1

Characteristics of studies	Study designs				
	All N = 62	Cohort n = 20	Case-control n = 16	Cross-sectional n = 25	Ecologic n = 1
Studies without adjustment for either confounders or effect modifiers	9	0	9	0	0
Studies with adjustment for either confounders or effect modifiers	53	20	7	25	1
Studies with adjustment for only confounders	40	16	6	17	1
Studies with adjustment for both confounders and effect modifiers	13	4	1	8	0
Studies without justification for the inclusion of confounders or effect modifiers	39	15	5	19	0
Studies with justification for the inclusion of confounders or effect modifiers	14	5	2	6	1
Studies with conceptual justification	7	2	1	3	1
Studies with empirical justification	10	3	2	3	0
Studies with both conceptual and empirical justifications	3	1	1	1	0
Approaches to treatment of confounders in the studies					
Studies with regression modeling	50	20	4	25	1
Studies with matching	2	0	2	0	0
Studies using ANOVA	1	0	1	0	0
Approaches to treatment of effect modifiers in the studies					
Studies using stratification	10	4	1	5	0
Studies using interaction	8	2	1	5	0
Studies using both stratification and interaction	5	2	1	2	0

ANOVA, analysis of variance.

Table 3 Summary of comprehensive set of confounders and effect modifiers that need to be considered in future epidemiologic studies

Confounding/effect modification	Variables
Confounders	
Primary confounders*	Maternal and child characteristics: maternal age, child's age, child's sex, family history of atopic disease, parity, birth weight, gestational age at birth, and mode of delivery Socioeconomic status: preferably, at least two parameters relating to education, income, occupation, or a measure of deprivation or social class Environmental exposures: use of antibiotics and paracetamol during pregnancy and early life, pets at home, smoking (maternal smoking, household, or environmental smoking exposure), home environment (dampness, mold, chemicals), exposure to day care during infancy Dietary factors: breastfeeding, and other relevant dietary factors depending on the research question
Secondary confounders†	Maternal BMI, living on a farm, race/ethnicity, place (or region) of birth, season of birth, and season of ascertainment of dietary factors, physical activity
Effect modifiers	Family history of atopic disease, season of birth or season of ascertainment of dietary factors, sex, race/ethnicity, place of residence, breastfeeding

*Always to be considered based on conceptual justification.

†To be considered based on empirical evidence (i.e., by appropriate statistical tests).

studies used either matching, regression modeling, or ANOVA. Effect modification was handled either by stratifying by the effect modifiers or by including interaction terms between the effect modifiers and the exposures of interest.

Confounding constantly poses great challenges to observational epidemiologic studies and, if not efficiently handled, invariably weakens the validity of conclusions (2–5). Effect modification, on the other hand, although not a bias aimed to be eliminated in a study (72), helps to identify whether the effect of an exposure on an outcome of interest varies across subgroups of the study population (70–72) and, in clinical practice, guides in more specific targeting of intervention to the subgroup of the population that needs it most (89).

Consequently, efficient consideration of confounding and effect modification is essential in order to reach a valid and reliable interpretation of findings from observational studies (2–5). As highlighted in our recent systematic review, available evidence on the role of diet during pregnancy, infancy, and childhood in the development of childhood atopic disease and asthma was largely inconclusive for several dietary factors, and this was largely attributed to the methodological weaknesses of the studies (7).

The present critical appraisal of these studies shows major inadequacies concerning the handling of confounding and effect modification, particularly as it relates to the process of selection and the criteria utilized for that purpose. Poor treat-

ment and report of confounding and effect modification has also been noted in other research contexts (5, 90, 91). The lack of consideration of confounding in the majority of the case-control studies considered in the present paper raises serious concerns about the conclusions of these studies, because the observed effect cannot be independently attributed to the dietary factors examined without taking into account the influence of other potential factors that usually confound the diet-disease relationship. It is also surprising that a majority of studies that considered confounding or effect modification gave no inclusion criteria for the selected variables. It is possible that some of these studies, *a priori*, had no criteria for selecting the variables, or might have used conceptual and/or empirical criteria, but failed to report them when published. While the inclusion of confounders and effect modifiers in a study must be justified, the criteria used for their selection must also be reported in the paper. A detailed recommendation for indicating such information in a research paper has been described elsewhere (6).

Efficient treatment of confounding and effect modification, beginning from selection to treatment in statistical analyses, is also addressed in most epidemiologic texts (72). Primarily, selection of the variables should be based on knowledge of the causal mechanism between diet and the development of allergy and asthma (conceptual justification) and secondarily on the evidence available from the data, which is usually assessed using appropriate statistical tests (based on *P*-values), percentage change in the main effect under study, stepwise regression models, or the more advanced techniques such as propensity scores or the shrinkage method (empirical justification) (72, 92). Unfortunately, of the studies that gave justification for inclusion, the majority based their criteria only on empirical evidence, ignoring the primary conceptual basis. The adjustment for confounding was suitably made in the cohort and cross-sectional studies, and in some case-control studies, by employing multiple regression modeling. However, regardless of the sophistication of the method used to adjust for confounding, findings may still remain invalid if appropriate steps have not been employed in confounder selection (72). As a result, the issue of residual confounding may remain an important concern (72). Although matching is one of the methods used to address the issue of confounding, its limitations, particularly for case-control studies, have been extensively addressed in the literature and authors should consider these recommendations when considering matching (72, 93). The selection of effect modifiers is mostly guided by prior knowledge of the effect under study (92). Handling effect modification by stratification is always determined by the adequacy of the sample size of a study (89). However, regardless of the sample size of a study, including interaction terms between the potential effect modifier(s) and the exposure of interest and performing appropriate statistical tests may be a good starting point to investigate the possible presence of effect modification (73). The statistical significance of such tests may give a clue as to whether the effect measure is modified by the potential effect modifier(s) (73). However, when such subgroup analyses are performed, appropriate steps should be taken to address the issue of

multiple tests of association so as to minimize the risk of generating false-positive findings.

We observed that the confounders and effect modifiers considered differed between studies. This lack of uniformity in the identification and inclusion of confounders or effect modifiers in studies of diet, allergies, and asthma is likely to have contributed to the heterogeneity of reported associations. Although we acknowledge that some confounding or effect-modifying variables may be specific to a study setting, it is important that, conceptually, a comprehensive set of variables be considered by studies investigating the effect of early life diet on the occurrence of atopic disease and asthma should be proposed to guide authors when planning their study and analysis. For this purpose, based on careful consideration of the reviewed studies and other related evidence (1, 62, 74–88), we have proposed several factors that should be taken into account, in the areas of maternal characteristics, birth measurements, socioeconomic characteristics, and environmental exposures. The confounders are grouped into primary (factors that are biologically relevant in the risk of allergies or that have been consistently established in previous studies) and secondary (factors with probable or insufficient evidence in previous studies) factors, indicating that the primary confounders must always be considered, while the secondary factors should be considered based on appropriate statistical tests. For effect modification, depending on the adequacy of the sample size of a study, we propose that the following variables should be considered as potential effect modifiers: family history of atopic disease, season of birth or season of ascertainment of dietary factors, race/ethnicity, place of residence, breastfeeding. We recommend employing tests for interaction, at least in the first place, before the consideration of stratification by the effect modifiers. We acknowledge that while these lists may not be exhaustive, the proposed factors, at present, constitute a core comprehensive set of important potential confounders and effect modifiers in this area of research.

A major challenge in nutritional epidemiology is the complex inter-relationships and interactions between dietary factors both within foods and dietary habits, which ultimately may confound the observed effect of interest (94). Consequently, including several dietary factors in the same statistical model could result in multicollinearity and hence distortion of the overall model performance. For nutrients, the observed effect may reflect interactions with other nutrients, as well as with other nonquantified nutrients. Although there are no straightforward approaches to dealing with this situation, there is a need to apply more advanced statistical techniques that can account for multicollinearity. If the analysis is based on the food level, suggestions that have been offered include (i) using food groups as the unit of dietary intake instead of using single foods and (ii) employing dietary pattern analysis, such as factor or cluster analysis (94). The situation may be less clear with nutrients considering the potential interactions between nutrients; however, appropriate statistical methods should be employed to explore such interactions. Our recent systematic review showed that the key dietary factors related to the risk of allergies in childhood include vitamins A, D, E, zinc, fruits,

and vegetables (7). With appropriate statistical methods, we suggest that these dietary factors should be considered for adjustment in subsequent dietary and nondietary studies. Furthermore, with appropriate statistical techniques, we also suggest that studies investigating the role of maternal diet should adjust for the dietary intake of the child and vice versa.

This is, as far as we are aware, the first study to address the issues of confounding and effect modification in studies investigating the possible effect of diet on the development of childhood asthma and atopic disease. We envisage that this groundwork will stimulate active discussions on the optimal strategies to consistently reduce the potential for bias in this area of research that has been mainly investigated using observational epidemiologic study designs. These observations and suggestions presented here could be the basis for further discussions and consensus on a core comprehensive set of potential confounders and effect modifiers that need to be considered when planning future epidemiologic studies. However, we note that considering confounding/effect modification-related issues will not address all of the challenges associated with observational studies, and a number of other factors, such as reverse causation, selection bias, information bias (regarding both the ascertainment of dietary intake and measurement of allergic outcomes), and sample size, still need to be considered. In addition to inadequate consideration of confounding, it is possible that differences in addressing this spectrum of factors might also be responsible for the inconsistent findings noted in previous studies. The studies included in this paper were those selected based on a set of rigorous inclusion criteria. Consequently, it is possible that the inadequacies regarding the handling of confounding and effect modification as highlighted in this paper might have been underestimated, considering that other studies with poorer quality were excluded. Regardless, we believe that the framework outlined in this paper is adequately applicable to all studies in this area of research.

Conclusions

This systematic review-based analysis provides evidence that the issues of confounding and effect modification have on the

whole been inadequately handled in observational epidemiologic studies investigating the role of diet in the development of childhood asthma and allergic disease. The selection of confounders and effect modifiers should be primarily based on conceptual justification and secondarily on empirical evidence. For effective handling of confounding and effect modification with consequent improvement in the methodological quality of studies in this area of research, there is a need to agree on a comprehensive set of variables that should be taken into account when planning future epidemiologic studies.

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Author contributions

AS conceived the idea for this work and oversaw all aspects of the data analysis, interpretation, and writing-up. UN and BIN undertook the analysis and drafted the report. GD contributed to the interpretation of data and the writing of the manuscript.

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Ethical approval

Ethical approval was not required.

Conflict of interest

All authors have no conflicts of interest.

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