

REVIEW ARTICLE

CD14 polymorphisms, microbial exposure and allergic diseases: a systematic review of gene–environment interactions

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Abstract

Asthma and allergy may develop as a result of interactions between environmental factors and the genetic characteristics of an individual. This review aims to summarize the available evidence for, and potential effects of, an interaction between polymorphisms of the *CD14* gene and exposure to microbes on the risk of asthma and allergic diseases. We searched PubMed, MEDLINE and Global Health databases, finding 12 articles which met inclusion criteria. Most studies reported a significant interaction between *CD14* polymorphisms and microbial exposure. When stratified by age at microbial exposure (early life vs adult life), there was evidence of a protective effect of gene–environment interaction against atopy in children, but not adults. We also found different effects of interaction depending on the type of microbial exposures. There was no strong evidence for asthma and eczema. Future studies should consider a three-way interaction between *CD14* gene polymorphisms, microbial exposures and the age of exposure.

While many risk factors for asthma have been explored, a current topic of much interest is the potential role of environmental endotoxins, a marker of microbial exposure. Endotoxins are lipopolysaccharides (LPS) from the cell wall of Gram-negative bacteria. They can induce the production of interleukin-12 (IL-12) resulting in promotion of Th-1 responses and inhibition of Th-2 responses in the host (1). They are present in both occupational and home environments as a component of dust (2, 3). Endotoxin levels can be directly measured by assaying dust samples, as well as estimated indirectly by survey measures including reporting of farming lifestyle and pet exposure. The protective effects of high microbial exposure against atopy, allergic rhinitis and to a lesser extent asthma have been well established (4–6). However, the aetiological pathways underlying these effects remain unclear.

Asthma and allergy may develop as a result of interactions between influences from the environment and genetic characteristics of an individual. Of the genes associated with asthma, one of the most widely studied is the monocyte differentiation

antigen 14 (*CD14*) gene. The *CD14* gene encodes for a protein which functions as a co-receptor for the toll-like receptor (*TLR*) and is one of the major components in the innate immune response (1). The *CD14* receptor is multifunctional with high specificity for LPS and together with *TLR4* forms a complex that activates the innate immune system through various pathways, releasing pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF- α). The *CD14* gene is located on chromosome 5q31.3, a region associated with asthma in genomewide linkage studies (7). While several genetic polymorphisms in the *CD14* gene have been examined in relation to allergic disease risk, a consistent link has not been established. A recent review found no evidence of a role for any single nucleotide polymorphism (SNP) in the *CD14* gene and the risk of childhood asthma among Caucasian children (8). On the other hand, a meta-analysis of the widely studied *CD14*–260 C>T promoter polymorphism and atopic asthma found a significant protective effect for carriers of the TT or CT genotype compared with the CC genotype (9). Some of these inconsistent results may be

related to a failure to account for gene and environment ($G \times E$) interactions in these associations (10–13).

Although a number of studies have examined the interaction between *CD14* polymorphisms and measures of microbial exposure on allergic disease risk, no systematic review has been conducted. Therefore, we have systematically examined the literature in this area to synthesize the evidence for a relationship between *CD14* polymorphisms and microbial exposure on allergic disease outcomes. We have assessed the quality of each study and further examined the effects of $G \times E$ interactions at different ages by stratifying the published results both by the type and by the times of exposure measurement and the times at which outcomes were reported.

Methods

Search strategy

A search for publications on the *CD14* gene and microbial exposure in relation to asthma and allergic diseases was conducted using three databases: MEDLINE, PubMed (both from 1950 to February 2013) and Global Health (from 1973 to February 2013).

Three groups of keywords with Medical Subject Headings (MeSH) terms were created using ‘OR’ or ‘AND’ within the group and using ‘AND’ to combine the 3 groups in PubMed and MEDLINE:

(i) Allergic disease terms: ‘asthma’ or ‘wheeze’ or ‘allergy’ or ‘IgE’ or ‘skin prick test’ or ‘asthma symptoms’ or ‘atopic dermatitis’ or ‘eczema’ or ‘allergic rhinitis’ or ‘hay fever’ or ‘rhinitis’

AND

(i) *CD14* terms: ‘Antigen, *CD14*’ or ‘*CD14*’ AND ‘polymorphism, genetics’ or ‘polymorphism, single nucleotide’

AND

(ii) Microbial exposure terms: ‘hygiene hypothesis’ or ‘farm*’ or ‘pets’ or ‘day care’ or ‘siblings’ or ‘bacterial infections’ or ‘endotoxin’ or ‘rural population’

The following groups of keywords were used to search relevant articles in the Global Health database:

(ii) Allergic disease terms: ‘asthma’ or ‘wheeze’ or ‘allergy’ or ‘IgE’ or ‘skin prick test’ or ‘asthma symptoms’ or ‘atopic dermatitis’ or ‘eczema’ or ‘allergic rhinitis’ or ‘hay fever’ or ‘rhinitis’

AND

(iii) *CD14* terms: ‘*CD14*’ or ‘*CD14* Antigen’ AND ‘genetic polymorphism’ or ‘single nucleotide polymorphism’ or ‘SNP’ or ‘polymorphism’

AND

(iv) Microbial exposure terms: ‘hygiene hypothesis’ or ‘farm*’ or ‘pets’ or ‘day care’ or ‘siblings’ or ‘bacterial infections’ or ‘endotoxin’ or ‘rural’

Inclusion criteria

All peer-reviewed original articles that examined the interaction between microbial exposure and polymorphisms of the *CD14* gene and allergic outcomes were included. Studies

reporting asthma, asthma symptoms, wheeze, eczema, atopic dermatitis, rhinitis, skin prick test and IgE measurements as outcomes were considered. Articles were only included if they had reported all of the following: (i) an outcome, (ii) an environmental microbial exposure and (iii) a *CD14* genetic polymorphism.

Exclusion criteria

Studies were excluded if they did not report polymorphisms of the *CD14* gene or did not report a gene–environment interaction (only reported gene–outcome and/or environment–outcome associations). Studies were also excluded if they were not in humans.

Selection of articles

A flow chart of the selection of eligible articles is shown in Fig. 1. Two authors (M.L. & B.C.) independently reviewed all titles and abstracts for inclusion. Disagreement was resolved by discussion and reference to the full text of the paper. If consensus could not be reached, a third author (S.D.) made a final decision.

Data extraction

For articles that met the inclusion criteria, study characteristics, including study design, setting, source of participants and their eligibility criteria; exposure and outcome definitions, genotyping methods and methods of statistical analysis including control of confounding were assessed. All data extraction was independently verified by a second person, and any disagreement was arbitrated by the third author (S.D.). The articles were assessed using the STREGA reporting guidelines (14).

Analysis

To assess the effects of $G \times E$ interactions, the SNP variations, the estimates and their precision, and the *P*-value for

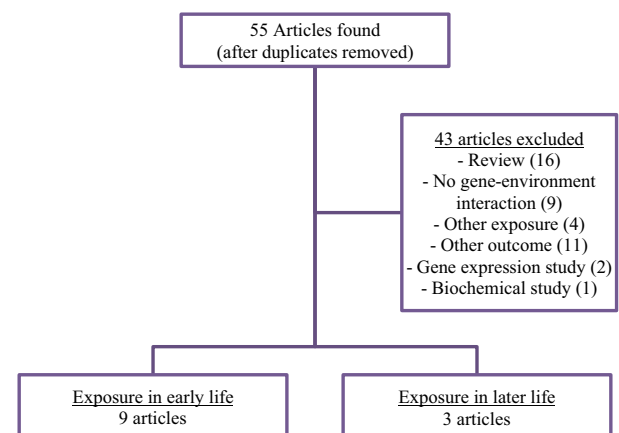


Figure 1 Summary of citations included in review.

G $\times$ E interactions were extracted and tabulated according to the type of microbial exposure and the age at which participants were exposed to environmental microbes (early life or adult life). No meta-analysis was conducted due to substantial heterogeneity among studies and the small number of studies in each category.

Results

Search results

Of the 98 articles identified, 43 were duplicates. Of the remaining 55 articles, a further 43 were excluded based on the inclusion/exclusion criteria, leaving 12 articles of which nine described microbial exposures in early life and three reported microbial exposures in adult life (Fig. 1).

Characteristics of included studies

All studies were published between 2006 and 2012. The study design, participant characteristics, measurement of exposure and outcome and genotyping methods extracted from each study are shown in Table 1. Of the 12 studies, four were prospective cohort studies (10, 11, 13, 15) and eight were cross-sectional studies (16–23), of which two were case–control studies (19, 20). Four studies were conducted in rural areas (16, 17, 20, 21) and eight in urban settings (10, 11, 13, 15, 18, 19, 22, 23). The number of participants ranged from 90 to 3,062, and participant age ranged from birth to 65 years.

Various methods were used to measure microbial exposures. These included direct measurement of endotoxin levels from dust (13, 15, 17, 21–23), parent-reported pet or barn animal exposure (11, 17), self-reported exposure to farming environment (18–21) and self-reported farm milk consumption (16). In seven studies, data on exposures were collected either by a self-completed or by a parent-completed questionnaire (11, 16–21). For the outcome, four studies used only objective measures (11, 17, 21, 22) such as serum IgE or skin prick testing, and the remaining eight used both an objective measure and a subjective measure such as self-reported asthma and eczema (10, 13, 15, 16, 18–20, 23).

Nine different SNPs of the *CD14* gene were reported. All *CD14* SNPs were in Hardy–Weinberg equilibrium. Only one study reported genotyping error rates (10) and another reported genotyping call rates (21). The remaining ten studies did not report genotyping error rates or call rates. Five studies did not adjust for any confounders (10, 13, 15, 22, 23), while six adjusted for age and/or sex (11, 16–19, 21). Some studies additionally adjusted for smoking habits or exposure to environmental tobacco smoke, presence of pets, severe respiratory infections in childhood and breastfeeding practices (Table 1).

Interaction between *CD14* polymorphisms and endotoxin exposure in children

The included studies predominantly observed protective effects of endotoxin exposure in early life among carriers of

the CC genotype of *CD14*–260 polymorphisms against atopy and eczema but not wheeze (Table 2). Three studies found a decreasing risk of atopy with increasing level of endotoxin among children with the *CD14*–260 CC genotype (13, 15, 17). Simpson and colleagues (13) reported a 30% decrease in the odds of sensitization for every log unit increase in endotoxin exposure among carriers of the *CD14*–260 CC genotype. This association was not observed among carriers of the CT or TT genotypes, and they reported modest evidence of G $\times$ E interaction ($P = 0.09$). Williams and colleagues also published similar results of decreasing levels of total IgE with increasing endotoxin levels among children with the *CD14*–260 CC genotype with modest evidence of G $\times$ E interaction ($P = 0.08$) (15). The ALEX study (17) looked at the same polymorphism and reported a decreasing risk of specific IgE with increasing tertiles of endotoxin exposure among carriers of the CC genotype. This study also found a significantly increased risk of elevated specific IgE in TT genotypes compared with the CC genotype among those with the highest tertile of endotoxin exposure (G $\times$ E interaction $P = 0.07$).

When examining eczema as the outcome, the MAAS birth cohort (13) reported a 27% decrease in the odds of eczema with every unit increase in measured endotoxin load with the *CD14*–260 CC genotype, but not the CT or TT genotypes. The G $\times$ E interaction approached statistical significance ($P = 0.05$). However, the same study found contrasting results when wheeze was examined as the outcome. Carriers of CC genotype had a higher risk of wheeze as they were exposed to higher level of endotoxin.

Interaction between *CD14* polymorphisms and endotoxin exposure in adult life

The studies of endotoxin exposure in adult life and *CD14* polymorphisms did not show a consistent pattern despite having reported some significant P -values for interaction terms (Table 2). Williams and colleagues (22) observed increasing total IgE levels with increasing endotoxin exposure among carriers of the CC genotype. However, among the TT genotype carriers, increasing endotoxin exposure was associated with a decreasing total IgE level. The P -value for the interaction between the *CD14*–260 SNP and endotoxin level suggested only a modest effect ($P = 0.063$). Conversely, the study by Zambelli-Weiner et al. (23) observed lower serum IgE levels in carriers of the *CD14*–260 TT genotype compared with carriers of the CT/CC genotype regardless of the level of endotoxin exposure.

The two studies that looked at asthma or wheeze as an outcome reported contrasting findings (21, 23). In the Barbados study (23), a protective effect against asthma of the *CD14*–260 TT genotype compared with the CC genotype was reported, but only at a low level of endotoxin. However at high levels of endotoxin exposure, the TT genotype increased the risk of asthma. In contrast, the Dutch study (21) reported a decreasing risk of wheeze among carriers of the TT genotype with increasing endotoxin level, but an increasing risk of wheeze among CC genotype carriers. The

Table 1 Characteristics of studies on CD14 polymorphisms and microbial exposure on allergic diseases

Study, design	Study design, setting	Population source, ethnicity	Clear inclusion and exclusion criteria	Ascertainment (A) of microbial exposure(s) and their definition (D)	Ascertainment (A) of outcome(s) and their definition (D)	Adequate description of genotyping methods	HWE described	Genotyping error rates and call rates	Population stratification	Appropriate statistical analysis	Adjustment for confounder(s)
Cohort study Simpson 2006 (13)	Prospective birth cohort (MAAS), urban	Population-based, European	✓	A: Endotoxin level D: ✓	A: Parental-reported wheeze and eczema, serum IgE level, SPT D: ✓	✗	Yes – in HWE	Not reported	N/A	✓	–
Williams 2008 (15)	Prospective birth cohort, urban	Population-based, White/African-American	✓	A: Endotoxin level D: ✓	A: Self-reported asthma, serum IgE level D: ✓	≈	Yes – in HWE	Not reported	Yes, by ethnicity	✓	–
Botterma 2008 (11)	Prospective birth cohort (PIAMA, KOALA and PREVASC), urban	Mix asthma-enriched and normal group, Caucasian	✓	A: Parental-reported exposure to pet D: ✗	A: serum IgE D: ✓	✓	Yes – in HWE	Not reported	All non-Dutch origin excluded	✓	Sex, family history, siblings, breastfeeding, ETS, pet exposure
Biagini-Myers 2010 (10)	Prospective birth cohort (CCAAPS), urban	High risk cohort, Caucasian	✓	A: Endotoxin level D: ✓	A: SPT, physician-diagnosed eczema D: ✓	≈	Yes – in HWE	Error rate <5%	N/A	✓	–
Cross-sectional studies Zambelli-Weiner 2005 (23)	Cross-sectional, urban	Asthma-enriched group, Afro-Caribbean	✓	A: endotoxin level D: ✓	A: ASQ, RHQ, SPT, lung function, serum IgE D: ✓	✓	Yes – in HWE	Not reported	N/A	✓	–
Eder 2005 (17)	Cross-sectional (ALEX), rural	Children from farming families, Caucasian	✓	A: Parental-reported exposure to pet/stable animals D: ✓	A: serum IgE D: ✓	✓	Yes – in HWE	Not reported	N/A	✓	Age, sex, study area

Table 1 (Continued)

Study, design	Study design, setting	Population source, ethnicity	Clear inclusion and exclusion criteria	Ascertainment (A) of microbial exposure(s) and their definition (D)	Ascertainment (A) of outcome(s) and their definition (D)	Adequate description of genotyping methods	HWE described	Genotyping error rates and call rates	Population stratification	Appropriate statistical analysis	Adjustment for confounder(s)
William 2006 (22)	Cross-sectional (WHEALS), urban	Pregnant women, White/African-American	✓	A: Endotoxin level D: ✓	A: Serum IgE level D: ≈	≈	Yes – in HWE	Not reported	Yes, by ethnicity	✓	–
Leynaert 2006 (18)	Cross-sectional (ECHRS II), urban	Population-based, French	✓	A: self-reported exposure to farm D: ≈	A: serum IgE, self-reported asthma and nasal allergies D: ≈	≈	Yes – in HWE	Not reported	N/A	✓	Age, sex, family history, smoking habits, pets in childhood, severe respiratory infections in childhood
Bieli 2007 (16)	Cross-sectional (PARSIFAL and ALEX), rural	Children from farming families, Caucasian	✓	A: parental-reported exposure to farm milk D: ✓	A: Parental-reported wheeze, parental-reported physician-diagnosed asthma, serum IgE D: ✓	✓	Yes – in HWE	Not reported	N/A	✓	Age, sex, being a farm child, study centre, ETS, maternal education, breastfeeding
Smit 2007 (20)	Case-control, rural	Farmers (cases) and ex-conscript (controls), Danish	✓	A: self-reported exposure to farm D: ✓	A: self-reported asthma, SPT D: ✓	≈	Yes – in HWE	Not reported	N/A	≈	Farm childhood, smoking habits, case-control status
Smit 2009 (19)	Case-control (EGEA), urban	High risk (asthmatics) and their family (controls), French	✓	A: Self-reported exposure to farm D: ✓	A: SPT, self-reported asthma D: ✓	✓	Yes – in HWE	Not reported	N/A	✓	Age, sex

Table 1 (Continued)

Study, design	Study design, setting	Population source, ethnicity	Clear inclusion and exclusion criteria	Ascertainment (A) of microbial exposure(s) and their definition (D)	Ascertainment (A) of outcome(s) and their definition (D)	Adequate description of methods	HWE described	Genotyping error rates and call rates	Population stratification	Appropriate statistical analysis	Adjustment for confounder(s)
Smit 2011 (21)	Cross-sectional, rural	Farmers, Dutch	✓	A: Self-reported exposure to farm, endotoxin level D: ✓	A: serum IgE, BHR D: ≈	≈	Yes – in HWE	Call rates >96%	N/A	✓	Age, sex, smoking habits, farm childhood

ASQ, Asthma Severity Questionnaire; BHR, bronchial hyperresponsiveness; ETS, environmental tobacco smoke; IgE, immunoglobulin E; N/A, not applicable; ✓, adequate; ✗, inadequate; ≈, moderate.

authors also reported a significant $G \times E$ interaction ($P = 0.006$). There were two other *CD14* polymorphisms investigated in one study (21): –1721A/G and –1247T/C. Both of these polymorphisms showed similar associations of decreasing risk of wheeze with increasing endotoxin level among the carriers of a homozygous minor allele.

CD14 polymorphisms and exposure to pets/animals in early life

Three studies examined the association between exposure to dogs/stable animals in early life and *CD14* polymorphisms and allergic outcomes (Table 3). We observed a consistent result of decreased serum IgE level in children with the TT genotype that were exposed to a dog or a cat. The ALEX study (17) reported decreasing specific IgE among carriers of the TT genotype who had been exposed to a dog or a cat compared with carriers of the CC genotype (17). Similar results were reported by Bottema and colleagues (11), who found a decrease in IgE level in children with the TT genotype who were exposed to a dog by age one year compared with those who were not exposed to a dog. Biagini-Myers et al. (10) examined the association between living with dogs and developing eczema in a birth cohort followed up at 1, 2 and 3 years of age. Among those who lived in homes with a dog, a significant decrease in the risk of eczema (up to 60% by age 3 years) was observed in children with the *CD14*/–260 CT/TT genotype compared to children with the CC genotype, as well as in children who were not exposed to a dog. The authors reported a statistically significant $G \times E$ interaction at both age 3 years ($P = 0.04$) and age 2 and 3 years combined ($P = 0.03$).

Four other *CD14* polymorphisms were explored in one study (11). Three of the *CD14* polymorphisms, –1619T/C, –1145T/C and 3'UTR C/A, showed lower IgE levels among those with homozygous minor alleles who were exposed to dogs. However, the –550C/T polymorphism showed increased level of IgE in those with TT genotype and who were exposed to dog compared with no exposure to dogs in the first year of life.

CD14 polymorphisms and farm-related exposure

Overall, studies of association between farm exposure and *CD14* polymorphisms and asthma and allergic diseases did not show any consistent effect (Table 4). Leynaert et al. (18) reported a significantly decreased risk of atopy and nasal allergies among carriers of the TT genotype of *CD14*/–260 exposed to a farming environment in childhood compared with the nonexposed carriers of the CC or CT genotypes. Smit and colleagues (20) also observed an association between the *CD14*/–260 SNP and atopy in Danish farmers born and raised on a farm. An additional T allele in this study was associated with a stronger protective effect against atopy when compared to those without farm childhood.

Smit and colleagues (19) reported a significant decrease in the risk of asthma among those who lived on a farm and were carriers of a CT or TT genotype of *CD14*/–260

Table 2 Studies examining the interactions between CD14 polymorphisms and endotoxin exposure on risk of allergic diseases

Study, year, country	Sample size, study design	Population, age, setting	SNP/rs-number,	Environmental exposure, type of scale	Outcome	G × E interaction (P-value)	Findings
Endotoxin exposure in early life							
Simpson 2006, UK (13)	N = 442, cohort	Birth cohort followed up at age 5, urban	–260 C>T (rs2569190)*	Endotoxin (living room), continuous	Sensitization = specific IgE >0.2 kU/l or SPT wheal ≥3 mm	0.09	↓ sensitization with increasing endotoxin load (OR = 0.70; 95% CI 0.55–0.89) among CC genotype
Williams 2008, USA (15)	N = 90, cohort	Birth cohort followed up at 6 months and 1 year, urban	–260 C>T (rs2569190)*	Endotoxin (bedroom floor), continuous	Total IgE level	0.08	↓ total IgE level with increasing endotoxin among CC genotype (P = 0.02)
Eder 2005, Austria and Germany (17)	n = 624, cross-sectional of ALEX	Children age 6–13, rural	–260 C>T (rs2569190)*	Endotoxin (from child's mattress), tertiles	Atopy = specific IgE ≥0.35 IU/ml	0.07	↑ risk of higher sIgE in TT genotype (OR = 7.0; 95% CI 1.36–36.02) vs CC genotype among those exposed to high endotoxin load (third tertile)
Simpson 2006, UK (13)	N = 442, cohort	Birth cohort followed up at age 5, urban	–260 C>T (rs2569190)*	Endotoxin (living room), continuous	Parental-reported recent wheeze	Not reported	↑ recent wheeze with increasing endotoxin load (OR = 1.31; 95% CI 1.00–1.73) among CC genotype
			–260 C>T (rs2569190)*	Endotoxin (living room), continuous	Parental-reported eczema	0.05	↓ eczema with increasing endotoxin load (OR = 0.73; 95% CI 0.56–0.95) among CC genotype
Endotoxin exposure in adult life							
Williams 2006, USA (22)	N = 517, cohort	Pregnant women age >21 in 2nd/3rd trimester, urban	–260 C>T (rs2569190)*	Endotoxin (bedroom floor), continuous	Total IgE level	0.063	↓ total IgE in CC genotype with increasing endotoxin level. ↑ total IgE in TT genotype with increasing endotoxin level.
Zambelli-Weiner, 2005, Barbados (23)	N = 747, cross-sectional	Asthmatics: 24.1 years, nonasthmatics: 40.5 years, urban	–260 C>T (rs2569190)*	Endotoxin (living room), binary	Serum total IgE level	Not reported	↓ total IgE regardless of level of endotoxin exposure in TT vs CC/CT genotype.
			–260 C>T (rs2569190)*	Endotoxin (living room), binary	Self-reported asthma	Not reported	↓ asthma in TT genotype (OR = 0.09; 95% CI 0.03–0.27) vs CC genotype at low endotoxin. ↑ asthma in TT genotype (OR = 11.66; 95% CI 1.03–31.7) than CC genotype at high endotoxin.

Table 2 (Continued)

Study, year, country	Sample size, study design	Population, age, setting	SNP/rs-number,	Environmental exposure, type of scale	Outcome	G × E interaction (P-value)	Findings
Smit 2011, Netherlands (21)	N = 408, cross-sectional	Farmers aged 18–65, rural	–260 C>T (rs2569190)*	Endotoxin (personal exposure), continuous	Self-reported current wheeze	0.006	↑ wheeze with increasing endotoxin (OR = 1.58; 95% CI 1.18–2.13) among CC/CT genotype. ↓ wheeze with increasing endotoxin (OR = 0.49; 95% CI 0.21–1.13) among TT genotype.
			–1721 A>G (rs2915863)	Endotoxin (personal exposure), continuous	Self-reported current wheeze	0.05	↑ wheeze with increasing endotoxin (OR = 1.50; 95% CI 1.12–2.00) among AA/AG genotype. ↓ wheeze with increasing endotoxin (OR = 0.62; 95% CI 0.22–1.75) among GG genotype.
			–1247 T>C (rs2569191)	Endotoxin (personal exposure), continuous	Self-reported current wheeze	0.007	↑ wheeze with increasing endotoxin (OR = 1.59; 95% CI 1.18–2.13) among TT/CT genotype. ↓ wheeze with increasing endotoxin (OR = 0.389; 95% CI 0.13–1.09) among CC genotype.

*CD14/–260C/T was previously known as CD14/–159C/T

Table 3 Studies examining the interactions between CD14 polymorphisms and exposure to pets/animals on risk of allergic diseases in children

Study, year, country	Sample size, study design	Population, age, setting	SNP/rs-number,	Environmental exposure, type of scale	Outcome	G × E interaction (P-value)	Findings
Eder 2005, Austria and Germany (17)	n = 624, cross-sectional of ALEX	Children age 6–13, rural	–260 C>T (rs2569190)*	Stable animal contact, binary	Atopy = specific IgE ≥0.35 IU/ml	0.018	↑ risk of higher sIgE in TT genotype (OR = 2.56; 95% CI 1.07–6.15) vs CC genotype among those exposed to stable animal contact
Biagini-Myers 2010, USA (10)	N = 762, birth cohort	Birth cohort recruited at 6–7 months, followed up at ages 1, 2 and 3 years, urban	–260 C>T (rs2569190)*	Living with dog(s), binary	Physician-diagnosed eczema	0.04 (age 3) 0.03 (age 2 and 3)	↓ eczema in CT/TT genotype by age 3 (OR = 0.56; 95% CI 0.33–0.96) and at both ages 2 and 3 (OR = 0.36; 95% CI 0.14–0.89) vs CC genotype and/or no dogs.
Eder 2005, Austria and Germany (17)	n = 624, cross-sectional of ALEX	Children age 6–13, rural	–260 C>T (rs2569190)*	Dogs and/or cats, binary	Atopy = specific IgE ≥0.35 IU/ml	0.018	↓ risk of higher sIgE in TT genotype (OR = 2.56; 95% CI 1.07–6.15) vs CC genotype among those exposed to dogs and/or cats contact
Bottema 2008, Netherlands (11)	N = 3,062, cohort (PIAMA, KOALA and PRESAVC)	Children aged 1–8 years, urban	–260 C>T (rs2569190)*	Dog exposure at first year, binary	Total and specific IgE level	P = 0.01 at age 4; P = 0.16 at age 8	↓ IgE level in TT genotype among those exposed compare to nonexposed
			–1619 T>C (rs2915863)	Dog exposure at first year, binary	Total and specific IgE level	0.03 at age 4 and 0.01 at age 8	↓ IgE level in CC genotype among those exposed compare to nonexposed
			3'UTR C>A (rs2563298)	Dog exposure at first year, binary	Total and specific IgE level	P = 0.05 at age 4; P = 0.80 at age 8	↓ IgE level in AA genotype among those exposed compare to nonexposed
			–1145 T>C (rs2569191)	Dog exposure at first year, binary	Total and specific IgE level	P = 0.005 at age 4; P = 0.10 at age 8	↓ IgE level in CC genotype among those exposed compare to nonexposed
			–550 C>T (rs57444455)†	Dog exposure at first year, binary	Total and specific IgE level	P = 0.00001 at age 4 and P = 0.03 at age 8	↑ IgE level in TT genotype among those exposed compare to non-exposed

*CD14/–260C/T was previously known as CD14/–159C/T

†CD14/–651C/T is also reported as CD14/–550C/T

Table 4 Studies examining the interaction between CD14 polymorphisms and farm-related exposure on risk of asthma and allergic diseases

Study, year, country	Sample size, study design	Population, age, setting	SNP/rs-number,	Environmental exposure, type of scale	Outcome	G × E interaction (P-value)	Findings
Allergic outcome in childhood							
Bieli 2007, Europe (16)	N = 533, ALEX study, cross-sectional	children age 7–13, rural	–1721 A>G (rs2915863)	Farm milk consumption in 1st year, binary	Asthma*	0.331	↓ asthma in farm milk consumption vs no farm milk among AA genotype (OR = 0.29; 95% CI 0.07–1.14) and GG genotype (OR = 0.75; 95% CI 0.18–3.1).
Bieli 2007, Europe (16)	N = 1,387, PARSIFAL, cross-sectional	children age 5–13, rural	–1721 A>G (rs2915863)	Farm milk consumption in 1st year, binary	Asthma*	0.004	↓ asthma in farm milk consumption (OR = 0.11; 95% CI 0.03–0.49) vs no farm milk consumption among AA genotype. ↑ asthma in farm milk consumption (OR = 1.14; 95% CI 0.46–2.86) vs no farm milk among GG genotype.
Allergic outcomes in adult life							
Leynaert 2006, France (18)	n = 600, cross-sectional	Adults, mean age 44.0 (SD 7.2), urban	–260 C>T (rs2569190)†	Self-reported having lived on farm in childhood (age ≤1 year), binary	Atopy = specific IgE ≥0.35 kIU/l;	0.381	↓ atopy in farm + TT genotype (OR = 0.21; 95% CI 0.05–0.93) vs nonfarm + CC/CT genotype
			–260 C>T (rs2569190)†	Self-reported having lived on farm in childhood (age ≤1 year), binary	Total IgE >100 IU/ml	0.613	↓ risk of increased total IgE in farm + TT genotype (OR = 0.42; 95% CI 0.12–1.51) vs nonfarm + CC/CT genotype
			–260 C>T (rs2569190)†	Self-reported having lived on farm in childhood (age ≤1 year), binary	Self-reported asthma	0.946	↑ asthma in farm + TT genotype (OR = 1.10; 95% CI 0.30–4.00) vs nonfarm + CC/CT genotype
			–260 C>T (rs2569190)†	Self-reported having lived on farm in childhood (age ≤1 year), binary	Nasal allergy = all symptoms of rhinitis	0.527	↓ nasal allergy in farm + TT genotype (OR = 0.26; 95% CI 0.07–0.94) vs nonfarm + CC/CT genotype
Smit 2007, Netherlands (20)	N = 100 cases, n = 88 controls, case-control	Farmers aged 19 years, rural	–260 C>T (rs2569190)†	Farm childhood (born and raised on a farm), binary	Atopy = SPT wheal ≥3 mm	0.313	Additive genetic T model OR = 0.21 (95% CI 0.06–0.73) in those with farm childhood compared with nonfarm childhood (additive T model OR = 0.45; 95% CI 0.20–1.03)
			–651 C>T (rs7444455)‡	Farm childhood (born and raised on a farm), binary	Atopy = SPT wheal ≥3 mm	0.232	Additive genetic T model OR = 7.10 (95% CI 1.55–32.59) in those with farm childhood compared with nonfarm childhood (additive T model OR = 2.43; 95% CI 1.01–5.83)

Table 4 (Continued)

Study, year, country	Sample size, study design	Population, age, setting	SNP/rs-number,	Environmental exposure, type of scale	Outcome	G × E interaction (P-value)	Findings
Smit 2009, France (19)	N = 825, case–control of EGEA	Mean age 47.1 (cases); 46.0 (controls), urban	–260 C>T (rs2569190) [†] +1341 C>A (rs2563298) +7444 C>T (rs778584) +7546C>T (rs778583)	Lived in country in childhood (age ≤16 years), binary Lived in country in childhood (age ≤16 years), binary Lived in country in childhood (age ≤16 years), binary Lived in country in childhood (age ≤16 years), binary	Self-reported asthma Self-reported asthma Self-reported asthma Self-reported asthma	0.95 0.029 0.72 0.055	↓ asthma in farm living (OR = 0.66; 95% CI 0.45–0.97) vs nonfarm living among TT/CT genotype ↓ asthma in farm living (OR = 0.44; 95% CI 0.26–0.73) vs nonfarm living among AC/AA genotype ↓ asthma in farm living (OR = 0.64; 95% CI 0.44–0.94) vs nonfarm living among CC/CT genotype ↓ asthma in farm living (OR = 0.47; 95% CI 0.28–0.76) vs nonfarm living among TT/CT genotype

*Physician-diagnosed asthma or recurrent asthmatic, spastic or obstructive bronchitis.

[†]CD14/–260C/T was previously known as CD14/–159C/T[‡]CD14/–651C/T is also reported as CD14/–550C/T

compared with those who had not lived on a farm in childhood, although the $G \times E$ interaction was not statistically significant ($P = 0.95$). The same study found similar results when other *CD14* polymorphisms were examined. Conversely, Leynaert and colleagues (17) found an increased risk of asthma among carriers of the TT genotype who had lived on a farm in childhood, although the effect was not statistically significant. Three other SNPs, +1341C/A, +7444C/T and +7546C/T, were reported in one study (19). For all three SNPs, there were significantly decreased risks of self-reported asthma among carriers of one or two minor alleles who had lived on a farm in childhood compared with those who had not lived on a farm.

Discussion

Interaction between CD14 polymorphisms and endotoxin

The main finding from our review was the apparent modification of the effect of early life endotoxin exposure on risk of atopy in childhood by the *CD14*/–260 polymorphism, with the CC genotype conferring protection in the setting of high endotoxin exposure. However, we did not observe a consistent pattern of association between *CD14* polymorphisms and endotoxin exposure on the risk of atopy in adults. The relationship with other allergic diseases including adult wheeze, adult asthma, childhood wheeze and childhood eczema could also not be established.

This suggests the possibility of different mechanisms of action for this biological interaction in children compared with adults. It is also known that the environmental and genetic risk factors associated with allergic diseases are different between children and adults (24–26). For example, the 17q21 locus was initially found to be associated with asthma from a genome-wide association study (GWAS) (27). When the same study was restricted to early-onset asthma, the risk was increased and was further augmented by exposure to environmental tobacco smoke (28). Kauffmann and Demenais (29) commented that both timing of the onset of disease and the timing of an exposure had a major role in the pathophysiology of asthma. The 2-dimensional view of $G \times E$ interactions could then be extended to a more complex 3-dimensional interaction (gene–environment–age at exposure). Furthermore, the age of outcome assessment would need to be considered when interpreting the findings. The current review stratified the included studies by timing of the microbial exposure (early vs later life), and we observed a difference in the risk of allergic diseases between children and adults (Fig. 2). Future studies could consider a three-way interaction such as gene–environment–age at exposure.

Interaction between CD14 polymorphisms and proxies of microbial exposures

Our results also showed a consistent finding of lower IgE levels among children with the minor homozygous allele of the *CD14*/–260 polymorphisms who had contact with a dog or a cat but not in children with CC or CT genotype. This finding

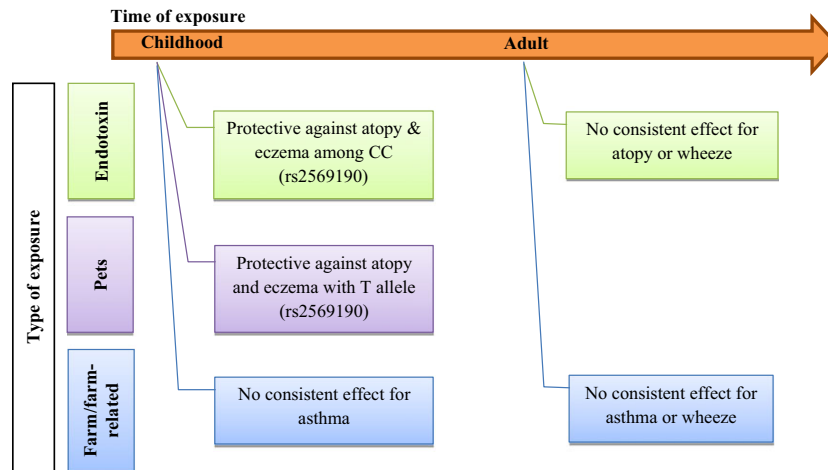


Figure 2 Effects of interaction between *CD14* polymorphisms and different types of environmental microbial exposures on allergic outcomes at different stages of life.

may explain why past studies looking at the effects of exposure to pets, particularly dogs and cats, on asthma and allergic diseases have had inconsistent results (30).

This result, however, is in contrast with the findings on the effects of endotoxin exposure. One reason may be that the activation of the inflammatory cascade within the cell depends on the binding of LPS to *CD14/TLR4* and it is influenced by several factors such as the type of LPS, the presence of LPS-binding proteins and the type of surfactant proteins in the lung (31). This may result in either a beneficial effect to the host by releasing adequate inflammatory response to eradicate the microbes or a detrimental by producing excessive inflammation resulting in severe allergies (31). Pets also interact with environmental micro-organisms and may transport these micro-organisms into dwellings when allowed indoors, particularly into bedrooms, thereby exposing their owners to larger but less specific exposures.

In contrast, the interaction between *CD14* polymorphisms and farm-related exposure was inconsistent. The types of microbes found in the farming environment may be different depending on the type of farming activity, that is, livestock farm and agricultural farm. It has been suggested that it is the diversity of exposure rather than the abundance of a particular species that may be important in conferring protection against allergic diseases (32). A recent European study found that children living on a farm were exposed to a higher number of bacterial or fungi groups and had a lower prevalence of atopy and asthma when compared to children living in rural area (33). More importantly, the study showed an inverse probability of asthma with an increasing number of bacterial or fungi group, supporting the evidence for an association between greater diversity of microbial exposure and a lower risk of asthma. Individuals growing up in a farm environment may additionally be exposed to other outdoor allergen such as air pollutants, pesticides and fungi that may have confounded the effect of a farming environment on asthma. Furthermore, almost half of the included studies used

self-reported proxy measures of microbial exposure, which may be less specific than objective measures such as direct measure of endotoxin exposure.

Adjustment for confounders

One reason for the heterogeneity of the results seen in this review is the inadequate control of potential confounders. For a factor to confound a $G \times E$ interaction, the variable has to be (i) associated with both the level of microbial exposure and (ii) the outcome, and (iii) the confounding effect of this factor needs to be greater in one polymorphism than another. The complexity in $G \times E$ interactions has made it difficult to determine whether any given variable is likely to be a potential confounder in a $G \times E$ interaction. Potential confounders should be examined in close detail as inappropriate adjustment may lead to imprecision in the risk estimates. In the current review, family history could be considered an ‘*a priori*’ confounder, as it could conceivably meet the above criteria, but this would need to be examined within each individual study.

Biological plausibility

CD14 is present in two forms: (i) on the surface membrane of monocytes/macrophages and to a lesser extent neutrophils (*mCD14*) and (ii) in the soluble form (*sCD14*) (34). The *sCD14* form is either secreted directly from intracellular vesicles or produced through shedding of the *mCD14* from the cell surface (1). *CD14* interacts with LPS and when activated forms a complex with *TLR4* and its cofactor, MD-2, on the surface of mast cells. This activated complex then triggers the transcription of pro-inflammatory cytokine genes such as $TNF-\alpha$, IL-1 and IL-6 through various pathways. The TT genotype of *CD14*/–260 has been associated with an increased level of *sCD14* compared with the CC genotype (35). It has been postulated that higher expression of *CD14* will stimulate Th1 responses resulting in a lower prevalence of allergies. This association of higher expression of *sCD14* among carriers of

the TT genotype has been demonstrated in children (13, 35, 36) but could not be established in adults (23). More studies are needed to evaluate the relationship between *CD14* expression and *CD14* genotypes in children and adults separately.

Strengths and limitations

Our review systematically evaluated the available evidence to date and provided moderate to strong evidence of interaction between *CD14* gene polymorphisms and microbial exposure in terms of allergic disease risk. The eligibility of the included articles was independently assessed by two reviewers, minimizing the risk of discarding or including articles incorrectly. We have included studies that reported various allergic outcome measures to strengthen our review.

There are several limitations to the current review. First, a meta-analysis of the results could not be performed due to heterogeneity of the microbial exposure, *CD14* polymorphisms and allergic outcomes in the included studies. Thus, we were unable to present a precise estimate of the effects. Second, the methodological differences between studies limited our ability to compare across studies. Third, most studies included participants of European ancestry, and only one study was conducted in an Afro-Caribbean population, thereby limiting the generalizability of the review. As with all reviews, the results from this study may have been influenced by publication bias. The *P*-values for interactions were presented in the tables. However, it is important to note that sample size, minor allele frequencies and the prevalence of environmental exposure may influence the statistical power to detect an interaction; therefore, ‘nonsignificant’ *P*-values of interaction should be interpreted with caution and should not necessarily be interpreted as evidence against a possible gene–environment interaction.

In conclusion, we found evidence of an interaction between *CD14* polymorphisms and microbial exposure on atopy, and this interaction was most apparent in children. We could not

observe a clear pattern of $G \times E$ interaction for asthma and eczema, possibly due to the small number of articles available. We also found some evidence that different types of $G \times E$ interaction depended on time of microbial exposure (early life vs later life), suggesting a different mechanism of biological interaction in children and in adults. We observed a relationship of decreased IgE level in those with a homozygous minor allele who were exposed to domestic pets, in particular dog and cat, suggesting that pet exposure may confer protection against atopy only in carriers of a homozygous minor allele. We recommend that future studies on endotoxin and *CD14* polymorphisms consider the 3-way interactions between gene–environment–age at exposure.

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Author contributions

ML was involved in identification of articles, extraction and interpretation of data, and drafting the manuscript. BC assisted in validating the search strategy. SD, MM, JB, CL and AL were involved in developing and refining the methodology. JB, SD and MM provided critical review and interpretation of data. All authors have reviewed and provided input to the manuscript drafts and approved the final version of the manuscript as submitted.

Conflicts of interest

All authors declare that they do not have any conflicts of interest.

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