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Interleukin 13, *CD14*, pet and tobacco smoke influence atopy in three Dutch cohorts: the allergenic study

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ABSTRACT: Studying gene–environment interactions may elucidate the complex origins of atopic diseases but requires large study populations. Pooling data from several cohort studies may help but may also obscure findings. Gene–environment interactions in atopy development were studied and the benefits of pooling data were evaluated.

Haplotype-tagging polymorphisms in the genes *interleukin (IL)13* and *CD14* were genotyped in 3,062 children from the following birth cohorts: the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study; the Prevention of Asthma in Children (PREVASC) study; and the Child, Parent, Health, Focus on Lifestyle and Predisposition (KOALA) study, and tested for association with total and specific immunoglobulin (Ig)E and interaction with tobacco smoke and pet exposure at ages 1, 2, 4 and 8 yrs by analysis of variance, Chi-squared tests and regression analyses.

At all ages, in *IL13*, minor alleles of rs1295685 and rs20541 were significantly associated with elevated IgE levels in pooled analyses. In *CD14*, the rs2569190-TT and rs2569191-CC genotypes associated with lower IgE and decreased risk of sensitisation at 4 and 8 yrs in children exposed to pets, with an opposite effect in nonexposed children. Findings for *IL13* and *CD14* were comparable in separate cohorts.

The present study indicates that atopy is importantly influenced by *interleukin 13* at age 1–8 yrs and by *CD14* in interaction with pet exposure at ages 4 and 8 yrs. Additionally, pooled data improved effect estimates and genetic effects could be detected in interaction with important environmental factors.

KEYWORDS: Atopy, *CD14*, environmental tobacco smoke, *interleukin 13*, pets

Atopy in childhood is a major risk factor for the development of persistent asthma [1]. Atopy and asthma are complex genetic diseases, *i.e.* they result from interplay between gene variations and environmental influences. Although multiple genes and various environmental factors have been identified as contributors in the development of atopy and asthma, their interactions have been scarcely investigated, so far. This is partially due to the fact that large cohorts are required to study gene–environment interactions [2].

In order to obtain sample sizes large enough to adequately study gene–environment interactions, pooling data from existing birth cohorts could be of crucial importance in the near future. However, cohorts that have been recruited independently from each other may be different in outcome prevalence, allele frequencies, ethnicity, sex, parental atopy and environmental factors. Pooling of data may, therefore, introduce bias and cause spurious results. Moreover, it may provide false-negative findings because signals from genes may be obscured due to heterogeneity of the data [3].

In the present study, the possibility of pooling data from different birth cohorts, to prospectively study gene–environment interactions in atopy, was investigated. Two candidate genes, *CD14* and *interleukin* (*IL*)*13*, were investigated in the present study, which consists of three well characterised Dutch birth cohorts. CD14 is a membrane receptor involved in the binding of bacterial components

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TABLE 1 Issues in pooling of data from separate cohort studies and proposed solutions

Issues in pooling data	Approach
Recruitment strategies	Stratification of analyses per cohort
Comparability of phenotype measurements	Selection of standardised phenotypes
Comparability of environmental exposures	Stratification of analyses per cohort and correction for confounding exposures in regression analysis
Different ethnicities	Evaluation of genetic effect separately in each ethnic group (in this study: exclusion of non-Dutch participants)
Allele frequencies	Checking of spurious effects caused by differences in allele frequencies between studies

Criteria are deduced from [25] and modified to apply to pooling data from cohort studies.

and capable of influencing the post-natal T-helper cell (Th) type 1–Th2 shift. Polymorphisms in *CD14* have been associated with atopy in several populations [4–6]. Interestingly, the association of a promoter polymorphism -159C/T (also known as -260C/T) has been found to be modified by exposure to pets [7, 8] and to environmental tobacco smoke (ETS) [9].

IL13 is a cytokine typically produced during Th2 responses and plays a crucial role in atopy and asthma [10]. Genetic variations in *IL13* have been associated with asthma and related phenotypes in numerous studies and in ethnically diverse populations living in variable environmental circumstances [11–14].

In the present study, it was investigated whether or not associations of *CD14* and *IL13* haplotype-tagging single nucleotide polymorphisms (SNPs) with serum total and specific immunoglobulin (Ig)E at ages 1, 2, 4, and 8 yrs could be identified in data both pooled from three birth cohorts and analysed separately. Additionally, knowledge of *CD14* gene–environment interactions is improved by investigating its interactions with ETS and pets, and dog and cat exposure separately in atopy development.

METHODS

Study populations

The present study includes three prospective Dutch birth cohorts of similar design: Prevention and Incidence of Asthma and Mite Allergy (PIAMA) [15]; Prevention of Asthma in Children (PREVASC) [16, 17]; and Child, Parent, Health, Focus on Lifestyle and Predisposition (KOALA) [18]. The three cohorts recruited children during pregnancy. The PIAMA study includes a natural history section and an intervention section, *i.e.* a double-blind placebo-controlled study on the primary preventive effect of the use of mattress covers. The PREVASC study addresses the primary prevention of asthma by implementing a multifaceted prenatally started intervention strategy in high-risk infants and includes a separate group of low-risk children followed without intervention for the natural history of asthma and atopy. The KOALA study includes children recruited among pregnant females, who were invited for a prospective cohort study on pregnancy-related pelvic-girdle pain, and a group of children recruited among pregnant females with alternative lifestyles, through organic food shops, anthropologic doctors and midwives, Steiner schools and magazines. The supplementary material summarises a description of the cohorts. Genetic studies were approved by local medical-ethics committees of participating institutes and all parents provided informed written consent.

Questionnaires

Parents completed annually distributed questionnaires, derived from the International Study of Asthma and Allergies in Childhood (ISAAC) [19] about allergic symptoms in the child. Also, information about general health, indoor environment, like ETS and pet exposure, socioeconomic characteristics, lifestyle, breast or bottle feeding and demographic factors was obtained by these questionnaires.

IgE measurements

Total and specific IgE levels were determined in capillary or venous blood collected at ages 1, 4, and 8 yrs in PIAMA, at ages 1, 2, and 4 yrs in PREVASC and at ages 1 and 2 yrs in KOALA (Sanquin Research, Amsterdam, the Netherlands). Total IgE levels were measured by radioimmunoassay as described previously [20–22] and expressed as $IU \cdot mL^{-1}$ (1 IU equals 2.4 ng of IgE). Specific IgE levels to mite (Der p1), cat (Fel d1), dog (Can f1), egg and milk were measured by radioallergosorbent testing. Sensitisation was defined as specific IgE concentration $\geqslant 0.35$ $IU \cdot mL^{-1}$ against food allergens (milk or egg) at 1 and 2 yrs and indoor allergens (house dust mite, cat and dog) at 4 and 8 yrs.

Genotyping

Haplotype-tagging SNPs were selected from publicly available databases of the International HapMap Project [23] or the Innate Immunity Program for Genetic Application [24], depending on the largest number of SNPs with a minor allele frequency $\geqslant 0.1$ available in each database. Additionally, the biomedical literature published up to October 2005 was screened for SNPs within the candidate genes known to have functional impact or to be associated with asthma or atopy. Information on DNA collection and genotyping are specified in the supplementary material.

Statistical methods

Association of the *CD14* and *IL13* genotypes and environmental-pet and -ETS exposure was assessed by two atopic phenotypes (logarithmically transformed total-serum IgE and sensitisation by analysis of variance and Chi-squared tests, respectively). Odds ratios (ORs) and 95% confidence intervals (CI) were calculated by logistic regression.

Gene–environment interactions of *CD14* SNPs with serum IgE, and sensitisation were tested by including an interaction term of genotype and ETS or pet exposure into linear and logistic regression models. Gene–environment analyses were

TABLE 2 Participant characteristics, for each birth cohort separately

Characteristics	PIAMA	PREVASC	KOALA	p-value
Participants in genetic study n	1037	374	1651	
DNA available	25.0	49.8	58.1	
Dutch origin#	95.1	95.7	95.2	
Male	51.2	49.2	50.6	0.80
Maternal education level#				<0.001
Low	19.0	8.1	8.6	
Intermediate	42.7	50.9	37.2	
High	38.3	40.9	54.3	
Family history#				
Atopy mother	66.5	51.4	33.3	<0.001
Asthma mother	16.7	31.4	8.8	<0.001
Atopy father	31.6	47.7	36.4	<0.001
Asthma father	7.4	21.9	10.0	<0.001
Intervention type	Mattress covers	Multifaceted	No	
No	57.3	68.3	100	
Placebo	23.9	0	0	
Active	18.8	31.7	0	
Environmental exposures#				
Breast feeding				<0.001
Never	14.4	22.7	14.5	
<3 months	35.1	23.3	20.2	
⩾3 months	50.5	54.0	65.3	
ETS at home first year	23.2	21.5	11.5	<0.001
Pet (dog and/or cat) first year	39.1	33.3	39.8	0.072
Dog first year	14.2	22.9	19.4	<0.001
Cat first year	28.7	12.9	24.5	<0.001
Older siblings at birth	48.5	59.9	58.1	<0.001
Total serum IgE IU·mL^{-1}				
1 yr	7.1 (2.0–17.0) 369	8.6 (3.5–19.4) 226	6.0 (2.6–12.5) 699	0.002
2 yrs	NA	11.7(4.2–28.7) 358	12.0 (3.7–38.0) 704	0.80
4 yrs	36.1 (12.0–101.0) 714	18.6 (8.8–49.0) 207	NA	<0.001
8 yrs	64.9 (23.0–240.0) 748	NA	NA	
Sensitisation to food allergens¶				
Egg				
1 yr	6.5 (355)	7.8 (167)	4.7 (674)	0.24
2 yrs	NA	8.2 (208)	4.7 (698)	0.056
Milk				
1 yr	27.2 (355)	NA	8.0 (690)	<0.001
2 yrs	NA	NA	14.5 (697)	
Sensitisation to indoor allergens+				
4 yrs	16.8 (709)	18.4 (206)	NA	0.58
8 yrs	26.8 (746)	NA	NA	

Data are presented %, geometric mean (interquartile range) n or % (n), unless otherwise stated. PIAMA: Prevention and Incidence of Asthma and Mite Allergy [15]; PREVASC: Prevention of Asthma in Children [16, 17]; KOALA: Child, Parent, Health, Focus on Lifestyle and Predisposition [18]; ETS: environmental tobacco smoke; Ig: immunoglobulin; NA: not available. #: definitions are presented in the online supplementary table E1; ¶: specific IgE ⩾0.35 IU·mL^{-1} to egg and/or milk allergens; +: specific IgE ⩾0.35 IU·mL^{-1} to house dust mite, cat and/or dog allergens.

adjusted for atopy of the mother, atopy of the father, sex, siblings, breast feeding, ETS and pet exposure. Pet exposure was defined as pet exposure at home in the first year of life. Categories were defined as exposure to either cat (excluding dog exposure), dog (excluding cat exposure) or both (including all children exposed to either cat or dog or both) and these categories were compared to children that were neither exposed to cat nor dog.

The approach to pooling data from cohort studies is described in table 1. Whether genetic and gene–environment effects were different between the cohorts was assessed by including an

TABLE 3 Single nucleotide polymorphisms selected for *CD14* and *IL13*, source of information and minor allele frequencies in the pooled and separate cohorts

Gene	Rs number	Alleles[#]	Genotype	Source	Pooled	PIAMA	PREVASC	KOALA
CD14	rs2563298	C/A	3′UTR	I.I.	0.27	0.28	0.29	0.27
CD14	rs2569190	C/T	-159C/T	I.I., lit	0.48	0.47	0.46	0.49
CD14	rs2569191	T/C	-1145T/C	I.I., lit	0.48	0.47	0.47	0.49
CD14	rs2915863	T/C	-1619T/C	I.I., lit	0.41	0.40	0.38	0.42
CD14	rs5744455	C/T	-550C/T	I.I., lit	0.23	0.23	0.24	0.23
IL13	rs1295685	C/T	3′UTR	lit, HM	0.20	0.20	0.20	0.20
IL13	rs20541	G/A	Arg130Gln	lit, HM	0.20	0.20	0.21	0.20
IL13	rs1881457	A/C	-1512A/C	lit	0.20	0.20	0.17	0.21
IL13	rs1800925	C/T	-1111C/T	lit	0.20	0.20	0.18	0.20

Allele frequencies were similar between the cohorts (Chi-squared test; $p>0.05$) and genotypes were in Hardy–Weinberg equilibrium ($p\geqslant0.01$). PIAMA: Prevention and Incidence of Asthma and Mite Allergy [15]; PREVASC: Prevention of Asthma in Children [16, 17]; KOALA: Child, Parent, Health, Focus on Lifestyle and Predisposition [18]; IL: interleukin; UTR: untranslated region. I.I.: innate immunity website [26]; lit: literature; HM: HapMap database [27]. [#]: major alleles first, followed by minor alleles.

interaction term with each of the cohorts into linear and logistic regression models. A p-value <0.05 was considered statistically significant.

RESULTS

Study population and genotyping

Characteristics and environmental exposures of 3,062 children participating in the genetic study are presented in table 2. Children who were not of Dutch origin (5.7%) were excluded from further analyses because they are likely to be non-Caucasian and inclusion may result in spurious genetic effects due to population stratification. The SNPs selected for *CD14* and *IL13*, data source and allele frequencies are presented in table 3.

Environmental exposures

Dog exposure was not significantly associated with serum IgE levels or sensitisation, neither in the pooled data nor in the separate cohorts. Cat exposure was associated with higher IgE levels at age 2 yrs in the pooled data, showing a trend in KOALA and PREVASC studies separately (p-values of 0.013, 0.060 and 0.087, respectively) and with decreased risk of sensitisation to indoor allergens at age 8 yrs (OR 0.5, 95% CI 0.4–0.8). ETS exposure was associated with lower serum IgE levels within the PIAMA cohort at ages 1 and 8 yrs ($p=0.01$ and $p=0.05$, respectively), but not in the pooled data. ETS exposure was not associated with sensitisation, neither in pooled data nor in separate cohorts.

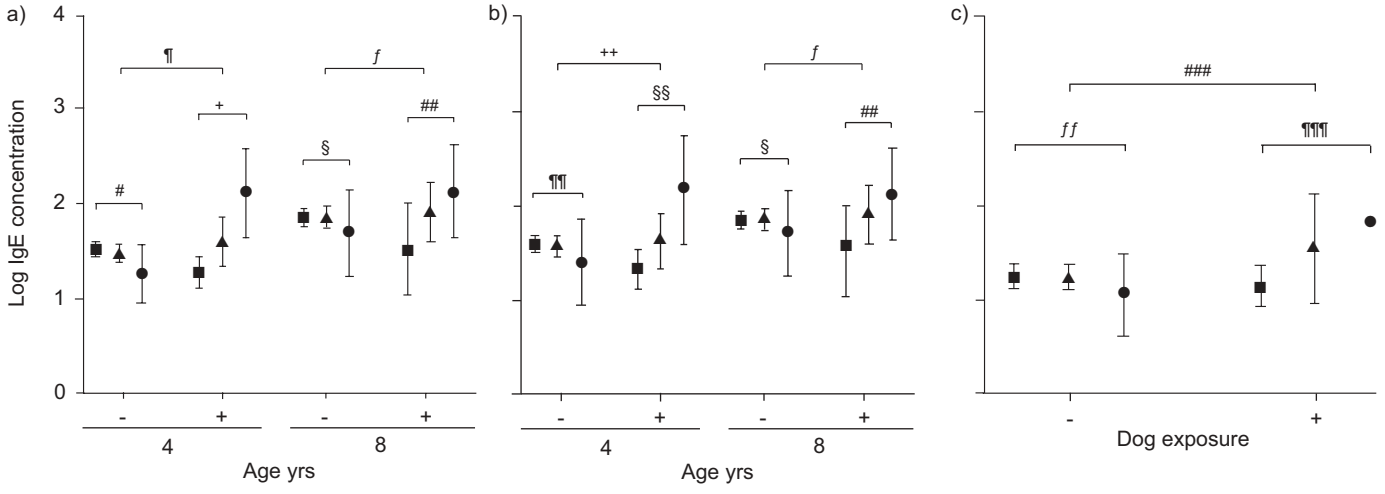

FIGURE 1. Interaction of *CD14* -550C/T genotypes in the presence (+) and absence (-) of dog exposure at home in the first year of life on total serum immunoglobulin (Ig)E levels (mean and 95% confidence intervals) at age 4 and 8 yrs in: a) pooled data; b) the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study [15]; and c) the Prevention of Asthma in Children (PREVASC) [16, 17] studies. IgE measurements were not available at age 4 yrs in the Child, Parent, Health, Focus on Lifestyle and Predisposition (KOALA) study [18] or at age 8 yrs in PREVASC and KOALA. ■: CC genotype; ▲: CT; ●: TT. [#]: $p=0.12$; [¶]: $p=0.000001$; [+]: $p=0.003$; [§]: $p=0.62$; [ƒ]: $p=0.03$; [##]: $p=0.3$; [¶¶]: $p=0.06$; [++]: $p=0.001$; [§§]: $p=0.015$; [ƒƒ]: $p=0.44$; [###]: $p=0.004$; [¶¶¶]: $p=0.2$.

TABLE 4 Influence of dog and cat exposure in interaction with *CD14* genotypes on total serum immunoglobulin (Ig)E levels at age 4 and 8 yrs

SNP	Genotype	No exposure[#]			Dog exposure[¶]			Cat exposure[+]			p-value interaction[§]	
		IgE[f]	n	p-value	IgE[f]	n	p-value	IgE[f]	n	p-value	Dog *versus* no exposure	Cat *versus* no exposure
Age 4 yrs												
3′UTR	CC+CA	30	481	0.33	29	104	0.11	29	177	0.07	0.05[##]	0.53
	AA	39	44		11	7		67	13			
-159C/T	CC+CT	29	431	0.05[##]	31	82	0.14	30	154	0.31	0.01[##]	0.59
	TT	40	99		18	28		41	36			
-1145T/C	TT+CT	28	429	0.05[##]	31	81	0.08	30	154	0.42	0.005[##]	0.46
	CC	40	99		17	30		38	38			
-1619T/C	TT+CT	29	422	0.13	30	83	0.05[##]	31	149	0.67	0.03[##]	0.54
	CC	40	69		14	18		36	27			
-550C/T	CC	33	279	0.12	19	64	0.003[##]	39	109	0.01[##]	0.00001[##]	0.19
	CT	30	202		39	37		23	74			
	TT	18	31		129	6		9	6			
Age 8 yrs												
3′UTR	CC+CA	73	402	0.35	54	64	0.42	59	155	0.37	0.80	0.38
	AA	55	34		20	2		86	17			
-159C/T	CC+CT	65	341	0.03[##]	55	52	0.68	59	141	0.24	0.16	0.63
	TT	99	92		44	14		85	32			
-1145T/C	TT+CT	65	344	0.05[##]	55	51	0.44	57	142	0.14	0.10	0.91
	CC	95	95		38	16		92	31			
-1619T/C	TT+CT	70	347	0.36	66	55	0.02[##]	57	138	0.17	0.01[##]	0.72
	CC	87	62		17	7		92	26			
-550C/T	CC	71	248	0.62	31	36	0.30	70	100	0.21	0.03[##]	0.27
	CT	72	168		82	24		45	67			
	TT	49	19		135	5		46	6			

In total, 110 children were exposed to both cat and dog and in these children, similar results were observed. SNP: single nucleotide polymorphism; UTR: untranslated region. [#]: neither cat nor dog exposure; [¶]: exposure to dog, with no cat exposure, at home in the first year of life; [+]: exposure to cat, with no dog exposure; [§]: from linear regression analyses adjusted for sex, maternal atopy, paternal atopy, siblings, breast feeding and environmental tobacco smoke exposure; [f]: geometric mean IgE values; [##]: statistically significant.

CD14

Total serum IgE and sensitisation

CD14 SNPs were not significantly associated with total serum IgE levels or sensitisation at any age when not taking environmental exposures into account, neither in the pooled data nor in the three cohorts separately.

Interaction with pet exposure

Dog/cat exposure

The *CD14* SNPs rs2569190, rs2569191, rs2915863, rs5744455 and rs2563298 (also known as -159C/T, -1145C/T, -1619C/T, -550C/T and 3′ untranslated region (UTR)) showed consistent and significant interaction with dog exposure, with respect to serum IgE levels at age 4 and 8 yrs (p-value for interaction at 4 yrs was 0.01, 0.005, 0.03, 0.00001 and 0.05, and at 8 yrs was 0.16, 0.10, 0.01, 0.03 and 0.80 for -159C/T, -1145C/T, -1619C/T, -550C/T and 3′UTR, respectively; fig. 1a and table 4). Similar direction of interaction was observed in the cohorts separately at 4 yrs in PIAMA (p=0.02, 0.007, 0.006, 0.006 and 0.09, respectively) and in PREVASC (p=0.33, 0.30, 0.97, 0.004 and 0.32, respectively). The comparison between genotype groups was not significant in PREVASC (fig. 1b and c). Additionally, a significant interaction with dog exposure was found for *CD14* -550C/T with respect to sensitisation to indoor allergens at 4 yrs (p=0.007, data not shown). Evaluation of the influence of cat exposure showed no statistically significant interaction of any *CD14* SNP with regard to serum IgE (table 4) or sensitisation (data not shown).

Dog and cat exposure combined

In *CD14*, the genotypes -159C/T and -1145T/C showed significant interactions with combined pet exposure with regard to serum IgE at age 4 yrs (p=0.04 and 0.01, respectively) and a trend for interaction at age 8 yrs (p=0.17 and 0.18, respectively; online supplement table E3). The interactions were not statistically significant in the separate cohorts at age 4 yrs, but were in the same direction in PIAMA (p=0.10 and 0.06, respectively) and PREVASC (p=0.21 and 0.12, respectively). A significant interaction with pet exposure was found for *CD14* -550C/T with respect to sensitisation to indoor allergens at age 4 yrs (p=0.02, data not shown).

The interaction effects of *CD14* with dog and pet exposure were in the same direction, *i.e.* homozygous genotypes for the

TABLE 5 Influence of dog and cat exposure in interaction with *CD14* genotypes on sensitisation to any allergen at age 8 yrs

SNP	Genotype	Sensitisation to any allergen[#]			p-value interaction[¶]	
		No pet exposure[+]	Dog exposure[§]	Cat exposure[f]	Dog *versus* no exposure	Cat *versus* no exposure
3′UTR	CC+CA	193/207 (0.93)	25/39 (0.64)	56/99 (0.57)	0.27	0.006[##]
	AA	10/24 (0.42)	1/1 (1.00)	9/7 (1.29)		
-159 C/T	CC + CT	153/187 (0.82)	23/29 (0.79)	59/81 (0.73)	0.04[##]	0.02[##]
	TT	50/41 (1.22)	3/11 (0.27)	9/23 (0.39)		
-1145 T/C	TT + CT	154/189 (0.81)	23/28 (0.82)	58/83 (0.70)	0.03[##]	0.02[##]
	CC	50/44 (1.14)	3/13 (0.23)	8/23 (0.35)		
-1619 T/C	TT + CT	159/188 (0.85)	25/30 (0.83)	56/81 (0.69)	0.09	0.10
	CC	34/27 (1.26)	1/6 (0.17)	8/18 (0.44)		
-550 C/T	CC	119/128 (0.93)	11/25 (0.44)	44/55 (0.80)	0.13	0.51
	CT	78/90 (0.87)	12/12 (1.00)	20/47 (0.43)		
	TT	6/13 (0.46)	3/2 (1.50)	3/3 (1.00)		

Data are presented as n sensitised/n not sensitised (proportion), unless otherwise stated. SNP: single nucleotide polymorphism; UTR: untranslated region. [#]: specific immunoglobulin E $\geqslant$0.35 IU·mL^{-1} to house dust mite, cat, dog, *Dactylis glomerata*, *Betula verrucosa*, *Alternaria alternata*, egg or milk allergens. [¶]: from logistic regression analyses adjusted for sex, atopy mother, atopy father, siblings, breast feeding and environmental tobacco smoke exposure; [+]: neither cat nor dog exposure; [§]: exposure to dog, with no cat exposure, at home in the first year of life [f]: exposure to cat, with no dog exposure; [##]: statistically significant.

minor alleles of -159C/T, -1145T/C and -1619T/C in a recessive model associated with lower IgE levels or lower risk of sensitisation in children with exposure, whereas these genotypes associated with higher IgE levels or increased risk of sensitisation in children without exposure (tables 4 and 5 and online supplement table E3). The effects were opposite for -550C/T, *i.e.* the minor T allele was associated with higher IgE levels or increased risk of sensitisation in children with exposure, whereas the T allele was associated with lower IgE levels and lower risk of sensitisation in children without pet exposure.

Sensitisation to any allergen

In order to compare the present data to a previous study [7], which defined sensitisation as positive specific IgE against multiple allergens in children aged 9 yrs, sensitisation to any of the allergens tested in the PIAMA cohort at age 8 yrs was evaluated (specific IgE $\geqslant$0.35 IU·mL^{-1} to house-dust mite, cat, dog, *Dactylis glomerata*, *Betula verrucosa*, *Alternaria alternata*, egg or milk allergens). *CD14* genotypes -159C/T and -1145T/C showed interaction with dog exposure with respect to sensitisation to any allergen at age 8 yrs (p=0.04 and 0.02, respectively; table 5). *CD14* genotypes -159C/T, -1145T/C and 3′UTRC/A showed interaction with cat exposure with respect to sensitisation to any allergen at age 8 yrs (p=0.02, 0.02 and 0.006, respectively; table 5). *CD14* genotypes -159C/T, -1145T/C, -1619T/C and 3′UTRC/A also showed interaction with combined pet exposure with respect to sensitisation to any allergen at age 8 yrs (p=0.002, 0.001, 0.03 and 0.002, respectively; online supplement table E4). Interactions were of similar direction as previously described.

Altering the reference categories

Evaluation of the effect of *CD14* SNPs on serum IgE levels and sensitisation to any allergen, in children with cat exposure, irrespective of dog exposure or children with dog exposure irrespective of cat exposure, and changing the reference category to children without cat exposure, irrespective of dog exposure or children without dog exposure, irrespective of cat exposure, did not substantially change direction of the results. The significance of the associations was stronger, since this analysis increased the numbers of children in each group (online data supplement table E5–E8).

Interaction with ETS exposure

In a recessive model, the AA genotype of *CD14* 3′UTRC/A was associated with lower mean IgE at age 4 yrs in children exposed to ETS (geometric mean of IgE was 14.8 (AA genotype) *versus* 29.0 IU·mL^{-1} (CC/CA genotype)), whereas this genotype was associated with higher IgE in children not exposed to ETS (geometric means 47.6 *versus* 29.6 IU·mL^{-1}, respectively), as reflected by a negative interaction (online supplement table E9). This effect was strongest in the PIAMA cohort and diminished when pooling the PIAMA and PREVASC cohorts together, since the effect was opposite in these two cohorts (p=0.01, 0.32 and 0.04 in PIAMA, PREVASC and PIAMA and PREVASC pooled, respectively). No gene–environment interactions of ETS exposure with *CD14* SNPs were found with respect to sensitisation to egg or milk allergens at ages 1 and 2 yrs, and indoor allergens at ages 4 and 8 yrs.

IL13

Associations with total serum IgE

In the pooled cohort, minor alleles of *IL13* SNPs rs20541 and rs1295685 (also known as Arg130Gln and 3′UTR) were associated with higher cross-sectional serum IgE levels at all ages (p=0.03–0.0000002; fig. 2a; online supplement table E2A). Minor alleles of rs1881457 and rs1800925 (also known as -1512A/C and -1111C/T) were significantly associated with

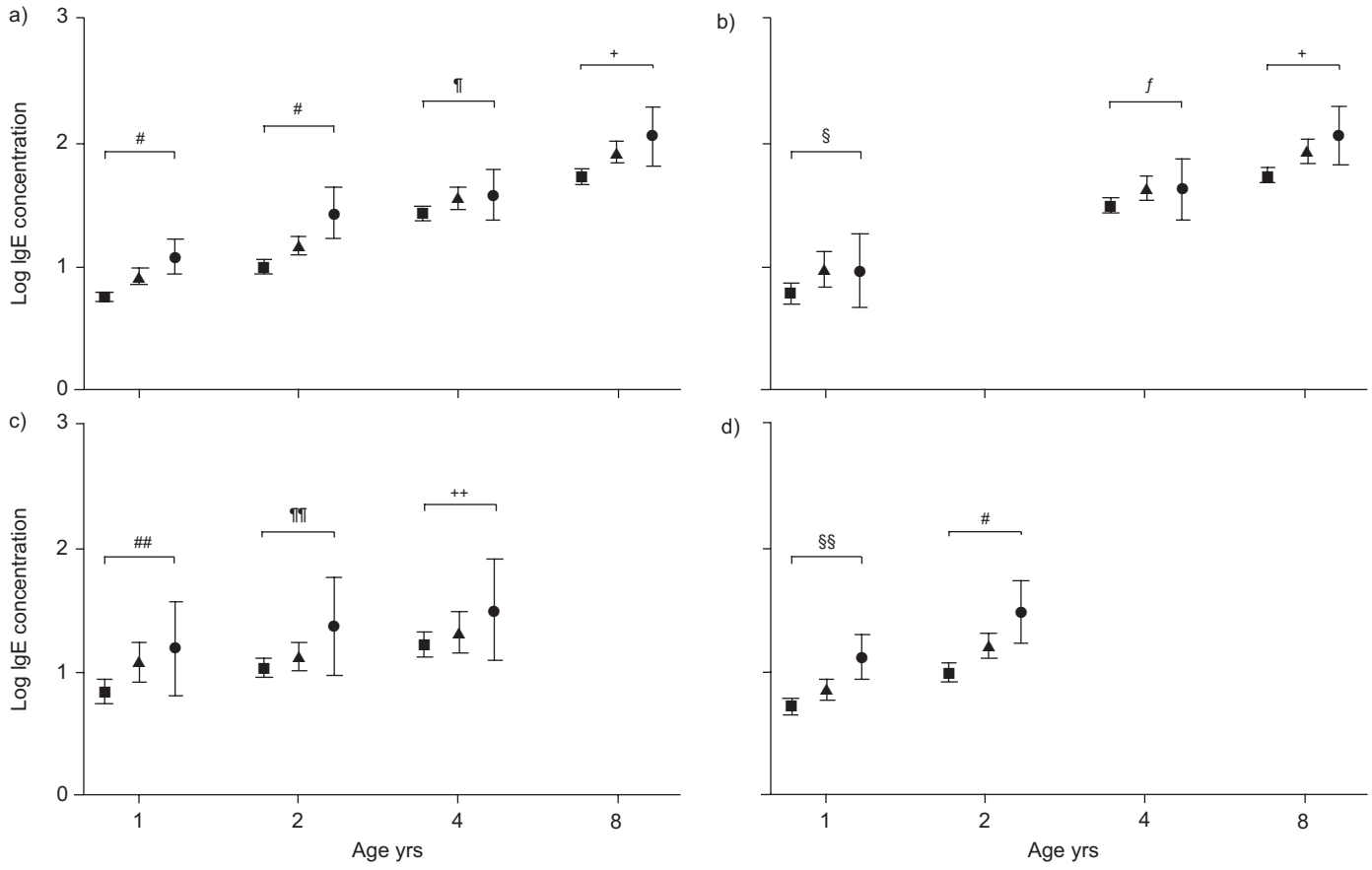

FIGURE 2. Association of *IL13* 3′UTR genotypes with logarithmised total serum immunoglobulin (Ig)E levels (mean and 95% confidence intervals) at ages 1, 2, 4 and 8 yrs in a) the pooled data, and b) the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) [15], c) the Prevention of Asthma in Children (PREVASC) [16, 17] and d) the Child, Parent, Health, Focus on Lifestyle and Predisposition (KOALA) [18] studies separately. IgE measurements were not available at age 2 yrs in PIAMA, 4 yrs in KOALA and 8 yrs in PREVASC and KOALA. Numerical data on all *IL13* single nucleotide polymorphisms and associations with total serum IgE levels are presented in online supplement table E1. ■: CC genotype; ▲: CT; ● TT. $^{\#}$: p<0.0001; ¶: p=0.028; $^{+}$: p=0.0004; §: p=0.031; ^{f}: p=0.075; $^{\#\#}$: p=0.006; ¶¶: p=0.04; $^{++}$: p=0.197; §§: p=0.0001.

higher serum IgE at ages 1 and 2 yrs (p=0.008–0.00007) and similar trends were observed in the same direction at ages 4 and 8 yrs.

The associations of *IL13* SNPs with serum IgE levels were consistent, *i.e.* they were also found in the separate cohorts with the same direction as the pooled data, although some associations did not reach statistical significance (fig. 2b–d, online supplement tables E2B–D).

Associations with sensitisation

IL13 SNPs were also associated with sensitisation. The SNPs -1512A/C and -1111C/T were significantly associated with sensitisation to egg at age 1 yr, and -1111C/T with sensitisation to egg at age 2 yrs. Multiplicative effects of the minor alleles were observed and ORs (95% CI) were 2.0 (1.3–3.0), 1.9 (1.3–2.9) and 1.7 (1.1–2.8) per minor allele. Significant associations of -1512A/C and -1111C/T were also found with sensitisation to indoor allergens at ages 4 and 8 yrs in the pooled data. A multiplicative effect of the minor alleles was observed, ORs (95% CI) for sensitisation at age 4 yrs were 1.6 (1.2–2.2) and 1.4 (1.1–2.0), respectively, per minor allele. At age 8 yrs, corresponding ORs (95% CI) were 1.3 (1.0–1.7) and 1.3 (1.0–1.7). Stratified analyses per cohort at age 4 yrs confirmed the associations with effects in the same direction in both PIAMA and PREVASC (fig. 3).

Effect of pooling

Whether genetic associations and gene–environment interactions were significantly different between studies, was evaluated by including interaction terms with the cohorts into the regression models. For *IL13* -1111C/T, a significantly different relationship with total IgE at age 1 yr was found between the PIAMA and PREVASC cohorts (p=0.04 for interaction with cohort). For *CD14* -1619T/C, the interaction between dog exposure and total serum IgE was found to be different between PREVASC and PIAMA (p=0.04). No further statistically significant differences were found between the cohorts and, therefore, the present results were not significantly affected by pooling data from three separate cohorts.

DISCUSSION

This is the first prospective cohort study describing association between *IL13* haplotype tagging SNPs and total and specific serum IgE at ages 1, 2, 4, and 8 yrs. In addition, a gene–environment interaction of *CD14* haplotype tagging SNPs with pet exposure was shown to be present with respect to total and

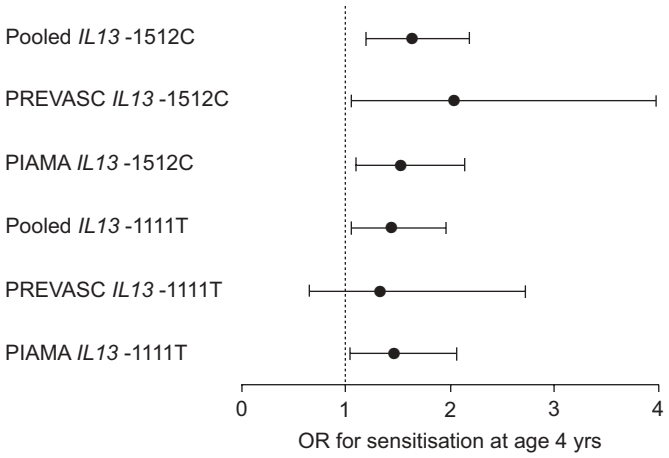

FIGURE 3. Minor alleles of *IL13* -1111C/T and -1512A/C increase the risk for sensitisation to indoor allergens (specific immunoglobulin (Ig)E $\geqslant$0.35 IU·mL^{-1} to house dust mite, cat or dog) at age 4 yrs. Odds ratios (OR) and 95% confidence intervals per minor allele are presented in pooled data and in the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) [15] and Prevention of Asthma in Children (PREVASC) [16, 17] studies separately. IgE measurements were not available at age 4 yrs in the Child, Parent, Health, Focus on Lifestyle and Predisposition study (KOALA) [18].

specific IgE at ages 4 and 8 yrs, but absent at ages 1 and 2 yrs. Highly significant and consistent associations of *IL13* and *CD14* polymorphisms with IgE levels and sensitisation in childhood were found by pooling data from three birth cohorts. This underlines the validity of the pooling strategy proposed in the present study (table 1), also in case of gene–environment interaction. The results confirm an important role of *IL13* and *CD14* polymorphisms in the development of atopy in childhood in a Dutch population [4, 11].

Associations of *CD14* promoter polymorphisms -159C/T and -1145T/C with IgE levels in 4-yr-old children and of 3′UTRC/A, -159C/T, -1149T/C, and -1619T/C with allergen sensitisation at age 8 yrs in interaction with pet exposure are consistent with results of EDER *et al.* [7], who showed interaction between the *CD14* -159C/T variant and animal contact in children with a mean age of 9 yrs. Similar to the present study, the -159T allele was associated with lower IgE levels and lower prevalence of elevated specific IgE in pet exposed children. EDER *et al.* [7] described an effect of dog and cat exposure combined. In the present study, this observation has been extended, by showing that the observed interactions of *CD14* genotypes are also significant for dog exposure separately with respect to serum IgE and sensitisation and for cat exposure with respect to sensitisation.

Children had decreased specific and total IgE levels at age 4 and 8 yrs by interaction of *CD14* SNPs and pet exposure, whereas this was not the case at age 1 and 2 yrs. O'DONNELL *et al.* [28] previously suggested that the influence of *CD14* -159C/T on atopy may be age specific, exerting an effect during mid-childhood that disappeared by early adulthood. The present data, for the first time, show that the interaction of *CD14* polymorphisms with environmental factors has an age-specific effect, *i.e.* an important influence on atopy in mid-childhood, but not in infancy. A study in adults did not find an interaction of pet exposure and the *CD14* -159C/T genotype with serum IgE levels [29], further indicating that interaction of *CD14* with pet exposure may have an age-specific effect on atopy development with a main influence in childhood.

The present study has also indicated an interaction between the *CD14* 3′UTR SNP and ETS exposure on serum IgE, confirming an interaction of *CD14* and ETS exposure previously described by CHOUDRY *et al.* [9], although the associated SNP was different.

Significant associations of the minor alleles of *IL13* SNPs with higher total and specific serum IgE confirm several studies that previously described associations at various ages during childhood [12–14]. The consistent results, at several cross-sectional time-points in all three birth cohorts, provide the best level of evidence that the gene under study contributes to atopy development.

Pooling data from three birth cohorts in the present study produced highly consistent results with more accurate effect estimates and higher significance levels in the pooled data, even when multiple environmental factors showed to be of importance. The consistency of the present results is striking when considering that replication of genetic associations has been proven to be difficult in previous years [3].

Notwithstanding this, some drawbacks of pooling were also noted. Interpretation of pooled data on environmental exposure should be performed cautiously when data acquisition is not performed under identical conditions. ETS exposure is one such example. Questionnaires in the PIAMA and PREVASC studies evaluated ETS exposure by asking whether parents (PREVASC) or parents and visitors (PIAMA) smoked in the house; whereas, in the KOALA study, parents were asked if any person smoked in the presence of the child (for full definitions, see online supplement table E1). Differences in formulation of questionnaires between the cohorts may have resulted in different apparent levels of ETS exposure between the cohorts. As a possible consequence, the interaction of ETS with the *CD14* 3′UTR SNP was not consistent between the cohorts. An alternative explanation is that the relatively low number of smoke-exposed children resulted in reduced power for elucidating any effect in the separate cohorts. Another difficulty encountered with pooling of data from birth cohorts may be the selection of phenotypes, as illustrated by the present definition of sensitisation. The prevalence of sensitisation is highly dependent on the number of allergens tested. The use of data from a cohort with a large number of tested allergens increases the number of cases available to study, which may help to obtain sufficient statistical power; in the present study, an example with *CD14* is provided. Interaction with pet exposure was more significant when sensitisation was defined as a positive test to any of the allergens tested in one cohort (the PIAMA cohort) compared with the definition of sensitisation to indoor allergens in the PIAMA and PREVASC cohorts jointly. However, no absolute contraindications for pooling of data were found, even when two of the three cohorts under study had implemented distinct interventions. The present authors recommend implementing precautions presented in table 1 in order to carefully evaluate if pooling resulted in spurious findings.

The results of the present study should be interpreted with an understanding of its limitations. First, as a result of recruitment strategies, the study represents a selected population with a relatively high number of children with atopic parents compared with the general population. However, adjustment of the analyses for atopy of the parents did not substantially change the direction or the significance of the results. Secondly, multiple comparisons have not been corrected for and, because associations of nine SNPs, two phenotypes and interaction with two types of environmental exposures were evaluated, one might argue that some of the present results may occur due to type-I statistical error. However, this is not very likely, since most of the present results were replicated in different age groups with similar directions of effects. Finally, the present authors explicitly chose to evaluate the effects of exposure to ETS and pets in the first year of life because environmental exposures may be biased by reverse causation, *i.e.* when assessing the effects of ETS and dog exposure at different time-points on atopy development, exposures may be influenced by the development of atopic disease. Exposure in the first year of life is unlikely to be influenced by atopic symptoms. However, the temporal relationship between exposure and disease remains uncertain. Additionally, it was impossible to distinguish between effects of *in utero* and post-natal environmental exposure, since prenatal exposure, *e.g.* maternal smoking, is highly correlated with post-natal exposure.

In conclusion, the present study confirms that both polymorphisms in the *CD14* and *interleukin 13* genes have an important role in atopy development during childhood and that *CD14* interacts with pet and environmental tobacco smoke exposure in an age-specific way. The successful identification of gene–environment interactions when pooling data from three well-characterised Dutch birth cohorts justifies a positive attitude towards future studies that use pooled data. These studies may improve insights into atopy and asthma pathogenesis and open new avenues for preventive and therapeutic strategies.

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