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IL13, *CD14*, pet and tobacco smoke influence atopy in 3 Dutch cohorts; The Allergenic study

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Abstract

Studying gene-environment interactions may elucidate the complex origins of atopic diseases, yet requires large study populations. Pooling data from cohort studies may help, but may also obscure findings. We studied gene-environment interactions in atopy development and evaluated benefits of pooling data.

Haplotype tagging polymorphisms of *Interleukin(IL)13* and *CD14* were genotyped in 3,062 children from birth cohorts PIAMA, PREVASC and KOALA, and tested for association with total and specific IgE and interaction with tobacco smoke and pet exposure at ages 1,2,4, and 8 years by analysis of variance, χ^2-tests, and regression analyses.

IL13: At all ages, minor alleles of rs1295685 and rs20541 were associated with elevated IgE levels in pooled analyses ($p<0.0001$-0.03). *CD14*: The rs2569190-TT and rs2569191-CC genotypes associated with lower IgE and decreased risk of sensitisation at 4 and 8 years in children exposed to pets, with an opposite effect in non-exposed children (p-value for interaction:0.001-0.04). Findings for *IL13* and *CD14* were comparable in separate cohorts.

This study indicates that atopy is importantly influenced by *IL13* at ages 1-8 years and by *CD14* in interaction with pet exposure at ages 4 and 8. Pooling data improved effect estimates and genetic effects could be detected in interaction with important environmental factors.

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Introduction

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 to 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].

To obtain sample sizes large enough to adequately study gene-environment interaction, pooling data of existing birth cohorts could become 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, gender, 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 this study we investigated whether it is possible to pool data from different birth cohorts to prospectively study gene-environment interactions in atopy. We investigated two candidate genes, *i.e. CD14 and Interleukin 13* (*IL13)*, in the Allergenic study, which consists of three well characterised Dutch birth cohorts. CD14 is a membrane receptor involved in binding of bacterial components and capable to influence the postnatal Th1/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 cell 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].

We investigated whether associations of *CD14* and *IL13* haplotype tagging single nucleotide polymorphisms (SNPs) with serum total and specific IgE at ages 1, 2, 4, and 8 years could be identified both in data pooled from our three birth cohorts and in these cohorts separately. Second, we expand current knowledge of *CD14* gene-environment interaction by investigating its interactions with ETS and pets, and dog and cat exposure separately in atopy development.

Methods

Study populations

The Allergenic study includes three prospective Dutch birth cohorts of similar design, *i.e.* PIAMA[15], PREVASC[16, 17] and KOALA[18]. The three cohorts recruited children during pregnancy. The PIAMA study includes a natural history part and an intervention part, *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 women who were invited for a prospective cohort study on pregnancy-related pelvic girdle pain and a group of children recruited among pregnant women with alternative lifestyles through organic food shops, anthropologic doctors and midwives, Steiner schools, and

magazines. The online repository summarizes a description of the cohorts. Genetic studies were approved by local medical ethics committees of participating institutes. All parents provided written informed consent.

Questionnaires
Parents completed annually distributed questionnaires derived from ISAAC[19] about allergic symptoms in the child. Also, information about general health, indoor environment like ETS and pet exposure, socio-economic 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 age 1, 4, and 8 years in PIAMA, at age 1, 2, and 4 in PREVASC, and at age 1 and 2 in KOALA (Sanquin Research, Amsterdam). Total IgE levels were measured by radioimmunoassay as described previously[20-22] and expressed as international units per milliliter (1 IU representing 2.4 ng of IgE). Specific IgE levels to mite (*Dermatophagoides pteronyssinus*), cat (*Fel d1*), dog (*Can f1*), egg and milk were measured by Radio Allergo Sorbent Test. We defined sensitisation as specific IgE $\geq$ 0.35 IU/ml against food allergens (milk or egg) at 1 and 2 years and indoor allergens (house dust mite, cat and dog) at 4 and 8 years.

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 $\geq$ 0.1 available in each database. Additionally, the biomedical literature until 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 online repository.

Statistical methods
We assessed whether association of the *CD14* and *IL13* genotypes, and environmental pet and ETS exposure was present with two atopic phenotypes, *i.e.* 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 adjusted for atopy of the mother, atopy of the father, gender, siblings, breastfeeding, 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), or 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 exposed to dog.
Our approach to pooling data from cohort studies is described in table 1. We assessed whether genetic and gene-environment effects were different between the cohorts by including an interaction term with each of the cohorts into linear and logistic regression models.
Calculations were performed using SPSS 14.0 statistical software and considered significant if $p<0.05$ (two sided).

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 from 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 sensitization, neither in the pooled data nor in the separate cohorts. Cat exposure was associated with higher IgE levels at 2 years of age in the pooled data, showing a trend in KOALA and PREVASC separately (p-values respectively 0.013, 0.060, 0.087) and with decreased risk of sensitisation to indoor allergens at age 8 (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 years (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.

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'UTR) showed consistent and significant interaction with dog exposure with respect to serum IgE levels at 4 and 8 years (p-values for interaction at 4 years: 0.01, 0.005, 0.03, 0.00001, and 0.05 respectively and at 8 years: 0.16, 0.10, 0.01, 0.03, and 0.80 respectively; see figure 1A and table 4). Similar direction of interaction was observed in the cohorts separately at 4 years, 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 (figure 1B-C). Additionally, a significant interaction with dog exposure was found for *CD14* -550C/T with respect to sensitisation to indoor allergens at 4 years (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: CD14 -159C/T and -1145T/C showed significant interactions with combined pet exposure with regard to serum IgE at 4 years (p=0.04, and 0.01 respectively) and a trend for interaction at 8 years (p=0.17, and 0.18 respectively, online supplement table E3). The interactions were not statistically significant in the separate cohorts at 4 years, 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 4 years (p=0.02, data not shown).

The interaction effects of *CD14* with dog and with pet exposure were in the same direction, *i.e.* homozygous genotypes for the minor alleles of -159C/T, -1145T/C, -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: To compare our data to a previous study by Eder *et al*[7], who defined sensitisation as positive specific IgE against multiple allergens in children aged 9 years, we evaluated sensitisation to any of the allergens tested in the PIAMA cohort at 8 years of age (*i.e.* specific IgE $\geq$ 0.35 IU/ml to house dust mite, cat, dog, *Dactylis glomerata*, *Betula verrucosa*, *Alternaria alternata*, egg, or milk allergens). *CD14* -159C/T and -1145T/C showed interaction with dog exposure with respect to sensitisation to any allergen at age 8 (p=0.04, and 0.02 respectively, table 5). *CD14* -159C/T, -1145T/C and 3'UTRC/A showed interaction with cat exposure with respect to sensitisation to any allergen at age 8 (p=0.02, 0.02, and 0.006 respectively, table 5). *CD14* -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 (p=0.002, 0.001, 0.03, and 0.002 respectively, online supplement table E4). Interactions were of similar direction as previously described results.

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 environmental tobacco smoke exposure
In a recessive model, the AA genotype of *CD14* 3'UTRC/A was associated with lower mean IgE at 4 years in children exposed to ETS (geometric mean IgE 14.8 (AA genotype) vs. 29.0 IU/ml (CC/CA genotype)), whereas this genotype was associated with higher IgE in children non-exposed to ETS (geometric means 47.6 vs. 29.6 IU/ml), 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 in PIAMA, p=0.32 in PREVASC, and p=0.04 in PIAMA and PREVASC pooled). 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 and indoor allergens at ages 4 and 8 years.

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, figure 2A, online supplement table E2A). Minor alleles of rs1881457 and rs1800925 (also known as -1512A/C and -1111C/T) were significantly associated with higher serum IgE at ages 1 and 2 years (p=0.008-0.00007) and similar trends were observed in the same direction at ages 4 and 8 years.
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, though some associations did not reach statistical significance (figures 2B-D, online supplement tables E2B-D).

Associations with sensitisation

IL13 SNPs also associated with sensitisation. The SNPs -1512A/C and -1111C/T were significantly associated with sensitisation to egg at age 1, and -1111C/T with sensitisation to egg at 2 years. We observed multiplicative effects of the minor alleles, ORs (95% CI) being respectively 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 4 and 8 years of age in the pooled data. We observed a multiplicative effect of the minor alleles, per minor allele ORs (95% CI) for sensitisation at 4 years were 1.6 (1.2-2.2) and 1.4 (1.1-2.0) respectively. At 8 years, corresponding ORs were 1.3 (1.0-1.7) and 1.3 (1.0-1.7). Stratified analyses per cohort at age 4 confirmed the associations with effects in the same direction in both PIAMA and PREVASC (figure 2).

Effect of pooling

We evaluated whether genetic associations and gene-environment interactions were significantly different between studies by including interaction terms with the cohorts into the regression models. For *IL13* -1111C/T, we found a significantly different relationship with total IgE at age 1 between the PIAMA and PREVASC cohort (p-value for interaction with cohort =0.04). 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). We further did not find any statistically significant differences between the cohorts and therefore conclude that our results were not significantly affected by pooling data of 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 years. In addition, we showed that gene-environment interaction of *CD14* haplotype tagging SNPs with pet exposure is present with respect to total and specific IgE at ages 4 and 8 years, but absent at ages 1 and 2 years. We found highly significant and consistent associations of *IL13* and *CD14* polymorphisms with IgE levels and sensitisation in childhood by pooling data from three birth cohorts. This underlines the validity of the pooling strategy proposed in this study (table 1), also in case of gene-environment interaction. The results confirm an important role of *IL13* and *CD14* polymorphisms in development of atopy in childhood in the Dutch population[4, 11].

Associations of *CD14* promoter polymorphisms -159C/T and -1145T/C with IgE levels in 4 year old children and of 3'UTRC/A, -159C/T, -1149T/C, and -1619T/C with allergen sensitisation at 8 years 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 years. Similar to our 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. We extend this observation 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 4 and 8 years by interaction of *CD14* SNPs and pet exposure, whereas this was not the case at 1 and 2 years. O'Donell and colleagues 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[25]. Our 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 [26] did not find an interaction of pet exposure and the *CD14* -159C/T genotype with serum IgE levels, further indicating that interaction of *CD14* with pet exposure may have an age specific effect on atopy development with a main influence in childhood.

Our study 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], though 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 this 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 our results is striking when considering that replication of genetic associations has been proven to be difficult in the previous years[3].

Notwithstanding this, we also noted some drawbacks of pooling. First, interpretation of pooled data on environmental exposure should be done cautiously when measurements of exposures are not performed exactly similar. We provide such an example with ETS

exposure. Questionnaires in PIAMA and PREVASC evaluated ETS exposure by asking whether parents (PREVASC); or parents and visitors (PIAMA) smoked in the house, whereas in KOALA parents were asked if any person smoked in the presence of the child (for full definitions we refer to online supplement table E1). Differences in formulation of questionnaires between the cohorts may have resulted in different 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 to elucidate 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 our 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. We provide such an example with *CD14*. 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 to the definition of sensitisation to *indoor allergens* in the PIAMA and PREVASC cohorts jointly. However, we found no absolute contra-indications for pooling of data, even when two of the three cohorts under study had implemented distinct interventions. We recommend implementing precautions presented in table 1, in order to carefully evaluate if pooling resulted in spurious findings.

The results of this study should be interpreted with an understanding of its limitations. First, as a result of recruitment strategies, this study represents a selected population with a relatively high number of children with atopic parents compared to 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. Second, we have not corrected for multiple comparisons, and because we tested for associations of 9 SNPs, 2 phenotypes, and interaction with 2 types of environmental exposures, one might argue that some of our results may result from type I statistical error. However, this is not very likely since most of our results were replicated in different age groups with similar directions of effects. Finally, we explicitly choose 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 relation between exposure and disease remains uncertain. Additionally, we could not distinguish between effects of *in utero* and postnatal environmental exposure, since prenatal exposure *e.g.* maternal smoking is highly correlated with postnatal exposure.

In conclusion, this study confirms that both *CD14* and *IL13* polymorphisms have an important role in atopy development during childhood and that *CD14* interacts with pet and ETS exposure in an age specific way. The successful identification of gene-environment interactions when pooling data from three well-characterized Dutch birth cohorts, justifies a positive attitude towards future studies that use pooled data from cohort studies. These studies may improve insights into atopy and asthma pathogenesis and open new avenues for preventive and therapeutic strategies.

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Figure legends

Figure 1. Interaction of *CD14* -550C/T genotypes with dog exposure at home in the first year of life on total serum IgE levels (mean and 95% CI) at 4 and 8 years of age in: **(A)** pooled data (Allergenic), and **(B)** PIAMA, **(C)** PREVASC. IgE measurements were not available at 4 years in KOALA and at 8 years in PREVASC and KOALA.

Figure 1A

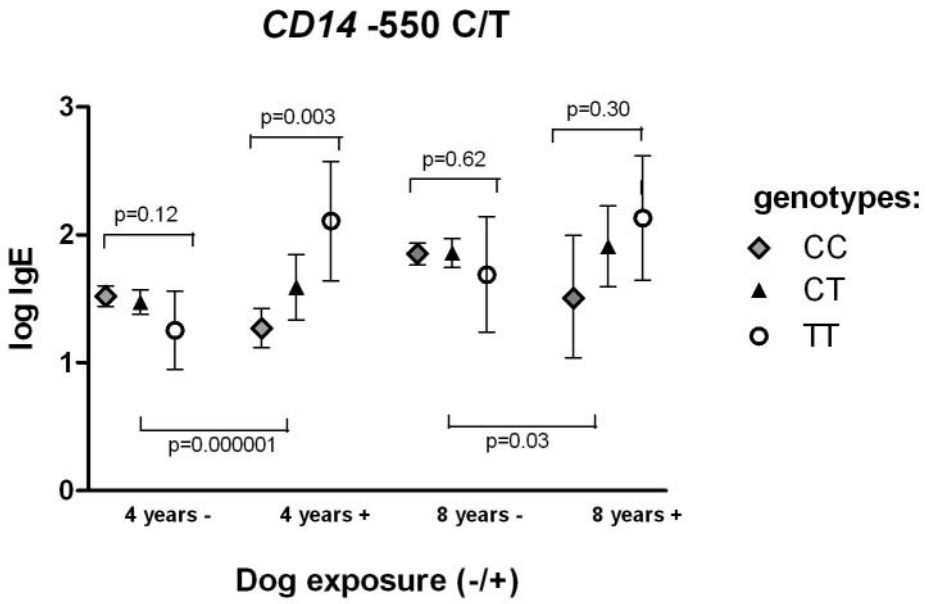


Figure 1B

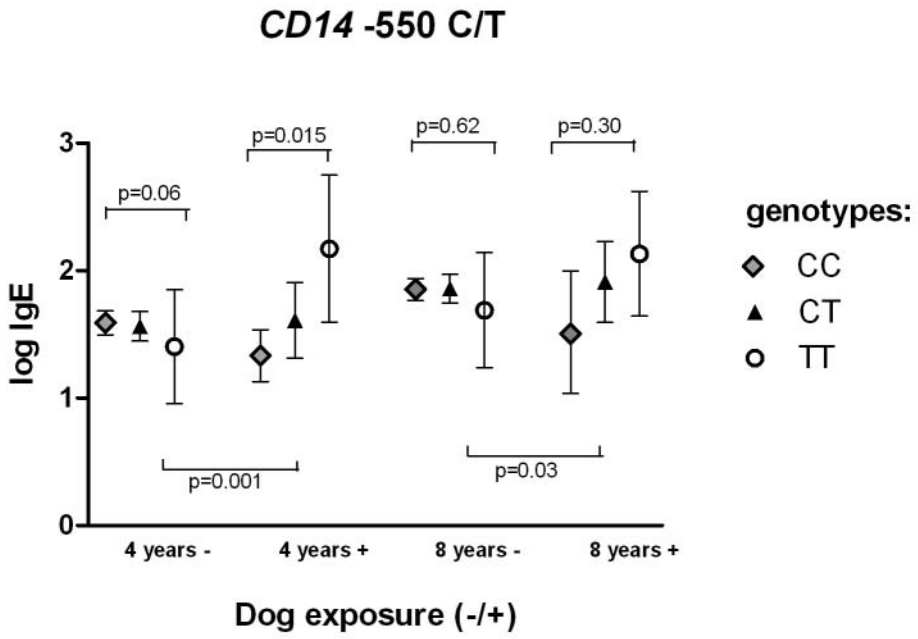


Figure 1C

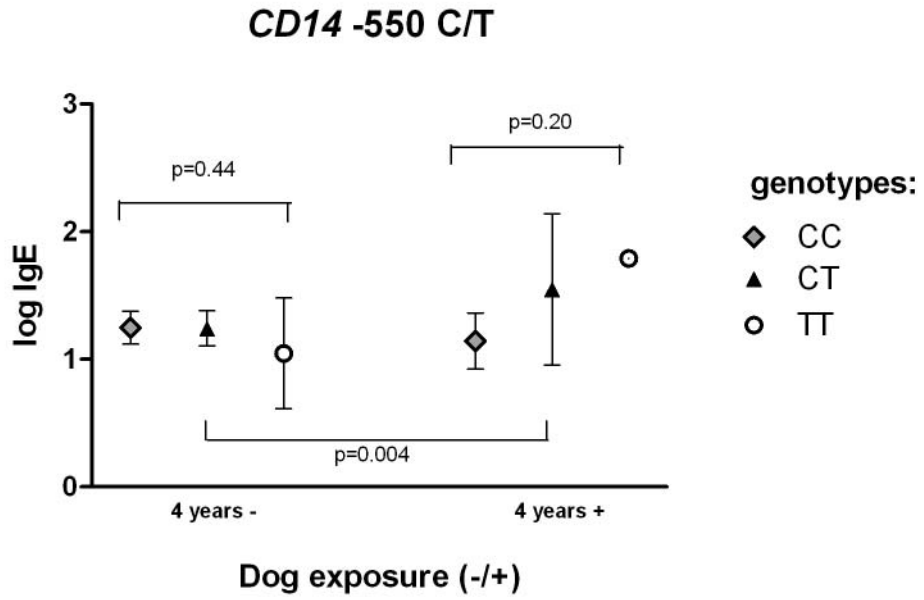


Figure 2 (A-D). Association of *IL13* 3'UTR genotypes with logarithmized total serum IgE levels (mean and 95% CI) at ages 1, 2, 4 and 8 years in: **(A)** pooled data (Allergenic), and **(B)** PIAMA, **(C)** PREVASC and **(D)** KOALA separately. IgE measurements were not available at 2 years in PIAMA, at 4 years in KOALA and at 8 years in PREVASC and KOALA. Numerical data on all *IL13* SNPs and associations with total serum IgE levels are presented in online supplement table E1.

Figure 2A

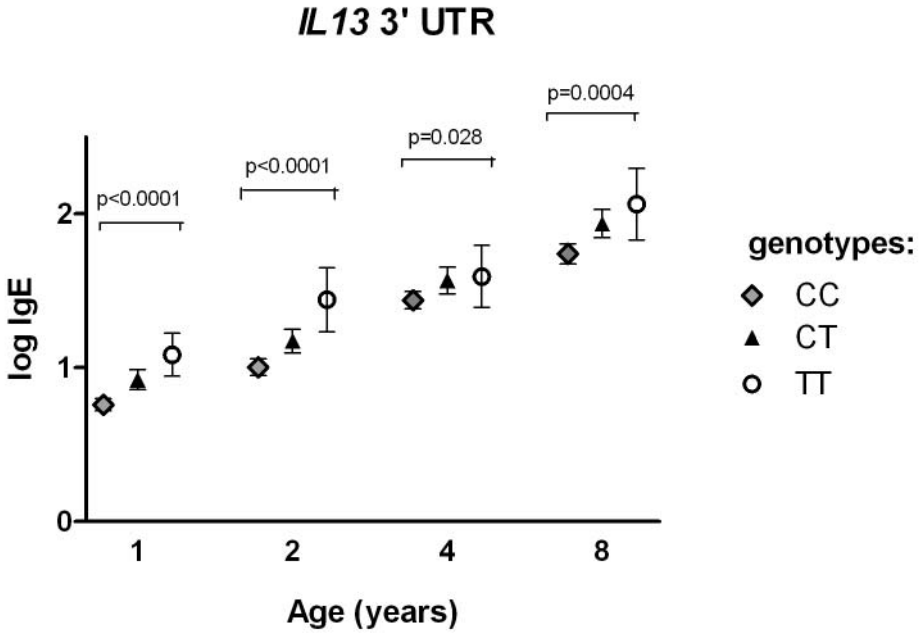


Figure 2B

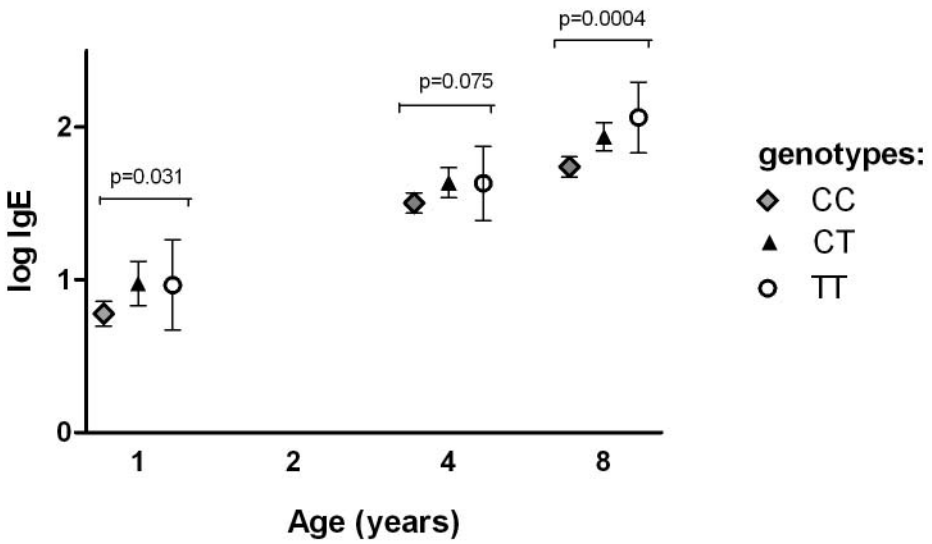
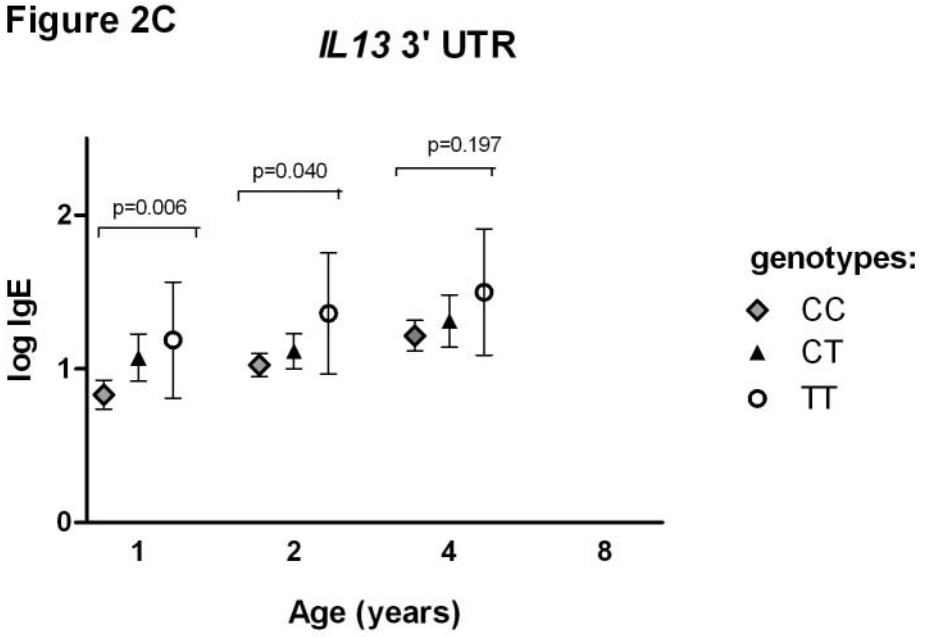


Figure 2C



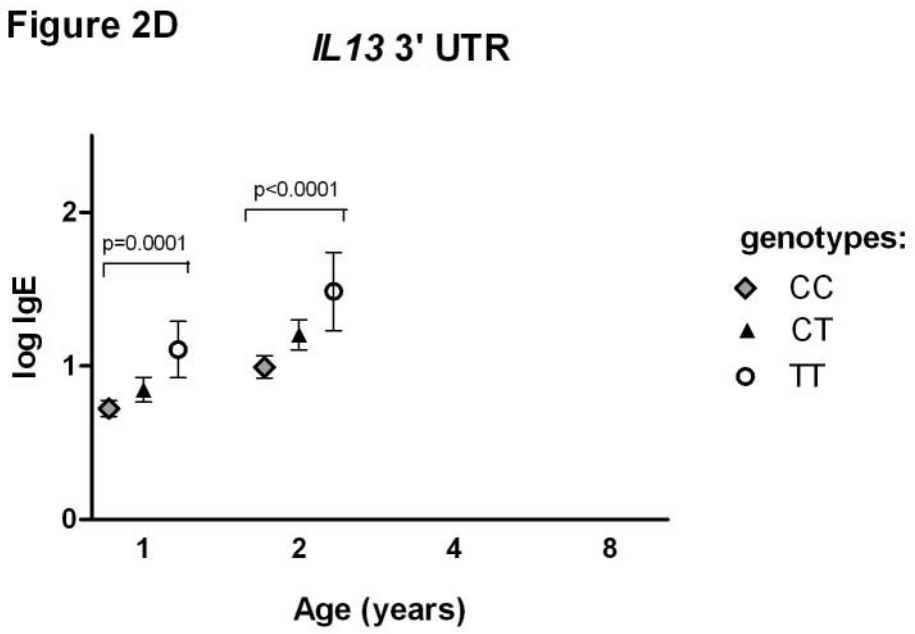


Figure 3. Minor alleles of *IL13* -1055C/T and -1512A/C increase the risk for sensitisation to indoor allergens (specific IgE ≥0.35 IU/ml to house dust mite, cat or dog) at 4 years of age. Odds ratios (OR) and 95% confidence intervals per minor allele are presented in pooled data (Allergenic) and in PIAMA and PREVASC separately. IgE measurements were not available at 4 years in KOALA.

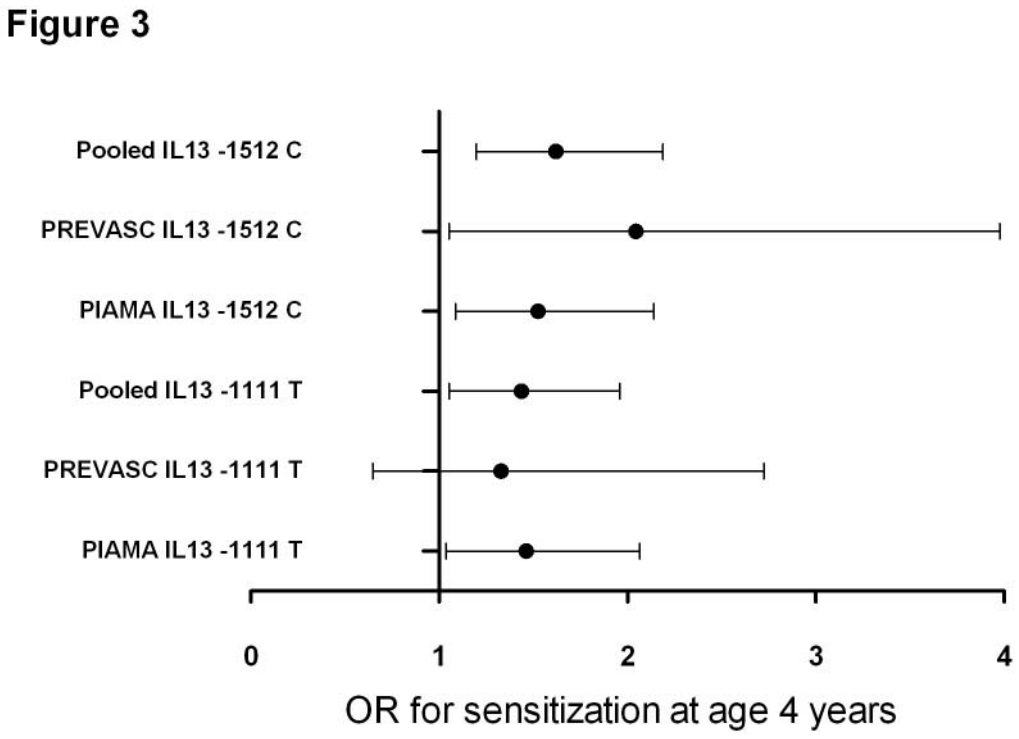


Table 1. Issues in pooling of data from separate cohort studies and proposed solutions.

Issues in pooling data	**Approach**
Recruitment strategies	Stratification of analyses per cohort
Comparability of phenotype measurements	Selection of standardized 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 [27] and modified to apply to pooling data from cohort studies.

Table 2. Participant characteristics, for each birth cohort separately.

Characteristics	PIAMA	PREVASC	KOALA	P
Participants in genetic study (number)	1,037	374	1,651	-
DNA available (% from total cohort)	25.0	49.8	58.1	-
Ethnicity (% Dutch origin)*	95.1	95.7	95.2	-
Boys (%)	51.2	49.2	50.6	0.80
Education level mother (%)*				**<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	Multifaceted	No	-
No (%)	covers	68.3	100	
Placebo (%)	57.3	0	0	
Active (%)	23.9	31.7	0	
	18.8			
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**
Presence older siblings at birth (%)	48.5	59.9	58.1	**<0.001**
Total serum IgE				
1 year (IU/ml) †	7.1	8.6	6.0	**0.002**
	(2.0-17.0)	(3.5-19.4)	(2.6-12.5)	
	N=369	N=226	N=699	
2 years (IU/ml) †	N.a.	11.7	12.0	0.80
		(4.2-28.7)	(3.7-38.0)	
		N=358	N=704	
4 years (IU/ml) †	36.1	18.6	N.a.	**<0.001**
	(12.0-101.0)	(8.8-49.0)		
	N=714	N=207		
8 years (IU/ml) †	64.9	N.a.	N.a.	-
	(23.0-240.0)			
	N=748			
Sensitisation to food allergens‡				
Egg, 1 year (% positive)	6.5, N=355	7.8, N=167	4.7, N=674	0.24
Egg, 2 years (% positive)	N.a	8.2, N=208	4.7, N=698	0.056
Milk, 1 year (% positive)	27.2, N=355	N.a	8.0, N=690	**<0.001**
Milk, 2 years (% positive)	N.a.	N.a.	14.5,	-

			N=697	
Sensitisation to indoor allergens§				
4 years (% positive)	16.8, N=709	18.4, N=206	N.a	0.58
8 years(% positive)	26.8, N=746	N.a	N.a	-

- = not tested; * Definitions of these variables are presented in the online supplement table E1; † Geometric mean (interquartile range); N = number of samples available; N.a. = not available; ‡ Specific IgE ≥0.35 IU/ml to egg and/or milk allergens; § Specific IgE ≥0.35 IU/ml to house dust mite, cat and/or dog allergens.

Table 3. SNPs selected for *CD14* and *IL13*, source of information and minor allele frequencies in the pooled (Allergenic) and separate cohorts.

Gene	Rs number	Alleles*	Synonym	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., L	0.48	0.47	0.46	0.49
CD14	rs2569191	T/C	-1145T/C	I.I., L	0.48	0.47	0.47	0.49
CD14	rs2915863	T/C	-1619T/C	I.I., L	0.41	0.40	0.38	0.42
CD14	rs5744455	C/T	-550C/T	I.I., L	0.23	0.23	0.24	0.23
IL13	rs1295685	C/T	3'UTR	H.M., L	0.20	0.20	0.20	0.20
IL13	rs20541	G/A	Arg130Gln	H.M., L	0.20	0.20	0.21	0.20
IL13	rs1881457	A/C	-1512A/C	L	0.20	0.20	0.17	0.21
IL13	rs1800925	C/T	-1111C/T	L	0.20	0.20	0.18	0.20

***** Major alleles first, followed by minor alleles;
‡ I.I.: innate immunity website (http://www.innateimmunity.net/data/homology); L: literature; H.M.: HapMap database (http://www.hapmap.org),
Allele frequencies were similar between the cohorts (χ^2 test; $p>0.05$) and genotypes were in Hardy-Weinberg equilibrium ($p\geq0.01$).

Table 4. Influence of dog and cat exposure in interaction with *CD14* genotypes on total serum IgE levels at age 4 and 8 years.

SNP	Genotype	No pet exposure†			Dog exposure†			Cat exposure†			P-value interaction Dog vs no exposure / cat vs no exposure ‡
		IgE*	N	P	IgE*	N	P	IgE*	N	P	
4 years											
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		
8 years											
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		

* Geometric mean IgE values; N= number of children per genotype; † Dog exposure = dog exposure, but no cat exposure at home in the first year of life; Cat exposure = cat exposure, but no dog exposure; No pet exposure = neither cat nor dog exposure; ‡ P-values for interaction from linear regression analyses evaluating dog exposure vs. no pet exposure / cat exposure vs. no pet exposure; adjusted for gender, atopy mother, atopy father, siblings, breastfeeding and ETS exposure; 110 children were exposed to both cat and dog, in these children similar results were observed.

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

SNP	**Genotype**	**Sensitisation to any allergen § yes / no* (proportion)**			**P-value interaction dog vs no exposure / cat vs no exposure ‡**
		No pet exposure †	Dog exposure †	Cat 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)	

§ Specific IgE ≥0.35 IU/ml to house dust mite, cat, dog, *Dactylis glomerata*, *Betula verrucosa*, *Alternaria alternata*, egg, or milk allergens. * Number of children sensitised / number of non-sensitised children; † Dog exposure = dog exposure, but no cat exposure at home in the first year of life; Cat exposure = cat exposure, but no dog exposure; No pet exposure = neither cat nor dog exposure; ‡ P-value for interaction from logistic regression analyses, evaluating dog exposure vs. no pet exposure / cat exposure vs. no pet exposure; adjusted for gender, atopy mother, atopy father, siblings, breastfeeding and ETS exposure.