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Genetics of asthma and atopy

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Chapter 8 | Association of a promoter polymorphism of the CD14 gene and atopy

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ABSTRACT

Atopy is generally considered to be caused by interaction of genetic and environmental factors. Recently, an association of a C to T transition in the promoter region of the CD14 gene on chromosome 5q31.1 and atopic phenotypes was reported in a population study of school children in the United States. The aim of the present study was to investigate the association of the C allele of the CD14/-159 with phenotypes of atopy and asthma in an adult Dutch population in which linkage of total serum IgE and bronchial hyperresponsiveness to chromosome 5q31-33 is present. We studied 159 probands with asthma and 158 spouses as controls. Phenotypes for asthma (e.g., bronchial hyperresponsiveness, physician's diagnosis) and for atopy (e.g., total serum IgE level, intracutaneous skin test, allergic rhinitis) were studied. In this population, homozygotes for the C allele had a higher number of positive skin tests and higher total serum IgE levels (in skin test positive individuals) and subsequently, more self reported allergic symptoms including rhinitis and hay fever, compared with subjects with CT and TT alleles. We conclude that the -159 C to T promoter polymorphism in the CD14 gene may result in expression of a more severe allergic phenotype.

INTRODUCTION

Atopic diseases, such as asthma, allergic rhinitis and eczema, are characterised by an elevated and prolonged immunoglobulin E (IgE) antibody response after exposure to ubiquitous, nonpathogenic allergens. Atopy is generally considered to be caused by the interaction of genetic and environmental factors.¹ There is concern about the worldwide rise of atopic diseases over the last decades and different hypotheses have been proposed to explain this increased prevalence.² Changes in environmental factors most likely play an important role, such as life style, diet, air pollution, allergen exposure and microbial environment.^{3,4} Recent studies have suggested that bacterial infections in infancy may protect against the development of allergy.⁵ In 1997, Holt and coworkers hypothesised that bacterial signals play a functional role in the maturation of the TH-1 type immune response, thereby suppressing the TH-2 type response, which may produce an atopic phenotype. Microbial products, such as lipopolysaccharides (LPS), can provide activation signals for TH-1 maturation.⁶

The pathway through which LPS may exert its function includes high- and low-affinity LPS receptors. An important high affinity receptor for LPS and other bacterial wall components is CD14, a 55-kd glycosylphosphatidylinositol anchored protein localized on monocytes, macrophages, and polymorphonuclear cells. Binding of LPS to CD14 is facilitated by lipopolysaccharide-binding protein. CD14 is also present as soluble CD14 (sCD14) in serum, where it acts like membrane bound CD14 in cells that do not express CD14.⁷ The gene encoding CD14 is a positional candidate gene for atopy, as it is localized on chromosome 5q31.1,⁸ a region that has been linked to asthma and atopic responses.⁹⁻¹⁴ In 1999, Baldini and coworkers reported the association of a polymorphism in the CD14 gene and atopy. In the promoter region of the CD14 gene, a C-to-T transition was identified at position -159 upstream from the major transcription site (CD14/-159). In a Caucasian subset (n=314) of a general population sample of 11-yr-old children from the United States, homozygotes with TT had higher serum levels of sCD14 than homozygotes with CC. In addition, among skin test positive children, homozygotes with the TT genotype had lower levels of serum total IgE and a lower number of positive skin prick tests, when compared to the pooled group of subjects carrying CC and CT.¹⁵ This study did not assess the effect of CD14 on asthma and allergic rhinitis.

The purpose of the present study was to investigate, whether the C allele of the CD14/-159 was associated with elevated total serum IgE levels and number of positive skin tests in an adult Dutch population, in which linkage of total IgE levels and bronchial hyperresponsiveness to chromosome 5q has been previously reported.^{9,12} In addition, we investigated whether this polymorphism was associated with allergic rhinitis and asthma phenotypes.

METHODS

Study population

Subjects were selected from a family study on the genetics of asthma in the Netherlands, which has been described in detail by Panhuysen and coworkers.¹⁶ Between 1962 and 1975, patients with asthma were evaluated for diagnosis of asthma and optimization of their treatment in Beatrixoord, Haren, the Netherlands. For inclusion in this study, from this first evaluation patients had to meet three criteria: (1) symptoms consistent with asthma; (2) age \leq 45 yr; (3) bronchial hyperresponsiveness to histamine ($PC_{20} \leq 32$ mg/ml using the de Vries 30 seconds inhalation method).¹⁷

From 1990 onwards, these probands were restudied together with their spouses, a minimum of two children and, if possible, grandchildren. In total, 200 two- and three generation families have been studied. Using a case-control approach, probands and their spouses were selected from these families. Data are presented from 159 probands and 158 spouses, from whom DNA was available at the time of this study.

Clinical assessment

The first evaluation (1962-1975) included the performance of intracutaneous skin tests with common aeroallergens, pulmonary function testing with a water-seal spirometer (Lode Spirograph, Groningen, the Netherlands), and testing for bronchial hyperresponsiveness with histamine, using the 30 seconds inhalation protocol as described by de Vries and coworkers.¹⁷

At the second evaluation (1990-1998), these measurements were repeated in the probands, and also performed in the relatives. Reversibility was tested by repeating spirometry 20 minutes after administration of 800 mg of salbutamol (albuterol). All participants were asked to stop pulmonary medication before the clinical testing if possible: inhaled corticosteroids were stopped for 14 d, inhaled long acting beta-mimetics and oral antihistamines 48 h, inhaled short acting beta-mimetics and anticholinergics 8 h. The asthma patients did not have an asthma exacerbation or require a course of oral prednisone in the 6 weeks prior to the study.

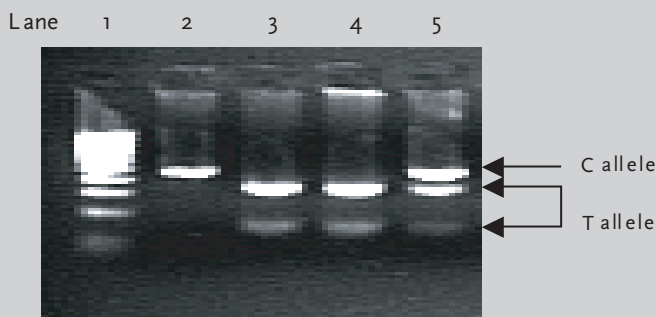
This evaluation further included a modified version of the British Medical Council questionnaire with additional questions on symptoms and therapy of asthma and allergy.¹⁶ By definition, a physician's diagnosis of asthma was present in the probands. In the spouses, it was present if the subject reported (1) being under current regular treatment for asthma, (2) having ever visited a general practitioner or specialist for asthma or (3) having ever used asthma medication. Allergic rhinitis was defined as a positive answer to one of the following questions: Do you have a runny or stuffed nose when you are near (1) animals (e.g., dogs, cats, horses), feathers (e.g., in pillows), a dusty part of the house, trees, grasses, and flowers? Hay fever was defined as a positive answer to the question: Did you ever have hay fever? Serum total IgE was measured in the first 92 families by solid phase immunoassay.¹⁶ In the second set of 108 families, serum IgE levels were mea-

sured by an enzyme linked fluorescent assay (Mini Vidas, Biomerieux Vitek Inc., Marcy, France). Skin testing was performed by an intracutaneous skin test with 16 common aeroallergens, a positive control, and a negative control. The following allergens were tested: mixed grass pollens, two mixed tree pollens, mixed weeds, house dust mite, storage mite, cat-, dog-, horse-, rabbit/guinea pig dander, feather mix, and five moulds (*Aspergillus Fumigatus*, *Alternaria Alternata*, *Cladosporium Herbarum*, *Penicillium Notatum*, *Botrytis Cineria*). (ALK-Abello, Nieuwegein, the Netherlands). A positive skin test was considered to be present if the largest wheal diameter was ≥ 5 mm. The Medical Ethics Committee of the University Hospital of Groningen and the University of Maryland approved this study. Written informed consents were obtained from all participants.

Molecular methods

DNA was extracted using standard protocols. (Puregene kit; Gentra Systems Inc., Minneapolis, MN). Genotyping of the CD14/-159 polymorphism was performed according to the protocol described by Baldini and coworkers.¹⁵ Briefly, polymerase chain reaction (PCR) was performed in 10 ml volumes consisting of 60 ng of DNA, 250 mM dNTP, 1.5 mM MgCl, 10X buffer (Life Technologies, Rockville, MD), 0.5 U of Taq polymerase and 0.1 mM of primer 5'GCCTCTGACAGTTTATGTAATC3' and primer 5'GTGCCAACAGATGAGGTTTCAC3'. Cycling conditions were 94°C for 3 minutes, 28 cycles of 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 30 seconds and a final extension of 72°C for 6 minutes. PCR amplified DNA was digested with 5 U of Ava II and 1 mL of the manufacturers buffer (New England Biolabs Inc., Beverly, MA) at 37°C for two h. Products were fractionated on 2.0% agarose gel. Ava II digests the PCR product only when the T allele is present. The uncut product is 497 bp while the digested products are 144 and 353 bp (figure 1) The results of this restriction fragment length polymorphism assay were confirmed by direct sequencing of the -159 promoter region of the CD14 gene in 32 patients and controls.

Figure 1



CD14/-159 restriction fragment assay. Electrophoresis gel showing size markers on lane 1. On lane 2, one band is visible for homozygotes carrying two C alleles. On lane three and four two bands for homozygotes carrying two T alleles, and on lane five a CT heterozygote is shown

Statistical methods

Three different genetic models were tested. To study a recessive model for the C allele, CC homozygotes were compared with CT heterozygotes and TT homozygotes. To study a recessive model for the T allele, TT homozygotes were compared with individuals with CT and CC. To study a codominant model, the three genotype groups were analyzed separately. Both parametric (T-test, ANOVA) and non-parametric analyses (Mann-Whitney, Kruskal Wallis test) were used to study phenotypic differences in each genotype group, depending on the normality of the distribution of the variables. The number of skin tests was not normally distributed, whereas serum IgE levels were log transformed to obtain a normal distribution. Linear and logistic regression analyses were used to test and correct for known confounding variables, such as age, sex and smoking. Results were considered significant if $p < 0.05$ (two-sided). All calculations were performed with the SPSS 8.0 statistical package.

Table 1. Baseline characteristics of the study population at the second evaluation

Characteristic	probands (n=159)	Spouses (n=158)
Male, %	63.5	36.7
Age, years (mean, range)	52.4 (37-76)	51.3 (34-76)
Smoking (none, ex-, current)	55/63/41	42/54/62
Asthma (doctor's diagnosis), %*	100	8.7
Allergic rhinitis (self reported), %*	59.1	19.6
Hay fever (self reported), %*	31.4	5.7
FEV ₁ % predicted (mean, SD)	67.7 (25.2)	97.8 (13.1)
Bronchial hyperresponsiveness (PC ₂₀ ≤ 32mg/ml), %	90.2	25.6
Skin test positivity (minimum 1 positive), %	79.9	29.1
Number of positive skin tests (median, range)	3.5 (0-13)	0 (0-9)
Serum total IgE, IU/ml (geometric mean, 95% CI)	97.3 (76.0-124.5)	27.4 (21.6-34.6)

Skin test was not available in 1 proband. IgE was not available in one other proband, FEV₁ not available in 3 probands. Testing of bronchial hyperresponsiveness was not performed in 29 individuals (27 probands, 2 spouses) due to low baseline lung function. All probands were hyperresponsive at the time of initial testing. PC₂₀ ≤ 32 mg/ml with 30 seconds inhalation protocol. * For definition see methods

RESULTS

Study population

Baseline characteristics of the study population at the second evaluation (1990 - 1998) are shown in table 1. Probands were predominantly male and their mean age was 52 years. All probands were hyperresponsive at initial testing. Although probands were not selected for atopy, 79.9% had atopy as measured by a positive skin test, compared to 29.1% of the spouses.

Genotype frequency of the CD14/-159 polymorphism

The genotype frequencies in probands were 32.1% for CC homozygotes, 47.8% for CT heterozygotes and 20.1% for TT homozygotes. In the spouses, these frequencies were 19.6%, 53.8%, and 26.6%, respectively.

Association of the C allele with total IgE levels, skin tests, hay fever and allergic rhinitis

Based on the report of Baldini et al.¹⁵, the primary analysis was the association of CD14/-159 and skin tests and serum total IgE levels. In the skin test positive population, CC homozygotes had significantly higher serum IgE levels compared to CT heterozygotes and TT homozygotes ($p=0.036$) The difference between the latter was not significant in skin test negative individuals (table 2).

Table 2. Association of the CD14/-159 promoter polymorphism and total serum IgE levels

Genotype	Skin test negative		Skin test positive	
	n	Total IgE levels	n	Total IgE levels
CC	31	20.3 (11.3-36.5)	51	162.6 (104.7-252.6) *
CT	71	20.8 (15.1-28.8)	88	94.5 (69.9-127.7)
TT	41	17.5 (12.0-25.4)	33	105.9 (66.8-167.7)

Total IgE levels are expressed as geometric mean (95% confidence interval of the mean). * $p < 0.05$ for CC versus CT and TT

In the skin test positive population as well as in the total population, CC homozygotes had a higher number of positive skin tests compared to CT heterozygotes and TT homozygotes. In skin test positive individuals, the median (mean) number of positive skin tests in individuals with CC was 5 (5.61), for CT 4 (4.19) and for TT 3 (3.76) (figure 2). The difference was statistically significant in a codominant model ($p=0.01$). This difference was also significant in a recessive model for the C allele ($p=0.008$) (figure 2).

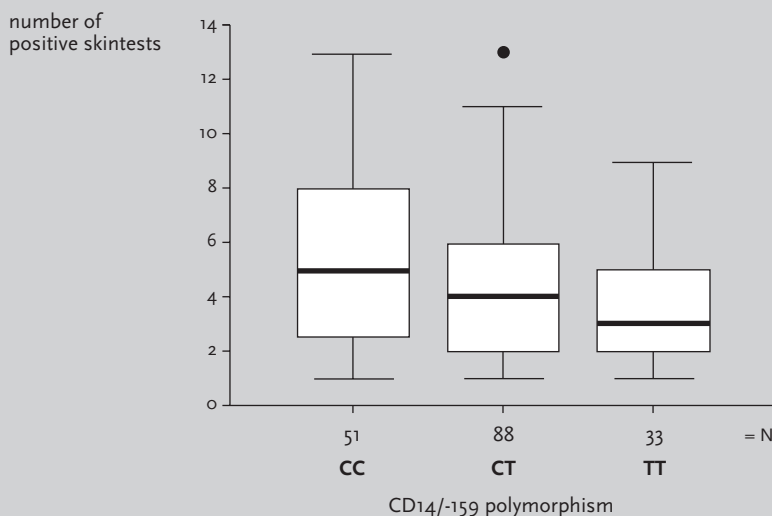
In a logistic regression analysis, CC homozygotes had a higher frequency of reporting hayfever (odds ratio (OR) 2.15, 95% confidence interval (CI) 1.18-3.92 and allergic rhinitis (OR 1.80, 95% CI 1.08-2.99), compared to individuals carrying CT and TT. After introduction of skin test positivity in three classes (0, 1-4, ≥ 5 positive skin tests) into this regression analysis, this association was no longer significant (allergic rhinitis: CD14 CC homozygotes OR 1.54, 95% CI 0.89-2.67; skin test positivity OR 2.53, 95% CI 1.88-3.43; hay fever: CD14 CC homozygotes: OR 1.78, 95% CI 0.92-3.45; skin test positivity OR 3.61, 95% CI 2.39-5.44).

Association of the C allele with asthma

The CD14/-159 genotype was not associated with doctor's diagnosed asthma and bronchial hyperresponsiveness to histamine, and peripheral blood eosinophilia ($p > 0.05$). In the total population of asthmatics and their spouses, the CD14 genotype showed borderline significance with prebron-

chodilator FEV₁ expressed as percentage of predicted in a codominant model (p=0.05) and in a recessive model for the C allele (p=0.015). The CD14/-159 was not associated with FEV₁ level when analyzed within the group of asthma patients. Reversibility to a bronchodilator expressed as the change in FEV₁ was not related to the CD14 genotype neither in the total nor in the asthmatic population.

Figure 2 Boxplot of number of positive skintests within skintest positive individuals.



This boxplot shows medians and interquartile ranges. P<0.01 for CC versus CT and TT (recessive model) ; p<0.05 for CC versus CT versus TT (codominant model).

DISCUSSION

This study confirms an association of the CD14/-159 promoter polymorphism with the number of positive skin tests and total serum IgE levels in skin test positive individuals. In this population selected for probands with asthma, as well as in the population studied by Baldini et al., the CD14/-159 genotype was not associated with atopy defined by at least one positive skin test.¹⁵ Therefore, the CD14/-159 genotype does not appear to represent a susceptibility gene for the development of atopy, yet it appears to produce a more severe atopic phenotype, that is, a higher total number of positive skin tests and a higher total IgE level in skin test positive individuals. In the population of children as described by Baldini et al., the C allele of the CD14/-159 polymorphism had a dominant effect on total IgE levels and the number of positive skin tests.¹⁵ In our data, the C allele is associated with a higher number of positive skin tests in a codominant model, and with higher total IgE levels and higher number of positive skin tests in a recessive model. The results confirm the importance of the C allele

influencing the expression and severity of the atopic phenotype. One might argue that this difference in the genetic model suggests that these data do not confirm the previous association study completely. However, we regard this finding as a confirmation, given the same direction of the phenotypical effects of the C allele (higher number of skin tests, higher serum IgE levels). The unresolved difference between these studies is the influence of having one C allele since the difference between the genetic models is the grouping of the heterozygotes. Possible explanations include differences in recruitment strategies of these populations, an age effect (mean age of our population is 52 years versus 14 years in the study of Baldini), or different gene-gene, and gene-environmental interactions in these different populations. We could not find a significant association of CD14/-159 with phenotypes of asthma, such as bronchial hyperresponsiveness and physician's diagnosis of asthma. These data fit our hypothesis that separate genes appear to regulate susceptibility to high total serum IgE levels and bronchial hyperresponsiveness on chromosome 5q.¹² Atopy and asthma, while separate disease entities, are closely related and it is difficult to distinguish their specific expression on the clinical phenotype. Several studies have shown that total serum IgE levels as well as the number of positive skin prick tests are associated with the presence of bronchial hyperresponsiveness, the development of asthma symptoms and a physician's diagnosis of asthma.^{18,19} Due to this close interrelation of asthma and atopy, alleles that are associated with atopy will be overrepresented in asthmatic individuals when compared with the general population. It is possible that genetic studies that separate the effects of atopic and asthmatic genotypes may increase our basic understanding of how allergic factors affect the development and progression of asthma.

A genetic association may be confounded by population admixture or can be observed as the result of linkage disequilibrium with another allele.²⁰ Since all individuals in our study are white and from a relatively homogeneous population in the northern part of the Netherlands, association due to population admixture seems very unlikely. Linkage disequilibrium as a possible confounder cannot be excluded. However, other polymorphisms in the promoter region of the CD14 gene have not been identified to date.^{15,21} The same promoter polymorphism was reported by Hubacek and coworkers in a study on myocardial infarction (-260 C to T transition). CD14/-159 is 260 base pairs upstream from the major translation region of CD14 (J. A. Hubacek, personal communication). Interestingly, in this study the T allele had a higher frequency in survivors of myocardial infarction compared to a control group indicating a possible role of CD14 in atherosclerosis.²²

Hall²³ suggested four criteria for a specific gene to become an established biological candidate gene in a complex disease: (1) consistent association; (2) location of the gene in a chromosomal area of linkage; (3) change in protein level or function by the mutation and, finally, (4) biological plausibility of the gene for the disease.

Two previous papers have reported association of CD14/-159 with serum total IgE levels.^{15,24} Apart from the paper by Baldini and coworkers, Gao and

coworkers²⁴ studied the association of this polymorphism in a British (n=300) and Japanese population (n=200). The CD14 genotype appeared to be associated with total IgE levels in RAST-negative individuals in a recessive model for the C allele in the British sample. This finding differed from the results in skin test-positive individuals in the US and the Netherlands. These authors concluded that CD14 might not be an atopy locus, but may act as a basic modifier of serum total IgE levels.

The CD14 gene is localized on chromosome 5q31.1, an area consistently reported to be linked to phenotypes of asthma and atopy. However, linkage analysis for a modifier effect within skin test positive family members has not been performed.^{9,12}

The functional role of CD14/-159 was indicated by the finding that CC homozygotes have lower levels of sCD14 and a lower density of CD14 on monocytes than TT homozygotes.^{15,22} This suggests that CD14/-159 could influence the transcription rate of the CD14 gene. CD14/-159 is near an SP1 binding site that has a major influence on monocyte specific expression of CD14, and near a CCAAT/enhancer-binding protein site that may play an important role in promoter activation of the CD14 gene during monocyte development.^{25,26} In addition, CD14/-159 is one basepair upstream from a putative AP-2 binding site.²⁵ More functional studies are needed to study the effect of CD14/-159 on promoter activity.

Finally, is CD14 a plausible biologic gene for atopy? CD14 is a multifunctional receptor and may play a role in different biological and pathophysiological processes: apoptosis, sepsis, and inflammatory diseases, such as atherosclerosis and atopy.^{5-7,27,28} There are several plausible explanations for a possible role of CD14 in atopy. CD14 on monocytes and polymorphonuclear cells functions as a receptor for LPS, thereby inducing mediator and cytokine release, including IL-6 and IL-8. Blockade of CD14 prevents release of LPS induced cytokines. Thus, CD14 may be involved in a pro-inflammatory pathway through the release of cytokines.⁷ In addition, Jabara and Vercelli²⁹ suggested a direct action of CD14 on monocytes, thereby inhibiting IgE production by B-lymphocytes. Finally, Holt and coworkers⁶ suggested that LPS or other bacterial wall products could stimulate antigen presenting cells (APCs) (e.g., dendritic cells) to produce IL-12 through the action of sCD14.³⁰ The source of LPS could be either the microbial flora from the gastro-intestinal tract, inhaled LPS from house dust, or bacterial infection.⁶ Subsequently, genetically determined higher levels of sCD14 in serum¹⁵, or a higher density of membrane bound CD14 could result in a stronger IL-12 signal of these APCs, which in turn may be a potent signal for Th1 maturation in early life. The interesting question is whether CD14-LPS interactions have a relevant role in the development of atopy. If this is true, the interaction of age, infectious exposure as well as environmental factors such as allergens needs to be investigated.

Besides the possible role of CD14 in the development of atopy, we studied CD14 genotype in relationship to asthma severity. Several studies have indicated that LPS inhalation in patients with asthma is associated with the severity of asthma, as assessed by medication use, clinical scores and an increased

bronchial responsiveness to histamine.³¹⁻³³ From studies of bronchoalveolar lavage, it has been suggested that CD14 mediated cell activation could play a role in the inflammatory response after allergen challenge.^{34,35} We asked if genetic variation in the CD14 gene could therefore play a modifying role in asthma severity. In the combined sample of cases and controls, evidence for an association of CD14/-159 with prebronchodilator FEV₁ levels was identified. This association was not statistically significant in the group of asthma patients only. One reason for this may be lack of power. We conclude that these analyses need to be repeated in larger sample size before definitive conclusions can be drawn.

In summary, in this study the association of a -159 C to T promoter polymorphism and atopy is confirmed. Homozygotes for the C allele had higher number of positive skin tests and higher total serum IgE levels (in skin test positive individuals) and subsequently, more self reported allergic rhinitis and hay fever.

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