

***IL-10* Gene Polymorphism Is Associated With Preschool Atopy and Early-Life Recurrent Wheezing After Bronchiolitis in Infancy**

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Summary. Background: Variations in the genes that regulate innate immunity responses may be associated with susceptibility to asthma or atopy after early-life bronchiolitis. The aim of this study was to evaluate the association between four different polymorphisms of the *IL-10* gene at rs1800871, rs1800872, rs1800890, and rs1800896, either alone or in combination, and post-bronchiolitis asthma or allergies at 5–7 years of age. Methods: Data on single nucleotide polymorphisms (SNP) of *IL-10* rs1800896 (–1082G/A), rs1800871 (–819C/T), rs1800872 (–592C/A), and *IL-10*rs1800890 (–3575T/A) were available for 135 children. Polymorphisms and their associations with asthma and allergies were studied in 135 preschool-aged children who had been hospitalized for bronchiolitis at age 0–6 months. Their parents were interviewed to record the children's history with asthma and allergies from infancy to the present. Results: At 6.4 years (mean), asthma was present in 17 children (12.6%), while recurrent wheezing during the first 7 years of life was present in 39 (28.9%) children. Fifty-three (39.3%) study participants had current atopy (atopic eczema or allergic rhinitis). Eight (72%) of 11 children with the *IL-10* rs1800896, *IL-10*rs1800871, and *IL-10* rs1800872 combination AA + CT + CA had current atopy ($P=0.02$ vs. 38% in other genotype combinations). Twenty-three (56%) children with the *IL-10* rs1800871C/T or *IL-10* rs1800872C/A genotype had present atopy versus 34 (38%) with other *IL-10* genotypes ($P=0.03$). Between 2 years and 3 years of age, 27% of ATA haplotype carriers had asthma versus 13.7% of other haplotype carriers ($P=0.02$). Conclusions: *IL-10* polymorphisms at rs1800871, rs1800872, rs1800890, and rs1800896 seem to be associated with elevated allergies and/or recurrent wheezing risk in later childhood, after early-life bronchiolitis. **Pediatr Pulmonol.** 2016; 9999:XX–XX. © 2016 Wiley Periodicals, Inc.

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INTRODUCTION

In earlier studies, asthma prevalence rates as high as 50% were reported after early-life bronchiolitis.^{1–3} Though several studies have been conducted on this issue, the mechanism that triggers the development of asthma after bronchiolitis is still, in many respects, obscure. Though atopy and asthma are connected, the link between bronchiolitis and atopic sensitization or clinical atopy is less evident.^{4,5} The well-known risk factors for asthma, like parental asthma, a patient's own early atopy and parental, especially maternal, smoking, have also been confirmed by post-bronchiolitis follow-up studies.^{6,7} Increasing evidence is accumulating that genetic variations in patients' immune systems are associated with the risk of developing asthma and atopy during childhood.^{8–10} Interestingly, children who present with severe early-life infections, like bronchiolitis, have differed from healthy children in terms of their immune responses.¹¹

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According to the hygiene hypothesis, lower microbial exposure may lead to insufficient maturation of the immune system during the early years of life. Clinically, this results in a higher prevalence of atopy later in life.⁵ On the contrary, severe infections in early childhood may trigger permanent alterations in immunity that favor Th2-oriented responses at the expense of Th1-oriented responses.¹² In addition, genetic predispositions to either strong, pro-inflammatory responses or weak, anti-inflammatory responses have been linked with a susceptibility to atopic diseases.⁵

Interleukin-10 (*IL-10*) is an anti-inflammatory cytokine that regulates both Th1 and Th2 immune responses.¹³ *IL-10* is the key regulator of immune responses that protect the host from abnormally strong immune responses, which, if not properly controlled, can lead to allergic inflammation and atopic diseases.¹⁴ Genetic variation in the promoter region of *IL-10* has been linked with *IL-10* production, which has raised interest in investigating the associations between variants of the *IL-10* gene and different disease outcomes.¹³ Previous studies have revealed several associations between *IL-10* gene single nucleotide polymorphisms (SNPs) and autoimmune disorders, allergic diseases, or asthma.^{8,9,15,16} The proximal promoter region *IL-10* SNPs rs1800896, rs1800871, and rs1800872 form a distinct haplotype. The ATA haplotype in particular is associated with low *IL-10* production.¹⁷ The carriage of the variant allele A in the distal promoter region rs1800890 has also been linked with low *IL-10*¹⁸ production.

Previously, we reported an association between the carriage of *IL-10* rs1800896 allele A and an increased risk for asthma at preschool age in this same post-bronchiolitis cohort.¹⁶ In addition, the *IL-10* rs1800896, rs1800871, and rs1800872 AA + CT + CA genotype and the ATA haplotype were associated with higher resistance and lower reactance, respectively, in baseline impulse oscillometry.¹⁹

Our hypothesis was that children who are low producers of *IL-10* are at an increased risk for the later development of allergies and/or asthma, following early-life bronchiolitis. The aim of the study was to evaluate the association between *IL-10* gene polymorphisms at rs1800871, rs1800872, rs1800890, and rs1800896, either alone or in combination, and post-bronchiolitis asthma or allergies at 5–7 years of age.

METHODS

Subjects

Previously healthy, full-term infants under 6 months of age who were hospitalized for bronchiolitis from December 1st, 2001 to May 31st, 2002, and from October 28th, 2002, to May 31st, 2004, at the Department of Pediatrics, Tampere University Hospital, Finland were enrolled in the study. The diagnosis of

bronchiolitis was based on clinical findings: rhinorrhea, cough, tachypnea, and diffuse wheezes and/or inspiratory crackles.²⁰ The follow-up visits were arranged between 2008 and 2010, when the children were 5–7.5 years of age. A total of 166 children participated in the follow-up study; 127 attended the clinical visit and 39 completed the questionnaire.²¹ Asthma and allergy-related symptoms were reviewed throughout their childhood with a structured questionnaire and interviews with their parents. The diagnosis of asthma and age of diagnosis were recorded. Current asthma was registered if (i) the child was on continuous inhaled corticosteroid (ICS) medication, or (ii) there had been at least one period of doctor-diagnosed wheezing, prolonged cough, or night cough without infection during the last 12 months and hyper-reactivity was detected in the exercise challenge test (ECT) by impulse oscillometry (IOS) under age 7 and by flow-volume spirometry (FVS) in study participants over age 7.²¹ In addition, if the child had at least three obstructive bronchitis episodes between 1 years and 6 years of age, which were verified by a doctor, and was on continuous inhaled steroid medication, the term “recurrent wheezing” was applied. The presence of current allergic rhinitis and atopic dermatitis was inquired about in the questionnaire and interview, and only doctor-diagnosed cases were registered with symptoms during the recent 12-month period. Current atopy was considered if the child had either allergic rhinitis or atopic dermatitis. In addition, only doctor-diagnosed cases of parental asthma, allergic rhinitis, or allergic conjunctivitis were registered. The etiology of bronchiolitis was studied upon admission by polymerase chain reaction (PCR) and the most common viral infection was respiratory syncytial virus (RSV), which was identified in 70.5% cases.²¹

Genotyping

In all, 135 blood samples were available for genetic analyses, including single nucleotide polymorphisms of *IL-10* rs1800896, rs1800871, rs1800872, and rs1800890 genes. Genotyping of the *IL-10* rs1800896 (–1082G/A) was performed using the ABI PRISM 7000 Sequence Detection System.²² The *IL-10* rs1800890 (–3575A/T) genotypes were identified by pyrosequencing, as described earlier.¹⁹ The detection of *IL-10* rs1800871 (–819 A/G) and rs1800872 (–592T/G) was performed using PCR and sequencing the amplified region; the SNPs at position –819 and –592 was detected simultaneously in one PCR reaction. The sequence of PCR primers was as follows: forward 5'-TAGGTCTCTGGGCCTTAGTT-3' and reverse 5'-AAGGCCAATTTAATCCAAGGTT-3', as described recently.¹⁹ The SNPs rs1800871 and rs1800872 are co-segregating and all four of the SNPs studied are highly linked.¹⁹

Ethics

The study was approved by the Ethics Committee of the Tampere University Hospital District. An informed consent was obtained from parents before enrollment both in infancy and at the 5- to 7.5-year control visit, including permission to study genes associated with susceptibility to asthma and allergies. The personal details of the patients were not given to the genetic laboratories.

Statistics

All statistical analyses were conducted using the SPSS package, version 21 (IBM Corp., Armonk, NY). Chi square and Fisher's exact tests were used for categorized variables, while the Student's *t*-test was utilized for normally distributed continuous variables and the Mann-Whitney test was used for non-normally distributed continuous variables. The results were expressed as frequencies, proportional frequencies, means, medians, and standard deviations (SD). $P < 0.05$ was considered to be statistically significant.

Deviations from Hardy-Weinberg (HW) equilibrium were calculated with the Haploview 4.2 program. $P > 0.05$ was considered to be statistically significant. First, all genotypes were analyzed independently regarding their associations with allergies or asthma. Thereafter, haplotype analyses were conducted combining *IL-10* rs1800896, rs1800871, and rs1800872 SNPs.

Logistic regression analyses were performed to control the potential confounding early-life factors. Early-life risk factors selected for adjusted analyses included age, gender, maternal atopy, and the etiology of bronchiolitis. The results were expressed as adjusted odds ratios (AOR) and their 95% confidence intervals (95%CI).

RESULTS

The mean age of the 135 children who participated in the clinical study was 6.4 years (SD 0.48), and 69 (51%) were male. Current asthma was present in 17 (12.6%) children, and recurrent wheezing between 1 year and 6 years of life was present in 39 (28.9%) children. Recurrent wheezing was most prevalent between 2 years and 3 years of life, when 23 (17.0%) children had suffered from doctor-diagnosed recurrent wheezing episodes. Current atopic dermatitis was present in 47 (34.8%) children and current allergic rhinitis had been present in 36 (26.7%) children during the follow-up visit conducted 12 months prior. A total of 53 (39.3%) study participants had current atopy (atopic eczema or allergic rhinitis).

The minor allele frequency for *IL-10* rs1800896 (G>A) was 0.44, as previously published,¹⁶ 0.21 for *IL-10* rs1800871 (C>T) and rs1800872 (C>A), and 0.39 for rs1800890 (A>T).

The *IL-10* rs1800890 genotype was A/A in 52 (38.5%) cases, A/T in 70 (52%) cases, and T/T in 17 (12.6%) cases. There were no significant associations between *IL-10* rs1800890 genotypes or allele frequencies and current atopic dermatitis, allergic rhinitis, or combined atopy (Table 1), nor with current asthma or recurrent wheezing episodes during childhood (Table 1).

The *IL-10* rs1800871/*IL-10* rs1800872 genotype was C/C (both) in 78 (58%) cases, C/T or C/A in 41 (30%) cases, and T/T or A/A in four (3%) cases. Twenty-six (57.8%) children with the variant *IL-10* rs1800871C/T or T/T, or as well with the variant *IL-10* rs1800872C/A or AA genotype, had current atopy versus 31 (33.4%) with wild *IL-10* rs1800871 or *IL-10* rs1800872 genotypes ($P = 0.02$) (Table 1). The OR was 2.56 (95%CI: 1.1–4.5) when adjusted for early-life risk factors, gender, age,

TABLE 1—The Prevalence of Atopic Dermatitis, Allergic Rhinitis, Atopy, Recurrent Wheezing During Childhood, or at the Follow-Up Regarding to *IL-10* rs1800890, rs1800871, and rs1800872 Single Nucleotide Polymorphisms

<i>IL10</i> genotypes and alleles	Atopic dermatitis (n = 47)	Allergic rhinitis (n = 36)	Atopy (n = 57)	Recurrent wheezing (n = 39)	Current asthma (n = 17)
<i>rs1800890</i>					
AA (n = 52)	20	14	22	16	8
AT (n = 70)	21	17	29	19	7
TT (n = 17)	6	5	6	4	2
T-allele (n = 104)	27	22	35	23	9
A-allele (n = 278)	41	31	51	35	15
<i>rs1800871</i> and <i>rs1800872</i>					
CC (n = 78)	25*	23	31**	24	11
CT/CA (n = 41)	18***	12	23** ¹	13	5
TT/AA (n = 4)	4	2	3	2	1
C-allele (n = 221)	43***	35	54***	37	16
T-/A-allele (n = 49)	22*	14	26** ²	15	6

¹aOR (gender, age, maternal asthma, etiology of bronchiolitis): 2.48, 95%CI: 1.15–5.4.

²aOR (gender, age, maternal asthma, etiology of bronchiolitis): 2.56, 95%CI: 1.1–4.5. All other associations non-significant.

* $P = 0.05$, ** $P < 0.05$, *** $P > 0.05$.

TABLE 2—The Prevalence of Atopic Dermatitis, Allergic Rhinitis, Atopy, Recurrent Wheezing During Childhood or Asthma at the Follow-Up Regarding *IL10* rs1800896, rs1800871, and rs1800872 Genotype Combination

<i>IL10</i> genotype combination	Atopic dermatitis (n = 47)	Allergic rhinitis (n = 36)	Atopy (n = 57)	Recurrent wheezing (n = 39)	Current asthma (n = 17)
GCC/GCC (n = 21)	7	5	6	6	2
GCC/GTA (n = 7)	4	2	4	1	1
GTA/GTA (n = 4)	1	2	1	0	0
GCC/ACC (n = 49)	12	13	19	13	6
GCC/ATA (n = 23)	7	5	11	8	3
ACC/ACC (n = 20/18)	7	4	6	5	2
ACC/ATA (n = 11)	7*	5	8*, ¹	4	2
ATA/ATA (n = 4)	2	0	2	2	1

¹aOR (gender, age, maternal asthma, etiology of bronchiolitis): 2.2, 95%CI: 1.4–4.8.

**P* = 0.02.

maternal asthma, and the etiology of bronchiolitis. However, there were no significant associations of *IL-10* rs1800871 or *IL-10* rs1800872 genotypes or allele frequencies with current asthma or asthma (Table 1).

We constructed genotype combinations from the three *IL-10* genes situated in the proximal promoter region of *IL-10* rs1800896, *IL-10* rs1800871, and *IL-10* rs1800872. Eight (72%) of 11 children with the combination AA (wild, homozygous) CT (variant, heterozygous) + CA (variant, heterozygous) (ACC/ATA) had current atopy (*P* = 0.02 vs. 38% in other genotype combinations) (Table 2). The OR, adjusted for gender, age, maternal asthma, and the RSV etiology of bronchiolitis, was 2.2 (95%CI: 1.4–4.8). However, none of the combined *IL-10* genotypes were associated with current asthma or with recurrent wheezing during childhood (Table 2). There were only four children who were homozygous for the variant *IL-10* rs1800871 and *IL-10* rs1800872 alleles (TT + AA), and so their role could not be analysed by statistical means.

In haplotype analyses, current atopic dermatitis, allergic rhinitis, or combined atopy had no significant associations with any of the investigated haplotypes of *IL-10* rs1800896, *IL-10* rs1800871, and *IL-10* rs1800872 (Table 3). Instead, carriage of the ATA haplotype, including the major *IL-10* rs1800896 allele A, the minor

IL-10 rs1800871 allele T, and the minor *IL-10* rs1800872 allele A, showed a significant association with recurrent wheezing at 1–3 years of age (Fig. 1 and Table 4). However, no such association was found with current asthma (Fig. 1 and Table 4). Between 1 year and 2 years of age, 21.6% of the ATA haplotype carriers had recurrent wheezing versus 10.5% of other haplotype carriers (*P* = 0.04). This finding was robust to adjustments with early-life risk factors, gender, age, maternal asthma, and the RSV etiology of bronchiolitis (OR: 2.2, 95%CI: 1.3–8.2). Further, between 2 years and 3 years of age, 27% of ATA haplotype carriers had asthma versus 13.7% of other haplotype carriers (*P* = 0.02, adjusted OR: 2.7, 95%CI: 1.2–7.2).

DISCUSSION

There were four main results in our present study investigating atopy and asthma prevalence after early-life

TABLE 3—The Prevalence of Atopic Dermatitis, Allergic Rhinitis, and Atopy at the Follow-Up at Preschool Age After Early-Life Bronchiolitis in Relation to *IL10* rs1800896, rs1800871, and rs1800872 Haplotype

<i>IL10</i> haplotype	Atopic dermatitis (n = 47)	Allergic rhinitis (n = 36)	Atopy (n = 57)
GCC (n = 100)	29	27	38
GTA (n = 11)	5	4	5
ACC (n = 79)	25	24	32
ATA (n = 37)	15	10	19

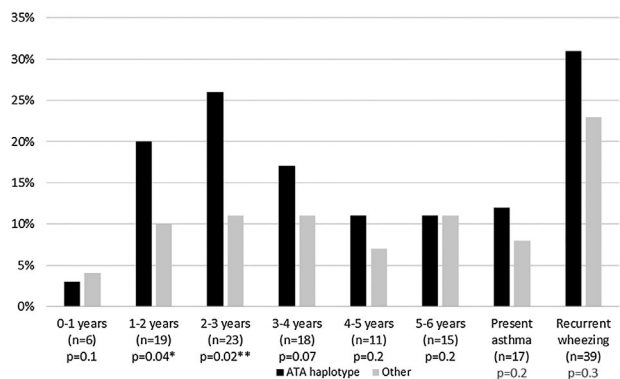


Fig. 1. Prevalence of recurrent wheezing during first 6 years of life in relation to ATA haplotype (*IL10* rs1800896, rs1800871, and rs1800872) versus other haplotypes. N = total cases of recurrent wheezing/age. * = aOR (gender, age, maternal asthma, etiology of bronchiolitis): 2.2, 95%CI: 1.3–8.2. ** = aOR (gender, age, maternal asthma, etiology of bronchiolitis): 2.7, 95%CI: 1.2–7.2.

TABLE 4—The Prevalence of Present Asthma at Preschool Age, Recurrent Wheezing During Childhood, and Recurrent Wheezing According to Age After Early-Life Bronchiolitis in Relation to *IL 10*rs1800896, rs1800871, and rs1800872 Haplotype

<i>IL10</i> haplotype	Asthma at follow-up (n = 17)	Recurrent wheezing during 0–6 years (n = 39)	Recurrent wheezing at 0–1 years (n = 6)	Recurrent wheezing at 1–2 years (n = 19)	Recurrent wheezing at 2–3 years (n = 23)	Recurrent wheezing at 3–4 years (n = 18)	Recurrent wheezing at 4–5 years (n = 11)	Recurrent wheezing at 5–6 years (n = 15)
GCC (n = 100)	13	26	4	9	13	12	9	13
GTA (n = 11)	1	1	0	0	0	0	0	1
ACC (n = 79)	12	21	4	11	13	10	6	8
ATA (n = 37)	6	12	1	8*	10**	7	5	5

* $P = 0.04$, aOR (gender, age, maternal asthma, etiology of bronchiolitis): 2.2, 95%CI: 1.3–8.2.

** $P = 0.02$, aOR (gender, age, maternal asthma, etiology of bronchiolitis): 2.7, 95%CI: 1.2–7.2.

bronchiolitis in relation to four *IL-10* SNPs. First, atopy was highly prevalent (72.7%) at preschool age among children with the combined *IL-10* rs1800896, rs1800872, and rs1800872 genotype AA+ CT+CA (ACC/ATA). The genotypes CT in *IL-10* rs1800871 and CA in rs1800872 are heterozygous, but the number of homozygous variant genotypes was too small for statistical analysis. Second, children with the variant *IL-10* rs1800871 CT and rs1800872 CA genotypes, and carriers of the variant alleles T and A, respectively, more often had current atopic dermatitis and combined atopy compared to those with wild genotypes or carriers of wild alleles. Third, recurrent wheezing was more common between 1 year and 3 years of age among *IL-10* rs1800896, rs1800872, and rs1800872 ATA haplotype carriers after hospitalization for bronchiolitis in infancy, but this trend seemed to disappear by school age. Fourth, the studied polymorphisms of *IL-10* rs1800890, rs1800872, or rs1800871 were not associated with increased asthma prevalence at preschool age or with recurrent wheezing during early childhood. The major and minor allele frequencies are comparable with Finnish and European population data.²³

In our present post-bronchiolitis study, children genetically determined to be low producers of *IL-10* had atopy significantly more often at preschool age than children with other genotypes. The children with the combined low-producing *IL-10* rs1800896, rs1800872, and rs1800872 ACC/ATA genotype exhibited a twofold increase in the prevalence of current atopy compared to other genotypes. The same polymorphisms investigated in the present study have previously been linked with atopy^{8,9,15} and asthma or wheezing symptoms.^{24,25} Recently, the *IL-10* rs1878672 polymorphism, which is closely linked to rs1800871, was associated with wheezing and atopic dermatitis at 3 years of age.⁸ However, our follow-up study is the only post-bronchiolitis cohort study that investigates the link between disease outcomes and genetic variations in the genes that encode cytokines and regulate immune responses.

The hygiene hypothesis posits that the bacterial and viral infections encountered during early life shift the balance of maturing immunity from infantile Th2-type immunity toward Th1-type immunity. Recently, the role of regulatory T-cells (Treg) and cytokines has also been emphasized, as early-life infections have been shown to increase the secretion of anti-inflammatory cytokines, mainly *IL-10*.⁸ During the first 2 years of life, *IL-10* is the key regulator of the shift from infantile Th2-type responses toward Th1-type, non-allergic immunity.¹³ Further, data derived from cord blood samples have demonstrated that *IL-10* seems to influence early immune maturation before clinical symptoms of allergic disease have developed.⁸ Thus, the upregulation of *IL-10* genes has been the key driver of decreased Th2-oriented responses.²⁶ Therefore, the suppressed secretion of allergy-related cytokines, like IL-4, IL-15, and IL-13, seems to be associated with phenotypes that are not related to allergies.⁵ In contrast, lower levels of secreted *IL-10* have been associated with increased Th2-type responsiveness, as well as with lowered immunological tolerance.¹³ In all, our finding of high atopy rates after severe early-life infections among children with potentially compromised immunity accords well with these earlier findings.

In our previous reports, we demonstrated that carriage of the *IL-10* rs1800896 minor allele A was significantly associated with preschool asthma and that combined low-producing variants of *IL-10* rs1800896, rs1800871, and rs1800872 genes were linked with obstructive lung function parameters.¹⁶ In the present study, children with the *IL-10* rs1800896, rs1800871, and rs1800872 ATA haplotype, the low *IL-10*-producing genetic variant, were more likely to wheeze during the first years of life, but at preschool age, there were no longer any significant differences between ATA or other haplotypes and asthma. Therefore, our finding that *IL-10* polymorphisms were significantly associated with changes in lung function parameters was somewhat surprising, as this association was not reported in clinical findings on asthma at preschool age. The probable explanation for this apparent

discrepancy in our results derives from the different phenotypes of young asthmatics. The majority of children who wheeze before reaching school age represent a group called “transient wheezers,” and this form of wheezing is not usually linked with atopy and more permanent forms of wheezing.²⁷ Hence, our finding regarding early wheezing and the ATA haplotype more likely derives from poorly controlled host-defense responses against viral pathogens than from atopy development and thus, more permanent forms of asthma. In lung tissue, *IL-10* is secreted mainly by alveolar macrophages and lower levels of secreted *IL-10* increase the risk of more invasive virus infections.²⁸ Therefore, the insufficient control of host-defense processes due to *IL-10* polymorphism might lead to chronic inflammation and hyper-reactivity in the airways, which causes a susceptibility to wheeze during the early years of life.

The limitations of the present study include its somewhat small sample size for a genetic study and the lack of a control group. However, under-powered statistical analyses due to small sample sizes are more likely to cause type II-errors than false-positive results. Moreover, we were able to control the potentially confounding factors, which, if not controlled, could easily lead to type-I errors, particularly in asthma studies. Furthermore, the prevalence of preschool asthma in a healthy Finnish population was well documented in earlier studies,²⁹ and the figure of 7–9% was surprisingly close to our 12.7% occurrence. In this study, our primary target was to identify risk factors for asthma and allergies in former bronchiolitis patients. From this point of view, the lack of a control group does not significantly diminish the value of our findings. The clear strengths of our study include its prospective design and unique cohort of children. All of the children were under 6 months of age when they were hospitalized due to bronchiolitis.

In conclusion, children with compromised immunity due to *IL-10* polymorphisms seem to be at an elevated risk for allergies and asthma in later childhood, after early-life bronchiolitis. However, we acknowledge that our present results should be interpreted with caution and need to be replicated with larger cohorts. The mechanisms driving these findings were derived from the dominance of Th2-type immune responses and also from insufficient host-defense responses due to genetic variations in the genes that encode *IL-10* cytokine production.

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