IL10 gene polymorphism is associated with preschool atopy and early-life asthma after bronchiolitis in infancy

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Abstract:

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background: Variations in genes regulating innate immunity responses may be associated with susceptibility to asthma or atopy after early-life bronchiolitis. The aim of the study was to evaluate the association between 4 different polymorphisms of the IL-10 gene at rs1800871, rs1800872, rs1800890 and rs1800896 either alone or in combinations, and post-bronchiolitis asthma or allergy at 5-to 7-years of age.

methods: Data on single nucleotide polymorphisms (SNP) of IL10 rs1800896 (-1082G/A), rs1800871 (-819C/T), rs1800872 (-592C/A) and IL10 rs1800890 (-3575T/A) were available for 135 children. Polymorphisms and their associations with asthma and allergy were studied in 135 preschool-aged children hospitalized for bronchiolitis at age 0-6 months. Parents were interviewed to record asthma and allergy from infancy to present.

results: At 6.4 years (mean) asthma was present in 17 (12.6%), and doctor-diagnosed asthma during the first seven years of life was present in 39 (28.9%) children. 53 (39.3%) study participants had current atopy (atopic eczema or allergic rhinitis). Eight (72%) of 11 children with the *IL10* rs1800896, *IL10* rs1800871 and *IL10* rs1800872 combination AA + CT + CA had current atopy (p=0.02 vs. 38% in other genotype combinations). 23 (56%) children with the *IL10* rs1800871 C/T or *IL10* rs1800872 C/A genotype had present atopy versus 34 (38%) with other *IL10* genotypes (p=0.03). Between 2 and 3 years of age, 27% of ATA haplotype carriers had asthma *vs.* 13.7% of other haplotype carriers (p=0.02).

conclusions: *IL10* polymorphisms at rs1800871, rs1800872, rs1800890 and rs1800896 seem to associate with elevated allergy and/or asthma risk in later childhood after early-life bronchiolitis

Key words:

Asthma, atopy, atopic dermatitis, bronchiolitis, genes, interleukin-10, polymorphism, RSV

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Introduction

In earlier studies, even up to 50% asthma prevalence rates have been reported after early-life bronchiolitis [1-3]. Despite of several studies, the mechanism how asthma develops 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 asthma risk factors, like parental asthma, own early atopy and parental, especially maternal smoking have been confirmed also in post-bronchiolitis follow-up studies[6, 7]. Increasing evidence is accumulating that genetic variations in immune system are associated with asthma and atopy risk 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].

According to the hygiene hypothesis, lower microbial exposures may lead to insufficient maturation of the immune system during the first years of life. Clinically, this will result in higher atopy prevalence in later life[5]. In contrary, severe early-life infections in early childhood may trigger permanent alterations in immunity favoring Th2-oriented responses in the expense of Th1-oriented responses[12]. In addition, genetic predisposition to either strong pro-inflammatory or weak anti-inflammatory responses have been linked with susceptibility to an atopic disease[5].

Interleukin 10 (IL-10) is an anti-inflammatory cytokine that regulates both the Th1- and Th2-type immune responses[13]. IL-10 is the key regulator of immune responses protecting the host from abnormally strong immune responses, which, if not properly controlled, can lead to allergic inflammation and atopic disease[14]. Genetic variation in the promoter region of IL-10 has been linked with IL-10 production, which has raised an interest to investigate associations between variants of the IL-10 gene and different disease outcomes[13]. Previous studies have revealed several

associations between IL10 gene single nucleotide polymorphisms (SNPs) and autoimmune disorders, allergic diseases or asthma [8, 9, 15, 16]. The proximal promoter region IL10 SNPs rs1800896, rs1800871 and rs1800872 form a distinct haplotype. Particularly, the ATA haplotype is associated with low IL-10 production[17]. Also, carriage of the variant allele A in the distal promoter region rs1800890 has been linked with low production of IL-10[18].

Previously, we reported an association between carriage of IL10 rs1800896 allele A and an increased asthma risk 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 study hypothesis was that children who are low producers of IL-10 are in an increased risk for later allergy and/or asthma after 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 combinations, and post-bronchiolitis asthma or allergy at 5 to 7 years of age.

Methods

Subjects

Previously healthy, full-term infants under 6 months of age hospitalized due to bronchiolitis from December 1st, 2001 to May 31st, 2002, and from October 28th, 2002, to May 31st, 2004, in 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 to 7.5 years of age. 166 children participated in the follow-up study; 127 attended the clinical visit and 39 completed the questionnaire[21]. Asthma and allergy related symptoms throughout the childhood were reviewed using a structured questionnaire and interviewing the parents. The diagnosis of asthma and and age when the diagnosis had been settled were recorded. Current asthma was registered 1) if the child was on continuous inhaled corticosteroid (ICS) medication, or 2) if 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) in less than 7 years old and by flow-volume spirometry (FVS) in more than 7 years old study participants[21]. Current allergic rhinitis and atopic dermatitis was asked in the questionnaire and interview, and only doctor-diagnosed cases were registered with symptoms during the recent 12 months. Current atopy was considered if the child had either allergic rhinitis or atopic dermatitis. Also, only doctor-diagnosed cases of parental asthma, allergic rhinitis or allergic conjunctivitis were registered. The etiology of bronchiolitis was studied on 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 *IL10* rs1800896, rs1800871, rs1800872 and rs1800890 genes. Genotyping of the *IL10* rs1800896 (-1082G/A) was performed by using the ABI PRISM 7000 Sequence Detection System[22]. The *IL10* rs1800890 (-3575A/T) genotypes were identified by pyrosequencing, as described earlier[19]. The detection of IL10 rs1800871 (-819 A/G) and rs1800872 (-592T/G) were performed using PCR and sequencing of the amplified region, the SNPs at position -819 and -592 were detected simultaneously in one PCR reaction. The sequence of of PCR primers was: forward 5´-TAGGTCTCTGGGCCTTAGTT-3´ and reverse 5´-AAGGCCAATTTAATCCAAGGTT-3´, as described recently[19] The SNPs rs1800871 and rs1800872 are co-segregating and all the studied four SNPs 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 years control visit, including permission to study genes associated with susceptibility to asthma and allergy. The personal details of the patients were not given to the genetic laboratories.

Statistics

All statistical analyses were done using SPSS package version 21 (IBM Corp. NY, USA). Chi square and Fisher's exact tests were used for categorized variables, and student's t-test for normally distributed and Mann-Whitney test for non-normally distributed continuous variables. The results are 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 Haploview 4.2 program. $P>0.05$ was considered to be statistically significant. First, all genotypes were analysed independently regarding their associations with allergies or asthma. Thereafter, haplotype analyses were conducted combining *IL10* rs1800896, rs1800871 and rs1800872 SNPs.

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

Results

The mean age of the 135 children participated in the clinical study was 6.4 years (SD 0.48), and 69 (51%) were males. Current asthma was present in 17 (12.6%) children, and doctor-diagnosed asthma ever that is during the first seven years of life was present in 39 (28.9%) children. Asthma was most prevalent between two and three years of life when 23 (17.0%) children had doctor-diagnosed asthma. Current atopic dermatitis was present in 47 (34.8 %) and current allergic rhinitis in 36 (26.7%) children that is during 12 months prior the follow-up visit. A total of 53 (39.3%) study participants had current atopy (atopic eczema or allergic rhinitis).

Minor allele frequency for *IL10* rs1800896 (G>A) was 0.44, as published previously[16], for *IL10* rs1800871(C>T) and rs1800872(C>A) 0.21, and for rs1800890 (A>T) 0.39.

The *IL10* rs1800890 genotype was A/A in 52 (38.5%), A/T in 70 (52%) and T/T in 17 (12.6%) cases. There were no significant associations between *IL10* rs1800890 genotypes or allele frequencies and current atopic dermatitis, allergic rhinitis or combined atopy (Table 1), nor with current asthma or asthma ever during childhood (Table 1).

The *IL10* rs1800871/ *IL10* rs1800872 genotype was C/C (both) in 78 (58%), C/T or C/A in 41 (30%) and T/T or A/A in 4(3%) cases. Twenty-six (57.8%) children with the variant *IL10* rs1800871 C/T or T/T, or as well with the variant *IL10* rs1800872 C/A or AA genotype had current atopy versus 31 (33.4%) with wild *IL10* rs1800871 or *IL10* rs1800872 genotypes (p=0.02) (Table1). The OR was 2.56, 95% Cl 1.1-4.5) when adjusted for early-life risk factors gender, age, maternal asthma and etiology of bronchiolitis. However, there were no significant associations of *IL10* rs1800871 or *IL10* rs1800872 genotypes or allele frequencies with current asthma or asthma ever (Table 1).

We constructed genotype combinations from the three IL10 genes situated in the proximal promoter region that is from *IL10* rs1800896, *IL10* rs1800871 and *IL10* rs1800872. Eight (72%) of 11 children with the combination AA (wild, homozygous) CT (variant, heterozygous) + CA (variant, hetezygous) (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 RSV etiology of bronchiolitis was 2.2 (95% Cl 1.4-4.8). However, none of the combined IL10 genotypes associated with current asthma or asthma during childhood (Table 2). There were only four children who were homozygous for the variant IL10 rs1800871 and IL10 rs1800872 alleles (TT + AA), and so, their role could not be analyzed 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 *IL10* rs1800896, *IL10* rs1800871 and *IL10* rs1800872 (Table 3). Instead, carriage of the ATA haplotype, including the major *IL10* rs1800896 allele A, the minor *IL10* rs1800871 allele T and the minor *IL10* rs1800872 allele A, showed a significant association with earlier asthma at 1-3 years of age (Figure 1, Table 4). However, no such association was found with current asthma or asthma ever (Figure 1, Table 4). Between 1 and 2 years of age, 21.6% of the ATA haplotype carriers had asthma *vs*. 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 RSV etiology of bronchiolitis (OR 2.2, 95% Cl 1.3-8.2). Further, between 2 and 3 years of age, 27% of ATA haplotype carriers had asthma *vs*. 13.7% of other haplotype carriers (p=0.02, adjusted OR 2.7, 95% Cl 1.2-7.2).

Discussion

There were four main results in our present study investigating atopy and asthma prevalence after early-life bronchiolitis in relation to four *IL10* SNPs. First, atopy was highly prevalent (72.7%) at preschool age among children with the combined *IL10* rs1800896, rs1800872 and rs1800872 genotype AA+ CT + CA (ACC/ATA). The genotypes CT in *IL10* rs*1*800871 and CA in rs1800872 are heterozygous, but the numbers of homozygous variant genotypes were too small for statistical analyses. Second, children with the variant *IL10* rs1800871 CT and rs1800872 CA genotypes, and carriers of the variant allelles T and A, respectively, had more often current atopic dermatitis and combined atopy when compared to those with wild genotypes or carriers of wild alleles. Third, asthma was more common between one and three years of age among *IL10* 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 *IL10* rs1800890, rs1800872 or 1800871 were not associated with increased asthma prevalence at preschool age or in early childhood. The major and minor allele frequencies are comparable with the Finnish and European population data[23]

In our present post-bronchiolitis study, children genetically determined to be low producers of IL-10 had significantly more often atopy at preschool age when compared to children with other genotypes. The children with the combined low producing *IL10* rs1800896, rs1800872 and rs1800872 ACC/ATA genotype had a two-fold increase in current atopy prevalence when compared to other genotypes. Previously, the same polymorphisms investigated in the present study have been linked with atopy[8, 9, 15] and asthma or wheezing symptoms[24, 25]. Recently, *IL10* rs1878672 polymorphism, which is in close linkage with rs1800871, was associated with wheezing and atopic

dermatitis at three years of age[8]. However, our follow-up study is the only post-bronchiolitis cohort study investigating the link between disease outcomes and genetic variations in genes encoding cytokines and regulating immune responses.

The hygiene hypothesis states that bacterial and viral infections encountered during early life shift the balance of maturing immunity from infantile Th2-type immunity towards 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 two years of life, IL-10 is the key regulator of the shift from infantile Th2-type responses towards Th1-type, non-allergic immunity[13]. Further, data derived from cord blood samples has demonstrated that *IL10* seems to influence the early immune maturation before clinical symptoms of allergic disease have developed[8]. Thus, up-regulation 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 not related to allergy[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 rate after severe early-life infection among children with potentially compromised immunity is well in accordance with these earlier findings.

In our previous reports, we demonstrated that carriage of *IL10* rs1800896 minor allele A was significantly associated with preschool asthma and that combined low producing variants of *IL10* 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 1800872 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 results, in which *IL10* polymorphisms associated significantly with changes in lung function parameters, but not in clinical findings of asthma at preschool age, are somewhat surprising. The probable explanation for this apparent discrepancy in our results derives from different phenotypes of young asthmatics. Majority of children that wheeze before school age represent a group called transient wheezers, and this form of wheezing is not usually linked with atopy and more permanent form of wheezing[27]. Hence, our finding regarding early wheezing and ATA haplotype derives more likely from poorly controlled host-defense responses against viral pathogens than from development of atopy, and thus, more permanent form of asthma. In lung tissue, IL-10 is secreted mainly by alveolar macrophages and lower levels of secreted IL-10 increase the risk for more invasive virus infection[28]. Thus, the insufficient control of host-defense processes due to *IL10* polymorphism might lead to chronic inflammation and hyper-reactivity in airways, which causes the susceptibility to wheeze during early years of life.

Limitations in our present study include somewhat small sample size for a genetic study and a lack of a control group. However, under-powered statistical analyses due to small sample size are more likely to cause type II errors than false-positive results. Moreover, we were able to control the potential confounding factors, which, if not controlled, could easily lead to type-I errors, particularly in asthma studies. Further, the preschool asthma prevalence in a healthy Finnish population has been 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 factors predicting asthma and allergy risk in the former bronchiolitis patients. From this view, the lack of a control group does not significantly diminish the value of our findings. The definite strengths of our study

include the prospective design of the study and the unique cohort of study children. All children were under six months of age when hospitalized due to bronchiolitis.

In conclusion, this study brings new insights into the mechanisms of asthma and allergy development after early-life bronchiolitis. Children with compromised immunity due to *IL10* polymorphisms seem to be in elevated allergy and asthma risk in later childhood after early-life bronchiolitis. Mechanisms for these findings derive from dominance of Th2-type immune responses and also from insufficient host-defense responses due to genetic variations in genes encoding IL-10 cytokine production.

Table 1. The prevalence of atopic dermatitis, allergic rhinitis, atopy, asthma during childhood, or at the follow-up regarding to *IL10* rs1800890, rs1800871 and rs1800872 single nucleotide polymorphisms.

IL10 genotypes and alleles	Atopic dermatitis n= 47	Allergic rhinitis n=36	Atopy n=57	Asthma ever n=39	Current asthma n=17
rs1800890					
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
rs1800871 and *rs1800872*					
CC n=78	25 *	23	31 **	24	11
CT / CA n=41	18	12	23 **,†	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 **,‡	15	6

* p=0.05, ** p<0.05, †aOR(gender, age, maternal asthma, etiology of bronchiolitis): 2.48, 95% Cl 1.15-5.4). ‡aOR 2.56(gender, age, maternal asthma, etiology of bronchiolitis), 95% Cl 1.1-4.5). All other associations non-significant.

Table 2. The prevalence of atopic dermatitis, allergic rhinitis, atopy, asthma during childhood or asthma at the follow-up regarding *IL10* rs1800896, rs1800871 and rs1800872 genotype combination.

IL10 genotype combination	Atopic dermatitis n= 47	Allergic rhinitis n=36	Atopy n=57	Asthma ever 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*, †	4	2
ATA/ATA n=4	2	0	2	2	1

*p=0.02, † aOR (gender, age, maternal asthma, etiology of bronchiolitis): 2.2, 95% Cl 1.4-4.8.

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

IL10 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

Table 4. The prevalence of present asthma, doctor-diagnosed asthma during childhood and asthma diagnose according to age at preschool age after early-life bronchiolitis in relation to *IL10* rs1800896, rs1800871 and rs1800872 haplotype

IL10 haplotype	Asthma at follow-up n=17	Asthma during 0-6 years n=39	Asthma at 0-1 years n=6	Asthma at 1-2 years n=19	Asthma at 2-3 years n=23	Asthma at 3-4 years n=18	Asthma at 4-5 years n=11	Asthma 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.

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