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***Interleukin-10 gene polymorphism rs1800896 is associated with post-bronchiolitis asthma at 11-13 years of age***

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**Short title:** *Interleukin-10* polymorphism and post-bronchiolitis asthma

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## ABSTRACT

**Aim** Interleukin-10 (IL-10) is an anti-inflammatory cytokine that is involved with bronchiolitis and asthma. We evaluated associations between four *IL-10* polymorphisms, namely rs1800871, rs1800872, rs1800890 and rs1800896, and post-bronchiolitis asthma in young adolescents.

**Methods** The cohort were 125 children hospitalised for bronchiolitis at Tampere University Hospital, Finland, in 2000-2004, at under six months of age. At 11-13 years, asthma diagnoses and asthma-presumptive symptoms, allergic rhinitis and use of inhaled corticosteroids (ICS) were registered. Data on the four polymorphisms and their genotypes, haplotypes and allele frequencies were analysed in relation to asthma, allergic rhinitis and asthma medication.

**Results** The variant *IL-10* rs1800896 genotype was associated with less persistent asthma at 5-7 and 11-13 years of age (4.3 versus 15.2%,  $p=0.04$ ) than the wild genotype and less ICS use during the previous 12 months (5.4 versus 18.2%,  $p=0.03$ ), as was the variant allele G. Allele A was associated with more persistent asthma and ICS use. The significant differences between the variant and wild genotypes were lost in adjusted logistic regression, but the direction of the association remained.

**Conclusion** *IL-10* rs1800896 gene polymorphism was associated with post-bronchiolitis asthma at 11-13 years of age in children hospitalised for bronchiolitis at under six months.

### Abbreviations

aOR, adjusted odds ratio; CI, confidence interval; FEV1, forced respiratory volume in one second; ; ICS, inhaled corticosteroids; IL-10, Interleukin 10; *IL-10*, *Interleukin 10* gene; RSV, respiratory syncytial virus; SNP, single nucleotide polymorphism; Th, T helper.

### Key notes

- Interleukin 10 (IL-10) is an anti-inflammatory cytokine that is involved with bronchiolitis and asthma.
- We evaluated *IL-10* polymorphisms rs1800871, rs1800872, rs1800890 and rs1800896 in 125 adolescents who had been hospitalised for bronchiolitis when they were under six months of age.
- Data on the four polymorphisms and their genotypes, haplotypes and allele frequencies showed that the *IL-10* rs1800896 variant polymorphism was protective for post-bronchiolitis asthma at 11-13 years of age.

### KEY WORDS

Asthma, Bronchiolitis, Genotypes. Interleukin 10, Polymorphisms.

## INTRODUCTION

Bronchiolitis is the most common respiratory tract infection that leads to infants being brought to the emergency department or admitted to hospital (1). Around 2-3% of infants are hospitalised for viral bronchiolitis during the first year of life and the percentage is fairly constant during this age span (1). Both virus-specific and genetic factors, especially those related to innate immunity, contribute to both disease severity and post-bronchiolitis outcomes, such as the development of asthma (2-4). The production of mediators of innate immunity, like interleukins and other cytokines, is genetically determined (5). An important immunological crossroad is the development of acquired immunity to either the T helper 1 cells (Th1) or the Th2 cells that are regulated by innate immunity (6). Rhinovirus-induced wheezing in infancy has been associated with Th2-oriented immunity and asthma in childhood more often than wheezing induced by other viruses (7).

Interleukin 10 (IL-10) is an anti-inflammatory cytokine that regulates the balance between Th1 and Th2 immune responses (8). The *IL-10* gene that regulates IL-10 production is highly polymorphic (9) and several *IL-10* single nucleotide polymorphisms (SNP) have been associated with atopy and wheezing in young children (10) and childhood asthma phenotypes (11). Four *IL-10* SNPs have been widely studied: rs1800896 (-1082A/G), rs1800871 (-819C/T) and rs1800872 (-592C/A) in the proximal promoter region (9,11-13) and rs1800890 (-3575T/A) in the distal promoter region (14) of the *IL-10* gene. In particular, three polymorphisms, namely rs1800871, rs1800872 and rs1800896, have been associated with the presence of asthma in children (11,15,16).

We previously carried out a prospective follow-up study up 166 children hospitalised for bronchiolitis at less than six months of age when 5-7 years of age (17,18). Earlier studies

from this cohort demonstrated that polymorphisms in the *IL-10* gene were associated with severe rhinovirus bronchiolitis (19) and with post-bronchiolitis asthma (20, 21) and lung function reduction (22) at 5-7 years of age. The aim of this prospective study was to evaluate any associations between the *IL-10* polymorphisms rs1800896, rs1800871, rs1800872 and rs1800890 with asthma when our post-bronchiolitis cohort reached 11-13 years of age.

## **MATERIALS AND METHODS**

In 2000-2004, 187 previously healthy, full-term infants were hospitalised for bronchiolitis at less than six months of age in the department of Paediatrics, Tampere University Hospital, Finland (17, 19). The diagnosis of bronchiolitis was based on clinical findings: rhinitis, short breathing, cough, and diffuse wheezes and, or, inspiratory crackles. During hospitalisation for bronchiolitis, nasopharyngeal aspirates were taken to determine the viral aetiology of the infection, including the respiratory syncytial virus (RSV), using antigen detection and polymerase chain reaction methods (17,19). Data on maternal asthma and atopic dermatitis at less than 12 months of age were registered during hospitalisation or at the control visit at 18 months of age (19).

### **Collecting data at 11-13 years of age**

The follow-up visits at the age of 11-13 years were held in 2014-2015 and 138 children (52% boys) attended (18) at a mean age of 11.7 years (range 10.2-13.2). Before the visit, the parents had completed structured questionnaires consisting of questions on doctor-diagnosed asthma and allergic rhinitis between the control visit at 5-7 years of age and the present visit at 11-13 years of age. Current use of asthma medication, current asthma-presumptive

symptoms and the presence of current asthma diagnoses were explored by the questionnaire, which was developed for this study. At the follow-up visit, the parents and children were interviewed to check the questionnaire data, the children were examined by a doctor and a bronchodilation test was performed (18). The test analysed the best forced expiratory volume in one second (FEV1) from three blows before and 15 minutes after they inhaled 400µg salbutamol using a 0.1mg/dos Ventolin Evohaler (GlaxoSmithKline, London, UK), measured with Vmax V62J Autobox flow-volume spirometry (Becton, Dickinson Co, New Jersey, USA). An increase of 12% or more in FEV1 was regarded as a positive test result and indicated a reversible airway obstruction.

### **Definitions of asthma and allergic rhinitis**

Current asthma was considered to be present if the child had used inhaled corticosteroids (ICS) regularly during the last 12 months. It was also present if the child had suffered from repeated wheezing, or from prolonged cough or night cough, for four weeks or more during the last 12 months and also showed an increase of 12% or more in FEV1 during the bronchodilation test (18). Current allergic rhinitis was considered to be present if it has been diagnosed by a doctor at any age and had been symptomatic during the last 12 months (18). Persistent asthma was defined as having asthma at both of the follow-up visits at 5-7 and 11-13 years of age.

### **Genetics**

A total of 125 blood samples were available for genetic analyses of the SNPs rs1800896, rs1800871 and rs1800872 and 124 were available for the SNP rs1800890 of the *IL-10* gene.

We carried out the genotyping of the *IL-10* rs1800896 (-1082A/G) SNP using the ABI PRISM 7000 Sequence Detection system (Thermo Fisher Scientific, Massachusetts, USA) for both polymerase chain reaction and allelic discrimination, as previously published (19). The *IL-10* rs1800890 (-3575A/T) genotyping was performed by pyrosequencing, as previously published (22). The *IL-10* rs1800871 (-819C/T) and rs1800872 (-592C/A) genotyping was performed using polymerase chain reaction and sequencing of the amplified region. The SNPs at positions -592 and -819 were simultaneously detected in one polymerase chain reaction (22).

*IL-10* rs1800896, rs1800871 and rs1800872 are situated close to each other in the proximal promoter region of the *IL-10* gene and form haplotypes (23). The currently available genetic programmes for haplotypes, like Haploview (Broad Institute, Massachusetts, USA), provide the frequencies of different haplotypes in the study population, but do not identify the individual haplotypes (24). Therefore, we constructed the theoretically possible haplotypes. The *IL-10* rs1800871 and rs1800872 SNPs were in full linkage (22), which means that the major C alleles always occur together and so do the minor T (rs1800871) and A (rs1800872) alleles. Therefore, only four *IL-10* rs1800896, rs1800871 and rs1800872 haplotypes could be constructed. These were the ACC (major allele) and the GCC, ATA and GTA (minor alleles).

## Statistics

The statistical analyses were performed using SPSS for Windows version 23.0 (IBM Corp, New York, USA). The chi-square test was used to analyse the associations between allele or haplotype frequencies and asthma outcomes or allergic rhinitis in the present cohort.

Logistic regression was used to analyse the genotypes between those with and without current asthma, persistent asthma, current ICS treatment for asthma and current allergic

rhinitis. The analyses were initially carried out as univariate analyses and then as multivariate analyses, adjusted for sex, age and early-life risk factors, such as non-RSV aetiology of bronchiolitis, maternal asthma, and atopic dermatitis at less than 12 months of age. The results were expressed as odds ratios (OR), or adjusted ORs (aOR), and 95% confidence intervals (95% CI).

The Hardy-Weinberg equilibrium was tested with the calculator produced by the manufacturer (Chang Bioscience Inc, California, USA), and all four *IL-10* SNPs of rs1800896 (-1082A/G), rs1800871 (-819C/T), rs1800872 (-592C/A) and rs1800890 (-3575T/A) were confirmed to fall within the equilibrium.

## **Ethics**

The Ethics Committee of Tampere University Hospital District approved the study. The parents gave their written informed consent before enrolling the children and at all control visits. The personal data of the study subjects were not given to the laboratories that performed the genetic studies, which were the Department of Medical Microbiology, Tampere University, Tampere, Finland, and the Laboratory of Immunology, National Public Health Institute, Turku, Finland

## **RESULTS**

There were 125 blood samples available for the genetic analyses of the SNPs rs1800896, rs1800871 and rs1800872. One sample was missing for SNP rs1800890 of the *IL-10* gene. The wild *IL-10* rs1800896 genotype AA was present in 33/125 (26.4%) and the variant genotype in 92/125 (73.6%) children, namely GA in 59 (47.2%) and GG in 33 (26.4%). The minor allele frequency (MAF) (G) was 0.500. The wild *IL-10* rs1800890 genotype AA was

present in 49/124 (39.5%) and the variant genotype in 75/124 (60.5%) children, namely AT in 57 (46.0%) and TT in 18 (14.5%). MAF (T) was 0.375. *IL-10* rs1800871 and rs1800872 were in full linkage and 85/125 (68%) children had the wild CC genotype, 34/125 (27.2%) had the variant CT or CA genotype and 6/125 (4.8%) had the variant TT or AA genotype. MAF (T or A) was 0.184.

Based on 125 children, current asthma was present in 15 (12.0%) children, persistent asthma in nine (7.2%) and 11 (8.8%) had used ICS during the last 12 months. The wild genotype (AA) of *IL-10* rs1800896 was associated with more persistent asthma and more use of ICSs during the last 12 months compared to the variant genotypes (AG or GG) (Table 1). MAF (allele G) was found in 33.3% of the children with current asthma and 52.3% without ( $p=0.052$ ), in 22.2% of the children with persistent asthma and 52.2% without ( $p=0.014$ ) and in 22.7% of the children with ICS use and 52.1% without ( $p=0.007$ ). These observations mean that the presence of the variant allele G, either homozygous or heterozygous, was associated with a decreased risk of post-bronchiolitis asthma. However, any asthma parameter did not have a significant association with the presence of wild or variant genotypes or with allele frequencies of *IL-10* rs1800871, rs1800872 or rs1800890 (Table 1).

When the constructed haplotypes of *IL-10* rs1800890, rs1800871 and rs1800872 were included in the analyses, the haplotype GTA, which only consisted of variant alleles, was not present in any of the children with persistent asthma or ICS use during the last 12 months (Table 2). In line with this, the haplotype that consisted of the variant G allele of the *IL-10* rs1800896 and the wild-type C alleles of the *IL-10* rs1800871 and rs1800872 was not associated with current asthma, persistent asthma or ICS use in any of the children (Table 2). The findings did not reach statistical significance but they do offer some additional evidence that the variant allele G of *IL-10* rs1800896 may be protective for asthma.

Current allergic rhinitis was present in 45/125 (36.0%) children. The *IL-10* rs1800871 and rs1800872 polymorphisms were associated with current allergic rhinitis, which means that the presence of the wild CC genotype was associated with less allergic rhinitis than the variant genotypes (Table 2). In line with this, MAF (allele T or A) occurred in 25.6% in children with allergic rhinitis versus 14.4% without allergic rhinitis ( $p=0.001$ ). However, allergic rhinitis had no significant associations with *IL-10* rs1800896 or rs1800890 polymorphisms (Table 2).

In the adjusted multivariate logistic regression, the statistical significances observed in the non-adjusted analyses were lost. The variant genotype of the *IL-10* rs1800896 was no longer significantly protective for the development of post-bronchiolitis asthma, though the aORs were still low, at 0.22 for persistent asthma and 0.32 for ICS use for asthma. Likewise, the *IL-10* rs1800871 and rs1800872 polymorphisms were no longer significantly associated with current allergic rhinitis, although the aOR was still high at 1.75 (Table 3).

## DISCUSSION

There were two main results to emerge from the present study, which focused on the association between four *IL-10* gene polymorphisms and asthma or allergic rhinitis at 11-13 years of age after bronchiolitis in early infancy. First, the variant genotype and the presence of the variant allele in *IL-10* rs1800896 were protective for asthma when it was assessed by two surrogates: persistent asthma from 5-7 to 11-13 years of age and the use of ICS medication for asthma during the last 12 months. The genotype finding lost its statistical significance when the analyses were adjusted for known early-life risk factors of post-bronchiolitis asthma, although the adjusted ORs remained low. Second, the polymorphisms

of *IL-10* rs1800871 and rs1800872 were associated with allergic rhinitis in the non-adjusted analyses.

The current results were in line with our previous observations from the same post-bronchiolitis cohort. The presence of the variant allele G of *IL-10* rs1800896 was protective for post-bronchiolitis asthma at 5-7 years of age, and, correspondingly, the wild-type allele A was associated with an increased asthma risk (20). The presence of the ATA haplotype of *IL-10* rs1800896, 1800871 and 1800872, consisting of the wild-type allele A of *IL-10* rs1800896, was associated with repeated post-bronchiolitis wheezing at 2-3 years of age (21) and with higher resistance and lower reactance in impulse oscillometry at 5-7 years of age (22). This pattern in impulse oscillometry indicated bronchial obstruction. During hospitalisation, the wild AA genotype was over-represented among infants with bronchiolitis caused by other viruses than RSV, mainly by rhinoviruses (19). Since rhinovirus-induced wheezing in early childhood has been associated with later asthma (7), this observation was in line with the observation from this cohort highlighting the role of the allele A, which predisposes patients to asthma, and role of allele G in protecting individuals from asthma (21, 25).

Our current and earlier results on the association between atopy and the four *IL-10* polymorphisms are conflicting. There were no significant associations between atopic dermatitis or allergic rhinitis and the *IL-10* rs1800896 polymorphism at 5-7 years of age (20). The combination of the wild genotype in rs1800896, the variant genotype in rs1800871 and the variant genotype in rs1800872 was associated with atopy at 5-7 years of age, but not with allergic rhinitis when analysed separately at that age (21). In the present study at 11-13 years

of age, the polymorphisms of *IL-10* rs1800871 and rs1800872 were associated with allergic rhinitis, but only in the unadjusted analyses.

Two meta-analyses have been published on the associations between *IL-10* rs1800896 (-1082A/G), rs1800871 (-819C/T) or rs1800872 (-592C/A) polymorphisms and asthma (26,27). The meta-analysis published in 2014 comprised 4,716 adults and children with asthma and 5,093 controls (26). This found that *IL-10* rs1800896 and rs1800872 polymorphisms were associated with asthma susceptibility in atopic children and adults. It also found that the wild genotype in *IL-10* rs1800896 and the variant genotype in *IL-10* rs1800872 were associated with an increased asthma risk (26). The result concerning the *IL-10* rs1800896 polymorphism was in agreement with the observations in the present study. The meta-analysis published in 2016 comprised 2,494 children with asthma and 2,160 controls (27) and included nine of the 11 paediatric studies in the 2014 meta-analysis (26). The conclusion was that there were no significant associations between the *IL-10* rs1800896, rs1800871 or rs1800872 polymorphisms and susceptibility to paediatric asthma (27).

The *IL-10* gene that encodes the structure and production of IL-10 is highly polymorphic (9). The *IL-10* polymorphisms rs1800871, rs1800872 and rs1800896, which were also investigated in the present study, have previously been linked with atopy (10), wheezing (11) and asthma (11,16). IL-10 production plays an important role in controlling the balance of the inflammatory and immunological responses by inhibiting the production of pro-inflammatory mediators (8). The ATA haplotype of *IL-10* rs1800896, rs1800871 and rs1800872, which contain the wild-type A in *IL-10* rs1800896, is a low IL-10 producing haplotype, and has been associated with severe asthma (13). The wild-type allele A of *IL-10* rs1800896 has been

associated with low IL-10 production (9). In infancy this may lead to a delayed shift of Th2-oriented immunity to Th1-oriented immunity (6), which may be one of the mechanisms behind childhood wheezing and asthma (10,28).

The main limitation of our present study was the small sample size for a genetic study. However, under-powered statistical analyses due to small sample sizes are more likely to cause false-negative than false-positive results. This highlights our positive findings on the association between the *IL-10* rs1800896 polymorphism and post-bronchiolitis asthma. The strengths of the present study were the prospective design, careful data collection and long follow up until 11-13 years of age. In addition, we studied all the four *IL-10* SNPs that have been associated with asthma and allergy in different populations in earlier studies. The homogeneity of the study population of the present study, which was ethnically Finnish children, is a clear benefit in genetic studies.

## CONCLUSION

We found preliminary evidence that the *IL-10* rs1800896 polymorphism was associated with asthma at 11-13 years of age in children hospitalised for bronchiolitis at less than six months of age. The variant genotype, and the presence of the variant allele, protected the children in this long-term post-bronchiolitis follow-up study from developing asthma.

## CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare

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**Table 1.** *Interleukin-10* rs1800896, rs1800871 and rs1800872 polymorphisms in the proximal region and *IL-10* rs1800890 polymorphism in the distal promoter region in relation to current asthma, persistent asthma, use of inhaled corticosteroids for asthma and the presence of allergic rhinitis. The detailed numbers and percentages relate to the genotypes in column one.

| Genotypes of the four <i>IL-10</i> genes | Current asthma<br>n=15 | Persistent asthma<br>n=9  | Inhaled cortico-steroids, last 12 months<br>n=11 | Current allergic rhinitis<br>n=45 |
|------------------------------------------|------------------------|---------------------------|--------------------------------------------------|-----------------------------------|
| <b>Rs1800896</b>                         |                        |                           |                                                  |                                   |
| AA (wild)<br>n=33 (%)                    | 6 (18.2)<br>p=0.20     | 5 (15.2)<br><b>p=0.04</b> | 6 (18.2)<br><b>p=0.03</b>                        | 13 (39.4)<br>p=0.39               |
| AG (heterozygous variant)<br>n=59 (%)    | 8 (13.6)               | 4 (6.8)                   | 5 (8.5)                                          | 21 (35.6)                         |
| GG (homozygous variant)<br>n=33 (%)      | 1 (3.0)<br>p=0.05      | 0<br>p=0.05               | 0<br>p=0.03                                      | 11 (33.3)<br>p=0.71               |
| <b>Rs1800871/ 1800872</b>                |                        |                           |                                                  |                                   |
| CC (wild)<br>n=85 (%)                    | 9 (10.6)<br>p=0.48     | 7 (8.2)<br>p=0.41         | 8 (9.4)<br>p=0.51                                | 27 (31.8)<br>p=0.15               |
| CT/CA (heterozygous variant)<br>n=34 (%) | 5 (14.7)               | 1 (2.9)                   | 2 (5.9)                                          | 13 (38.2)                         |
| TT/AA (homozygous variant)<br>n=6 (%)    | 1 (16.7)<br>p=0.54     | 1(16.7)<br>p=0.37         | 1 (16.7)<br>p=0.43                               | 5 (83.3)<br><b>p=0.02</b>         |
| <b>Rs1800890</b>                         |                        |                           |                                                  |                                   |
| AA (wild)<br>N=49 (%)                    | 6 (12.2)<br>p=0.97     | 4 (8.2)<br>p=0.51         | 5 (10.2)<br>p=0.67                               | 20 (40.8)<br>p=0.32               |
| AT (heterozygous variant)<br>N= 57 (%)   | 8 (14.0)               | 4 (7.0)                   | 5 (8.8)                                          | 19 (33.3)                         |
| TT (homozygous variant)<br>N=18 (%)      | 1 (5.6)<br>p=0.32      | 1 (5.6)<br>p=0.61         | 1 (5.6)<br>p=0.50                                | 5 (27.8)<br>p=0.32                |

*IL-10*; *Interleukin-10* gene

**Table 2.** *Interleukin-10* rs1800896, rs1800971 and rs1800972 haplotypes, constructed theoretically, in relation to three definitions of asthma and the presence of allergic rhinitis. The detailed numbers and percentages relate to the haplotypes in column one.

| Haplotype                     | Current asthma<br>n=15 | Persistent asthma<br>n=9 | Inhaled corticosteroids, last 12 months<br>n=11 | Current allergic rhinitis<br>n=45 |
|-------------------------------|------------------------|--------------------------|-------------------------------------------------|-----------------------------------|
| <b>GCC</b><br><b>n=73 (%)</b> | 7 (9.6)<br>p=0.33      | 4 (5.5)<br>p=0.49        | 5 (6.8)<br>p=0.36                               | 25 (34.2)<br>p=0.63               |
| <b>GTA</b><br><b>n=26 (%)</b> | 3 (11.5)<br>p=0.62     | 0<br>p=0.11              | 0<br>p=0.07                                     | 10 (38.5)<br>p=0.77               |
| <b>ACC</b><br><b>n=88</b>     | 13 (14.8)<br>p=0.14    | 8 (9.1)<br>p=0.19        | 10 (11.4)<br>p=0.11                             | 31 (35.2)<br>p=0.78               |
| <b>ATA</b><br><b>n=14</b>     | 3 (21.4)<br>p=0.22     | 2 (14.3)<br>p=0.27       | 3 (21.4)<br>p=0.11                              | 8 (51.7)<br>p=0.08                |
| <b>GCC/GCC</b><br><b>n=23</b> | 0<br><b>p=0.04</b>     | 0<br>p=0.15              | 0<br>p=0.09                                     | 5 (21.7)<br>p=0.08                |
| <b>GCC/GTA</b><br><b>n=8</b>  | 1 (12.5)<br>p=0.65     | 0<br>p=0.54              | 0<br>p=0.47                                     | 4 (50.0)<br>p=0.31                |
| <b>GTA/GTA</b><br><b>N=2</b>  | 0<br>p=0.77            | 0<br>p=0.86              | 0<br>p=0.83                                     | 2 (100)<br>p=0.13                 |
| <b>GCC/ACC</b><br><b>n=42</b> | 6 (14.3)<br>p=0.58     | 4 (21.4)<br>p=0.35       | 5 (11.9)<br>p=0.38                              | 16 (61.9)<br>p=0.73               |
| <b>ACC/ACC</b><br><b>n=20</b> | 3 (15.0)<br>p=0.44     | 3 (15.0)<br>p=0.16       | 3 (15.0)<br>p=0.25                              | 6 (30.0)<br>p=0.54                |
| <b>ACC/ATA</b><br><b>n=10</b> | 2 (20.0)<br>p=0.34     | 1 (10.0)<br>p=0.54       | 2 (20.0)<br>p=0.21                              | 5 (50.0)<br>p=0.34                |
| <b>ATA/ATA</b><br><b>n=4</b>  | 1 (25.0)<br>p=0.40     | 1 (25.0)<br>p=0.26       | 1 (25.0)<br>p=0.31                              | 3 (75.0)<br>p=0.13                |
| <b>ACC/GTA</b><br><b>n=16</b> | 2 (12.5)<br>p=0.61     | 0<br>p=0.28              | 0<br>p=0.21                                     | 4 (25.0)<br>p=0.25                |

*IL-10*; interleukin10 gene

**Table 3.** Multivariate logistic regression: variant *IL-10* rs1800896 and rs1800871/1800872 genotypes in relation to three definitions of asthma and allergic rhinitis.

| Clinical outcome                                                 | <i>IL-10</i> variant genotype rs1800896 | <i>IL-10</i> variant genotype rs1800871/ rs1800872 |
|------------------------------------------------------------------|-----------------------------------------|----------------------------------------------------|
| <b>Current asthma</b><br>aOR (95% CI)                            | 0.61 (0.18-2.05)                        | 1.58 (0.44-5.59)                                   |
| <b>Persistent asthma</b><br>aOR (95% CI)                         | 0.22 (0.04-1.16)                        | 0.65 (0.09-4.96)                                   |
| <b>Inhaled corticosteroids in last 12 months</b><br>aOR (95% CI) | 0.32 (0.08-1.27)                        | 0.86 (0.17-4.38)                                   |
| <b>Current allergic rhinitis</b><br>aOR (95% CI)                 | 1.08 (0.43-2.73)                        | 1.75 (0.72-4.23)                                   |

*IL-10*; interleukin10 gene

The analyses were adjusted for sex, age and early-life risk factors of non-respiratory syncytial virus aetiology of bronchiolitis, asthma in mothers and atopic dermatitis in children at less than 12 months of age.

The results are expressed as adjusted odds ratios (aOR) and 95% confidence intervals (95% CI).

The definitions of current asthma and persistent asthma are presented in the methods.