CD14/Toll-like receptors interact with bacteria and regulatory T-cells in the development of childhood asthma

To the Editor:

The susceptibility to asthma development in childhood is influenced by genetic as well as environmental factors, and interactions between these factors [1–3]. However, at present, their exact role is still largely undetermined. Genetic variations in the innate immune system may lead to different adaptive immune responses to bacteria and may therefore vary the development of asthma [2, 4, 5]. We performed a prospective longitudinal study in preschool children, in which we determined polymorphisms in Toll-like receptors (TLRs) and CD14, the presence of bacteria, and the proportion of regulatory T-cells (Treg) all in relation to an asthma diagnosis at 6 years of age. We hypothesise that specific genetic variants in genes that affect the innate immune system influence the response to bacteria and the recruitment of Treg in preschool children, leading to an increased likelihood of asthma at 6 years of age.

The Asthma DEtection and Monitoring (ADEM) study is a long-term prospective case–control study. A detailed protocol of this study has previously been published [6]. A total of 202 children who had experienced at least two wheezing episodes during their lifetime (International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire) [7] and 50 children without wheezing episodes were included at 2–4 years of age. The children were randomly selected from a random population sample in Limburg, the Netherlands, based on the presence or absence of recurrent wheeze [6]. During the initial visit, saliva or buccal cells (DNA), nasal and throat swabs (bacterial cultures), and blood (Treg) were collected. Participants were genotyped for six single nucleotide polymorphisms in TLR2, TLR4, TLR9 and CD14 (Sequenom Inc., Hamburg, Germany). Nasal and throat swabs were analysed for colonisation with Haemophilus (para)influenzae, Streptococcus pneumoniae and Staphylococcus aureus. Treg (CD4+CD25highCD127−) was determined by flow cytometry. First, we tested the assumption that the CD4+CD25highCD127− phenotype is a valid proxy marker for FoxP3 positive Treg (CD4+CD25highFoxP3+) in a paediatric population. FoxP3 is a transcription factor closely related to the suppressive function of Treg cells as demonstrated previously in adults. We assessed FoxP3 positive Treg in samples from the first 24 study participants at preschool age (four asthmatics, 13 transient wheezers, and seven healthy children diagnosed at 6 years of age). The correlation between CD4+CD25highCD127− and FoxP3 positive Treg in this study sample was high (R=0.86, p<0.01). We therefore concluded that the CD4+CD25highCD127− phenotype was a valid surrogate for FoxP3 positive Treg in our study population. At 6 years of age, diagnosis (healthy, transient wheeze or true asthmatic) was determined by two experienced paediatricians in the field of respiratory medicine and a computer algorithm (91% level of agreement) based on recurrent wheeze at inclusion, respiratory symptoms, lung function (reversibility to a β2-agonist and/or bronchial hyperresponsiveness), and the use of asthma medication, as previously described [8]. Nominal regression analysis with reference category asthma was performed. Consequently, an OR >1 demonstrated a decreased asthma risk and an OR <1 demonstrated an increased asthma risk. The following interactions between genetic variants and bacterial colonisation on a diagnosis were tested based on biological conceivability: TLR2 (rs3804099 and rs4696480) with gram-positive bacteria (S. pneumoniae and S. aureus); TLR4 (rs2737190) and CD14 (rs2569190) with gram-negative bacteria (H. (para)influenzae). In addition, interaction between all the genetic variants (including TLR9 rs187084 and rs5743836) and Treg in relation to asthma development was tested. In the presence of an interaction, stratified analysis per genotype was performed with reference category asthma.

Of the 247 children analysed at 6 years of age, 76 were diagnosed with asthma, 122 had transient wheeze and 49 were characterised as healthy controls. Atopy, eczema and the use of β2-agonists and inhaled corticosteroids were significantly different between the groups, with the highest percentages in the asthmatic group. No significant independent associations were found between asthma diagnosis and genetic variants, bacteria and Treg. Correction for possible confounders (type of child care, number of siblings, sex, smoking exposure, exposure to furry pets, age at inclusion, inhaled corticosteroid use) did not alter these results. However, several significant interactions with asthma at 6 years of age were found (fig. 1). In the stratified analysis, children with the AA genotype of TLR4 rs2737190 were found to have an increased risk of
developing asthma when colonised with *H. parainfluenzae* at preschool age (OR 0.25 (95% CI 0.07–0.89) compared with the control group and OR 0.32 (95% CI 0.12–0.84) compared with those with transient wheeze). In addition, the GG genotype increased the risk of asthma when colonised with *H. parainfluenzae* compared with the control group (OR 0.07 (95% CI 0.01–0.82)). The CD14 rs2569190 GG genotype showed a positive association between colonisation of *H. influenzae* at preschool age and asthma development compared with the transient wheeze group (OR 0.20 (95% CI 0.05–0.82)). In comparison with healthy children, a higher proportion of Treg at preschool age was associated with the risk of childhood asthma development (AA genotype of TLR2 rs4696480: OR 0.38 (95% CI 0.17–0.86); GG genotype of TLR9 rs187084: OR 0.55 (95% CI 0.34–0.92)).

At present, the exact role of genetic variants in TLRs and CD14 in the aetiology of childhood asthma is still largely undetermined, but interest is increasing. Bacteria’s disruption of the respiratory epithelium’s barrier function triggers the immune system, which might predispose the airways to asthma development [9, 10]. The extent and nature of the effect of bacteria on the immune system can be influenced by genetic variants in immune receptors [1, 2]. Indeed, we demonstrated several interactions between genetic variants and bacteria, suggesting an increased risk of developing asthma in the presence of specific genotypes combined with colonisation of the upper airways by a particular bacteria species. Furthermore, a higher proportion of Treg at preschool age was found to be associated with the development of asthma at 6 years of age in the presence of certain genotypes in immune response genes. These interactions might explain the conflicting findings between studies on this topic [2, 3]. However, it is still unclear whether the observed interactions are primary or secondary factors in the development of the disease. It might also be possible that children with asthma and a particular genotype are simply at a higher risk of being colonised with certain pathogens.

Contrary to most studies, the present study has a longitudinal design. This enabled us to investigate the effect of parameters on asthma development in early life. We achieved a high follow-up success rate (98%). In addition, fewer incorrect diagnoses were expected as the diagnosis was based on both expert opinion and a computer algorithm, with reassessment in inconclusive cases. To reduce the influence of viral exposures, children were clinically stable at the time of assessment. We chose not to correct for multiple testing as our

![Figure 1](image-url)
study was hypothesis-generating. When we corrected for multiple testing, by using the false discovery rate, the interactions between TLR4 rs2737190 and H. parainfluenzae, CD14 rs2569190 and H. influenzae, and CD14 rs2569190 and H. parainfluenzae were no longer be significant. It should be noted that the presented results are based on secondary analysis, and therefore the study was relatively small for this kind of analysis. Consequently, our power to demonstrate associations or interactions was limited. Further larger studies are needed to confirm our findings.

In conclusion, we hypothesise that at preschool age, the effect of bacterial colonisation of the upper airways and serum Treg on asthma development at 6 years of age may be affected by genetic variants in the TLRs and CD14 genes. We reason that the combination of genetic predisposition and bacterial exposure at preschool age leads to an altered immune response, as demonstrated by the Treg response, which in turn leads to the development of asthma at 6 years of age.

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