Dual responses of CD14 methylation to distinct environments: a role in asthma and allergy

To the Editor:

Gene–environment interactions are believed to cause an increased prevalence of asthma and allergic diseases in Western Countries, in comparison to Eastern Countries. To date, research has been inconclusive about which specific genetic and environmental risk factors are important, how the epigenetics/genetics interact with the environment, and which pathogenic mechanisms underlie the development of asthma and allergy. Inconsistencies in research outcomes are mainly attributed to substantial variations in genetic backgrounds and environmental conditions between different populations. Furthermore, a poor understanding of epigenetics might partially account for the observed disparities.

The Finnish and Russian Karelian populations are ideal for the study of the influence of Western and Eastern environments/lifestyles on allergic conditions [1–3]. These two populations were separated at the time of the Second World War and belong to the same ethnic group, thus having a similar genetic background [4, 5]. The Finnish Karelians live westernised lifestyles and have a higher prevalence of allergic disease than the Russian Karelians who maintain a traditional rural lifestyle [3, 5–7].

CD14 is a pattern-recognition receptor for environmental lipopolysaccharides (LPS) and other bacterial wall-derived components. Engagement of the CD14-LPS complex could induce immune cells toward Th1 cytokine production to activate innate host defence system. By studying the Karelian population, we previously found that the Russian and Finnish environments exerted opposite effects of the *CD14* genotypes on the risk of allergic diseases in adult women and children [2, 8]. Certain gene–environment interactions during the development of allergic diseases are thought to be governed by *CD14* methylation [9]. In our recent study, we showed higher levels of *CD14* methylation in the Finnish Karelian compared to the Russian Karelian children [10]. However, the variations in methylation of this candidate gene cannot explain the contrasts in asthma and allergy between these two groups [10]. Thus, we hypothesised that *CD14* DNA methylation is regulated differentially in response to the environment, and, as such, interacts with its genotype to regulate the development of allergic diseases.

A total of 500 Karelian children were included in the study, 250 from Russia and 250 from Finland. There were no significant differences in age and gender between the two groups. In accordance with previous studies [5–7], Finnish children had a higher prevalence of allergic diseases, including asthma, rhinitis, conjunctivitis, hay fever, itchy rash, atopic eczema and atopy, compared to their Russian counterparts ($p<0.001$).

Compared to Finnish children, children living in Russia are more often exposed to farm animals, family pets and passive smoking. These factors are positively associated with endotoxin levels, which are regarded as a proxy for high microbial burden. CD14 is critical for endotoxin-dependent signal transduction, acting as a key player in protecting against allergic responses to the environment [11]. We stratified the geographic location of Karelian children to examine the environmental influence on *CD14* methylation and the association between epigenetic methylation and diseases. Three CpG sites in *CD14* amplicon 5 (Amp5Site1, Amp5Site2 and Amp5Site3) were selected for the present study, as they are in the promoter region and are of particular interest when comparing Karelian children residing in Russia and Finland [10]. In Karelian children living in Russia, reduced levels of CpG methylation were observed at Amp5Site1, due to indoor smoking (figure 1a); at Amp5Site2, due to contact with hens (figure 1b); and at Amp5Site3,

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This study shows that *CD14* DNA methylation is differentially regulated by environmental factors
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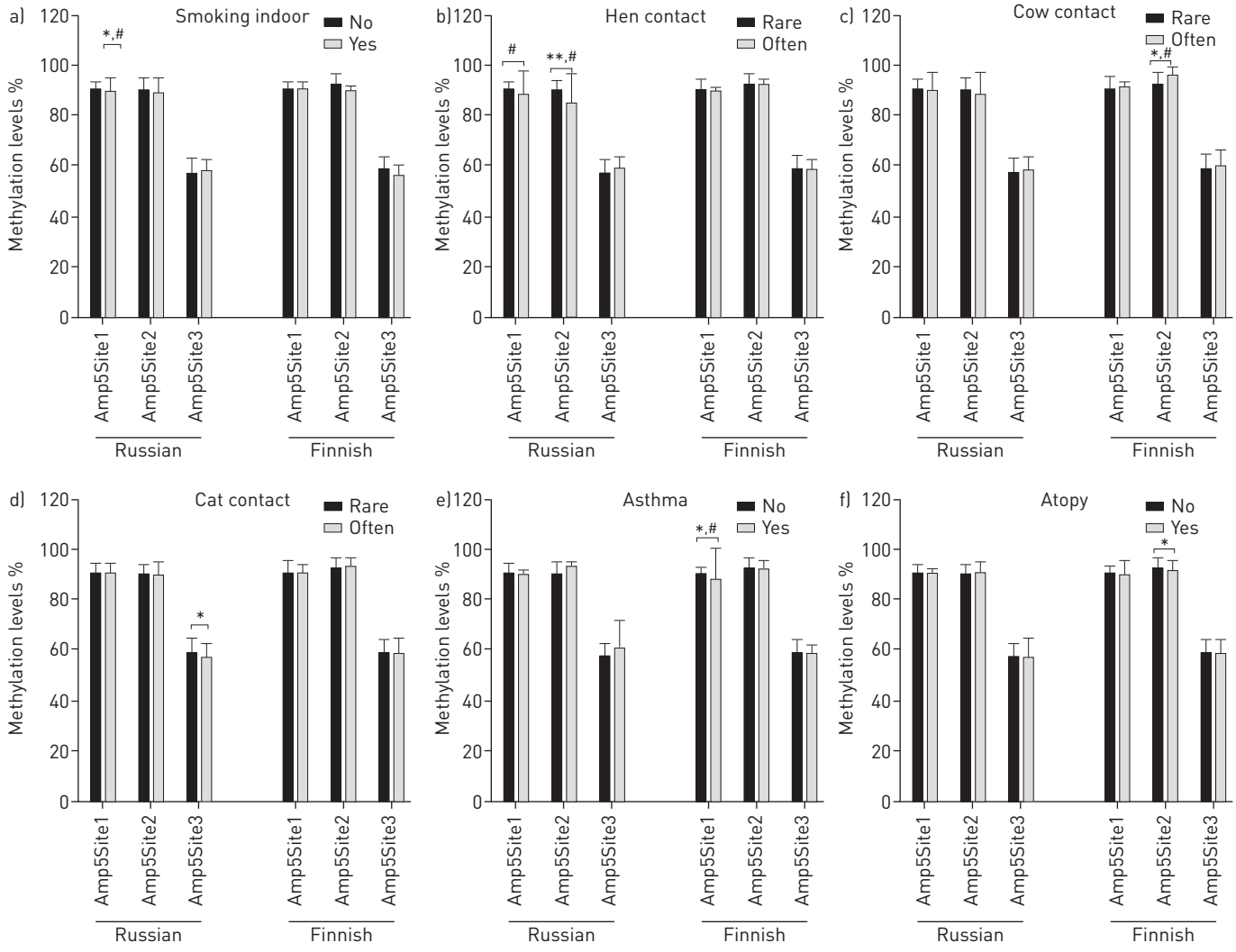

FIGURE 1 *CD14* DNA methylation and environmental factors/diseases. DNA methylation levels of Amp5Site1, Amp5Site2 and Amp5Site3 in the *CD14* promoter region were compared in Karelian children exposed to a) passive smoking indoors, or contact with b) hens, c) cows and d) cats within the last 12 months, and in Karelian children e) with/without asthma or f) with/without atopy in the two locations, Russia and Finland. Data are presented as mean±SD. *: p<0.05; **: p<0.01; #: p<0.05 after adjusting for age, sex, IgE and *CD14* genotypes.

due to contact with cats (figure 1d). In contrast, the Amp5Site2 methylation levels in Finnish children were significantly increased by contact with cows (figure 1c). Finnish children with asthma, rhinitis or atopy had significantly lower methylation levels at both Amp5Site1 and Amp5Site2, compared to local children without these disorders. After adjusting for age, gender, IgE and *CD14*–550 and *CD14*-260 genotypes, the identified environmental influence attained significance, except for that of contact with cats on methylation levels at Amp5Site3. Association of *CD14* methylation with asthma was significant in Finnish children (figure 1e), whereas a marginal association (p=0.088) was revealed between atopy and methylation status at Amp5Site2, after controlling the confounding effects (figure 1f). Thus, the same types of environmental factors influence *CD14* DNA methylation levels in opposite directions in Russia and Finland. This further highlights the complex role of CD14 in immune-related diseases and explains the insignificant contribution of variations in *CD14* methylation to asthma and allergy gradients between Finnish and Russian children.

The endotoxin switch is an emerging concept that merges with the 'hygiene hypothesis' and considers microbial load as a major determinant of CD14-mediated gene–environment interactions [12]. Based on this theory, the influence of CD14 on allergen-induced immune responses depends on the quantity and/or quality of the relevant microbial load. Using 16S rRNA gene sequencing, Pakarinen *et al.* [13] found major disparities between Russian and Finnish house dust, in microbial quantity and diversity, both of which were much greater in Russia. Therefore, the opposite changes in DNA methylation that we observed

are probably due to differences in bacterial diversity or microbial load, particularly considering the ethnic homogeneity of the two populations under investigation.

Apart from the environmental factors, methylation levels in *CD14* are influenced by age [14] and gender [9], and are associated with IgE [10] and *CD14* gene polymorphisms [14]. In the present study, we employed a general linear model to establish the association between *CD14* methylation and asthma in Finland, after adjusting for these confounders. Consequently, it is unlikely that gene polymorphisms contribute to the DNA methylation-mediated allergic response. On the contrary, we speculate that DNA methylation is a critical mechanism in modulating the genetic effects associated with asthma and allergy. We observed that the allele C of *CD14*-550 was associated with low methylation levels at Amp5Site3 of the *CD14* promoter region, but allele C of *CD14*-260 was associated with high methylation levels. Moreover, the association between genetic variants and DNA methylation showed a similar pattern in both Russian and Finnish children. Polymorphisms of both *CD14*-550 and *CD14*-260 reportedly influence soluble CD14 levels. For example, the C allele in *CD14*-550 is associated with a higher serum CD14 level than the T allele [14], whereas the C allele in *CD14*-260 is associated with low serum CD14 levels [15]. The associations that we observed in *CD14* methylation and *CD14* polymorphisms are in line with these observations, indicating that the effect of genetic variants on serum CD14 level might be regulated *via* *CD14* methylation. One limitation of our study is the relatively small sample size derived, after stratification by genotype. Therefore, we are unable to establish a reliable genotype–phenotype association model to assess the effects of DNA methylation. This theory requires further investigation.

In agreement with our hypothesis, our data suggest that the regulatory mechanisms of *CD14* methylation responding to environmental factors differ between Russia and Finland. We also demonstrated that *CD14* methylation status is significantly associated with *CD14* polymorphisms. These findings provide a novel insight into CD14 and the pathogenesis of asthma and allergy, as well as a possible explanation for inconsistencies in prior genetic association studies. This study also has the limitations of a lack of measurement of the endotoxin levels in each region in the initial study design, and a lack of direct evidence in relation to downstream CD14 protein expression and subsequent Th1/Th2 cytokine profiles. These findings will help to define the exact regulatory mechanisms of CD14 in response to environmental LPS in the development of asthma and allergy.

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