The candidate gene approach in asthma: what happens with the neighbours?

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In the last few decades, multiple genes important in asthma and atopy development have been identified. A successful approach has been to investigate candidate genes, that is, genes with a biologically plausible function.[1] This approach has also been applied by Zhu *et al*[2] in a previous issue of this journal. They analysed *IL18R1*, an interesting candidate gene for asthma and atopy, and provided replicated evidence in three European populations that SNPs located in *IL18R1* were associated with asthma.[2] Specifically, SNP rs1420099, rs1362348, and rs1974657 were associated with asthma in these three populations.

IL-18 receptor is a key immunoregulator; the gene product of *IL18R1* forms the alpha chain of the IL-18 receptor.[3] Binding of IL-18 to the IL-18 receptor can stimulate Th1 as well as Th2 cytokine release.[4] These findings may indeed point towards a role of IL18R1 in the pathophysiology of asthma.[2] *IL18R1* is localized in the IL1 receptor cluster on chromosome 2q12. In its close vicinity reside *IL1R2*, *IL1RL*, *IL1RL2*, *IL1RL1*, and *IL18RAP*. We have recently undertaken a candidate gene approach analysing genes located in a region of strong linkage disequilibrium (LD) in this gene cluster, that is, *IL18R1*, as well as *IL1RL1* and *IL18RAP*, in two Dutch asthma and one Dutch rhinitis cohort.[5] We reported replicated evidence for association of SNPs in this gene cluster with asthma phenotypes in our two Dutch asthma populations. For *IL18R1*, four SNPs were associated with asthma and bronchial hyperresponsiveness in a combined analysis of the two asthma cohorts ($P<0.05$); these SNPs, that is, rs12999364, rs1558627, rs2270297, and rs1035130, were not genotyped by Zhu *et al*. Furthermore, we found significant associations with SNPs in *IL1RL1* and *IL18RAP*. A haplotype from SNPs in *IL1RL1* and *IL18R1* was significantly associated with bronchial hyperresponsiveness. Strong LD was detected between SNPs in the three genes in this region.

IL1RL1 encodes the receptor for IL-33, which is located on mast cells, Th2 cells, regulatory T cells, and macrophages, and is also present in serum in a soluble form.[6–8] IL1RL1 is a member of the Toll-like receptor superfamily and can either stimulate or inhibit Th2 responses by influencing TLR pathway signalling.[9–12] There is increasing evidence that this gene is important in atopic diseases such as eczema and asthma; interestingly, a recent large genome-wide association study also indicated *IL1RL1* to be important in asthma, and thus *IL1RL1* is also a plausible candidate gene for asthma.[5,13,14]

In their paper, Zhu *et al* mentioned the limitation of not analysing SNPs located in *IL18R2* (also known as *IL18RAP*). SNPs in *IL1RL1* and *IL18RAP*, next to *IL18R1*, may also contribute to the genetic association signal on chromosome 2q12. We suggest that genetic association studies in regions with strong LD may not be conclusive as to which gene or genes are causal in disease development. It would therefore be of interest to investigate also *IL1RL1* and *IL18RAP* in the populations described by Zhu *et al*. Moreover, we suggest the investigation of this region in populations with different LD characteristics and to perform functional studies. Our observations imply that, once positive genetic associations are identified, it is worthwhile to take a look at the neighbouring genes.

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Reply to Reijmerink *et al*

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We appreciate the comments from Reijmerink *et al* on our IL18R1 genetic association results published in the *European Journal of Human Genetics*.[1] As we had pointed out in the paper, ours was a

candidate gene study specifically exploring the hypothesis of genetic association between IL18R1 and asthma, as IL18R1 is a plausible candidate based on the available biological information. The observation that the SNPs in IL1RL1, IL18RAP and IL18R1 are associated with asthma in the Dutch asthma cohorts is interesting. We did not discuss the possibility of association with this gene cluster in our paper, as we were focused on IL18R1's association with asthma.

A recent GWAS study to identify sequence variants affecting blood eosinophil counts reported significant association with the LD block containing IL1RL1, IL18R1, IL18RAP and SLC9A4, and this region is significantly associated with asthma ($P=5.5\times10^{-12}$) in a collection of 10 different populations.[2] This study, along with the results pointed out by Reijmerink *et al*[3] and our study,[1] highlights the importance of this region in asthma-related phenotypes. As IL1RL1, IL18R1, IL18RAP and SLC9A4 are in tight linkage disequilibrium, we agree with Reijmerink *et al*'s suggestion to take a closer look at this region in large asthma populations. Considering the LD pattern in this gene cluster, genotyping and analyzing additional SNPs may not resolve the questions raised here. We suggest deep sequencing of this region in a large population to identify functional variants and also suggest undertaking functional studies to pinpoint the biological association with the disease. We agree with the comments of Reijmerink *et al* on the significance of this region and highlight the importance of examining the flanking regions in all candidate gene studies. Recent efforts in setting up large databases for genetic association studies in asthma (GABRIEL consortium) and other diseases will be helpful to solve some of these questions.

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