# Polymorphisms in the novel gene acyloxyacyl hydroxylase (*AOAH*) are associated with asthma and associated phenotypes

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**Background: The gene encoding acyloxyacyl hydroxylase (AOAH), an enzyme that hydrolyzes secondary fatty acyl chains of LPS, is localized on chromosome 7p14-p12, where evidence for linkage to total IgE (tIgE) concentrations and asthma has been previously reported.**

**Objective: We hypothesized that variants in AOAH are associated with asthma and related phenotypes. Because both AOAH and soluble CD14 respond to LPS, we tested for gene-gene interaction.**

**Methods: We investigated the association between 28 single nucleotide polymorphisms throughout the AOAH gene and asthma, concentrations of tIgE, the ratio of IL-13/IFN-γ, and soluble CD14 levels among 125 African Caribbean, multiplex asthmatic pedigrees (n = 834). Real-time PCR was used to assess whether AOAH cDNA expression differed with AOAH genotype.**

**Results: Significant effects were observed for all 4 phenotypes and AOAH markers in 3 distinct regions (promoter, introns 1-6, and the intron 12/exon 13 boundary/intron 13 region) by means of single-marker and haplotype analyses, with the strongest evidence for a 2-single-nucleotide-polymorphism haplotype and log[tIgE] ($P = .006$). There was no difference in AOAH expression levels by AOAH genotype for any of the markers. Comparing genotypic distributions at both the AOAH marker rs2727831 and *CD14*(−260)C>T raises the possibility of gene-gene interaction ($P = .006$-$.036$).**

**Conclusion: Our results indicate that polymorphisms in markers within the AOAH gene are associated with risk of asthma and associated quantitative traits (IgE and cytokine levels) among asthmatic subjects and their families in Barbados, and there is an interactive effect on tIgE and asthma concentrations between an AOAH marker and the functional *CD14*(−260)C>T polymorphism.**

**Clinical implications: AOAH is a novel innate immunity candidate gene associated with asthma and related phenotypes in an African ancestry population. (J Allergy Clin Immunol 2006;118:70-7.)**

***Key words:*** *CD14, acyloxyacyl hydroxylase, association, asthma, total IgE, soluble CD14, family-based association test*

The interface between innate (natural) immunity and the more recently evolved acquired (adaptive) immune response might be critical in the development and manifestation of several allergic diseases. Chronic exposure to domestic endotoxin (soluble fragments of bacterial LPS) appears to influence the risk for asthma.[1,2] Several innate immunity receptors that bind endotoxin have been proposed as candidate genes for allergic airway disease. For example, *CD14* is a cell-surface antigen preferentially expressed on mature monocyte cells, which initiate antimicrobial host defense responses[3] characterized by release of $T_H1$-type inflammatory cytokines (eg, TNF-α, IL-1, IL-6, and INF-γ) and subsequently send inhibitory signals to $T_H2$ lymphocytes (eg, IL-4 and IL-13). Baldini et al[4] demonstrated that the −159 (subsequently designated −260 [rs2569190])C>T allele of the *CD14* gene was significantly associated with lower serum total IgE (tIgE) levels, a finding replicated by several groups.[5-10] Significant associations have also been observed between this

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*Abbreviations used*
AOAH: Acyloxyacyl hydroxylase
FBAT: Family-based association test
LD: Linkage disequilibrium
OR: Odds ratio
sCD14: Soluble CD14
SNP: Single nucleotide polymorphism
tIgE: Total IgE

marker and asthma.[11-13] LeVan et al[14] showed this polymorphism is functional, resulting in higher soluble CD14 (sCD14) concentrations. To date, the *CD14*(−260)C>T polymorphism is one of the most reproducible associations for asthma and its associated traits.

Another interesting candidate, but one for which relatively little is known, is acyloxyacyl hydroxylase (AOAH), a highly conserved,[15] eukaryotic lipase that releases secondary acyl chains from the LPS found on cell walls of gram-negative bacteria.[16-18] A unique leukocyte enzyme, AOAH is potentially an important host defense molecule because deacylated LPS is at least 100-fold less potent than intact LPS.[19] *In vitro* assays show deacylated LPS is an effective LPS antagonist.[20,21] Recently, it was demonstrated that AOAH also appears to modulate B-cell proliferation and polyclonal antibody production in response to gram-negative bacterial infection.[22]

AOAH is located on chromosome 7p14-p12,[23] a region where evidence for linkage to asthma, bronchial hyperreactivity, and tIgE concentrations has been reported.[24-26] Collectively, these observations suggest that AOAH is a candidate gene in the LPS signaling pathway for tIgE levels in particular and possibly for other allergic diseases, including asthma.

## METHODS

### Study subjects

Nuclear and extended asthmatic families were recruited in Barbados as a part of an ongoing asthma genetics study (described elsewhere[27,28]). Patients with a positive family history of asthma (≥1 asthmatic siblings) were referred by physicians cooperating with coinvestigators at the University of the West Indies. Blood samples were collected from all subjects for serum extraction of DNA for genotyping, and total RNA was collected from a subset (n = 30) for expression experiments.

Asthma and tIgE concentrations were measured, as described previously.[27,29] IgE values were log transformed to minimize skewness and then adjusted for age and sex. Soluble CD14 levels were measured with a commercially available ELISA kit supplied by Biosource (Europe S.A.), and concentration was determined by means of extrapolation from a standard curve estimated from a panel of standards with known concentrations. The minimum detectable concentration was estimated to be 1 ng/mL and defined as the sCD14 concentration corresponding to the average OD of 20 replicates of the zero standard +2 SD. Serum IL-13 and IFN-γ levels were measured by means of ELISA with specific anti-IL-13 and anti-IFN-γ mAbs obtained from BD Biosciences (San Diego, Calif), according to the manufacturer's instructions. Serum IL-13 and IFN-γ levels were log transformed to approximate a normal distribution. Family-based association tests (FBATs) on the qualitative phenotype asthma and the quantitative traits were performed on 834 persons from 125 pedigrees (both nuclear and extended).

All subjects provided verbal and written consent, as approved by the Johns Hopkins Bayview Hospital Institutional Review Board and the Ministry of Health in Barbados.

### Genotyping

A total of 29 single nucleotide polymorphisms (SNPs) from the gene encoding AOAH (accession no. NT_007819.13) were genotyped in these data. Of these, 26 were validated SNPs selected from Applied Biosystems (Foster City, Calif) by using SNPBrowser software 3.0 (www.allsnps.com/snpbrowser), a freely available Applied Biosystems tool. Of these, one nonsynonymous variant (Gly/Ala) was not polymorphic in this African Caribbean population. Two additional SNPs that were part of an initial pilot study on AOAH were also included. The final panel comprised 28 SNPs, all of which were intronic, with the exception of four 5′ UTR SNPs; this, however, reflects the lack of reported SNPs in exons in this gene (~1% exonic).

DNA was extracted by using standard protocols. When necessary, whole-genome amplification of DNA was prepared by OmniPlex Technology (Rubicon Genomics Inc, Ann Arbor, Mich). Genetic screening for the *CD14*(−260) variant (rs2569190) was performed as previously described[29] through PCR amplification and restriction digest (described elsewhere[4]). Genotyping of the AOAH SNPs was performed by using the TaqManR probe-based, 5′ nuclease assay with minor groove binder chemistry (Applied Biosystems). Genotyping quality was high, with an average completion rate of 97%, with no discordances on repeat genotyping of a random 10% of the sample and a low rate of Mendelian inconsistencies.

### Quantitative RT–PCR analysis

Reverse transcription was performed by using total RNA isolated from human PBMCs from subjects selected on the basis of their rs2727831 (AOAH IVS12-355C>T) genotype (10 CCs, 10 CTs, and 10 TTs) and processed with the Applied Biosystems High-Capacity cDNA Archive kit first-strand synthesis system for RT-PCR, according to the manufacturer's protocol. Quantitative RT–PCR was performed by using the TaqMan assay system from Applied Biosystems. All PCR amplifications were carried out on an Applied Biosystems Prism 7300 Sequence Detection System by using a fluorogenic 5′ nuclease assay (TaqMan probes). Probes and primers were designed and synthesized by Applied Biosystems. Relative gene expressions were calculated by using the $2^{-Ct}$ method, in which Ct indicates cycle threshold, the fractional cycle number at which the fluorescent signal reaches a detection threshold.[30] The normalized ΔCt value of each sample was calculated by using a total of 3 endogenous human control genes (*GAPDH*, *ACTB*, and *PGK1*). Fold change values are presented as $Averagefoldchange = 2^{-(AverageCt)}$ for genes in treated samples relative to control samples. Error bars represent the SEM for multiple biologic replicates (10 for each group).

### Statistical analyses

Clinical characteristics of the study population, including mean age, proportion by sex, proportion of asthmatic subjects, and mean values with SDs for serum measures and quantitative traits considered (log[tIgE], sIL-13, sIFN-γ, the IL-13/IFN-γ ratio, and sCD14), were calculated for all subjects with available data who were genotyped for AOAH, for founder members of the population, and for asthmatic and nonasthmatic subjects by using STATA 8.2 (StataCorp, College Station, Tex). Equality of means was tested between asthmatic and nonasthmatic subjects for serum measures and quantitative traits by