

TABLE I. Predictors of outcome of repeated OFC

Initial OFC N = 112	Failed repeated OFC N = 66	Passed repeated OFC N = 46
Mild reaction n = 39	64.1% (n = 25)	35.9% (n = 14)
Mod reaction n = 46	47.8% (n = 22)	52.2% (n = 24)
Severe reaction n = 27	70.4% (n = 19)	29.6% (n = 8)
Challenge dose (%)	39.7	41.5
Mean age (y)	4.8	5.1
Interval (y)	2.9	2.8
IgE (kU/L)	2.2	1.8

Initial OFC (milk) N = 46	Failed repeated OFC (milk) N = 31	Passed repeated OFC (milk) N = 15
Mild reaction n = 20	75% (n = 15)	25% (n = 5)
Mod reaction n = 13	30.8% (n = 4)	69.2% (n = 9)
Severe reaction n = 13	92.3% (n = 12)*	7.7% (n = 1)*
Challenge dose (%)	31.6	32.7
Mean age (y)	4.7	4.2
Interval (y)	2.9	2.3
IgE (kU/L)	2.7	1.7

Initial OFC (egg) N = 38	Failed repeated OFC (egg) N = 20	Passed repeated OFC (egg) N = 18
Mild reaction n = 6	50% (n = 3)	50% (n = 3)
Mod reaction n = 22	54.5% (n = 12)	45.5% (n = 10)
Severe reaction n = 10	50% (n = 5)	50% (n = 5)
Challenge dose (%)	53.3	35.4
Mean age (y)	2.6	5.9
Interval (y)	2.6	2.9
IgE (kU/L)	1.3	1.5

Initial OFC (peanut) N = 28	Failed repeated OFC (peanut) N = 15	Passed repeated OFC (peanut) N = 13
Mild reaction n = 13	53.8% (n = 7)	46.2% (n = 6)
Mod reaction n = 11	54.5% (n = 6)	45.5% (n = 5)
Severe reaction n = 4	50% (n = 2)	50% (n = 2)
Challenge dose (%)	38.8	63.6
Mean age (y)	5.4	5.1
Interval (y)	3.2	3.0
IgE (kU/L)	2.4	2.2

Mod, Moderate.

*P = .002.

milk-specific IgE level at the time of the repeat OFC than those who failed their repeat milk OFC. Because this was a retrospective chart review of a clinical population rather than a designed research protocol, uncontrolled factors such as seasonality, patient maturity, and family/patient decisions of whether and when to repeat OFC likely had some impact. However, with the possible exception of those who have severe symptoms to their initial milk OFC, the circumstances of the initial failed OFC are

poorly correlated with the outcomes of repeat OFCs. This suggests patients who have not had known reactions in the interim should be considered candidates for a repeat OFC, regardless of the circumstances of the initial OFC.

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Altered epigenetic regulation and increasing severity of bronchial hyperresponsiveness in atopic asthmatic children

To the Editor:

Environmental influences are believed to shape clinical outcomes in part through epigenetic mechanisms that alter susceptibility to or maintenance of chronic diseases, such as asthma and allergies.¹ Better understanding of the roles of epigenetics in these processes is key to understanding the pathogenesis of chronic inflammatory conditions, as well as to developing prophylactic strategies to reduce their incidence or modulate their intensity. Mechanisms controlling gene expression include methylation of DNA and modifications (eg, acetylation, methylation, and phosphorylation) of histone tails, actively modifying what is known as the "histone code."² Recent evidence suggests that the *in vivo* balance of histone deacetylase (HDAC) and histone acetyltransferase (HAT) activities provides an important mechanism that governs gene expression potential.^{2,3} One limitation of studies to date that seek to assess the effect of (de)acetylation in human subjects is that many focus solely on HDAC or HAT activity when the net ratio of the 2 is thought to be the most relevant outcome.

We recently reported that experimental alteration of the endogenous HDAC/HAT balance in healthy children changes recall T_H1/T_H2 cytokine responses by CD4⁺CD45RO⁺ (antigen-experienced/memory) cells.⁴ Others, using whole cell lysates,

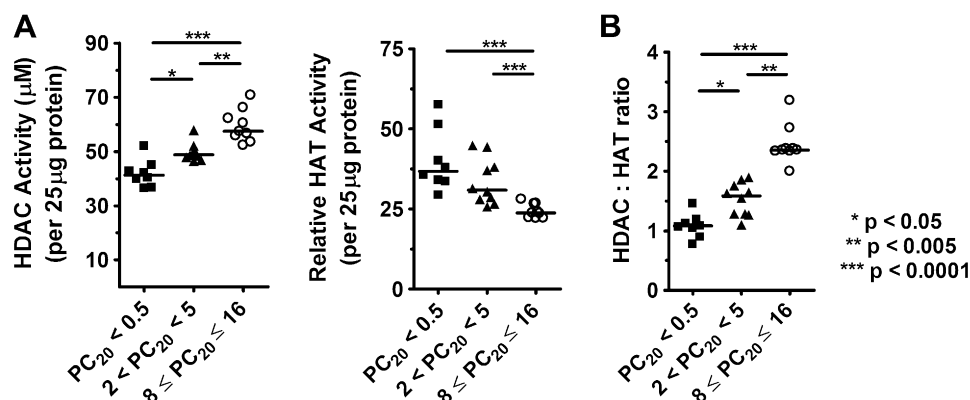


FIG 1. Relationship between endogenous HDAC and HAT activities and BHR in atopic asthmatic and atopic nonasthmatic children. **A**, Endogenous HDAC and HAT activities were measured directly *ex vivo* in nuclear lysates of PBMCs from atopic children with severe, mild, or no detectable BHR. **B**, Paired HDAC/HAT ratios for each subjects were calculated, with medians (bars) shown. Significance was assessed by using Mann-Whitney tests, yielding the following: * $P < .05$, ** $P < .005$, or *** $P < .0001$.

observed reduced endogenous HDAC expression and activity in adults with severe, but not those with mild, asthma.^{5,6} Together, these data suggest a link between ongoing epigenetic regulation, immunity, and physiologic responses in human subjects. However, the prevalence and nature of epigenetic differences in healthy versus atopic asthmatic individuals, their relationship with ongoing immune and physiologic processes, and ultimately clinical outcomes remain ill defined and controversial.

Bronchial hyperresponsiveness (BHR) is widely linked to asthma severity in children.⁷ We therefore asked whether any relationship exists between levels of endogenous HDAC or HAT functional activity in atopic but otherwise healthy versus atopic asthmatic children who exhibit different severities of BHR. Twenty-seven age-matched children were randomly selected from the Study of Asthma, Genes, and the Environment population-based case-control cohort.⁸ Atopic asthmatic children ($n = 18$) were determined to be asthmatic according to a pediatric allergist's diagnosis, had positive skin prick test responses to at least 1 of the common allergens tested, and had PC₂₀ values of less than 5 mg/mL. Atopic nonasthmatic children ($n = 9$, 8 mg/mL < PC₂₀ ≤ 16 mg/mL) were included as control subjects. BHR was assessed by measuring PC₂₀ values.

Because the level of histone acetylation is dependent on the net balance of HDAC and HAT activity, we independently assessed levels of both of these directly *ex vivo* in nuclear (rather than whole cell) PBMC lysates (Fig 1, A). HDAC and HAT activities were measured with colorimetric assay kits (BioVision, Mountain View, Calif), according to the manufacturer's protocol. Statistical comparisons were made by using Mann-Whitney analysis with Graph Pad Prism 4.0 software (San Diego, Calif). Highly significant reductions in HDAC activity were evident among children with mild or severe allergic asthma relative to atopic nonasthmatic control subjects ($P < .0001$). Interestingly, allergic asthmatic subjects exhibit significantly enhanced HAT activity. For both measures, the intensity of these alterations was progressively related to the degree of BHR, as measured based on PC₂₀ values. Consequently, the net *in vivo* histone (de)acetylation activity (expressed as the ratio of HDAC/HAT activity) is skewed toward increased histone acetylation in allergic asthmatic subjects, with the intensity of increased cellular acetylation activity increasing with BHR severity. Although markedly reduced HDAC/HAT activity

ratios (median, approximately 250% reduced) were most apparent in atopic asthmatic children with severe BHR (Fig 1, B), a continuum is evident in which these ratios decrease with increasing severity of BHR ($P < .0001$). This indicates most increased cellular acetylation activity among atopic asthmatic children with severe BHR. Taken together, these data reveal a significant and continuous relationship between altered epigenetic controls of gene expression potential (here, net HDAC/HAT activity) and increased severity of BHR across the spectrum of atopic asthma in children.

This relationship raises the speculative possibility that endogenous HDAC activities, HAT activities, or both play a role in driving the progression of BHR severity and that re-equilibrating such epigenetic controls might prove clinically beneficial.

One important limitation to this study is its cross-sectional nature. Although the outcomes examined exhibit highly significant relationships, detailed longitudinal cohort studies will be required to temporally define their origins and their continued interaction in the clinical manifestation of chronic inflammatory disease. Similarly, it will be important to determine to what extent differentially altered epigenetic regulation is a contributor to rather than caused by increased BHR.

In summary, this report demonstrates, for the first time, relationships between the intensity of an endogenous epigenetic control (here, the balance of HDAC and HAT activities) in healthy and atopic asthmatic children with the physiologic intensity of BHR. These data reinforce the importance of attaining a better understanding of the interplay in subjects of different clinical phenotypes between distinct patterns of epigenetic regulation, altered immune capacity, and resulting physiologic alterations.

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