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KJIM

A polymorphism in the histone deacetylase 1 gene is associated with the response to corticosteroids in asthmatics

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Background/Aims: Recent investigations suggest that histone deacetylase 1 (*HDAC1*) and *HDAC2* may be target molecules to predict therapeutic responses to corticosteroids. We evaluated the effects of variation in *HDAC1* and *HDAC2* on the response to corticosteroids in asthmatics.

Methods: Two single nucleotide polymorphisms (SNPs) were selected after resequencing *HDAC1* and *HDAC2*. For the first analysis, we evaluated the association between those SNPs and asthma severity in 477 asthmatics. For the second analysis, we evaluated the effects of these SNPs on lung function improvements in response to corticosteroid treatment in 35 independent adult asthmatics and 70 childhood asthmatics.

Results: We found that one SNP in *HDAC1* (rs1741981) was significantly related to asthma severity in a recessive model (corrected $p = 0.036$). Adult asthmatics who were homozygous for the minor allele of rs1741981 showed significantly lower % forced expiratory volume in 1 second (%FEV_1) increases in response to systemic corticosteroids treatment compared with the heterozygotes or those homozygous for the major allele (12.7% ± 7.2% vs. 37.4% ± 33.7%, $p = 0.018$). Similarly, childhood asthmatics who were homozygous for the minor allele of rs1741981 showed significantly lower %FEV_1 increases in response to inhaled corticosteroid treatment compared with the heterozygotes or those homozygous for the major allele (14.1% ± 5.9% vs. 19.4% ± 8.9%, $p = 0.035$).

Conclusions: The present study demonstrated that rs1741981 in *HDAC1* was significantly associated with the response to corticosteroid treatment in asthmatics.

Keywords: Asthma; Glucocorticoids; Histone deacetylase 1; Pharmacogenomics; Polymorphism

INTRODUCTION

Asthma, a chronic airway inflammatory disease, is an important source of morbidity and mortality worldwide [1]. Current guidelines recommend corticosteroid treatment for the management of asthma [2-4]. Endogenous corticosteroid levels and therapeutic responses to exogenous corticosteroids are known to be influenced by genetics, with heritability ranged from 0.40 to 0.56 [5-7]. This suggests a pharmacogenetic basis for the intraindividual variability of corticosteroid responsiveness in asthmatics [8].

Histone deacetylase (HDAC) plays a key role in the regulation of inflammatory genes by removing acetyl

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groups from histones [9,10]. It has been reported that conditional deletion of *HDAC1* in T cells resulted in enhanced airway inflammation and increased Th2 cytokine production [11]. Moreover, HDAC is involved in the mechanism of action of corticosteroids. For example, recruitment of HDAC2 to activated inflammatory genes is an important mechanism of inflammatory gene repression by corticosteroids and HDAC2 activity is reduced in some diseases in which patients showed poor responses to corticosteroids treatment [12]. Together, HDAC1 and HDAC2 may be target molecules to predict therapeutic responses to corticosteroids. However, to date, any genetic effects of *HDAC1* and *HDAC2* on the response to corticosteroids in asthmatics have remained unknown.

In the present study, we evaluated associations between asthma severity, which is related to reduced responsiveness to corticosteroids [13], and single nucleotide polymorphisms (SNPs) in *HDAC1* and *HDAC2* in adult asthmatics. Then, we further assessed the effects of those SNPs on responsiveness to corticosteroids, as measured in terms of the change in lung function, in independent adult and childhood asthmatics.

METHODS

All subjects enrolled in this study provided written informed consent. The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (H-1110-130-384).

Primary analysis

In total, 477 asthmatics were enrolled at Seoul National University Hospital, Seoul, Korea. The diagnosis of asthma was made at least 6 months prior to enrollment, according to current guidelines [2-4]: episodic symptoms including wheezing, coughing, and dyspnea plus a positive bronchodilator response (an increase in forced expiratory volume in 1 second [FEV_1], from baseline that was more than 200 mL and more than 15% of the prebronchodilator value), or a provocative concentration of methacholine causing a 20% reduction in FEV_1 (PC_{20}) ≤ 16 mg/mL [2-4]. Enrolled asthmatics were treated with conventional medications based on the The Global Initiative for Asthma guideline according to their asthma control status [2]. Asthma severity was determined based on lung function and the medication use index needed to obtain control, as described previously [14,15].

Secondary analysis

To generalize the results obtained in the primary analysis, we enrolled adult (treatment-naïve 35 subjects, systemic corticosteroid group) and childhood asthmatics (treatment-naïve 70 subjects, inhaled corticosteroid group) in a secondary analysis. Adult asthmatics visited our clinic first with dyspnea and decreased lung function (FEV_1 ≤ 80% predicted value) and were diagnosed with asthma based on positive bronchodilator responses. They were treated with short-term systemic steroids alone for prompt relief of their symptoms (oral prednisolone 15 mg, twice per day for 7 days). Childhood asthmatics enrolled at the Asan Medical Center, Seoul, Korea, were diagnosed with asthma according to current treatment guidelines [2-4]. All were treated with inhaled corticosteroids for 8 weeks according to their asthma control status. Treatment responses in both groups were measured by the degree of increase in FEV_1: $\%FEV_1$ increase = (FEV_1 after treatment - FEV_1 at baseline)/FEV_1 at baseline × 100.

Genotyping

The assessment of the SNPs in *HDAC1* and *HDAC2* was described in our previous study [16]. Briefly, after isolating genomic DNA from the peripheral blood of 24 healthy Korean subjects using the QIAamp DNA blood kit following the manufacturer's protocol (Qiagen, Hilden, Germany), we amplified 2 kb of the 5'-upstream region in the promoter and all exons, including the exon-intron boundaries, of *HDAC1* and *HDAC2* by polymerase chain reaction (PCR; reference genome sequences; NM_004964.2 [*HDAC1*] and NM_001527.3 [*HDAC2*]). Amplified PCR products were sequenced using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in both directions according to standard protocols. After sequencing of *HDAC1* and *HDAC2*, we identified five SNPs in *HDAC1* and 14 SNPs in *HDAC2* (Supplementary Tables 1 and 2). Among these, two SNPs (rs1741981 in *HDAC1* and rs58677352 in *HDAC2*) were selected for scoring after considering minor allele fre-

quencies (higher than 5%) and location (in exons and 5', near the gene). Scoring was conducted with the high throughput single base-pair extension method (SNP-IT assay) using an SNPstream25K system, which was customized to automatically genotype DNA samples in 384-well plates and to provide a colorimetric readout (Orchid Biosciences, Princeton, NJ, USA) as described previously [17].

Statistical analyses

An association analysis, based on a case-control design, was performed for each SNP using a genetic model approach: an allele model (A [major allele] vs. B [minor allele]), a dominant model (AA vs. AB + BB), and a recessive model (BB vs. AA + AB). Hardy-Weinberg equilibrium was tested using 2 × 2 tests. All statistical analyses were performed with the R version 2.15.3 (http://www.R-project.org/). p values of less than 0.05 were considered to indicate statistical significance.

RESULTS

The characteristics of the asthmatics enrolled in the primary and secondary analyses are shown in Tables 1 and 2, respectively. Rs1741981 in *HDAC1* and rs58677352 in *HDAC2* were in Hardy-Weinberg equilibrium. The primary analysis revealed that rs1741981 was significantly related to asthma severity in a recessive model (p = 0.006) (Table 3). This relationship was still significant after the Bonferroni correction (multiplied by 6 [2 SNPs × 3 models]; corrected p = 0.036). However, rs58677352 showed no relationship with asthma severity (data not shown). As rs1741981 showed a significant relation with asthma severity in a recessive model, the secondary analysis was done using the same (recessive) model. Adult asthmatics with the CC genotype of rs1741981 (n = 12) showed significantly lower %FEV_1 increases in response to systemic corticosteroid treatment compared with those with the CT or TT genotype (12.7% ± 7.2% vs. 37.4% ± 33.7%, p = 0.018) (Fig. 1A).

Table 1. Characteristics of asthmatics enrolled in the primary analysis

Characteristic	Mild	Moderate to severe
No.	134	343
Male sex	44 (32.8)	122 (35.7)
Age, yr	47.3 ± 17.0	50.2 ± 16.3
Basal FEV1 predicted, %	95.2 ± 15.8	78.6 ± 13.5
PC20-methacholine[a], mg/mL	6.9 ± 1.2	4.7 ± 1.1
Atopy	66 (49.3)	157 (45.8)

Values are presented as number (%) or mean ± SD.
FEV_1, forced expiratory volume in 1 second.
[a]Geometric mean.

Table 2. Characteristics of asthmatics enrolled in the secondary analysis

Characteristic	Adult	Childhood
No.	35	70
Male sex	17 (48.5)	34 (48.6)
Age, yr	56.7 ± 12.1	10.2 ± 1.4
Basal FEV1 predicted, %	57.1 ± 12.9	78.9 ± 7.4
PC20-methacholine[a], mg/mL	NA	9.7 ± 1.3
Atopy	10 (28.6)	52 (74.3)

Values are presented as number (%) or mean ± SD.
FEV_1, forced expiratory volume in 1 second; NA, not applicable.
[a]Geometric mean.

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The same tendency was observed in childhood asthmatics. Subjects with the CC genotype of rs1741981 (n = 15) showed significantly lower %FEV_1 increases in response to inhaled corticosteroid treatment compared with those with the CT or TT genotype (14.1% ± 5.9% vs. 19.4% ± 8.9%, p = 0.035) (Fig. 1B). The combined p value, calculated from the one-sided p values of the secondary populations using Stouffer z-transform test [18], was 0.0019. However, rs58677352 showed no relationship with lung function improvement in response to corticosteroid treatment adults or childhood asthmatics (data not shown).

DISCUSSION

The present study demonstrated that rs1741981 in *HDAC1* is significantly associated with the response to corticosteroid treatment in asthmatics. To our knowledge, this is the first report of a genetic association between *HDAC1* and response to corticosteroids in asthmatics.

Table 3. Genotype frequency of rs174198 in *HDAC1* and rs58677352 in *HDAC2* among asthmatics in the primary analysis

Genotype	Frequency		*p* value		
	Mild	Moderate to severe	Dominant	Recessive	Allele
HDAC1 rs174198					
CC	17 (12.7)	83 (24.5)	0.203	0.006	0.014
CT	75 (56.0)	171 (50.4)			
TT	42 (31.3)	85 (25.1)			
HDAC2 rs58677352					
AA	3 (2.2)	15 (4.4)	0.887	0.406	1.000
AG	51 (38.1)	119 (34.7)			
GG	80 (59.7)	209 (60.9)			

Values are presented as number (%). Rs174198 genotyping failed in four moderate to severe asthmatics.

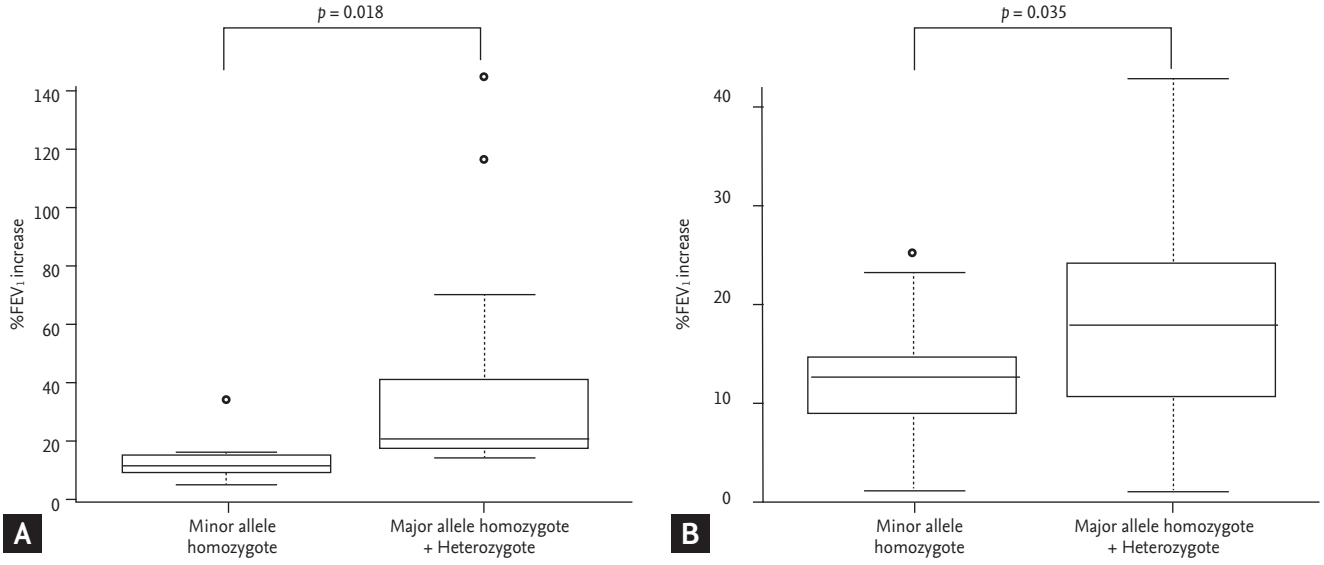

Figure 1. Forced expiratory volume in 1 second (FEV_1) improvement in response to corticosteroid treatment according to the genotype of rs174198 in *HDAC1* in the secondary analysis. (A) Adult asthmatics treated with oral prednisolone 15 mg, twice per day for 7 days. (B) Childhood asthmatics treated with inhaled corticosteroids according to their asthma control status. %FEV_1 increase = (FEV_1 after treatment - FEV_1 at baseline) / FEV_1 at baseline × 100.

Corticosteroids are among the most effective treatments for asthma [19], and reduce inflammation via glucocorticoid receptor-mediated recruitment of HDAC2 [20]. Because reduced *HDAC2* expression was observed in bronchial biopsies of asthmatic patients [21] and HDAC2-mediated deacetylation of the corticosteroid receptor enabled nuclear factor-κB suppression in airway epithelial cell line and alveolar macrophages [22], the discussion of the corticosteroid response in asthma has to date focused on HDAC2 alone. However, several investigators have argued that corticosteroid action in asthma is explained partially by inhibition of T cell activation [23-25]. Recently, it has been reported that that T cell-specific loss of *HDAC1* resulted in enhanced allergic airway inflammation, by modulating cytokine production [11]. Moreover, HDAC1 was localized within most airway cells and infiltrating inflammatory cells in the lung in a murine asthma model, and the HDAC inhibitor TSA attenuated allergic airway inflammation in mice by reducing T cell infiltration and Th2 cytokine production [26]. Taken together, HDAC1 may play an important role in mediating the anti-inflammatory action of corticosteroids. Accordingly, our data provide pharmacogenetic evidence that a genetic polymorphism in *HDAC1* can predict the response to corticosteroid treatment in asthmatics.

Functional variants strengthen the reliability of results of a pharmacogenetic study. Rs1741981 is located in the 5' near gene region of *HDAC1*. We used a web-based tool to evaluate the functional relevance of rs1741981. The Functional Single Nucleotide Polymorphism (F-SNP; http://compbio.cs.queensu.ca/F-SNP/) database integrates information obtained from 16 bioinformatics tools and databases about the functional effects of SNPs [27]. For rs1741981, F-SNP used TFSearch (http://www.cbrc.jp/research/db/TFSEARCH.html) [28] and Consite (http://asp.ii.uib.no:8090/cgi-bin/CONSITE/consite) [29] tools. Both tools computationally predict changes in transcription factor binding according to the allele of re1741981 (T allele vs. C allele) (Fig. 2 and Supplementary Fig. 1). F-SNP indicated 0.268 for the FS score of rs1741981. The FS score is a functional SNP scoring system provided by F-SNP to distinguish features of disease-related SNPs versus neutral SNPs [30]. The median FS score for neutral

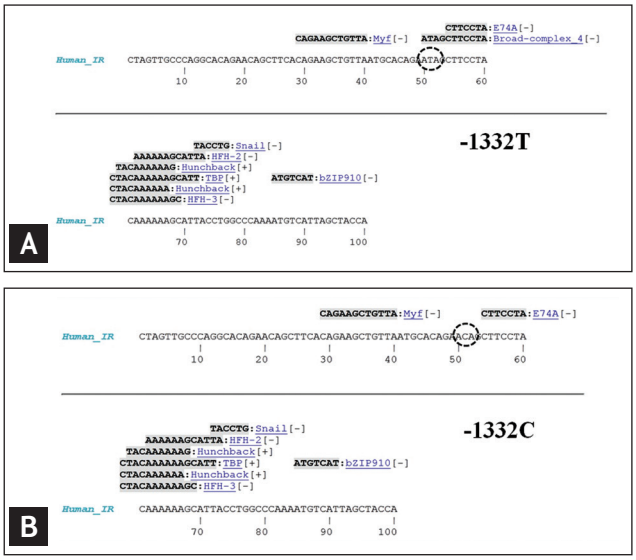

Figure 2. Changes in transcription factor binding according to the allele of rs1741981 in *HDAC1*. (A) -1332T. (B) -1332C. Predicted results from Consite (http://asp.ii.uib.no:8090/cgi-bin/CONSITE/consite).

SNPs is 0.1764, whereas for disease-related SNPs, the median rises to 0.5 [30]. Thus, rs1741981 may be functionally important.

There are several potential limitations to our results. First, sufficient statistical power, conferred by a large sample size, is an important aspect of genetic studies. The small number of patients enrolled in the present study limits the scope of our results. Second, subsequent replication studies should consider as many clinical and demographic confounding factors as possible and should be performed in diverse ethnic backgrounds. Third, haplotype approaches for *HDAC1* would result in more precise associations. For example, one previous study showed the advantage of examining both individual SNPs and haplotypes to further pin-point a potential causal SNP [31]. Finally, although a computational method suggested that rs1741981 may be functionally important, further mechanistic studies are needed to clarify the precise role of rs1741981 and *HDAC1* in the response to corticosteroid treatment.

Conflict of interest

No potential conflict of interest relevant to this article is reported.

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KEY MESSAGE

1. In the present study, we identified rs1741981 in *HDAC1*, which showed a significant relationship with asthma severity.
2. We also found that this genetic variation was associated significantly with lung function improvements in response to systemic corticosteroid treatment in adult asthmatics and in response to inhaled corticosteroids treatment in childhood asthmatics.
3. This is the first report of a significant association between a genetic variation in *HDAC1* and the response to corticosteroid treatment in asthmatics.

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