Genetic risk factors for decreased bone mineral accretion in children with asthma receiving multiple oral corticosteroid bursts

Heung-Woo Park, MD, PhD,[a,b] Bing Ge, MSc,[c] Szeman Tse, MDCM,[a,d] Elin Grundberg, PhD,[e] Tomi Pastinen, MD, PhD,[c,e,f] H. William Kelly, PharmD,[g] and Kelan G. Tantisira, MD, MPH[a,h] *Boston, Mass, Seoul, Korea, Montreal, Quebec, Canada, and Albuquerque, NM*

Background: Long-term intermittent oral corticosteroid (OCS) use in children with asthma leads to significant decreases in bone mineral accretion (BMA).

Objective: We aimed to identify genetic factors influencing OCS dose effects on BMA in children with asthma.

Methods: We first performed a gene-by-OCS interaction genome-wide association study (GWAS) of BMA in 489 white participants in the Childhood Asthma Management Program trial who took short-term oral prednisone bursts when they experienced acute asthma exacerbations. We selected the top-ranked 2000 single nucleotide polymorphisms (SNPs) in the GWAS and determined whether these SNPs also had *cis*-regulatory effects on dexamethasone-induced gene expression in osteoblasts.

Results: We identified 2 SNPs (rs9896933 and rs2074439) associated with decreased BMA and related to the tubulin γ pathway. The rs9896933 variant met the criteria for genome-wide significance ($P = 3.15 \times 10^{-8}$ in the GWAS) and is located on the intron of tubulin folding cofactor D *(TBCD)* gene. The rs2074439 variant ($P = 2.74 \times 10^{-4}$ in the GWAS) showed strong *cis*-regulatory effects on dexamethasone-induced tubulin γ gene expression in osteoblasts ($P = 8.64 \times 10^{-4}$). Interestingly, we found that BMA worsened with increasing prednisone dose as the number of mutant alleles of the 2 SNPs increased.

Conclusions: We have identified 2 novel tubulin γ pathway SNPs, rs9896933 and rs2074439, showing independent interactive effects with cumulative corticosteroid dose on BMA in children with asthma receiving multiple OCS bursts. (J Allergy Clin Immunol 2015;136:1240-6.)

Key words: *Asthma, bone mineral density, child, corticosteroids*

Bone mineral accretion (BMA) is defined as the accrual in bone mineral density (BMD) over time. Because adults normally lose BMD as they age, the achievement of BMD that occurs during childhood and adolescence is regarded as an important factor contributing to osteoporosis.[1] Therefore factors affecting BMA during this critical period might also increase the risk of osteoporosis later in life. The adverse effects of oral corticosteroids (OCSs) on bone health have been well known for many years, and even intermittent OCS use can affect BMA in childhood. Our group previously reported that frequent OCS use in children with asthma led to significant decreases in BMA.[2,3]

Advances in genomic technology enable us to integrate gene expression data with genotype data. Through this integration, we significantly increase the possibility of identifying functional genetic variants underlying disease pathogenesis. For example, we observed dexamethasone-specific *cis*-regulation of tenascin C *(TNC)* expression in the primary osteoblast.[4] We then performed an association study among 6 expression single nucleotide polymorphisms (SNPs) within the *TNC* locus and examined increases in lung function after 2 months of inhaled corticosteroid (ICS) treatment in children with asthma. Finally, we demonstrated that this heritable *cis*-regulation of *TNC* expression explained the difference in response to ICSs by showing that 4 of 6 SNPs were significantly associated with lung function increases.[4] Using a cellular expression quantitative trait locus (eQTL) approach, we have also recently identified several SNPs associated with ICS response at a genome-wide level.[5]

The purpose of the present study was to identify genetic factors with the potential to substantially modify the effects of OCSs on BMA in children with asthma receiving multiple intermittent OCS courses over time. We initially performed a genome-wide association study (GWAS) of OCS-induced BMA changes observed in children with asthma participating in the Childhood Asthma Management Program (CAMP) trial.[5] They were followed for a mean of 4.3 years and took short-term oral prednisone bursts when they experienced acute exacerbations. To refine the potential for these SNPs to have a functional effect on bone, we determined whether the top-ranked SNPs had *cis*-regulatory

From [a]the Channing Division of Network Medicine and [h]the Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston; [b]the Department of Internal Medicine, Seoul National University College of Medicine; [c]McGill University and Genome Quebec Innovation Centre, Montreal; [d]the Department of Pediatrics, Sainte-Justine University Health Center, University of Montreal; the Departments of [e]Human Genetics and [f]Medical Genetics, McGill University, Montreal; and [g]the Department of Pediatrics, University of New Mexico Health Sciences Center, Albuquerque.

Supported by grants U01 HL65899, R01 HL092197, and R01 NR013391. The Childhood Asthma Management Program is supported by contracts NO1-HR-16044, 16045, 16046, 16047, 16048, 16049, 16050, 16051, and 16052 with the National Heart, Lung, and Blood Institute and General Clinical Research Center grants M01RR00051, M01RR0099718-24, M01RR02719-14, and RR00036 from the National Center for Research Resources.

Disclosure of potential conflict of interest: H.-W. Park, S. Tse, and K. G. Tantisira have received research support from the National Institutes of Health. H. W. Kelly has received research support from the National Heart, Lung, and Blood Institute (NHLBI; the Childhood Asthma Management Program was supported by contract NO1-HR-16051 with the NHLBI and General Clinical Research Center grant M01RR0099718-24 from the National Center for Research Resources) and has received consultancy fees from GlaxoSmithKline, AstraZeneca, Merck, and Novartis. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication October 9, 2014; revised April 2, 2015; accepted for publication April 3, 2015.

Available online May 27, 2015.

Corresponding author: Kelan G. Tantisira, MD, MPH, Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Ave, Boston, MA 02115. E-mail: kelan.tantisira@channing.harvard.edu.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2015.04.014

Abbreviations used
APPL1: Adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1
BMA: Bone mineral accretion
BMD: Bone mineral density
CAMP: Childhood Asthma Management Program
eQTL: Expression quantitative trait locus
F-SNP: Functional Single Nucleotide Polymorphism
GWAS: Genome-wide association study
ICS: Inhaled corticosteroid
LD: Linkage disequilibrium
OCS: Oral corticosteroid
PAK2: p21 protein–activated kinase 2
SNP: Single nucleotide polymorphism
TBCD: Tubulin folding cofactor D
TNC: Tenascin C
TUBG1: Tubulin γ 1

effects on corticosteroid-induced gene expression in osteoblast cells. Finally, noting that 2 of the associated variants mapped to the same pathway, we tested the combined effects of the 2 SNPs on BMA.

METHODS

The CAMP trial was approved by the institutional review boards of the participating institutions, and informed consent was obtained from the participating children and their parents.

CAMP BMA measurements

A total of 1041 children with mild-to-moderate asthma aged 5 to 12 years were randomized to budesonide, nedocromil, or placebo and followed for a mean of 4.3 years in the CAMP trial.[6] For asthma exacerbations, oral prednisone bursts were prescribed per protocol as follows: 2 mg/kg per day (up to 60 mg) for 2 days followed by 1 mg/kg per day (up to 30 mg) for 2 days, with an option to continue dosing if improvement was insufficient. BMD measurements (in grams per square centimeter) of the lumbar spine (L1-L4) were performed yearly during the study period by means of dual-energy x-ray absorptiometry with the Hologic (Waltham, Mass) QDR-1500 (at 6 centers) or the Lunar (Madison, Wis) DPX (at 2 centers). Hologic dual-energy x-ray absorptiometry machines were further divided by the use of pencil-beam or fan-beam measurements. Detailed methods to convert Lunar measures to Hologic values and to adjust deviations between pencil- and fan-beam measurements were described in our previous report.[3] BMA (in grams per square centimeter per year) represents the average gain of BMD over time and was calculated as follows:

(BMD at 4 years' follow-up − BMD at baseline)/4 years.

A 4-year follow-up was selected because this approximated the end of the trial. BMD z scores were calculated by using CAMP internal references.[2]

GWAS of BMA

Genotyping was done with the HumanHap550v3 BeadChip or the Infinium HD 140 Human610-Quad BeadChip (Illumina, San Diego, Calif). SNPs showing significant deviation from Hardy-Weinberg equilibrium ($P < .001$), SNPs with low minor allele frequency (<0.05), and SNPs with high rates of missing data (>0.01) were excluded. Using a linear regression gene-environment interaction model as implemented in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/),[7] we performed a GWAS to test the following model:

BMA ~ Genotype (additive genetic model) + Cumulative dose of OCS + Genotype*cumulative dose of OCS + Covariates.

TABLE I. Baseline characteristics of subjects enrolled in CAMP

Total no.	489
Male sex, no. (%)	302 (61.7)
Age (y), mean (SD)	8.8 (2.1)
Height (cm), mean (SD)	132.0 (19.8)
BMI (kg/m^2), median (IQR)	17.1 (15.8-19.7)
BMD (g/cm^2), median (IQR)	0.782 (0.683-0.935)
Vitamin D (ng/mL), median (IQR)	36.6 (28.8-47.9)
Tanner stage, no. (%)	
I/II/III/IV/V	353 (72.2)/100 (20.4)/24 (4.9)/11 (2.2)/1 (0.3)
Cumulative dose of prednisolone (mg), median (IQR)	360 (120-900)
BMA (g/cm^2/y), median (IQR)	0.038 (0.024-0.063)

BMI, Body mass index; *IQR*, interquartile range.

Based on our previous report, age, sex, BMD, height, body mass index, serum vitamin D concentration, and Tanner stage measured at baseline were included as covariates.[3] Because we focused on the interactive effect between genotype and cumulative OCS dose on BMA, we reported P values from the "genotype*cumulative dose of OCS" term. Only the data from white children were used in the present study because of potential issues with population stratification.

Cis-regulatory effect on corticosteroid-induced gene expression in osteoblasts

We used data from our previous study.[4] In brief, expression profiling of human primary bone cells derived from 113 donors (51 female and 62 male donors undergoing total hip or knee replacement at the Uppsala University Hospital, Uppsala, Sweden) and stimulated with dexamethasone (100 nmol/L) for 24 hours was measured by using Illumina HumRef-8v2 BeadChips. Genotyping was done with the Illumina HapMap550K Duo chip, as previously described.[4] We performed an association test between corticosteroid-induced gene expression and SNPs by using a linear regression model implemented in SNPTEST software (http://www.stats.ox.ac.uk/~marchini/software/gwas/gwas.html).[8] An additive effect of SNP was assumed, and 2 covariates (year of birth and sex) were included in a regression model. *Cis*-regulatory SNPs were defined when SNPs were located in a 1-Mb window flanking the target gene or within the target gene itself, and their P values were less than .05.

Statistical analysis

Pearson correlation and simple linear regression analysis were done to evaluate relations between the cumulative dose of OCS and BMA, according to the genotypes. Multiple linear regression models were constructed to test effects of cumulative OCS dose on BMA adjusted by baseline covariates. All analyses were performed with R (version 3.0.2) software (www.r-project.org).

RESULTS

A total of 489 white subjects were included in the GWAS of OCS-induced BMA. Table I summarizes baseline characteristics. The median number of OCS bursts was 3 (range, 0-27). The cumulative dose of OCS showed a significantly negative association with BMA after adjustment for covariates (β coefficient $= -3.036 \times 10^{-6}$ and $P = 7.96 \times 10^{-5}$, see Table E1 in this article's Online Repository at www.jacionline.org). A total of 44,319 SNPs with P values of less than .05 in GWASs were identified. Table II shows 9 SNPs that met the threshold for genome-wide significance ($P < 1 \times 10^{-7}$) for the interaction of genotype with corticosteroid dose on BMA. Quantile-quantile and Manhattan plots for the interaction results