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Original Article

Correlation between eosinophil count, its genetic background and body mass index: The Nagahama Study

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DNA, deoxyribonucleic acid; GWAS, genome-wide association study; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HLA, human leukocyte antigen; LDL, low-density lipoprotein; MDC1, mediator of DNA damage checkpoint protein 1; MHC, major histocompatibility complex; PCA, principal component analysis; SNP, single-nucleotide polymorphisms; T-Chol, total cholesterol; WBC, white blood cells

ABSTRACT

Background: Obesity affects the pathogenesis of various chronic diseases, including asthma. Research on correlations between obesity/BMI and eosinophilic inflammation in asthma has yielded contradictory results, which could be partly ascribed to the absence of epidemiological data on the correlations. We aimed to elucidate the correlations between blood eosinophil count, its genetic backgrounds, and BMI in the general population.

Methods: This community-based Nagahama study in Japan enrolled 9789 inhabitants. We conducted self-reporting questionnaires, lung function tests, and blood tests in the baseline and 5-year follow-up studies. A genome-wide association study (GWAS) was performed in 4650 subjects at the baseline and in 4206 of these at the follow-up to determine single-nucleotide polymorphisms for elevated blood eosinophil counts. We assessed the correlations between BMI and eosinophil counts using a multifaceted approach, including the cluster analysis.

Results: Eosinophil counts positively correlated with BMI, observed upon the interchange of an explanatory variable, except for subjects with the highest quartile of eosinophils ($\geq$200/μL), in whom BMI negatively correlated with eosinophil counts. GWAS and human leukocyte antigen (HLA) imputation identified rs4713354 variant (*MDC1* on chromosome 6p21) for elevated eosinophil counts, independent of BMI and IgE. Rs4713354 was accumulated in a cluster characterized by elevated eosinophil counts (mean, 498 ± 178/μL) but normal BMI.

Conclusions: Epidemiologically, there may be a positive association between blood eosinophil counts and BMI in general, but there was a negative correlation in the population with high eosinophil counts. Factors other than BMI, particularly genetic backgrounds, may contribute to elevated eosinophil counts in such populations.

Introduction

Obesity, a major public health concern, is a critical risk factor for the onset of asthma and severe disease with altering response to therapy, especially glucocorticosteroids[1]; this is primarily ascribed to mechanical and physiological changes, such as a reduction in the functional residual capacity and airway calibers, but also to

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systemic inflammation.[1–3] Traditionally, systemic inflammation in obesity is considered to be associated with macrophages and neutrophils, also true for asthma.[4]

Meanwhile, in severe asthma, Desai *et al.* reported higher eosinophil counts in the airway submucosa and sputum interleukin-5 levels in obese asthmatics compared with leaner counterparts.[5] Reportedly, anti-interleukin-5 antibody was the most effective in obese severe asthmatics with relatively increased blood eosinophils ($\geq$150/μL) and reversible airways.[6] Previously, epidemiological studies have reported that overweight or obesity is a risk factor for blood eosinophil >2% in a population with normal lung function.[7,8] In addition, metabolic syndrome is associated with increased white blood cell (WBC) count, including eosinophils.[9,10] Thus, eosinophilia can coexist with overweight or obesity. Conversely, higher blood eosinophil count correlates with leaner patients with asthma.[11] Moreover, higher serum periostin levels, another marker of eosinophilic/type 2 inflammation,[12,13] are related to the lower BMI in the general population[14–16] and patients with asthma.[16–18] Considering these conflicting findings overall, the correlations between BMI/obesity and eosinophilic/type-2 inflammation might not be unidirectional. Indeed, at least, two distinct phenotypes with different age onset are reported in obese asthma.[2] A study reported that obese uncontrolled asthma was characterized by earlier-onset asthma, higher levels of exhaled nitric oxide, and more severe airway hypersensitivity, compared with obese controlled asthma.[19] Likewise, among two groups of asthmatics with eosinophilic airway inflammation and relative steroid insensitivity, one group was characterized by high BMI.[20] To elucidate these complicated and heterogeneous correlations between BMI/obesity and eosinophilic inflammation in asthma,[21] evidence on their correlations in the general population is essential.

This large-scale, general population study aims to elucidate the correlations between BMI/obesity and blood eosinophil counts by a multifaceted and longitudinal approach. We hypothesized that an increase in BMI generally contributes to an increase in blood eosinophil counts; however, non-eosinophilic inflammation might overwhelm in subjects with severe obesity. Meanwhile, subjects with eosinophilia would exhibit low or normal BMI, in whom eosinophils could be determined by other factors such as genetic backgrounds[22] rather than by BMI.

Methods

Genotyping and genome-wide association study and human leukocyte antigen imputation

We extracted the deoxyribonucleic acid (DNA) from peripheral blood samples by the phenol–chloroform method and analyzed using a series of BeadChip DNA arrays as follows: Human610k-Quad, HumanOmni2.5-4, HumanOmni2.5-8, HumanOmni2.5s, and HumanExome (Illumina, San Diego, CA). In addition, the genotyping quality was assured by excluding single-nucleotide polymorphisms (SNPs) with a call rate <99%, minor allele frequency <0.01, or extreme deviation from the Hardy–Weinberg equilibrium ($P < 1.0 \times 10^{-7}$) in each DNA array, followed by combining genotypes for the common SNPs among all the DNA arrays. We confirmed the cryptic relatedness and ethnicity using the identity-by-descent estimates and the principal component analysis (PCA) using the PLINK ver. 1.9 software, respectively. For the PCA, we used SNPs independent of one another among genotypes, including those obtained by our genome-wide association study (GWAS) and from HapMap data (http://www.hapmap.org) of Utah residents with Northern and Western European ancestry from the CEPH collection (CEU), Japanese in Tokyo, Japan + Han Chinese in Beijing, China (JPT + CHB), and Yoruba in Ibadan, Nigeria (YRI). Among these independent SNPs, the SNPs of the first and second principal components were applied to ethnic discrimination. Next, we repeated the PCA using independent SNPs from our genotype data and JPT + CHB data to discriminate the subjects with Japanese ancestry from those with non-Japanese East-Asian ancestry.

The imputation included 4615 variants—3848 SNPs, 645 HLA amino acids, 53 two-digit HLA alleles, and 69 four-digit HLA alleles. The pan-Asian panel ($n = 530$) was used as a reference. For the accuracy of the imputation, we excluded imputed SNPs with R^2_{dosage} <0.8 from the analysis. In this study, we considered $P \leq 1.08 \times 10^{-5}$ (0.05/4615, Bonferroni correction) as statistically significant.

Cluster analysis

For the cluster analysis, we used the following five variables: total cholesterol (T-Chol), smoking history (current- or ex-smoking vs never smoking), high-density lipoprotein (HDL), hemoglobin A1c (HbA1c), and blood eosinophil counts; these variables were reduced from the following 12 variables according to the PCA: age, gender, smoking history, BMI, blood neutrophil counts, blood eosinophil counts, serum total IgE, T-Chol, HDL, low-density lipoprotein (LDL), and HbA1c levels.

Study participants and measurements

The Nagahama Prospective Genome Cohort for Comprehensive Human Bioscience (The Nagahama Study) is a community-based prospective cohort study[23,24] in which subjects were enrolled from the general population of Nagahama (125,000 inhabitants) in the Shiga Prefecture, Central Japan, from November 2008 to November 2010.

The inclusion criteria were as follows: age 30–74 years, able to live independently, and no current serious diseases or physical impairment. Overall, 9804 residents voluntarily participated in the study and underwent self-reported questionnaires, physical examinations, lung function test, and blood tests, including glycolipid metabolisms, serum total IgE levels, and genetic analyses, at enrollment. The presence of asthma was determined with the use of a self-reported questionnaire by an affirmative response to the question “Have you ever experienced an asthmatic episode since becoming an adult?” Of the 9804, 14 withdrew consent later, and 1 was excluded because of the missing blood eosinophil data. Thus at the baseline, the total number of current cohort participants was 9789. After 5 years of the baseline, the participants in this Nagahama cohort were invited to a follow-up assessment, from November 2013 to November 2015. Overall, 8308 subjects participated in the follow-up study. Excluded from the follow-up assessment were individuals who had migrated from Nagahama. We performed examinations similar to those at the baseline, except for the serum total IgE measurement. This study confirms the standards of the Declaration of Helsinki. The Ethics Committee of the Kyoto University Graduate School of Medicine (Kyoto, Japan) and the Nagahama Municipal Review Board approved this study protocol (Registry ID G0278). We obtained written informed consent from all the participants.

Genotyping and genome-wide association study and human leukocyte antigen imputation

In the genome-wide association study (GWAS) for elevated blood eosinophil counts, 99,065 single-nucleotide polymorphisms (SNPs) were genotyped for 4650 subjects at the baseline, of whom 16 were estimated as non-Japanese eastern Asian ethnicity by the principal component analysis (PCA; see

Supplementary Methods). Of these, the GWAS was repeated at the follow-up study in 4206 subjects. The detailed methods are described elsewhere[25] and in the Supplementary Methods. The correlations between the SNPs and eosinophil counts were assessed by the linear regression analysis under an additive genetic model adjusted for age and gender. We considered $P \leq 5 \times 10^{-7}$ (0.05/99,065, Bonferroni correction) in the baseline study as statistically significant in this study. In addition, we performed the human leukocyte antigen (HLA) imputation using the SNP2HLA for variants located within the major histocompatibility complex (MHC) region—chr 6: 29–34 Mbp on build 36.[26] A validation study in an independent population (n = 1254) was conducted to assess a possible correlation between eosinophil counts and a genetic variant. This population was also part of the Nagahama study, but was newly examined for genetic backgrounds in addition to the analysis of the 4650 subjects in the discovery cohort.

Statistical analysis

In this study, JMP version 12 (SAS Institute Inc., Tokyo, Japan) was used to perform the statistical analyses.

First, we used the regression analysis to examine the distributions of blood granulocyte counts (dependent variables) against BMI (independent variable). Then, the distribution of BMI (dependent variable) against blood eosinophil counts (independent variable) was assessed. Using data at the baseline and follow-up examinations, the correlations between changes in blood eosinophil counts and changes in BMI during the follow-up period were examined by the regression analysis. The distribution of serum total IgE concentrations and peripheral blood absolute eosinophil counts were skewed, which were logarithmically transformed for the regression analysis.

We then used the K-means method to perform the cluster analysis, and the best cluster number was evaluated according to the cubic clustering criterion (SAS Institute Inc.). We assessed the differences in characteristics among clusters by ANOVA, Wilcoxon's rank-sum test, and χ^2 test, appropriately. The data are presented as means ± SDs, if not otherwise specified.

Detailed methods are described in the Supplementary Methods.

Results

Participants' characteristics

Table 1 summarizes the characteristics of the 9789 participants. The average BMI was 22.3 ± 3.3 kg/m^2, peripheral blood neutrophil counts were 3396 ± 1203/μL, and peripheral blood eosinophil counts were 155 ± 128/μL (Table 1).

Table 1
Participants' characteristics at the baseline.

Indices	
Age, years	53.6 ± 13.4
Sex, male/female, %	33/67
BMI, kg/m^2	22.3 ± 3.3
Smoking history, never/ex/current, %	65/20/15
Asthma[†], +/−, %	4/96
Allergic rhinitis[†], +/−, %	35/65
Blood neutrophil counts,/μL	3396 ± 1203
Blood eosinophil counts,/μL	155 ± 128

The data at enrollment are presented in this table. The data are presented as means ± SDs.
[†] Determined by self-reporting questionnaires.

Associations between blood indices, asthma and BMI

Blood neutrophil counts positively correlated with BMI ($P = 2.2 \times 10^{-21}$; Supplementary Fig. 1); this correlation remained after the adjustment for age, gender, smoking history, and blood WBC counts ($P = 4.7 \times 10^{-19}$). When a quadratic equation that also fit the data was applied, coefficients of both BMI and BMI^2 were positive (data not shown), suggesting that blood neutrophil counts increased in an accelerated manner as BMI increased. Similarly, blood eosinophil counts positively correlated with BMI ($P = 1.1 \times 10^{-51}$; Fig. 1); this correlation remained after the adjustment for confounders ($P = 9.6 \times 10^{-20}$; Table 2), including serum total IgE that was also positively related to BMI (Supplementary Fig. 2). However, when a quadratic equation was applied, a coefficient of BMI^2 was negative, and the vertex of the quadratic equation was located at around 40 kg/m^2 of BMI and 171/μL (95% confidence interval: 139–211/μL) of eosinophils. Among the metabolic indices, while serum HbA1c and LDL levels positively, HDL and T-Chol levels negatively correlated with blood eosinophil counts (Supplementary Table 1). Lastly, higher BMI was associated with the presence of self-reported asthma, after the adjustment for age, gender, smoking history, blood neutrophil counts, eosinophil counts, and serum total IgE [estimate (95% confidence interval), 0.037 (0.006–0.067), $P = 0.016$].

Distribution of BMI against blood eosinophil counts

Next, we assessed BMI distribution against blood eosinophil counts. As anticipated, BMI positively correlated with eosinophil counts ($P = 1.1 \times 10^{-51}$; Supplementary Fig. 3), even after the adjustment for age, gender, smoking history, blood WBC counts and total IgE level ($P = 7.3 \times 10^{-20}$; Table 3A). However, when we divided all the subjects into quartiles based on blood eosinophil counts, BMI negatively correlated with blood eosinophil counts in the highest quartile group of eosinophils (eosinophil ≥ 200/μL) ($P = 0.0099$; Fig. 2), which was confirmed after the correction of confounders ($P = 0.001$; Table 3B). When the analysis was confined to subjects with self-reported asthma and elevated eosinophil counts (≥200/μL) (n = 174), negative correlation between BMI and blood eosinophil counts, albeit marginally ($P = 0.056$), was observed.

Cluster analysis and longitudinal observation

The cluster analysis of all the participants yielded nine clusters, the number of which was determined according to the cubic

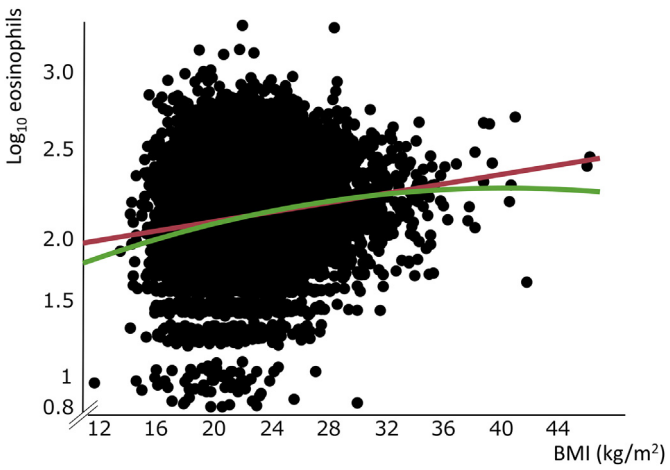

Fig. 1. The distribution of blood eosinophil counts against BMI. Red line, the linear regression line; green curve, the quadratic regression curve.

Table 2
The contribution of the BMI to blood eosinophil counts†, adjusted by age, gender, smoking history, blood WBC counts, and serum total IgE level.

Indices	Estimate (95% CI)	*P*
BMI, kg/m^2	0.01 (0.007–0.011)	<0.0001
Age, by 10-year old increase	−0.01 (−0.02 to −0.01)	<0.0001
Gender (male)	0.02 (0.01–0.03)	<0.0001
Smoking history (current or ex)	0.02 (0.01–0.03)	<0.0001
WBC counts, by 2000/μL increase	0.09 (0.08–0.09)	<0.0001
Serum total IgE, IU/mL†	0.11 (0.1–0.12)	<0.0001
	$R^2 = 0.14$, $P < 0.0001$	

WBC, white blood cells.
† Log-transformed.

Table 3A
The contribution of blood eosinophil counts to BMI, adjusted by age, gender, smoking history, blood WBC counts, and serum total IgE level.

Indices	Estimate (95% CI)	*P*
Eosinophil counts†	0.95 (0.74–1.1)	<0.0001
Age, by 10-year old increase	0.37 (0.32–0.41)	<0.0001
Gender (male)	0.73 (0.65–0.82)	<0.0001
Smoking history (current or ex)	−0.17 (−0.25 to −0.09)	<0.0001
WBC counts, by 2000/μL increase	0.57 (0.49–0.65)	<0.0001
Serum total IgE, IU/mL†	0.03 (−0.08 to 0.14)	<0.0001
	$R^2 = 0.11$, $P < 0.0001$	

WBC, white blood cells.
† Log-transformed.

clustering criterion. Since one cluster was too small to analyze (n = 27), we excluded it from the analysis. Finally, we included eight clusters for the further analysis (Fig. 3). Of the eight clusters, one was characterized by eosinophilia (498 ± 178/μL), the presence of self-reported asthma, and normal BMI (22.4 ± 3.3 kg/m^2; Cluster 5 in Fig. 3 and Supplementary Table 2). In the "eosinophilic" cluster, BMI exhibited a negative correlation with blood eosinophil counts ($P = 0.049$), in contrast to other 7 clusters.

Further, we conducted a follow-up study in 8308 participants after 5 years. The average changes in BMI and blood eosinophil counts were +0.1 ± 4.6 kg/m^2 and −11 ± 122/μL, respectively. We assessed the correlations between changes in BMI and those of eosinophil counts. In all the participants, the changes in BMI positively correlated with changes in eosinophils ($P = 1.5 \times 10^{-7}$; Table 4). This correlation persisted after the adjustment for age, gender, smoking history, and WBC counts at the follow-up. Meanwhile, the changes in BMI were not related to changes in eosinophils in "eosinophilic" cluster ($P = 0.8$; Table 4).

GWAS and HLA imputation for blood eosinophil counts

In the baseline study, the GWAS identified 15 SNPs with *P* value $\leq 5 \times 10^{-7}$ on chromosome 6p21 in the MHC region, which correlated with eosinophil counts (Supplementary Table 3). Among these, rs2394392 exhibited the lowest *P* value (2.38×10^{-8}). In the follow-up study, all of the 15 SNPs exhibited statistical significance with $P \leq 0.003$ (0.05/15, Bonferroni correction). Previously reported SNPs of rs1420101 located in *IL1RL1* on 2q11, rs4857855 in *GATA2* on 3q21, and rs4143832 in *IL5* on 5q23[22] were unrelated with higher eosinophil counts in this study.

To further localize the potential variants responsible for increased blood eosinophils in the MHC region, we conducted the HLA imputation and identified rs4713354 in *MDC1* on 6p21.33, coding mediator of deoxyribonucleic acid (DNA) damage checkpoint 1 (MDC1) protein, with the lowest *P* value ($\beta = 0.14$; $P = 2.17 \times 10^{-8}$) (Fig. 4). According to the validation study, we found a significant and positive correlation between the incidence of the C allele of rs4713354 and eosinophil counts ($\beta = 0.14$; $p = 0.02$). The frequencies of alleles A and C in the validation study were 77.7% and 22.3%, which were similar to those in the discovery cohort, 78.4% and 21.6%, respectively. All genotyped data were in Hardy–Weinberg equilibrium. In addition, we identified 2 two-digit HLA alleles, 3 four-digit HLA alleles, and 5 amino acid variants, all for HLA class I. However, none of them exceeded the *P* value of rs4713354 (Supplementary Table 4), and when conditioning on rs4713354, other SNPs, HLA alleles, and amino acid variants lost the statistical significance.

Lastly the impact of the risk C allele of rs4713354 on increased eosinophil counts remained significant after the adjustment for BMI, smoking history, WBC counts, and serum total IgE in addition to age and gender ($P = 2.92 \times 10^{-9}$). The C allele of rs4713354 was accumulated in the "eosinophilic" cluster with allele frequency of 27%, while frequency of C allele was 22% in all the participants. Frequency of subjects having C allele was 47% in this cluster, whereas it was 38% in all the participants (Supplementary Table 2).

Discussion

To the best of our knowledge, this is the first study to elucidate nonlinear, nonuniform correlations between BMI and eosinophil counts in the general population, which might provide epidemiological evidence to comprehend conflicting findings in asthma. Corroborating the established evidence that obesity correlates with chronic low-grade neutrophilic inflammations,[27] blood neutrophil counts elevated in an accelerated manner as BMI increased. This

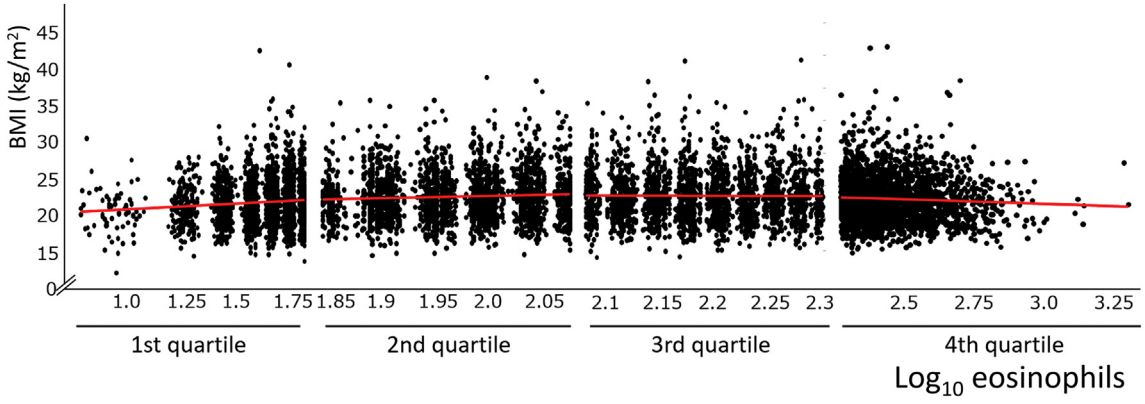

Fig. 2. The distribution of BMI were negatively related to subjects in the fourth quartile of blood eosinophil counts (≥200/μL). Red line, the linear regression line.

Table 3B
The contribution of blood eosinophil counts[†] to BMI in each quartile of blood eosinophil counts.

Eosinophil range	First Quartile	Second Quartile	Third Quartile	Fourth Quartile
	0–70/μL	70–120/μL	120–200/μL	≥200/μL
	$n = 2461$	$n = 2454$	$n = 2427$	$n = 2447$
Estimate (95% CI)	1.5 (0.9–2.1)	2.7 (0.8–4.5)	−1.3 (−3.3 to 0.8)	−1.6 (−2.5 to −0.6)
P	<0.0001	0.005	0.2	0.001

Adjusted by age, gender, smoking history, white blood cell counts, serum total IgE.
[†] Log-transformed.

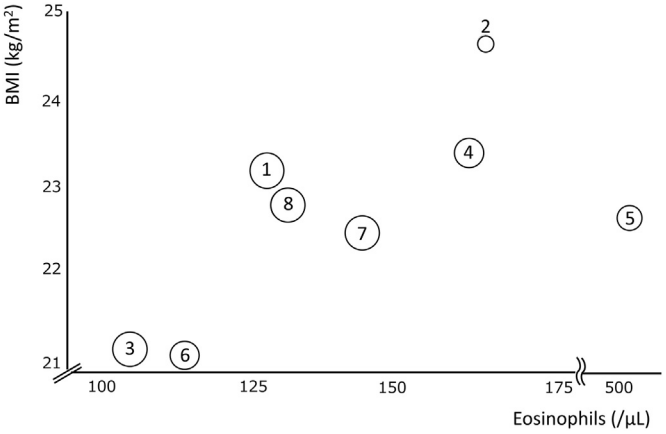

Fig. 3. The distribution of eight clusters on the axes of BMI and blood eosinophil counts. Circle size, the number of subjects in each cluster. Circle numbers, the cluster numbers in Supplementary Table 2.

study revealed that eosinophil counts also increased as BMI increased until reaching a plateau at around 40 kg/m^2, whereas in subjects with eosinophilia (eosinophil ≥ 200/μL), eosinophil counts negatively correlated with BMI. Finally, GWAS and HLA imputation identified a correlation between the rs4713354 variant and elevated eosinophil counts.

In spite of the traditional recognition that obesity is independent of type-2/eosinophilic pathways, some smaller epidemiological studies[9,10] demonstrated positive correlations between blood eosinophil counts and BMI or metabolic syndrome. In a larger sample size, we reported that blood eosinophil counts generally increased as BMI increased, which corroborates a recent report from the Netherlands.[8] In addition, consistent with a previous study, glycolipid metabolites correlated with higher blood eosinophil counts in this study.[10] This epidemiological evidence substantially supports the possible coexistence of obesity, metabolic changes, and eosinophilic inflammation. One of the potential mechanisms underlying the positive correlation between blood eosinophil counts and BMI could be attributed to elevated leptin levels in overweight/obese subjects, although we did not measure adipokines, including leptin in this study. Reportedly, leptin is released from adipocytes and exerts various effects on inflammation and immune system,[28] such as the suppression of the apoptosis of eosinophils,[29,30] as well as neutrophils, and the promotion of type-2 inflammations.[31] Briefly, overweight/obese and

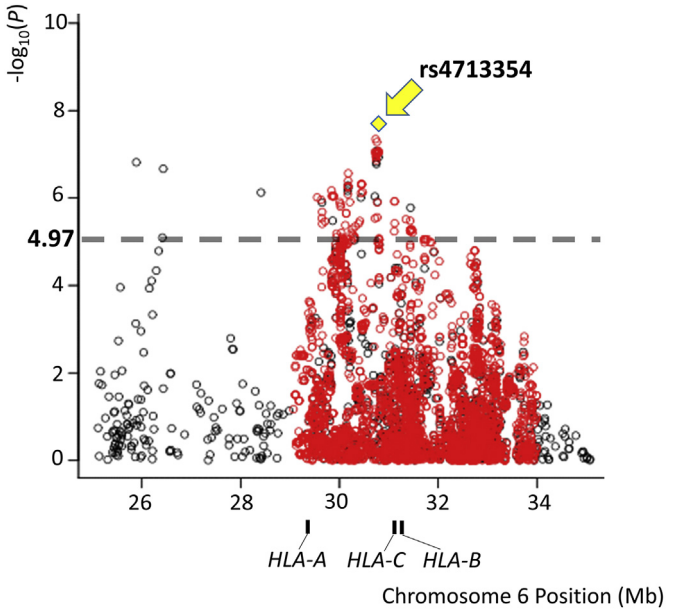

Fig. 4. Correlation plots between variants and increased peripheral eosinophil counts. White circles, representing $-\log_{10}$ (*P* values) of SNPs identified in the baseline and follow-up GWAS. Red circles, representing each variant including SNPs, HLA and amino acids identified in the HLA imputation. Diamond, rs4713354, which exhibited the lowest *P* value. Dotted line, the threshold for the imputation significance, $-\log_{10}(1.08 \times 10^{-5}) = 4.97$. HLA, human leukocyte antigen.

Table 4
Contributions of changes in BMI to those in eosinophils[†].

Indices	All participants ($n = 8211$)		Eosinophilic cluster ($n = 511$)	
	Multivariate analysis		Multivariate analysis	
	Estimate (95% CI)	*P*	Estimate (95% CI)	*P*
⊿BMI[†]	0.99 (0.6–1.4)	<0.0001	0.14 (−0.87 to 1.2)	0.8
Age[‡], by 10-year old increase	3.7 (2.1–5.3)	<0.0001	1.5 (−2.7 to 5.7)	0.49
Gender (male)	2.5 (−0.3 to 5.4)	0.08	2.2 (−4.8 to 9.2)	0.54
Smoking history (current or ex)[‡]	0.7 (−2.3 to 3.5)	0.64	−4 (−11 to 2.9)	0.25
⊿WBC[†]	−0.4 (−0.5 to −0.3)	<0.0001	−0.5 (−0.7 to 0.3)	<0.0001

WBC, white blood cells.
[†] Defined as data [(at baseline − at follow-up)/at baseline] × 100 (%).
[‡] Data at the follow-up study.

its metabolic changes could contribute to mild-to-moderate increment in eosinophil counts, which is replaced by accelerated neutrophilic inflammation in severe obese subjects, as is repeatedly reported in obese asthma.[4,32]

When assessing the impact of blood eosinophil counts on BMI, blood eosinophil counts negatively correlated with BMI in subjects with eosinophilia or the "eosinophilic" cluster. The negative correlation was also observed, when the analysis was confined to subjects with self-reported asthma. This epidemiological finding supports previous studies reporting negative correlations of BMI with blood eosinophil counts in asthma[11] or with serum periostin levels in the general population[14,15] and in patients with asthma.[16,18] In this study, the mechanisms underlying the negative correlation between eosinophil counts and BMI in subjects with high eosinophils remain unclear. As a potential mechanism, although speculative, Brestoff *et al.* recently reported that eosinophils and type-2 cytokines induced M2-macrophages in the adipose tissue, which induced differentiation of white adipocytes into beige adipocytes. Furthermore, epinephrine/norepinephrine from beige adipocytes shifts the energy storage to emanation in the mitochondria and, then, leads to the suppression of obesity.[33]

In the GWAS, we determined several risk variants in the MHC region for increased eosinophil counts, which is consistent with some previous studies.[22,34] After the HLA imputation, rs4713354 (position 30793399) of *MDC1* that codes the MDC1 protein exhibited the strongest correlation with increased eosinophil counts. The MDC1 protein plays a role in the DNA maintenance by recruiting repair proteins to the site of DNA damage and comprises antiapoptotic properties. When considering that eosinophils have the DNA repair machinery,[35] variants of *MDC1* might affect the lifespan of eosinophils; however, there is no conclusive evidence for the correlation until further investigation with larger sample size and functional analyses are conducted. Albeit, *MDC1* might be merely a tag SNP for eosinophilia, and there could be an eosinophilia-associated haplotype specifically to the Japanese population, as described later. In this study, we determined several variants in class I HLA alleles and amino acids for elevated eosinophil counts. Of interest, among the variants, 3 four-digit class I HLA alleles, that is, HLA_A_3303, HLA_C_1403, and HLA_B_4403, reportedly form a low frequency of long-range haplotype in the Japanese population.[36] In addition, risks of these class I HLA alleles for increased eosinophil counts disappeared when conditioning on rs4713354, suggesting the presence of genetic linkage of rs4713354 with this haplotype. Thus, the identification of rs4713354 and this haplotype that ranges approximately between 31.2 and 33.0 Mbp and includes nearly 120 genes may facilitate in efficiently searching candidate genes for eosinophilia in other ethnicities.

This study has several limitations. First, this study focused on the Japanese population that comprises fewer obese subjects than Caucasians or African Americans. However, the Japanese are known to be susceptible to an increment in BMI and prone to developing obesity-related conditions with lower BMI than Caucasians. For example, in diabetes mellitus, the impact of BMI on its morbidity is two-fold higher in Japanese Americans than in Caucasians.[37] Similarly, the visceral and abdominal fat deposition is higher in Asian females than in Caucasian females with similar BMI levels.[38] Thus, lower BMI in this study does not severely detract from our main message. Second, the fluctuation of blood eosinophil counts should be considered. However, large sample size and repeated measurements of eosinophil counts at the baseline and follow-up, through which several SNPs were identified, might have minimized its effects. Third, we did not take into account parasite infection, another important factor for increased blood eosinophil counts. Because subjects with parasitic infections may influence the correlations between eosinophils and BMI, these findings should be carefully applied to populations in areas as Africa, South America, and South Asia, where parasitic infections continue to be public concerns.[39] Despite these limitations, this study is important because it provides basic and fundamental associations between BMI and eosinophil counts.

In conclusion, this study elucidates nonlinear, nonuniform correlations between BMI and blood eosinophil counts in a large general population. An increase in BMI usually contributes to an increase in both neutrophil and eosinophil counts. Meanwhile, there is a population with eosinophilia in which genetic backgrounds might be more crucial than BMI. The findings of this study might build on the limited evidence on correlations between BMI and granulocyte counts in the general population.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.alit.2019.05.012.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

HS analyzed and interpreted the data and wrote the draft. HM conceived and designed the study, collected, analyzed, and interpreted the data, wrote and edited the manuscript, and has the accountability for all aspects of the work. YIz, TN and YK collected, and analyzed the data. YIs and CM analyzed the data and revised the work critically. TO collected and analyzed the data and revised the manuscript critically. II revised the work critically. KM collected the data and revised the work critically. KC provided overall supervision, and critically revised the manuscript. SM revised the work critically. TK, YT and FM contributed to the design of the Nagahama cohort study, recruited subjects, acquired the funding, performed genetic analysis and critically revised the manuscript. TH provided overall supervision, and critically revised the manuscript.

References

1. Dixon AE, Holguin F, Sood A, Salome CM, Pratley RE, Beuther DA, et al. An official American Thoracic Society Workshop report: obesity and asthma. *Proc Am Thorac Soc* 2010;**7**:325–35.
2. Holguin F, Bleecker ER, Busse WW, Calhoun WJ, Castro M, Erzurum SC, et al. Obesity and asthma: an association modified by age of asthma onset. *J Allergy Clin Immunol* 2011;**127**:1486–93. e2.
3. Gibson PG. Obesity and asthma. *Ann Am Thorac Soc* 2013;**10**(Suppl):S138–42.

4. Telenga ED, Tideman SW, Kerstjens HA, Hacken NH, Timens W, Postma DS, et al. Obesity in asthma: more neutrophilic inflammation as a possible explanation for a reduced treatment response. *Allergy* 2012;**67**:1060–8.
5. Desai D, Newby C, Symon FA, Haldar P, Shah S, Gupta S, et al. Elevated sputum interleukin-5 and submucosal eosinophilia in obese individuals with severe asthma. *Am J Respir Crit Care Med* 2013;**188**:657–63.
6. Ortega HG, Yancey SW, Mayer B, Gunsoy NB, Keene ON, Bleecker ER, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. *Lancet Respir Med* 2016;**4**:549–56.
7. DiSantostefano RL, Hinds D, Le HV, Barnes NC. Relationship between blood eosinophils and clinical characteristics in a cross-sectional study of a US population-based COPD cohort. *Respir Med* 2016;**112**:88–96.
8. Amini M, Bashirova D, Prins BP, Corpeleijn E, Bruinenberg M, Franke L, et al. Eosinophil count is a common factor for complex metabolic and pulmonary traits and diseases: the LifeLines Cohort Study. *PLoS One* 2016;**11**:e0168480.
9. Shim WS, Kim HJ, Kang ES, Ahn CW, Lim SK, Lee HC, et al. The association of total and differential white blood cell count with metabolic syndrome in type 2 diabetic patients. *Diabetes Res Clin Pract* 2006;**73**:284–91.
10. Babio N, Ibarrola-Jurado N, Bullo M, Martinez-Gonzalez MA, Warnberg J, Salaverria I, et al. White blood cell counts as risk markers of developing metabolic syndrome and its components in the PREDIMED study. *PLoS One* 2013;**8**:e58354.
11. Caspard H, Tran TN, Ambrose C. Population-based evaluation of blood eosinophil counts and association with patient characteristics in US adults with asthma [abstract]. *J Allergy Clin Immunol* 2018;**141**:AB103.
12. Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013;**132**:305–12. e3.
13. Izuhara K, Conway SJ, Moore BB, Matsumoto H, Holweg CT, Matthews JG, et al. Roles of periostin in respiratory disorders. *Am J Respir Crit Care Med* 2016;**193**:949–56.
14. Caswell-Smith R, Hosking A, Cripps T, Holweg C, Matthews J, Holliday M, et al. Reference ranges for serum periostin in a population without asthma or chronic obstructive pulmonary disease. *Clin Exp Allergy* 2016;**46**:1303–14.
15. James A, Janson C, Malinovschi A, Holweg C, Alving K, Ono J, et al. Serum periostin relates to type-2 inflammation and lung function in asthma: data from the large population-based cohort Swedish GA(2)LEN. *Allergy* 2017;**72**:1753–60.
16. Kimura H, Konno S, Makita H, Taniguchi N, Goudarzi H, Shimizu K, et al. Serum periostin is associated with body mass index and allergic rhinitis in healthy and asthmatic subjects. *Allergol Int* 2018;**67**:357–63.
17. Matsusaka M, Kabata H, Fukunaga K, Suzuki Y, Masaki K, Mochimaru T, et al. Phenotype of asthma related with high serum periostin levels. *Allergol Int* 2015;**64**:175–80.
18. Sunadome H, Matsumoto H, Petrova G, Kanemitsu Y, Tohda Y, Horiguchi T, et al. IL4Ralpha and ADAM33 as genetic markers in asthma exacerbations and type-2 inflammatory endotype. *Clin Exp Allergy* 2017;**47**:998–1006.
19. Sutherland ER, Goleva E, King TS, Lehman E, Stevens AD, Jackson LP, et al. Cluster analysis of obesity and asthma phenotypes. *PLoS One* 2012;**7**:e36631.
20. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, et al. A transcriptome-driven analysis of epithelial brushings and bronchial biopsies to define asthma phenotypes in U-BIOPRED. *Am J Respir Crit Care Med* 2017;**195**:443–55.
21. Peters U, Dixon AE, Forno E. Obesity and asthma. *J Allergy Clin Immunol* 2018;**141**:1169–79.
22. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 2009;**41**:342–7.
23. Izuhara Y, Matsumoto H, Nagasaki T, Kanemitsu Y, Murase K, Ito I, et al. Mouth breathing, another risk factor for asthma: the Nagahama Study. *Allergy* 2016;**71**:1031–6.
24. Matsumoto H, Izuhara Y, Niimi A, Tabara Y, Nagasaki T, Kanemitsu Y, et al. Risks and cough-aggravating factors in prolonged cough. Epidemiological observations from the Nagahama cohort study. *Ann Am Thorac Soc* 2017;**14**:698–705.
25. Muro S, Tabara Y, Matsumoto H, Setoh K, Kawaguchi T, Takahashi M, et al. Relationship among chlamydia and mycoplasma pneumoniae seropositivity, IKZF1 genotype and chronic obstructive pulmonary disease in a general Japanese population: the Nagahama study. *Medicine (Baltimore)* 2016;**95**:e3371.
26. Jia X, Han B, Onengut-Gumuscu S, Chen WM, Concannon PJ, Rich SS, et al. Imputing amino acid polymorphisms in human leukocyte antigens. *PLoS One* 2013;**8**:e64683.
27. Marzullo P, Minocci A, Giarda P, Marconi C, Tagliaferri A, Walker GE, et al. Lymphocytes and immunoglobulin patterns across the threshold of severe obesity. *Endocrine* 2014;**45**:392–400.
28. Abella V, Scotece M, Conde J, Pino J, Gonzalez-Gay MA, Gomez-Reino JJ, et al. Leptin in the interplay of inflammation, metabolism and immune system disorders. *Nat Rev Rheumatol* 2017;**13**:100–9.
29. Conus S, Bruno A, Simon HU. Leptin is an eosinophil survival factor. *J Allergy Clin Immunol* 2005;**116**:1228–34.
30. Wong CK, Cheung PF, Lam CW. Leptin-mediated cytokine release and migration of eosinophils: implications for immunopathophysiology of allergic inflammation. *Eur J Immunol* 2007;**37**:2337–48.
31. Zheng H, Zhang X, Castillo EF, Luo Y, Liu M, Yang XO. Leptin enhances TH2 and ILC2 responses in allergic airway disease. *J Biol Chem* 2016;**291**:22043–52.
32. Scott HA, Gibson PG, Garg ML, Wood LG. Airway inflammation is augmented by obesity and fatty acids in asthma. *Eur Respir J* 2011;**38**:594–602.
33. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, et al. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* 2014;**519**:242–6.
34. Okada Y, Hirota T, Kamatani Y, Takahashi A, Ohmiya H, Kumasaka N, et al. Identification of nine novel loci associated with white blood cell subtypes in a Japanese population. *Plos Genet* 2011;**7**:e1002067.
35. Salati S, Bianchi E, Zini R, Tenedini E, Quaglino D, Manfredini R, et al. Eosinophils, but not neutrophils, exhibit an efficient DNA repair machinery and high nucleolar activity. *Haematologica* 2007;**92**:1311–8.
36. Okada Y, Momozawa Y, Ashikawa K, Kanai M, Matsuda K, Kamatani Y, et al. Construction of a population-specific HLA imputation reference panel and its application to Graves' disease risk in Japanese. *Nat Genet* 2015;**47**:798–802.
37. Maskarinec G, Erber E, Grandinetti A, Verheus M, Oum R, Hopping BN, et al. Diabetes incidence based on linkages with health plans: the multiethnic cohort. *Diabetes* 2009;**58**:1732–8.
38. Lim U, Ernst T, Buchthal SD, Latch M, Albright CL, Wilkens LR, et al. Asian women have greater abdominal and visceral adiposity than Caucasian women with similar body mass index. *Nutr Diabetes* 2011;**1**:e6.
39. Coulter JBS. Global importance of parasitic disease. *Curr Paediatrics* 2002;**12**:523–33.