Gene Polymorphisms of *NLRP3* Associated With Plasma Levels of 4β-Hydroxycholesterol, an Endogenous Marker of CYP3A Activity, in Patients With Asthma

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Inflammation decreases the activity of cytochrome P450 3A (CYP3A). Nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain containing 3 (NLRP3) is responsible for regulating the inflammatory response, and its genetic polymorphisms have been linked to inflammatory diseases such as asthma. However, there have been few studies on the effect of NLRP3 on CYP3A activity. We aimed to investigate the association between polymorphisms in the *NLRP3* gene and plasma 4β-hydroxycholesterol (4βOHC), an endogenous marker of CYP3A activity, in patients with asthma. In this observational study including 152 adult asthma patients, we analyzed 10 *NLRP3* gene single-nucleotide polymorphisms (SNPs). Plasma 4βOHC levels were measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS). The results showed that five SNPs were associated with significantly lower plasma 4βOHC concentrations. Among these SNPs, rs3806265, rs4612666, rs1539019, and rs10733112 contributed to a significant increase in plasma IL-6 concentrations. Moreover, a multivariate regression model showed that the rs3806265 TT, rs4612666 CC, rs1539019 AA, and rs10733112 TT genotypes were significant factors for decreased plasma 4βOHC, even after including patient background factors and *CYP3A53 (rs776746) gene polymorphisms as covariates. These results were also observed when plasma 4βOHC concentrations were corrected for cholesterol levels. We conclude that *NLRP3* gene polymorphisms are involved in increasing plasma IL-6 concentrations and decreasing plasma 4βOHC concentrations in patients with asthma. Therefore, *NLRP3* gene polymorphisms may be a predictive marker of CYP3A activity in inflammatory diseases such as asthma.**

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ In certain medical conditions characterized by inflammation, activity of the cytochrome P450 3A (CYP3A) enzyme is reduced. This is caused by an increase in the levels of inflammatory cytokines in the blood, which negatively impacts enzyme function.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ NLRP3, a member of the NOD-like receptor family with a pyrin domain, plays a critical role in regulation of immune responses in inflammatory diseases such as asthma. However, the impact of NLRP3-mediated inflammatory regulatory mechanisms on fluctuations in CYP3A activity remains unclear.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Genetic polymorphisms in the *NLRP3* gene were found to affect the levels of plasma interleukin-6 and plasma 4β-hydroxycholesterol, which is a marker for CYP3A activity, in patients with asthma.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ *NLRP3* genetic polymorphisms may function as markers for interindividual variability in CYP3A activity in patients with inflammatory diseases, such as asthma.

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The cytochrome P450 (CYP) 3A family plays a crucial role in metabolizing a wide range of drugs.[1] CYP3A activity can be affected by several factors, including inflammation.[2–4] Cytokines and other inflammatory mediators are known to be related to the variability of drug metabolism. Indeed, increased inflammation levels are suggested to attenuate drug-metabolizing activity, including CYP3A.[4] Additionally, a decrease in CYP3A activity has been observed in several diseases, including rheumatoid arthritis,[5–7] inflammatory bowel disease,[8,9] and type 2 diabetes mellitus.[10,11] Chronic inflammation in these diseases contributes to decreased plasma levels of 4β-hydroxycholesterol (4βOHC) and the 4βOHC/cholesterol ratio,[8–10,12] an endogenous marker of CYP3A activity.[13–15] However, the detailed mechanisms of CYP3A attenuation in inflammatory diseases are poorly understood, and there is no biomarker for predicting CYP3A activity variability in clinical practice.

NOD-like receptor family pyrin domain containing 3 (NLRP3) is now widely accepted as a critical component in regulation of inflammatory/immune responses.[16] NLRP3 is a cytosolic pattern-recognition receptor that detects bacterial, viral, and fungal pathogens and endogenous danger signals. Following ligand detection, NLRP3 is involved in the formation and activation of the NLRP3 inflammasome, which promotes release of the proinflammatory cytokines interleukin 1β (IL-1β) and IL-18, stimulating downstream inflammatory responses. Although NLRP3 is essential for the immune defense mechanism, the hyperactivation of NLRP3 is involved in the initiation and progression of inflammatory diseases.[17] Furthermore, studies have shown that gene polymorphisms of *NLRP3* are closely related to the activity and risk of inflammatory disease.[18,19]

Asthma is a well-known health issue that affects a wide range of the population.[20,21] It is characterized by chronic airway inflammation, and several inflammatory mechanisms are involved in its pathology.[22] Although eosinophilic inflammation is a well-recognized cause of inflammation, neutrophilic inflammation is also key to asthma pathology.[23,24] Recent studies have reported the role of the NLRP3 inflammasome in the pathogenesis of asthma and suggested an association between eosinophilic and neutrophilic inflammation.[25,26]

The NLRP3 inflammasome is increasingly being recognized as a critical inflammatory molecule in several diseases, including asthma. However, few studies have focused on the regulation of the NLRP3 inflammasome in the variability in CYP activities. We hypothesized that *NLRP3* gene polymorphisms affect the inflammatory status of asthma and that this difference causes interindividual variability in CYP3A activity. This study aimed to investigate the association between *NLRP3* polymorphisms and plasma levels of 4βOHC in patients with asthma.

MATERIALS AND METHODS

Study subjects

The study conducted at Shizuoka General Hospital, Shizuoka, Japan, was an observational study that was cross-sectional in nature. The study included subjects who met the following criteria: over 20 years of age, had asthma diagnosed by a physician, and had no exacerbations in the preceding 8 weeks. The diagnosis of asthma was made according to Global Initiative for Asthma guidelines. The research study received approval from the ethics committee of Shizuoka General Hospital (approved number SGH15-01-55). Prior to participating in the study, all individuals provided written informed consent.

Measurements

The study obtained peripheral blood plasma samples from each subject to determine the plasma levels of 4βOHC by liquid chromatography–tandem mass spectrometry (LC–MS/MS) according to a previous study,[27,28] with minor modifications. A 50-μL plasma sample was saponified with potassium hydroxide in methanol, and picolinoyl derivatization was completed to obtain the sample for analysis. After each procedure, supported liquid extraction was performed to clean up the samples using an ISOLUTE SLE+ cartridge (Biotage Japan, Tokyo, Japan). Calibration curves were prepared over the 5–150-ng/mL concentration range with an internal standard of 4βOHC-d7 (Toronto Research Chemicals, Toronto, ON, Canada). An enzymatic method was used to determine total plasma cholesterol levels using a LabAssay cholesterol kit (FUJIFILM Wako Chemicals, Osaka, Japan). Plasma cytokine levels of IL-1β and IL-6 were analyzed by using a ProQuantum immunoassay kit (Thermo Fisher Scientific, Waltham, MA, USA).

Genotyping

We selected single-nucleotide polymorphisms (SNPs) in the *NLRP3* gene based on a minor allele frequency of more than 10% and linkage disequilibrium for the Japanese population in 1000 Genomes Project data using LDlink 5.0 (https://ldlink.nci.nih.gov/). The following SNPs were genotyped by polymerase chain reaction-restriction fragment length polymorphism analysis: *NLRP3* (rs12048215, rs10754555, rs3806265, rs7525979, rs3806268, rs4612666, rs1539019, rs10754558, rs10733112, and rs4353135) and *CYP3A5* (*3, rs776746).

Statistical analysis

We analyzed the associations between *NLRP3* SNPs and plasma concentrations of 4βOHC and cytokines using linear regression analysis. The regression coefficient (β) and 95% confidence interval (CI) were estimated in additive, dominant, and recessive models. In the additive model, homozygous, heterozygous, and alternative homozygous genotypes were coded as 0, 1, and 2, respectively. Similarly, each genotype was coded as 0, 2, and 2 in the dominant model and as 0, 0, and 2 in the recessive model. For multivariate linear regression analysis, covariates were selected by the forward and backward stepwise procedure. Continuous variables are summarized as medians and interquartile ranges and categorical variables as frequencies and percentages. We performed all statistical analyses using R version 4.3.2 (R Foundation for Statistical Computing, Vienna, Austria) and considered a P value less than 0.05 to indicate statistical significance.

RESULTS

Subject characteristics and genotypes

A total of 152 patients with asthma were included in this study. **Table 1** describes the demographic and clinical characteristics and measurement results of the study subjects. The detailed characteristics of these subjects have been reported in our previous study.[29] We observed that 51% of the study subjects were carriers of at least one *CYP3A5* *1 allele and were considered *CYP3A5* expressors. The allele frequencies of 10 analyzed SNPs in *NLRP3* are shown in **Table 2**. The genotype frequency of all SNPs did not deviate significantly from the Hardy–Weinberg equilibrium. Linkage disequilibrium analysis was performed, and pairwise r^2 values between SNPs are shown in **Figure 1**. This analysis revealed that rs3806265 and rs3806268 were in almost complete linkage disequilibrium ($r^2 = 0.99$). For this reason, we examined only rs3806265 data in further analysis.

The proportion of severe asthma was higher in patients with the rs1539019 AA genotype than in patients with the AC/CC genotype (8/25, 32% for the AA genotype; 17/127, 13%

for the AC/CC genotype; $P = 0.035$ by Fisher's exact test). Furthermore, patients with the rs1539019 AA genotype were prescribed higher doses of inhaled corticosteroids (mean ± standard deviation; 684 ± 356 μg/day for the AA genotype; 525 ± 300 μg/day for the AC/CC genotype; $P = 0.020$ by t test). Other demographic and clinical characteristics were not affected by *NLRP3* SNPs.

Associations between *NLRP3* SNPs and plasma 4βOHC concentration

We analyzed the influence of *NLRP3* SNPs on plasma concentrations of 4βOHC in additive, dominant, and recessive models using linear regression analysis (**Table 3**). In the additive model, rs3806265, rs7525979, rs4612666, rs1539019, and rs10733112 were significantly associated with plasma concentrations of 4βOHC. Among these SNPs, rs3806265, rs7525979, rs4612666, and rs1539019 also showed a significant association in the dominant model, and rs10733112 showed significance in the recessive model. These results indicate that plasma concentrations of 4βOHC were significantly decreased with the rs3806265 TT genotype, rs7525979 CC genotype, rs4612666 CC genotype, rs1539019 AA genotype, and rs10733112 CC/CT genotype (**Figure 2a**). These associations were also observed for the 4βOHC/cholesterol ratio (**Table S1**).

Table 1 Characteristics of study subjects

N	152
Age (years)	66 (50, 74)
Sex, male	71 (47)
BMI (kg/m^2)	23.0 (20.7, 25.3)
Total cholesterol (mg/dL)	163.6 (143.2, 186.4)
Peripheral blood neutrophil (cells/μL)	3,586 (2,988, 4,662)
Peripheral blood eosinophil (cells/μL)	316 (194, 506)
CYP3A5 rs776746	
*1/*1	9 (6)
*1/*3	69 (45)
*3/*3	74 (49)
Plasma 4βOHC (ng/mL)	21.43 (16.60, 29.37)
Plasma 4βOHC/cholesterol ratio (×10^{-5})	1.31 (0.98, 1.74)
Plasma IL-6 (pg/mL)	3.01 (1.54, 5.17)
Plasma IL-1β (pg/mL)	0.44 (0.28, 0.73)

Data are shown as median (interquartile range) or frequency (percentage). 4βOHC, 4β-hydroxycholesterol; BMI, body mass index; IL-1β, interleukin 1β; IL-6, interleukin 6.

Associations between *NLRP3* SNPs and plasma cytokine levels

Associations between *NLRP3* SNPs and plasma concentrations of IL-6 were evaluated by linear regression analysis (**Table 4**). Among SNPs influencing the plasma concentrations of 4βOHC, rs3806265, rs4612666, rs1539019, and rs10733112 were significantly associated with IL-6 concentrations. Plasma concentrations of IL-6 were significantly increased or tended to be increased with the genotypes that decreased 4βOHC concentrations (rs3806265 TT genotype, rs4612666 CC genotype, rs1539019 AA genotype, and rs10733112 CC/CT genotype, **Figure 2b**). In contrast, rs7525979 was not associated with the IL-6 concentration. Among other SNPs, rs4353135 affected plasma concentrations of IL-6, and patients with the rs4353135 GG/GT genotype showed an increase in IL-6 (**Table 4**). We also evaluated the influence of *NLRP3* SNPs on plasma concentrations of IL-1β, but with no significant differences among genotypes (**Table S2**).

Haplotype analysis

Four SNPs, rs3806265, rs4612666, rs1539019, and rs10733112, were found to be associated with plasma 4βOHC and IL-6 concentrations. To assess the effect of these SNPs on plasma 4βOHC and IL-6 concentrations, we scored the genotypes that affected plasma levels and classified the haplotypes into four categories by combining the scores. Strong linkage disequilibrium was observed for rs3806265 and rs4612666, so their scores were calculated using the following formula: (rs3806265 TT or rs4612666 CC)*1 + (rs1539019 AA)*1 + (rs10733112 CC/CT)*1. The scores were divided into four levels based on 0, 1, 2, and 3. Patients with

Table 2 Allele frequencies of *NLRP3* polymorphisms

		Allele	Allele frequency (%)		Genotype frequency, *n* (%)		
SNP ID	Location	1/2	1	2	1/1	1/2	2/2
rs12048215	Intron 3	A/G	79	21	92 (61)	55 (36)	5 (3)
rs10754555	Intron 3	C/G	62	38	54 (36)	82 (54)	16 (11)
rs3806265	Intron 3	T/C	61	39	57 (38)	72 (47)	23 (15)
rs7525979	Exon 5	C/T	84	16	107 (70)	42 (28)	3 (2)
rs3806268	Exon 5	A/G	62	38	58 (38)	71 (47)	23 (15)
rs4612666	Intron 7	C/T	57	43	51 (34)	71 (47)	30 (20)
rs1539019	Intron 8	A/C	40	60	25 (16)	71 (47)	56 (37)
rs10754558	Exon 11	G/C	37	63	17 (11)	77 (51)	58 (38)
rs10733112	3′Flanking	C/T	47	53	38 (25)	68 (45)	46 (30)
rs4353135	3′ Flanking	G/T	38	62	25 (16)	64 (42)	63 (41)

higher scores had lower plasma 4βOHC and higher plasma IL-6 concentrations. The median plasma 4βOHC concentration for patients with a score of 0 was 26.7 ng/mL, whereas the median for patients with a score of 4 was 18.0 ng/mL, indicating a 32% reduction (**Figure S1**).

Predicting model for plasma 4βOHC concentrations

We determined the demographic and clinical factors influencing plasma 4βOHC concentrations through univariate linear regression analysis (**Table S3**). Male sex, higher body mass index (BMI), higher peripheral blood neutrophils, and the *CYP3A5**3/*3 allele were significantly associated with lower plasma concentrations of 4βOHC. Moreover, increased total cholesterol contributed to the higher 4βOHC concentrations. Plasma concentrations of IL-6 tended to be significantly associated with 4βOHC concentrations ($P = 0.068$). By using these factors and each SNP of *NLRP3* as candidate factors, we applied a multivariate linear regression model for predicting plasma 4βOHC concentrations by a stepwise selection procedure (**Table S5**). Significant associations of rs3806265, rs4612666, rs1539019, and rs10733112 with plasma 4βOHC were maintained in the multivariate model; however, rs7525979 showed no significant association after adjusting for covariates (β, −0.151; 95% CI, −0.344 to 0.042; $P = 0.126$). The model of rs3806265 had a relatively higher coefficient of determination (R^2) and explained 34.7% of the individual variability in plasma 4βOHC concentrations. The contributions of the rs3806265 TT, rs4612666 CC, rs1539019 AA, and rs10733112 TT genotypes to individual variability were estimated to be 3.07–3.41%. These estimated values were comparable to those of *CYP3A5**3/*3.

We also determined the effects of rs3806265, rs4612666, rs1539019, and rs10733112 on the plasma 4βOHC/cholesterol ratio following univariate (**Table S4**) and multivariate regression analyses (**Table S5**). The model of rs3806265 had a higher coefficient of determination ($R^2 = 31.9\%$). Among these SNPs, the rs3806265 TT genotype and rs4612666 CC genotype could explain ~4% of the individual variability in the plasma 4βOHC/cholesterol ratio. These contributions were also comparable to those of *CYP3A5**3/*3.

Finally, multiple linear regression analysis was conducted using the allele scores derived from the haplotype analysis (**Table 5**). Despite adjustment for covariates, the increase in allele scores proved to be a significant factor in the decrease in plasma 4βOHC concentrations and the plasma 4βOHC/cholesterol ratio.

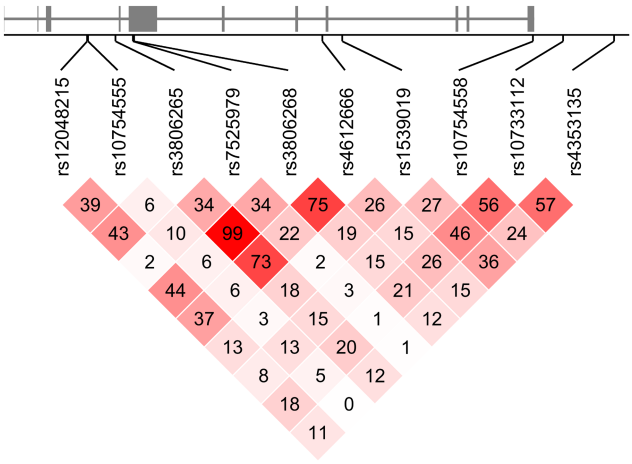

Figure 1 Linkage disequilibrium (LD) plots for the 10 analyzed polymorphisms of the *NLRP3* gene. LD coefficient (r^2) values are shown in the figure, with strong correlations highlighted in red.

DISCUSSION

The main finding of this study is that *NLRP3* polymorphisms influence plasma concentrations of 4βOHC, an endogenous marker of CYP3A activity, in patients with asthma. We found that the *NLRP3* rs3806265 TT, rs4612666 CC, rs1539019 AA, and rs10733112 CC/CT genotypes were associated with decreasing plasma 4βOHC concentrations and, at the same time, affected increasing plasma concentrations of IL-6. These results suggest that

Table 3 Influence of *NLRP3* polymorphisms on logarithm (log2)–transformed plasma 4β-hydroxycholesterol (4βOHC) concentrations

	Allele	Additive model			Dominant model			Recessive model		
SNP ID	1/2	β	(95% CI)	*P* value	β	(95% CI)	*P* value	β	(95% CI)	*P* value
rs12048215	A/G	0.038	(−0.143, 0.219)	0.681	0.029	(−0.074, 0.132)	0.584	−0.031	(−0.313, 0.252)	0.830
rs10754555	C/G	0.028	(−0.131, 0.188)	0.727	0.010	(−0.096, 0.115)	0.860	0.037	(−0.127, 0.201)	0.659
rs3806265	T/C	**0.158**	**(0.014, 0.301)**	**0.033**	**0.118**	**(0.015, 0.220)**	**0.026**	0.078	(−0.063, 0.218)	0.280
rs7525979	C/T	**0.270**	**(0.076, 0.465)**	**0.007**	**0.148**	**(0.040, 0.256)**	**0.008**	0.190	(−0.171, 0.551)	0.304
rs4612666	C/T	**0.162**	**(0.023, 0.300)**	**0.023**	**0.127**	**(0.022, 0.232)**	**0.019**	0.083	(−0.043, 0.209)	0.198
rs1539019	A/C	**0.177**	**(0.036, 0.318)**	**0.015**	**0.163**	**(0.030, 0.297)**	**0.018**	0.091	(−0.013, 0.194)	0.088
rs10754558	G/C	0.092	(−0.063, 0.247)	0.246	−0.004	(−0.164, 0.156)	0.964	0.083	(−0.019, 0.186)	0.114
rs10733112	C/T	**0.139**	**(0.005, 0.273)**	**0.044**	0.050	(−0.066, 0.166)	0.399	**0.136**	**(0.029, 0.244)**	**0.014**
rs4353135	G/T	0.075	(−0.065, 0.215)	0.294	0.045	(−0.091, 0.181)	0.516	0.054	(−0.048, 0.156)	0.297

Coefficients (β) and 95% confidence intervals (CIs) are estimated using linear regression analysis. Allele of 1/1, 1/2, and 2/2 are converted to 0, 1, and 2 in the additive model, 0, 2, and 2 in the dominant model, and 0, 0, and 2 in the recessive model.
Bold indicates statistical significance.

NLRP3 polymorphisms enhance inflammation levels in asthma and that this enhanced inflammation attenuates CYP3A activity. To our knowledge, this is the first report of *NLRP3* polymorphisms affecting interindividual variability in CYP3A activity.

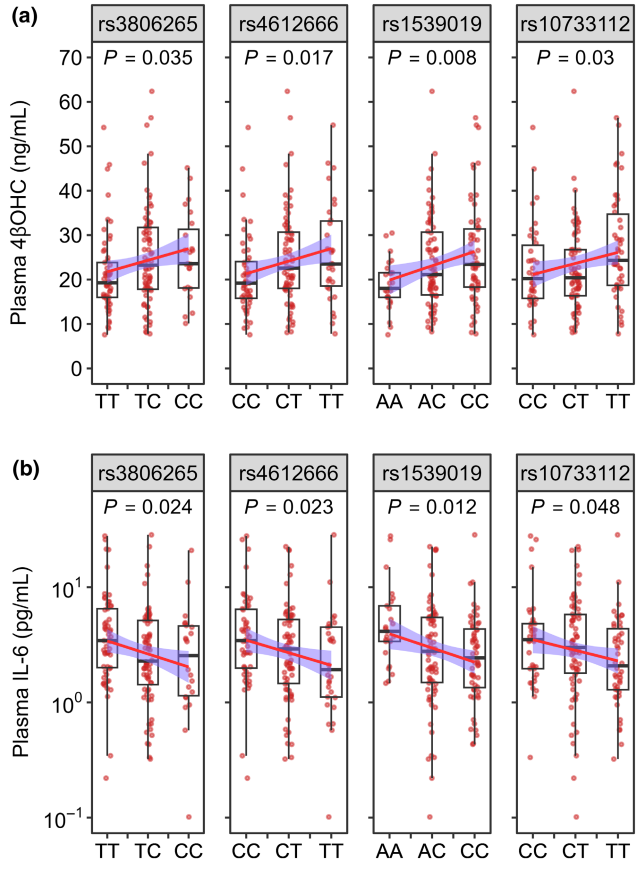

Figure 2 Influence of *NLRP3* polymorphisms on plasma 4β-hydroxycholesterol (4βOHC) and interleukin-6 (IL-6) concentrations. Boxes and thick lines in the box indicate the interquartile range (IQR) and median. The red solid line and blue area represent the linear regression line and 95% confidence interval of the slope, respectively.

In clinical practice, *NLRP3* polymorphisms might be a valuable marker for predicting variability in CYP3A activity in patients with inflammatory disease. However, the effects of *NLRP3* polymorphisms on CYP3A activity should be validated in a cohort including those with asthma and other inflammatory diseases.

The NLRP3 inflammasome plays a fundamental role in the initial process of inflammatory and immune responses. It involves synthesis of the proinflammatory cytokines IL-1β and IL-18 by induction of caspase-1 activation.[30] These cytokines trigger downstream inflammatory responses, such as elevated IL-6 levels.[31] In a previous study, an asthma mouse model revealed the contribution of NLRP3 to pulmonary inflammation and cytokine production.[32] In addition, asthma patients have higher expression levels of NLRP3 and caspase-1 than healthy subjects.[33,34] This study is the first to show an association between *NLRP3* polymorphisms and plasma IL-6 levels in patients with asthma. Previous studies reported increased plasma IL-6 levels in patients with severe asthma.[35] In this report, a high IL-6 level was defined as a plasma concentration of IL-6 above 3.1 pg/mL according to a comparison between healthy cohorts and patients with asthma.[35] Of the 152 participants in our study, 74 (49%) were classified as having high IL-6 levels. These findings suggested that a greater proportion of the patients in our study exhibited an elevated inflammatory phenotype. In this study, there was a trend toward a significant relationship between plasma IL-6 concentrations and plasma 4βOHC and the 4βOHC/cholesterol ratio ($P = 0.068$, $P = 0.020$) in univariate linear regression analysis; however, IL-6 was not included in the multivariate model. This might be attributed to the fact that increasing plasma IL-6 concentrations is one of the mechanisms for the decrease in plasma 4βOHC by *NLRP3* SNPs, including rs3806265, rs4612666, rs1539019, and rs10733112. In contrast, we did not observe a relationship between *NLRP3* SNPs and the plasma IL-1β concentration in this study. Elevated NLRP3 activity is believed to contribute to increased IL-1β production. IL-1β is a key player in the initial phase of the inflammatory response,[36] and high levels of this cytokine are found in bronchoalveolar lavage fluid and sputum, which reflect local

Table 4 Influence of *NLRP3* polymorphisms on logarithm (log2)–transformed plasma interleukin 6 (IL-6) concentrations

	Allele	Additive model			Dominant model			Recessive model		
SNP ID	1/2	β	(95% CI)	*P* value	β	(95% CI)	*P* value	β	(95% CI)	*P* value
rs12048215	A/G	−0.261	(−0.663, 0.140)	0.205	−0.146	(−0.375, 0.083)	0.215	−0.180	(−0.809, 0.448)	0.575
rs10754555	C/G	−0.066	(−0.424, 0.291)	0.716	0.017	(−0.219, 0.253)	0.889	−0.179	(−0.544, 0.185)	0.336
rs3806265	T/C	**−0.376**	**(−0.699, −0.052)**	**0.024**	**−0.278**	**(−0.506, −0.05)**	**0.018**	−0.184	(−0.502, 0.134)	0.259
rs7525979	C/T	−0.165	(−0.626, 0.296)	0.484	−0.139	(−0.386, 0.108)	0.273	0.688	(−0.291, 1.667)	0.171
rs4612666	C/T	**−0.363**	**(−0.674, −0.053)**	**0.023**	**−0.242**	**(−0.477, −0.007)**	**0.045**	−0.246	(−0.529, 0.037)	0.091
rs1539019	A/C	**−0.409**	**(−0.722, −0.095)**	**0.012**	**−0.434**	**(−0.729, −0.139)**	**0.004**	−0.176	(−0.407, 0.056)	0.139
rs10754558	G/C	−0.324	(−0.667, 0.018)	0.065	−0.148	(−0.503, 0.208)	0.417	−0.227	(−0.456, 0.001)	0.053
rs10733112	C/T	**−0.306**	**(−0.607, −0.005)**	**0.048**	−0.187	(−0.447, 0.074)	0.162	−0.231	(−0.473, 0.010)	0.063
rs4353135	G/T	**−0.356**	**(−0.667, −0.046)**	**0.026**	−0.218	(−0.524, 0.088)	0.164	**−0.253**	**(−0.478, −0.028)**	**0.029**

Coefficients (β) and 95% confidence intervals (CIs) are estimated using linear regression analysis. Allele of 1/1, 1/2, and 2/2 are converted to 0, 1, and 2 in the additive model, 0, 2, and 2 in the dominant model, and 0, 0, and 2 in the recessive model.
Bold indicates statistical significance.

Table 5 Multivariate linear regression model for plasma 4β-hydroxycholesterol (4βOHC) concentrations and plasma 4βOHC/cholesterol ratio

	Plasma 4βOHC concentrations				Plasma 4βOHC/cholesterol ratio			
Factors	β	(95% CI)	*P* value	Partial R^2 (%)	β	(95% CI)	*P* value	Partial R^2 (%)
	Model R^2 = 36.1%				Model R^2 = 34.4%			
Intercept	4.868	(4.335, 5.400)			2.469	(2.174, 2.763)		
NLRP3 allele score[a]	−0.143	(−0.228, −0.020)	0.001	5.8	−0.139	(−0.221, −0.057)	0.001	6.0
CYP3A5 *3/*3	−0.188	(−0.356, −0.051)	0.028	2.6	−0.222	(−0.383, −0.060)	0.007	3.9
Sex, male	−0.311	(−0.484, −0.138)	<0.001	6.6	−0.203	(−0.364, −0.042)	0.014	2.3
BMI > 23 kg/m^2	−0.392	(−0.565, −0.219)	<0.001	8.6	−0.431	(−0.592, −0.271)	<0.001	13.8
Neutrophil (per 1,000 cells/μL)	−0.136	(−0.199, −0.074)	<0.001	8.2	−0.123	(−0.183, −0.063)	<0.001	8.3ln
Total cholesterol (per 100 mg/dL)	0.425	(0.160, 0.689)	0.002	4.3				

Coefficients (β) and 95% confidence intervals (CIs) are estimated using linear regression analysis. Plasma 4βOHC concentrations were transformed into logarithm (log2) form.
[a]*NLRP3* allele score was calculated with the following formation: (rs3806265 TT or rs4612666 CC)*1+(rs1539019 AA)*1+(rs10733112 CC or CT)*1. The scores were divided into four levels by 0, 1, 2, and 3.

inflammation in asthmatic patients.[37,38] However, the plasma IL-1β concentrations were relatively low. Therefore, unlike plasma IL-6 concentrations, the relationships between plasma IL-1β concentrations and asthma pathology and severity remain unclear. Thus, variability in NLRP3 activity could not be adequately assessed using plasma IL-1β concentrations.

Several investigators have demonstrated that *NLRP3* polymorphisms are related to inflammatory disease susceptibilities, including psoriasis,[39] food-induced anaphylaxis,[19] and aspirin-induced asthma.[19] A recent meta-analysis also indicated that associations between *NLRP3* polymorphisms and autoimmune disease risks differ among races.[18] In the Japanese population, Hitomi *et al.*[19] reported that the *NLRP3* rs3806265 T allele, rs4612666 C allele, and rs10733112 C allele are associated with susceptibility to food-induced anaphylaxis in pediatric patients. Additionally, the rs4612666 C allele was shown to be associated with susceptibility to aspirin-induced asthma in adult patients.[19] An *in vitro* study was also used to demonstrate that the rs4612666 C allele contributed to an increase in *NLRP3* mRNA expression.[19] Among the SNPs analyzed in this study, two were synonymous variants located in exon regions, and the others were located in noncoding regions. Although the effects of these SNPs on *NLRP3* function have not been fully elucidated, the result of the present study suggested that they may be involved in the pathogenesis of inflammation. Specifically, in our study alleles of these SNPs implicated in disease susceptibility increased IL-6 levels and decreased CYP3A activity, supporting the hypothesis that they contribute to enhanced inflammation. Therefore, polymorphisms in the *NLRP3* gene may be related to the degree of inflammation and modification of CYP3A activity in patients with inflammatory diseases, which is similar to the findings of this study. In contrast, NLRP3 hyperactivation is less commonly induced in individuals without inflammatory diseases; thus, SNPs in *NLRP3* are unlikely to influence CYP3A activity in healthy individuals and patients without inflammatory disease.

In this study, we used the plasma 4βOHC concentration as a marker of CYP3A activity. We hypothesized that heightened inflammation would lead to decreased CYP3A activity and subsequently lower plasma 4βOHC concentrations. Although CYP3A is expressed in both the liver and intestine and plays a crucial role in drug metabolism, plasma 4βOHC levels may be more indicative of hepatic CYP3A activity.[40] In a previous report on IL-6 treatment of cultured human hepatocytes, IL-6 was shown to decrease the expression of CYP3A4 at both the mRNA and protein levels,[41,42] suggesting that increased cytokine production contributes to the decline in CYP3A activity. The plasma 4βOHC concentration is affected by factors such as sex, BMI, cholesterol levels, and genetic variations that affect CYP3A activity.[14,40] In the present study, we observed an association between plasma 4βOHC concentrations and neutrophil counts. This association may be attributed to the correlation between elevated plasma IL-6 concentrations and increased neutrophil counts in patients with asthma.[35] Another possible explanation is that NLRP3 expression is more prominent in neutrophils and macrophages.[16,17] Because the concentration of plasma 4βOHC is influenced by various factors, it is challenging to make direct comparisons with previous reports. In previous studies, the plasma levels of 4βOHC in patients with type 2 diabetes mellitus were found to be, on average, ~50% lower than those in nondiabetic subjects.[10] In the present study, the plasma 4βOHC concentration decreased by ~30% among the haplotypes. Another study investigated the effects of CYP3A inhibitors on plasma 4βOHC levels. The results showed that plasma 4βOHC concentrations were 23% lower 5 days after ketoconazole administration[43] and 18% lower after 4 weeks of antiretroviral therapy.[44] These results suggest that the enhanced inflammatory response caused by *NLRP3* gene polymorphisms appears to inhibit CYP3A activity. However, further research is needed to determine the impact of the observed reduction in plasma 4βOHC concentrations on the pharmacokinetics of CYP3A-substrate drugs.

In conclusion, genetic polymorphisms in *NLRP3*, a molecule associated with inflammatory responses, are responsible for increased

plasma IL-6 concentrations and decreased plasma 4βOHC concentrations in asthma patients. Increased activity of NLRP3 by genetic polymorphisms might lead to enhanced inflammation in asthma patients, decreasing CYP3A activity. In addition to *CYP3A5* gene polymorphisms, it may be beneficial to evaluate inflammation-related gene polymorphisms such as NLRP3 in patients with inflammatory diseases to estimate CYP3A activity alterations.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

K.H. and T.K. analyzed the data and wrote the manuscript. K.H. and K.I. designed the research. K.H., T.K., Y.S., T.S., and T.S. performed the research.

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