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Letter to the Editor

Association between the *NOS2* pentanucleotide repeat polymorphism and risk of postoperative recurrence of chronic rhinosinusitis with nasal polyps in a Japanese population

Dear Editor,

Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common airway inflammatory disease associated with asthma and has a high rate of postoperative recurrence.[1] In western countries, most cases of CRSwNP have been characterized as a Th2 cytokine-dominant inflammation with eosinophilic infiltration and are categorized as eosinophilic chronic rhinosinusitis (ECRS).[1] We previously proposed a diagnostic algorithm for classifying the severity of ECRS according to the results of a large-scale epidemiological survey called the Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis (JESREC) Study.[2] In Japan, this diagnostic algorithm has been widely used for diagnosis of ECRS in patients with CRSwNP.[3]

Nitric oxide (NO) plays various roles in airway defense mechanisms in the nasal cavity.[4] NO is catalyzed by nitric oxide synthase (NOS); thus far, three NOS isoforms have been identified: constitutive isoforms (nNOS and eNOS; gene names *NOS1* and *NOS3*, respectively) and the inducible isoform (iNOS; gene name *NOS2*). In airway epithelial cells, *NOS2* is mainly expressed upon an inflammatory response.[4] High expression of *NOS2* was observed in nasal polyps (NP) in comparison with control tissues, but no significant differences were observed between the expression levels of *NOS1* and *NOS3* in NP and those in control tissues.[5] Moreover, upregulation of *NOS2* and deposition of oxidized NO metabolites were observed in NP from patients with ECRS; furthermore, the deposition was colocalized with eosinophil accumulation.[6] A pentanucleotide (CCTTT)n repeat polymorphism was previously identified in the *NOS2* promoter region.[7] In Japanese patients with asthma, the expression levels of *NOS2* in peripheral blood mononuclear cells (PBMCs) gradually increased as the pentanucleotide repeat numbers decreased, and patients carrying $\leq$ 11 repeats had high rates of asthma exacerbation.[7] Therefore, it is speculated that the pentanucleotide repeat polymorphism in the *NOS2* promoter may be associated with *NOS2* expression in NP and with postoperative recurrence in patients with CRSwNP, especially in those with ECRS. In the present study, we investigated the relationship between the *NOS2* pentanucleotide repeat polymorphism and its effects on *NOS2* expression in the NP of patients with CRSwNP and ECRS. Furthermore, we examined the association between the *NOS2* pentanucleotide repeat polymorphism and risk of postoperative recurrence in a multicenter cohort. The Methodology details appear in the Supplementary Material.

To investigate whether *NOS2* expression levels in NP are related to the pentanucleotide repeat polymorphism, 63 patients with CRSwNP who had previously undergone functional endoscopic sinus surgery (FESS) were enrolled at the Department of Otorhinolaryngology Head & Neck Surgery, University of Fukui (*NOS2* expression group). The patients' characteristics are shown in Table 1. The pentanucleotide repeat number of the *NOS2* promoter region ranged from 9 to 21 repeats (Supplementary Fig. 1A). The association between the sum of the pentanucleotide repeats and the *NOS2* expression levels in NP is shown in Supplementary Figure 1B. *NOS2* expression levels in NP gradually increased as the sum of pentanucleotide repeat numbers decreased (P = 0.014 by the Jonckheere–Terpstra test, Supplementary Fig. 1B). When patients with CRSwNP were subdivided into ECRS and non-ECRS (Supplementary Table 1), according to the diagnostic criteria proposed by the JESREC Study,[2] expression of *NOS2* was higher in patients with ECRS than in those with non-ECRS ($P < 0.001$ by the Wilcoxon rank sum test, Supplementary Fig. 1C). There was a positive correlation between number of eosinophils in NP and *NOS2* expression ($P < 0.001$ and $r = .482$ by the Spearman's rank correlation coefficient). The expression levels of *NOS2* gradually increased as the sum of pentanucleotide repeat numbers decreased in patients with ECRS, but not with non-ECRS (P = 0.039 for ECRS and P = 0.63 for non-ECRS by the Jonckheere–Terpstra test, Supplementary Fig. 1C). There was no statistically significant trend between pentanucleotide repeats numbers and number of eosinophils in NP (P = 0.15 by the Jonckheere–Terpstra test). Subsequently, we classified the patients according to the *NOS2* pentanucleotide repeat polymorphism following the definition given by Hirai *et al.*: short alleles (S) with $\leq$11 repeats, whilst long alleles (L) comprise those with >11 repeats.[7] The expression levels of *NOS2* in NP gradually increased as the number of the pentanucleotide repeat polymorphism decreased; therefore, those with the S/S genotype had the highest levels of expression (P = 0.023 by the Jonckheere–Terpstra test, Supplementary Fig. 1D), followed by those with the S/L genotype, then those with the L/L genotype. When patients with CRSwNP were subdivided into ECRS and non-ECRS, there was no statistically significant trend in the *NOS2* expression levels among the S/S, S/L and L/L genotypes (P = 0.078 in patients with ECRS and P = 0.63 in patients with non-ECRS by the Jonckheere–Terpstra test, Supplementary Fig. 1D).

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Table 1
Comparison of the characteristics of patients with the S/S genotype with those of patients with the S/L + L/L genotypes.

Characteristic	*NOS2* expression group				Multicenter group			
	All patients (n = 63)	S/S (n = 20)	S/L + L/L (n = 43)	*P* value	All patients (n = 191)	S/S (n = 35)	S/L + L/L (n = 156)	*P* value
Age (mean ± SD)	55.0 ± 15.8	56.3 ± 16.9	54.4 ± 15.4	.668	53.7 ± 13.7	50.2 ± 13.9	54.4 ± 13.6	.128
Sex (male, %)	44 (69.8)	14 (70.0)	30 (69.8)	1.00	134 (70.2)	25 (71.4)	109 (69.9)	1.00
Eosinophils in peripheral blood (%, median, range)	3.9 (.1–19.9)	4.9 (.5–10.3)	3.9 (.1–19.9)	.901	5.2 (.2–26.0)	5.6 (.9–15.3)	5.1 (.2–26.0)	.138
Eosinophils in nasal polyps (/HPF, median, range)	25 (0–394)	41 (0–103)	20 (0–394)	.974	52 (0–523)†	68 (0–171)†	49 (0–523)†	.433
CT shadow								
Ethmoid ≥ maxillary (%)	49 (77.8)	17 (85.0)	32 (74.4)		166 (86.9)	31 (88.6)	135 (86.5)	
Ethmoid < maxillary (%)	14 (22.2)	3 (15.0)	11 (25.6)	.518	25 (13.1)	4 (11.4)	21 (13.5)	1.00
Complication								
Asthma (%)	18 (28.6)	5 (25.0)	13 (30.2)	.770	68 (35.6)	15 (42.9)	53 (34.0)	.334
Aspirin intolerance (%)	6 (9.5)	0 (.0)	6 (14.0)	.171	21 (11.0)	7 (20.0)	14 (9.0)	.073
Diagnosis								
ECRS (%)	44 (69.8)	16 (80.0)	28 (65.1)		158 (82.7)	32 (91.4)	126 (80.8)	
Non-ECRS (%)	19 (30.2)	4 (20.0)	15 (34.9)	.377	33 (17.3)	3 (8.6)	30 (19.2)	.214
Postoperative Recurrence (%)	–	–	–		49 (25.7)	13 (37.1)	36 (23.1)	.036

CT, computed tomography; ECRS, eosinophilic chronic rhinosinusitis. *$P < 0.05$.
† Among 191 patients in multicenter group, 100 patients (14 patients with S/S genotype and 86 patients with S/L + L/L genotype) were available.

Next, we examined the association between *NOS2* pentanucleotide repeat polymorphisms and risk of postoperative recurrence in a multicenter cohort from five university hospitals; our analysis included 191 CRSwNP patients who had previously undergone FESS (multicenter group). The patients' characteristics are shown in Table 1, and the association results of other risk factors for postoperative recurrence is described in the Supplementary Material. Postoperative recurrence was defined as the occurrence of condition with NP or purulent discharge in the middle meatus lasting for more than four weeks after the surgery, which was confirmed by otorhinolaryngologists by use of a nasal endoscope. No significant differences were found in terms of age, sex, ECRS diagnosis, or JESREC-based postoperative recurrence risk factors among patients with the S/S genotype and those with the S/L + L/L genotypes.[2] The number of pentanucleotide repeats in the *NOS2* promoter region ranged from 5 to 20 (Fig. 1A). A Kaplan–Meier plot consisting of postoperative recurrence in patients with CRSwNP is shown in Figure 1B. CRSwNP patients with the S/S genotype had a higher risk of postoperative recurrence than did those with the S/L + L/L genotypes ($P = 0.036$ by the log-rank test, Fig. 1B). When we focused on patients with ECRS (Supplementary Table 2), those carrying the S/S genotype had a higher risk of postoperative recurrence but not with non-ECRS ($P = 0.032$ for ECRS and $P = 0.57$ for non-ECRS by the log-rank test, Fig. 1C). No association was observed between the pentanucleotide polymorphisms and other risk factors associated with postoperative recurrence such as eosinophils in NP and asthma ($P > 0.05$, Table 1).

Limitation should be noted in the present study. First, most of the patients were those with ECRS, and it is difficult to reach a valid conclusion with small sample size for non-ECRS. Second, few studies have been conducted to examine the relationship between CRSwNP and *NOS2* pentanucleotide repeats polymorphisms. So far, two studies have been performed to examine the association between the *NOS2* pentanucleotide repeats polymorphisms and the presence of NP.[8,9] In contrast to our results, the results of both of those studies showed that the presence of NP gradually increased as the pentanucleotide repeat numbers increased. However, those studies did not report on the status of chronic rhinosinusitis or the clinical prognosis, therefore, it is unclear whether different environmental backgrounds and phenotypes examined may result in an inverted association.

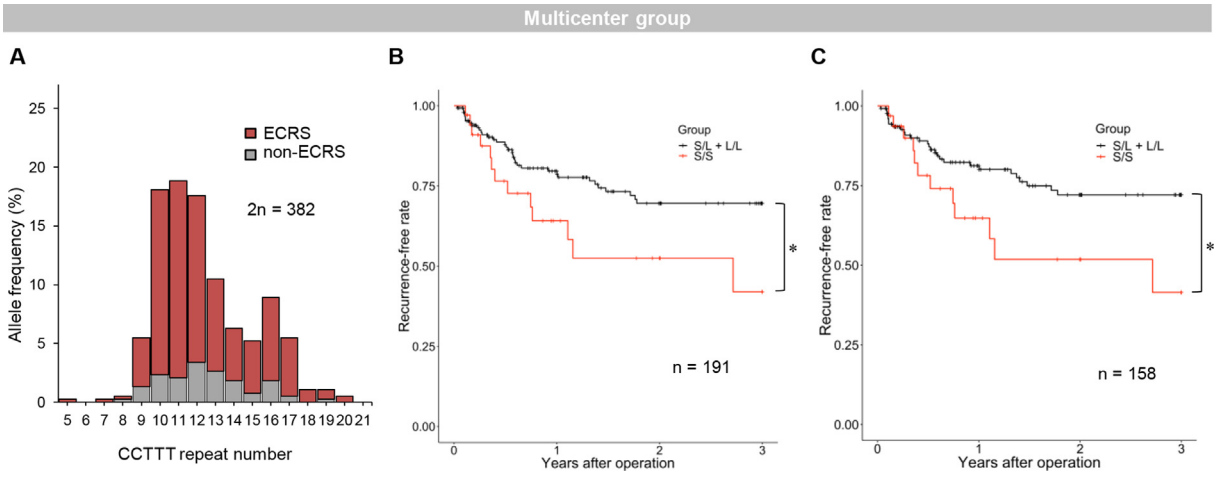

Fig. 1. **A**, Allelic frequency of the *NOS2* pentanucleotide repeat polymorphism in the multicenter group. **B**, Kaplan–Meier curves of the recurrence-free rate in the multicenter group (S/S genotype vs S/L + LL genotypes). **C**, Kaplan–Meier curves of the recurrence-free rate in patients with ECRS (n = 158) of the multicenter group (S/S genotype vs S/L + LL genotypes). ECRS, eosinophilic chronic rhinosinusitis. *$P < 0.05$.

In conclusion, to the best of our knowledge, this is the first study to demonstrate that the short pentanucleotide repeat polymorphism of the *NOS2* promoter increases expression of *NOS2* in NP and that this polymorphism is associated with the risk of postoperative recurrence in patients with CRSwNP and ECRS. Our results may indicate that *NOS2* pentanucleotide polymorphism is one of the shared genetic risk factors for CRSwNP and asthma. Further study is needed to determine the role of *NOS2* in the pathogenesis of CRSwNP in various populations.

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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.2020.04.005.

Conflict of interest

The authors have no conflict of interest to declare.

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