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Nitric oxide synthase-2 (CCTTT)n polymorphism is associated with local gene expression and clinical manifestations in patients with chronic rhinosinusitis

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Abstract

Introduction: Nitric oxide (NO) is synthesized through NO synthase (NOS). The proximal NOS2 gene promoter contains the pentanucleotide CCTTT repeat polymorphism. We examined whether CCTTT repeats are associated with NOS2 expression in the sinonasal tissues and clinical manifestations in patients with chronic rhinosinusitis.

Methods: Mucosal specimens were obtained from the ethmoid sinus and inferior turbinate of 30 eosinophilic chronic rhinosinusitis (ECRS) and 28 non-ECRS patients. CCTTT repeats were classified into short alleles (S), with less than or equal to 14, and long alleles (L), with more than 14. The subjects were classified into the L/S + L/L and S/S groups.

Results: In ECRS, the NOS2 mRNA levels of the ethmoid sinus mucosa were significantly higher in the L/S + L/L group than in the S/S group (median, 1.66 and 0.77, respectively). On the ther hand, ECRS patients showed no significant difference in the NOS2 mRNA level of the inferior turbinate between the L/S + L/L group and the S/S group (median, 0.63 and 0.88, respectively). In ECRS, preoperative SNOT-22 were significantly higher in the L/S + L/L group than in the S/S group, whereas the former group showed a lower postoperative recurrence risk.

Conclusion: CCTTT repeat polymorphism in the NOS2 promotor gene may be a useful indicator to evaluate ECRS severity and prognosis.

Keywords

sinusitis, isoforms, nitric oxide, nasal polyps, nitric oxide synthase type II, pentanucleotide repeats, polymorphism

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Introduction

Nitric oxide (NO) is an important biomarker of eosinophilic airway inflammation. The human paranasal sinuses are the major source of NO production and contribute largely to NO levels in the nasal cavity.[1] NO is an essential biological messenger in the nasal airways, and it is involved in several components of the immune response; these include bacteriocidal activities, antiviral

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activities, modulation of mucociliary clearance via effects on ciliary beat frequency, and regulation of alveolar circulation in the lung.[2-6] On the other hand, inappropriately high NO concentrations can result in toxic effects, such as septic shock and pain.[7] The beneficial and detrimental actions of NO are highly concentration-dependent. Therefore, the regulation of NO production is important for maintaining its physiological functions and controlling its deleterious effects. Furthermore, factors that regulate NO levels can serve as biomarkers of eosinophilic airway inflammation.[8]

Nitric oxide is synthesized through the action of NO synthase (NOS) on L-arginine. There are three isoforms of NOS; two of them, NOS1 (neuronal NOS) and NOS3 (endothelial NOS), are constitutively expressed. The second isoform (NOS2) is inducible and is thus termed inducible NOS.[9] NOS2 is not constantly present in cells, and is only expressed when the cell is induced or stimulated, typically by proinflammatory cytokines and/or bacterial lipopolysaccharide.[7,10]

Previous studies have shown that NOS2 and NOS3 are responsible for most NOs produced in the nasal sinuses.[11] NOS2 is found in inflammatory cells, such as macrophages, as well as sinus mucosal epithelium.[12] The gene encoding NOS2 is located at chromosome 17q11.2-q12, which comprises 27 exons (with the transcription start site in exon 2 and stop codon in exon 27).[13] The proximal NOS2 gene promoter contains pentanucleotide microsatellites,[14] including a polypyrimidine microsatellite (CCTTT) at position −2.6 kb.[15] As it has been reported that differences in NOS2 expression depend on the number of CCTTT repeats,[16] NOS2 pentanucleotide CCTTT repeat polymorphism may potentially influence various clinical features in chronic rhinosinusitis (CRS) patients.

Chronic rhinosinusitis is classified into two categories in western countries, that is, CRS with nasal polyp (CRSwNP) and CRS without nasal polyp (CRSsNP). Eosinophilic chronic rhinosinusitis (ECRS) has been proposed as a subtype of CRSwNP in Japan, and it is associated with severe eosinophilic infiltration and intractable clinical courses.[17–19] In contrast to ECRS, CRSsNP or non-ECRS is thought to be caused by neutrophil-dominant inflammatory cell infiltration.[20,21] A multicenter large-scale epidemiological study (Japanese Epidemiological Survey of Refractory ECRS Study: JESREC Study) established a diagnostic algorithm for ECRS in 2015.[22]

Based on previous reports that NO production by NOS2 isoforms is dependent on the number of CCTTT repeats in Japanese asthmatic patients,[23] we hypothesized that certain similarities in sinonasal tissues might affect the prognosis of ECRS. The elucidation may also imply clinical significance in applying NO levels as a useful biomarker for sinus eosinophil inflammation.

Materials and methods

Subjects and tissues samples

This was a retrospective cohort study. All procedures contributing to this work complied with the ethical standards of the Helsinki Declaration of 1975, revised in 2013. The study protocol was approved by the Institutional Review Board at the Hiroshima University School of Medicine on 11 June 2018 (Approval No. Hi-136-2). Written informed consent was obtained from all patients before their participation.

Patients with or without ECRS, who underwent endoscopic sinus surgery in Hiroshima Medical University Hospital between October 2016 and August 2019, were enrolled in this study. A total of 30 ECRS and 28 non-ECRS patients (31 males and 27 females) were recruited. The diagnosis of CRS was based on computed tomography (CT) scanning and patient history, clinical symptoms, and endoscopic findings. The diagnosis of AR was based on clinical history, presence of nasal symptoms together with positive nasal eosinophils, and positive allergen-specific IgE antibody. ECRS was diagnosed based on previously established criteria.[22] The criteria for diagnosing ECRS are shown in Figure 1(a) (JESREC score). A typical example of a histological image obtained from an ECRS patient is shown in Figure 1(b). The indications for surgery were no improvement in post-nasal drip and nasal congestion after medical treatment including low-dose macrolide therapy. Patients were excluded if they had nasal papilloma, fungal sinusitis, or odontogenic maxillary sinusitis. None of the patients had received topical or systemic steroids for at least 4 weeks prior to the surgery. The surgical procedures were performed by board-certified otorhinolaryngologists (KT, ST, and TI). There was no bias in the surgical skill. Post-surgical treatment included nasal irrigation in all cases. Low-dose macrolide therapy was prescribed to non-ECRS patients. Topical nasal steroids and short-term oral steroids were administered to ECRS patients. CT images were graded according to the Lund-Mackay system.[24] Postoperative recurrence of the disease was defined according to that in the JESREC study,[22] that is, the presence of nasal polyps or nasal symptoms in nasal endoscopy, which continues for more than 1 month during postoperative follow-up. SNOT-22 questionnaire was used as an outcome tool for quality of life (QOL) measures.[25] The judgment of olfactory disorder was made based on the question item “Loss of smell or taste” in SNOT-22. Nasal polyp scoring was quoted from the paper by Parasher et al.[26]

(a)

Factor	Score
Side affected: both sides	3 points
With nasal polyps	2 points
CT changes: ethmoid/maxillary ≥ 1	2 points
Peripheral blood eosinophil count (%)	
2< and ≤ 5%	4 points
5< and ≤ 10%	8 points
10%<	10 points

(b)

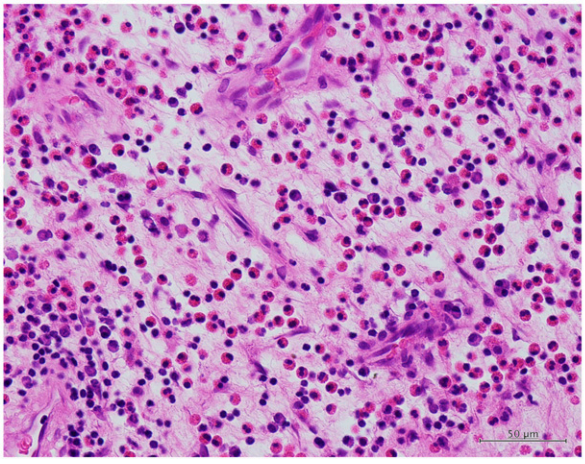

Figure 1. (a) The criteria for diagnosing ECRS (JESREC score). When the score is 11 points or higher, the ECRS diagnosis is highly probable. A definite diagnosis of ECRS requires microscopic examination of the density of eosinophil infiltration in nasal polyp/paranasal sinus tissues, that is, an average of more than 70 eosinophils/HPF. (b) A typical histological image obtained from ECRS patients stained with hematoxylin and eosin. More than 70 eosinophils/HPF were observed at a magnification of ×400.

Genotyping the pentanucleotide CCTTT repeat polymorphism in the NOS2 promoter region

Peripheral blood was collected from all patients for genotyping of the pentanucleotide CCTTT repeat in the NOS2 promoter region. The pentanucleotide CCTTT repeat of the NOS2 promoter region was analyzed using polymerase chain reaction (PCR). Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood using the PAXgene® Blood DNA kit (Qiagen, Hilden, Germany). Genomic DNA was amplified using a PTC-200 thermal cycler (MJ Research, Waltham, MA) and the following primers: forward, 5′-ACCCCTGGAAGCCTACAACTGCAT-3′; and reverse, 5′-GCCACTGCACCCTAGCCTGTCTCA-3′. The forward primer was labeled at the 5′ end with the fluorescent dye 6-FAM. The following PCR conditions were applied: initial denaturation at 95°C for 10 min; amplification for 30 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min; and final extension at 72°C for 7 min. The samples were directly sequenced by the Macrogen Japan Corporation using an automated DNA sequencer (ABI3730XL, Applied Biosystems, Foster City, CA, USA). The sizes of the CCTTT repeats were calculated with the Peak Scanner (Applied Biosystems).[27] The NOS2 pentanucleotide CCTTT repeat polymorphism was classified into two alleles based on length: short alleles (S), with a length less than or equal to 14 repeats, and long alleles (L), with a length greater than 14 repeats. The study subjects were consequently categorized into two genotype groups: (1) genotype L/S + L/L and (2) genotype S/S. The classification of genotypes was based on the study conducted by Sato et al.[23] They have shown that phenotypes of Japanese patients with bronchial asthma can be also classified based on the genotyping employed in the study. For example, the mean FeNO level before treatment was significantly higher in asthmatic patients with the L/S and L/L genotype compared to those with the S/S genotype. In addition, the distribution of NOS2 promoter microsatellite alleles was similar to their study.

Reverse transcription (RT)-PCR analysis

Mucosal specimens were obtained from the ethmoid sinus, inferior turbinate, uncinate process, and nasal polyps (if any) at the time of the surgery; specimens were obtained from both sides when CRS was present bilaterally. They were immersed in RNAlater® solution (Ambion, Austin, TX), and quantitative PCR analysis was performed using an ABI Prisms 7300 system (Applied Biosystems). Cellular ribonucleic acid (RNA) was isolated using RNeasy mini kits (Qiagen). Total RNA was then reverse-transcribed to complementary DNA using a High Capacity RNA-to-complementary DNA kit (Applied Biosystems), according to the manufacturer's instructions. Gene expression was measured on a real-time PCR system using TaqMan Gene Expression Assays (Life Technologies, Carlsbad, CA). PCR primers specific for the NOS2 gene (Hs01075529_m1) were used. Primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Hs03929097_g1) were used as a reference. PCR cycles were run in triplicates for each sample. Amplification of the PCR products was quantified by the number of cycles, and the results were analyzed using the comparative cycle threshold (Ct) method ($2^{-\Delta\Delta Ct}$). The quantities of target gene expression are presented as relative rates compared to the expression of the reference gene GAPDH (ratio: target gene/GAPDH expression).

Statistical analysis

The Wilcoxon rank-sum test, Fisher exact test, and the student's t-test were performed to analyze differences between the S/S and S/L + L/L groups and between ECRS and non-ECRS patients. Relapse-free survival curves of postoperative recurrence were generated using the Kaplan-Meier method and analyzed using the log-rank test. Values of $p < 0.05$ were considered statistically significant. Statistical analysis was carried out with JMP Pro ver. 14 (SAS Institute Inc., Cary, NC, USA).

Results

Patient characteristics

The distribution of the NOS2 pentanucleotide CCTTT repeat polymorphism is shown in Figure 2. The number of microsatellite repeats ranged from 9 to 21. The number of patients with genotypes S/S, L/S, and L/L was 35, 17, and 6, respectively. The baseline characteristics of the study population are summarized in Table 1. There were no significant differences in age, body mass index, smoking, bronchial asthma, allergic rhinitis, NSAIDs hypersensitivity, blood eosinophils, immunoglobulin E, oral fractional exhaled NO (FeNO), tissue eosinophils, or olfactory disorder between patients classified with the L/L + L/S and

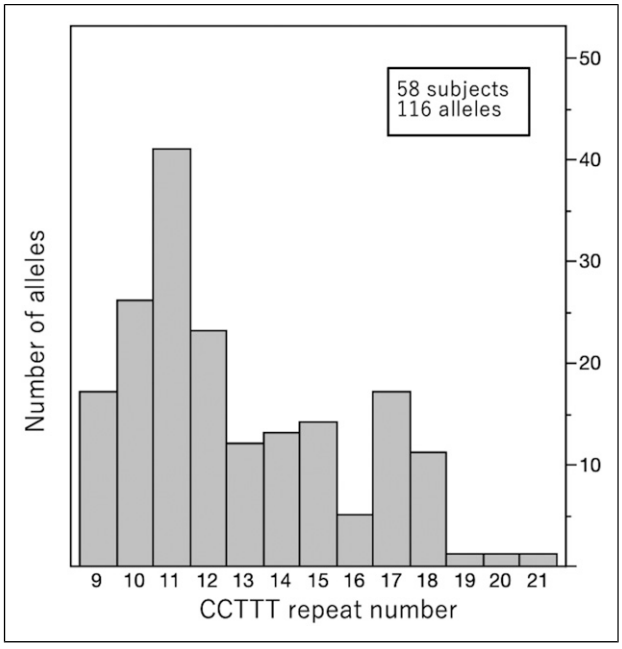

Figure 2. Distribution of NOS2 promoter microsatellite alleles in Japanese ECRS and non-ECRS patients (58 subjects, 116 alleles). ECRS: eosinophilic chronic rhinosinusitis; NOS2: nitric oxide synthase-2.

S/S genotypes in either the ECRS or non-ECRS group. On the other hand, the mean CT score is significantly higher in the ECRS group than in the non-ECRS group ($p < 0.001$). Further, the mean SNOT-22 scores were significantly higher in the L/S + L/L genotype group than in the S/S genotype group in ECRS patients ($p = 0.038$).

NOS2 expression levels in the ethmoid sinus mucosa, inferior turbinate, and nasal polyps

Among patients with ECRS, the median NOS2 mRNA level (relative to GAPDH) of the nasal polyps and ethmoid sinus mucosa was significantly higher in the L/S + L/L genotype group (median, 1.66; interquartile range (IQR), 0.85–3.65) than in the S/S genotype group (median, 0.77; IQR, 0.44–1.66) ($p = 0.044$) (Figure 3). There was no significant difference in the median NOS2 mRNA level of the nasal polyps and ethmoid sinus mucosa between the L/S + L/L genotype group (median, 1.08; IQR, 0.05–2.57) and the S/S genotype group (median, 0.82; IQR, 0.59–2.02) among patients without ECRS ($p = 0.898$). There was no significant difference in the median NOS2 mRNA level (relative to GAPDH) of the inferior turbinate between the L/S + L/L genotype group (median, 0.63; IQR, 0.32–10.24) and the S/S genotype group (median, 0.88; IQR, 0.32–1.71) among patients with ECRS ($p = 0.871$). There was no significant difference in the median NOS2 mRNA level (relative to GAPDH) of the inferior turbinate between the

Table 1. Background and baseline characteristics of the study population.

	ECRS			Non-ECRS		
Characteristic	L/L + L/S	S/S	*p* value	L/L + L/S	S/S	*p*-value
Number (male/female)	12 (7/5)	18 (9/9)	0.722	11 (4/7)	17 (11/6)	0.246
Age (mean ± SD)	53.3 ± 10.6	54.1 ± 14.3	0.873	53.5 ± 14.8	51.4 ± 18.9	0.748
BMI (kg/mm^2) (mean ± SD)	23.6 ± 3.8	22.1 ± 2.8	0.225	22.9 ± 3.9	22.6 ± 2.7	0.855
Smoking (%)	2 (16.8)	2 (11.1)	1	1 (9.1)	3 (17.7)	1
Bronchial asthma (%)	6 (50.0)	7 (38.9)	0.711	2 (18.1)	2 (11.8)	1
Allergic rhinitis (%)	10 (83.3)	8 (44.4)	0.058	8 (80.0)	12 (70.6)	0.678
NSAIDs hypersensitivity (%)	2 (16.8)	2 (11.1)	1	0 (0)	0 (0)	—
Blood eosinophils (%) (median, range)	6.8 (5.6–8.8)	6.5 (5.4–9.4)	0.799	1.5 (0.3–3.5)	1.7 (1.6–3.5)	0.323
IgE (IU/mL) (median, range)	177.0 (101.0–319.0)	104.6 (36.0–242.3)	0.334	142 (64.0–369.0)	159 (30.4–437.5)	0.841
CT score (mean ± SD)	16.0 (11.8–22.0)			5.0 (3.0–11.0)		<0.001*
	15.9 ± 5.8	16.9 ± 6.2	0.652	8.2 ± 7.8	6.6 ± 4.6	0.503
Oral FeNO (median, range)	23.0 (9.0–36.0)	24.5 (15.0–43.5)	0.567	15.5 (11.5–21.6)	8.5 (7.5–18.5)	0.134
Tissue eosinophils (cells/HPF) (median, range)	91.5 (71.5–245.8)	130.5 (81.0–205.0)	0.446	13 (10.0–45.3)	10 (6.0–22.0)	0.222
SNOT-22 (median, range)	47.0 (36.0–60.0)	23.0 (16.0–39.5)	0.038*	37.5 (26.5–57.5)	28.0 (13.75–64.25)	0.267
Olfactory disorder (%)	11 (91.7)	15 (83.3)	0.632	1 (9.1)	6 (35.3)	0.191
Polyp score (median, range)	4.0 (0.5–5.75)	5.0 (2.75–6.0)	0.282	3.0 (0–4.0)	0 (0–1.0)	0.025*

Data are shown as mean (SD), median (range (25–75%)), or number (%).
ECRS: eosinophilic chronic rhinosinusitis; BMI: body mass index; IgE: immunoglobulin E; CT: computed tomography; FeNO: fractional exhaled nitric oxide; HPF: high power field (×400); SNOT-22: sinonasal outcome test-22; L: long allele; S: short allele; SD: standard deviation.
*$p < 0.05$.

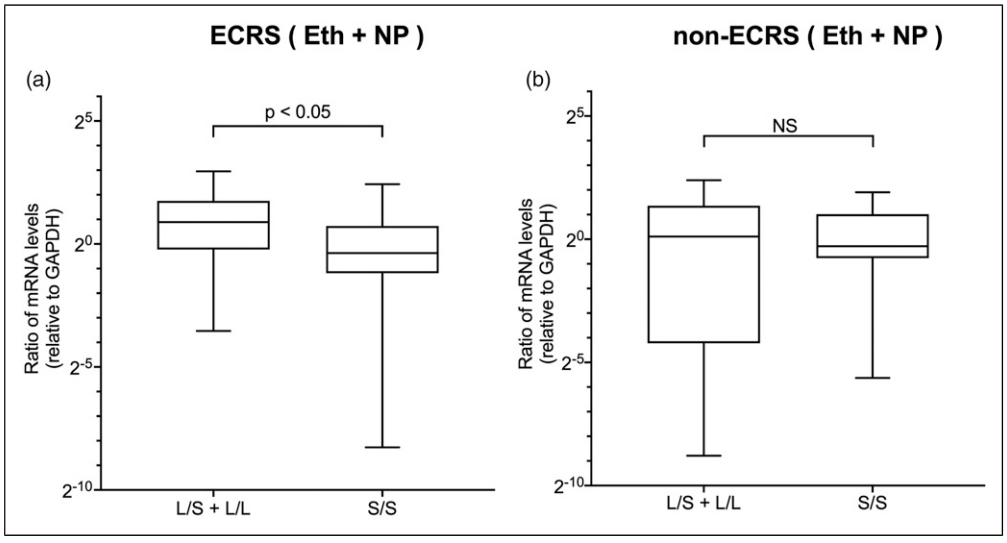

Figure 3. Comparison of mRNA expression in ethmoid sinus mucosa and nasal polyps between the L/S + L/L genotype group and S/S genotype group for (a) ECRS and (b) non-ECRS patients. NOS2 mRNA levels are normalized to GAPDH mRNA levels. The box signifies the 25th and 75th percentiles, and the median is represented by a horizontal line within the box. Eth: ethmoid sinus mucosa; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; L: long allele; mRNA: messenger ribonucleic acid; NP: nasal polyp; NS: not significant; S: short allele.

L/S + L/L genotype group (median, 0.32; IQR, 0.05–0.67) and the S/S genotype group (median, 0.33; IQR, 0.18–0.87) among patients without ECRS ($p = 0.317$) (Figure 4).

Recurrence-free rate in ECRS patients

A Kaplan–Meier plot of postoperative recurrence against time after operation in ECRS patients is shown in Figure 5. Twelve patients with the L/S + L/L genotype and 18 patients with the S/S genotype were included. The number of recurrence cases was 5 out of 20 in the L/S + L/L genotype group and 11 out of 18 in the S/S genotype group. There was no significant difference in postoperative recurrence between the L/S + L/L genotype and S/S genotype groups ($p = 0.135$). However, there was a trend for a lower risk of postoperative recurrence in the L/S + L/L genotype group. We further analyzed the recurrence rate of all the ECRS patients (L/S + L/L+ S/S). The rate was 42.1% (16 out of 38 cases) and compatible with the overall recurrence rate of in ECRS patients reported by the original JESREC study.[22]

Discussion

In this study, we analyzed the association between the NOS2 (CCTTT)n polymorphism and local gene expression and its effects on clinical manifestations in patients with CRS. The NOS2 pentanucleotide CCTTT repeat polymorphism has been studied in various diseases such as rheumatoid arthritis,[28] atrial fibrillation,[29] human immunodeficiency virus infection,[30] asthma, and atopy.[23,31-34] For example, Batra et al. reported an association between a CCTTT 12 repeat polymorphism and high serum immunoglobulin E and NO levels in patients with asthma.[32] However, only a few studies have addressed the effects of CCTTT polymorphism in patients with CRS.

The number of microsatellite repeats in the present study ranged from 9 to 21. The distribution was skewed to the right, with (CCTTT)10, (CCTTT)11, and (CCTTT)12 being the three most common alleles. Konno et al. also reported that the number of microsatellite repeats ranged from 8 to 21 in the Japanese population (141 non-atopic healthy controls, 102 atopic healthy controls, 56 non-atopic asthmatic subjects, and 198 atopic asthma subjects).[31] The finding of a bimodal distribution pattern for the allelic frequency of the pentanucleotide CCTTT repeat in the NOS2 promoter region in the present study is supported by previous studies conducted in Japan.[23,34]

The classification of genotypes was based on the study conducted by Sato et al., who found that the mean FeNO level before treatment was significantly higher in asthmatic patients with the L/S and L/L genotype compared to those with the S/S genotype. There was also a significant positive correlation between FeNO levels before treatment and longer (the longest allele out of two) CCTTT microsatellite repeats. The number of CCTTT repeats in the NOS2 promoter region was associated with higher FeNO levels before treatment, reflecting the importance of the NOS2 genotype in the clinical measurement of FeNO in this patient group.[23] The lack of a significant difference in FeNO between the different genotype groups in the present study may be due to the following reasons. First, patients with asthma who comprised half of the ECRS patients had already been medically treated. Second, non-ECRS patients had a low asthma complication rate. This may

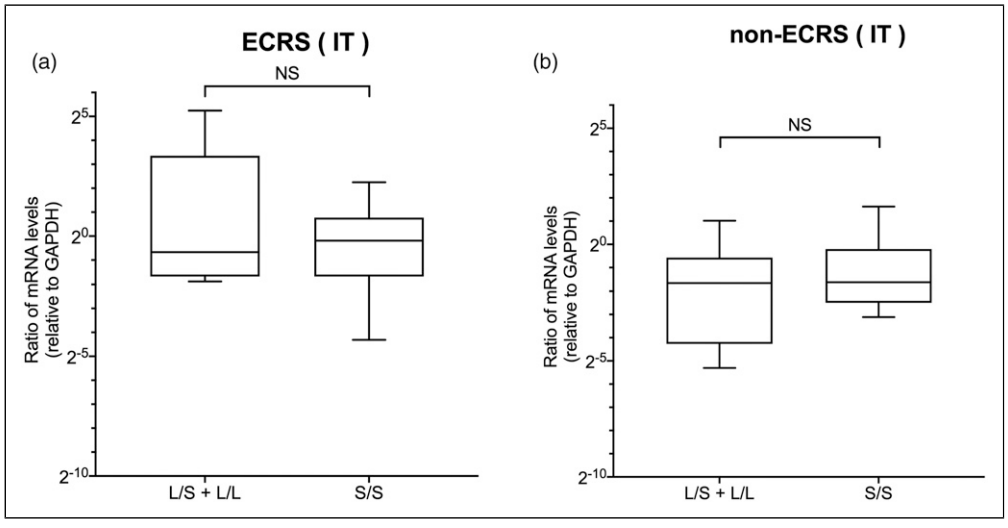

Figure 4. Comparison of mRNA expression in the inferior turbinate between the L/S + L/L genotype group and the S/S genotype group for (a) ECRS and (b) non-ECRS patients. NOS2 mRNA levels are normalized to GAPDH mRNA levels. The box signifies the 25th and 75th percentiles, and the median is represented by a horizontal line within the box. GAPDH: glyceraldehyde 3-phosphate dehydrogenase; IT: inferior turbinate; L: long allele; mRNA: messenger ribonucleic acid; NS: not significant; S: short allele.

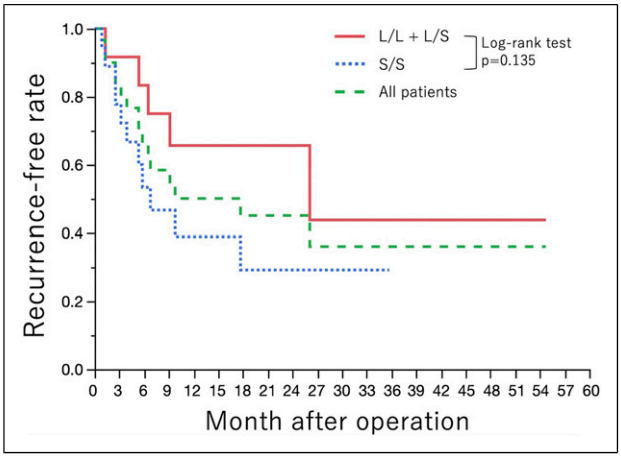

Figure 5. Kaplan–Meier curve of the recurrence-free rate in ECRS patients (L/S + L/L genotype group vs S/S genotype group). Postoperative recurrence was defined according to the JESREC study,[22] that is, the presence of nasal polyps or nasal symptoms, which continues for more than 1 month, by nasal endoscopy. ECRS: eosinophilic chronic rhinosinusitis; L: long allele; S: short allele.

suggest that oral FeNO levels tended to be higher in the L/L+L/S group. On the other hand, Hirai et al. reported that NOS2 mRNA expression levels in peripheral blood mononuclear cells gradually decreased as CCTTT repeat number increased. A genotype classification based on an allele length cutoff of 11 repeats (S, shorter than or equal to 11 repeats; L, longer than 11 repeats) indicated an association between short allelic length (S/S and S/L) and a higher risk of asthma exacerbation.[33] Due to technical reasons, we could not measure sinus NO levels in this study. Several methods have been proposed for nasal NO measurement. However, it remains challenging to apply nasal NO levels in reference data on sinonasal conditions with reliable clinical relevance.[35,36]

Pascual et al. investigated the relationship between the number of CCTTT repeats and the frequency of nasal polyposis in a case-control study conducted among white patients (150 asthma patients, 46 polyposis patients, and 98 controls) in Spain. The overall distribution of alleles was evaluated in the control and patient groups, and analyses were conducted with different cutoffs for CCTTT repeat length (11, 12, 13, 14, and 15 repeats). They found that nasal polyposis was associated with a progressive increase in the number of CCTTT repeats, with a specific cutoff at 15 repeats.[27]

Thus, the number of CCTTT repeats in the promoter region of NOS2 is likely related to the inflammatory process in nasal polyposis; this is consistent with the present study results and further highlights the importance of genotype stratification.

Eosinophilic chronic rhinosinusitis has been described as an intractable chronic sinus inflammation accompanied by the infiltration of numerous activated eosinophils in the paranasal sinus mucosa and nasal polyps.[24] We believe CCTTT repeats may affect not only asthmatics but also patients with ECRS; this is because ECRS is an eosinophilic airway inflammatory disease that is frequently associated with asthma. A previous study demonstrated a significantly greater upregulation of NOS2 and IL-5 mRNA expression in the ethmoid mucosa of ECRS patients compared to non-ECRS patients.[37] Another study reported a significantly higher upregulation of NOS2 mRNA in the nasal polyps and ethmoid mucosa of CRSwNP patients than that of CRS patients without nasal polyps.[38] Several reports have indicated that nasal FeNO tends to be lower in patients with acute and chronic sinusitis due to hampered ventilation of gaseous NO through occluded sinus ostia and increases NO absorption via inflamed sinus mucosa.[39-42] While significantly lower nasal FeNO levels were found in untreated non-ECRS patients compared to normal subjects, there was no significant difference compared to ECRS patients; this may have been attributed to increased NO production in the inflamed paranasal sinus mucosa, despite occluded sinus ventilation. High NO levels in ECRS patients seem to reflect NOS2 upregulation.[37] Noda et al. observed a positive immunoreactivity of NOS2 in both epithelial cells and submucosal inflammatory cells.[43] Thus, several lines of evidence indicate that there is a significant upregulation of NOS2 mRNA in ECRS patients, leading to high NO levels. In the present study, we reported that NOS2 mRNA levels (relative to GAPDH) of the nasal polyps and ethmoid sinus mucosa were significantly higher in the L/S + L/L genotype group compared to the S/S genotype group in ECRS patients. The results indicate that the difference in iNOS genotypes should be considered when using NO levels as a biomarker for eosinophil inflammation in human sinonasal pathways. Our results also suggest that iNOS polymorphism may be used to predict the risk of postoperative recurrence. The assessment of CCTTT repeats and NOS2 mRNA and FeNO levels may aid the diagnosis and classification of diseases associated with eosinophilic inflammation.

In contrast to the ethmoid sinus mucosa, no significant difference in the median NOS2 mRNA level of the inferior turbinate was observed between patients with the L/S + L/L genotype and S/S genotype. Indeed, ECRS patients usually show severe inflammation around the ethmoid sinus, with the region around the inferior turbinate being unaffected. This may be related to differences in the physiological function between the inferior turbinate and ethmoid sinus.

Controversies remain regarding the relationship between the number of CCTTT repeats and NOS2 mRNA expression. Kidoguchi et al. classified NOS2 pentanucleotide repeat polymorphism in CRSwNP patients according to the criteria established by Hirai et al. (i.e. S

defined by ≤11 repeats, and L defined by >11 repeats). They found that CRSwNP patients with the S/S genotype had the highest NOS2 mRNA expression levels in nasal polyps, followed by those with the S/L genotype and L/L genotype.[44] The difference in results compared to the present study may be attributed to differences in patient enrollment and cutoffs for allele length.

Eosinophilic chronic rhinosinusitis is a chronic inflammatory disease associated with a high symptom burden and poor health-related QOL. We have shown that preoperative SNOT-22 scores were highly affected in the L/S + L/L genotype group than in the S/S genotype group (p = 0.038). SNOT-22 is a questionnaire most suitable for evaluating clinical manifestations.[25] We consider that this supports that CCTTT repeat polymorphism can be a tool for predicting ECRS severity from a QOL point of view.

We found no significant difference in postoperative recurrence between patients with the L/S + L/L genotype and those with the S/S genotype; nevertheless, there was a trend for a lower risk of postoperative recurrence in the L/S + L/L genotype group. The dual activity (beneficial vs detrimental) of NO has previously been established. It has also been shown that nasal NO increases significantly due to medical and surgical therapies for CRS; this suggests that treatment may result in the recovery of normal NO production via the ciliated epithelium.[42,43] Since the L/S + L/L genotype group exhibited higher levels of NOS2 mRNA and a trend for a lower risk of postoperative recurrence, NO may have beneficial effects in lowering the risk of recurrence after operation. The number of CCTTT repeats may serve as a potential biomarker for the prediction of postoperative ECRS recurrence.

There are some limitations to our study. First, due to the small sample size, we could not demonstrate significant differences in postoperative recurrence between genotype groups. Second, this study only included Japanese patients; hence, caution should be taken when extrapolating our results to other ethnic groups. Third, we could not measure NO levels in the maxillary sinus mucosa because of ethical and technical issues. As initially reported by Lundberg et al.,[1] high NO production levels up to 3000–25,000 ppb were observed in the maxillary sinus. However, the amount of NO levels contributed by other paranasal sinuses to the human airways remains unknown. Fourth, the relationship between CCTTT repeats and proinflammatory cytokines that induce NOS2 expression needs to be assessed further. For example, Recent studies have shown that polymorphism in the TNF-alpha gene might be a risk factor for NP pathogenesis.[45]

Conclusions

The present study results indicated that ECRS patients with longer pentanucleotide repeat polymorphisms have a higher expression of NOS2 mRNA in the ethmoid sinus mucosa and nasal polyps. The results indicate that differences in iNOS genotypes should be considered when using NO as a biomarker for eosinophil inflammation in human sinonasal pathways. The NOS2 pentanucleotide CCTTT repeat polymorphism may be an indicator of ECRS severity and prognosis. Confirmation of our findings may provide a better understanding of the genetic significance of this polymorphism in ECRS. Further studies are required to confirm the utility of assessing the number of CCTTT repeats to predict ECRS prognosis.

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Ethics approval

Ethical approval for this study was obtained from the Institutional Review Board at the Hiroshima University School of Medicine on 11 June 2018 (Approval Number Hi-136-2).

Informed consent

Written informed consent was obtained from all subjects before the study.

Data accessibility

The datasets used and analyzed in the study are available from the authors on reasonable request.

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