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Original article

Comparison of gene expression profiles in eosinophilic esophagitis (EoE) between Japan and Western countries

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Abbreviations:

ALOX15, arachidonate-15 lipoxigenase;
 CAPN14, calpain 14; CCL26, C–C chemokine
 ligand 26; CCR3, C–C chemokine receptor
 type 3; CDH26, cadherin-like 26;
 CLC, Charcot-Leyden crystals;
 EG, eosinophilic gastritis; EGE, eosinophilic
 gastroenteritis; EGID, eosinophilic
 gastrointestinal disorders; EoE, eosinophilic
 esophagitis; FLG, filaggrin;
 GI, gastrointestinal; PMCH, pro-melanin-
 concentrating hormone; POSTN, periostin;
 TGF- β 1, transforming growth factor- β 1;
 TSLP, thymic stromal lymphopoietin

ABSTRACT

Background: The prevalence rate of eosinophilic esophagitis (EoE) between Japan and Western countries is quite different. Although multiple factors, including the genetic background, lifestyle and dietary habits, may account for the difference, the pathogenic mechanism of EoE has not been fully clarified in Japanese. To elucidate whether EoE's pathogenic mechanisms differ between those populations, we performed transcriptome analysis of esophageal biopsy specimens from Japanese EoE patients and compared the identified gene signatures with published microarray data for EoE patients in the US.

Methods: We prospectively enrolled adult Japanese EoE patients ($n = 4$) according to the 2011 consensus guidelines for diagnosis of EoE. Age-matched healthy volunteer subjects ($n = 4$) were also enrolled as controls. We assessed the gene expression profiles of esophageal biopsies using microarray technology and then compared the identified gene signatures with earlier data generated in the US.

Results: Of 42,545 transcripts represented on the microarray, 385 were differentially expressed between the EoE and control samples (≥ 2 fold change and adjusted p -value of < 0.05). Our microarray data showed strong overlapping with the data from US patients with EoE. An EoE-specific-transcript signature is typically composed of IL-13-inducible and eosinophil-related genes, including eotaxin-3/C–C chemokine ligand 26 (CCL26).

Conclusions: This transcriptome study suggests that the pathogenic mechanisms of EoE in Japan and Western countries are similar. Our findings may contribute to a better understanding of the pathogenesis of EoE and to more accurate diagnosis of this disease in Japanese individuals.

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Introduction

Eosinophilic gastrointestinal disorders (EGID) are clinicopathologically characterized by massive eosinophilic infiltration within

the gastrointestinal (GI) tract.¹ They are classified according to the site of infiltration as eosinophilic esophagitis (EoE), gastritis (EG), gastroenteritis (EGE), enteritis and colitis.² In Japan, the prevalence of EoE is reportedly quite low (estimated to be 0.01%),^{3,4} and much less than that of EGE.⁵ In contrast, the prevalence of EoE in Western countries, including the US and Europe, has been increasing in recent years, is now estimated to be 0.44–1%^{6,7} and is much higher than that of EGE.⁸ Notably, the prevalence of IgE-mediated food allergies (except for EGID) is increasing in Japan and shows a similar tendency to that in Western countries.^{9,10} Thus, the reason for the approximate 50– to 100–fold difference in the prevalence rates of EoE between Japan and Western countries is unclear.

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Multiple factors, including the genetic background, lifestyle and dietary habits, may account for the difference in EoE between Japan and Western countries. To date, studies have suggested that EoE is more common in Caucasians than other racial groups.^{11,12} Interestingly, EoE may have different clinical presentations and endoscopic findings between racially distinct populations. Moreover, dietary habits, especially a Mediterranean diet, are reportedly associated with development of allergic diseases.¹³ The traditional diet of Japan is characterized by low fat content, even when consuming protein and dietary fiber, and it tends to prevent inflammatory bowel disease and colorectal cancer. In contrast, Western diets tend to promote those diseases. Since we have experienced EoE patients in spite of the Japanese genetic background and traditional Japanese dietary habits, the question arises as to whether EoE in Japan and Western countries is a different disease or not. A previous study of the pathogenic mechanism of EoE analyzed esophageal tissues by the transcriptome approach and found that eotaxin-3/C–C chemokine ligand 26 (CCL26) played a crucial role in inducing selective recruitment of eosinophils into the esophageal epithelium.¹⁴

In order to elucidate whether the pathogenic mechanism of EoE in Japan is similar to that in Western countries, we performed transcriptome analysis of esophageal biopsy specimens from Japanese EoE patients and compared the identified gene signatures with microarray data that have been published for EoE patients in the US.

Methods

We prospectively enrolled adult Japanese EoE patients ($n = 4$) who lived in the countryside and followed a traditional Japanese lifestyle and dietary habits. After granting informed consent, they underwent upper GI endoscopy due to their clinical symptoms. Diagnosis of EoE was defined as >15 eosinophils/HPF persisting in the distal esophagus even after proton pump inhibitor therapy, absence of treatment with oral or systemic steroids, in accordance with the 2011 consensus guidelines,¹⁵ and exclusion of other possible causes of esophageal eosinophilia. Age-matched healthy volunteer subjects ($n = 4$) were also enrolled as controls. Control specimens were defined as having <1 eosinophils/HPF, with no history of treatment with oral or systemic steroids or EoE. All esophageal biopsies were obtained from the Department of Gastroenterology, Shimane University Hospital (Shimane, Japan).

The study was performed according to a protocol approved by the institutional review board of National Center for Child Health and Development, Tokyo, Japan. Samples were placed in RNeasy[®] solution (QIAGEN, Valencia, CA, USA) at room temperature after biopsy and then stored at -80°C until gene expression profiling.

Microarray analysis (Agilent Technologies, Santa Clara, CA, USA) was performed according to the manufacturer's instructions. Briefly, total RNA was extracted with an RNeasy Micro kit (Qiagen) and then evaluated with an Agilent Bioanalyzer and an RNA 6000 Nano kit (Agilent Technologies). The gene expression profiles were assessed using microarray technology with Agilent SurePrint G3 Human GE 8 x 60k. Data analysis was performed using GeneSpring software ver. 12.5 (Agilent Technologies). To normalize variation in the staining intensity between microarrays, the average difference for all genes on a given microarray was divided by the median of all measurements on that microarray, and genes in EoE patient specimens that showed a significant difference in signal intensity compared with the same genes in the control specimens ($P < 0.05$, t test) were considered to be up-regulated or down-regulated. Hierarchical clustering was performed using the gene expression data, contrasting the EoE and control specimens (see Fig. 1). The differentially expressed genes of EoE in Japan and the US were compared by systematic analysis using the NextBio search engine (<http://www.nextbio.com/b/nextbio.nb>)¹⁶ (see Fig. 2).

Results

Characteristics of the subjects

The clinical and histopathological characteristics of all subjects are summarized in Table 1. All patients with EoE had a history of dysphagia, a common symptom observed among Japanese patients with EoE.⁵ The most frequent endoscopic finding in patients with EoE was linear furrows, the diagnostic usefulness of which was recently reported.¹⁷ Besides abnormal endoscopic findings, evidence of marked eosinophilic inflammation was observed in all patients with EoE.

Microarray analysis of differentially expressed genes in esophageal biopsies

Esophageal biopsy specimens from individual patients were subjected to whole-genome transcript expression profile analysis.

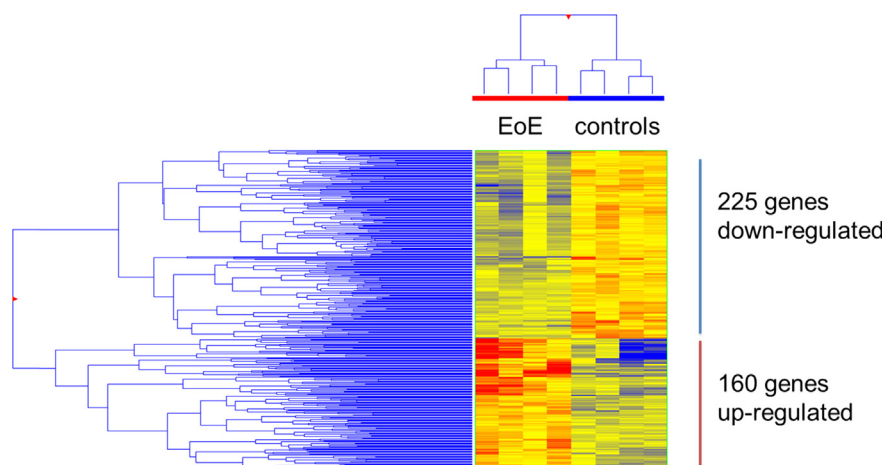


Fig. 1. Microarray analysis of differentially expressed genes in esophageal biopsies. The 385 genes differentially expressed ($P < 0.05$, fold difference ≥ 2) in the EoE group compared with control subjects are shown in a heatmap image. Up-regulated genes are represented in red and down-regulated genes in blue.

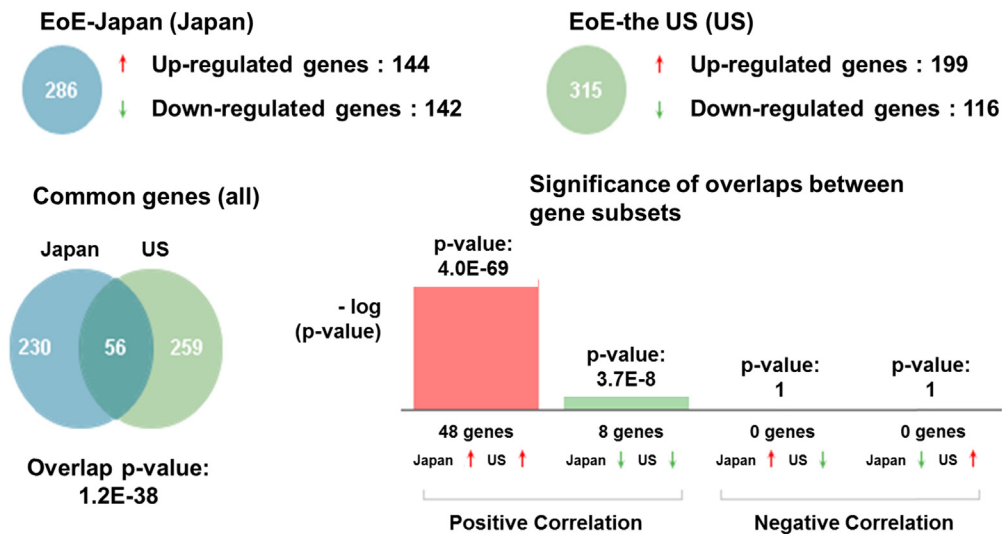


Fig. 2. Comparison of gene expression profiles in EoE between Japan and the US. NextBio was used to compare our list of dysregulated genes to those of Blanchard et al.,¹⁴ using the same fold difference (≥ 2 fold difference). Venn diagrams compare the number of genes identified as up-regulated and down-regulated in EoE across both datasets.

Of the 42,545 transcripts represented on the microarrays, 385 were differentially expressed (≥ 2 fold difference and adjusted p-value of < 0.05) in the Japanese EoE patients versus the normal control biopsy specimens (see [Supplementary Table 1](#)). Hierarchical clustering of the signal intensities of the individual transcripts in each group showed high similarity of transcript expression patterns among the EoE patients. Of those 385 transcripts, 160 were expressed more abundantly and 225 were expressed less abundantly in the EoE patients compared with the control group (Fig. 1). In agreement with a US study of EoE patients,¹⁴ cadherin-like 26 (CDH26), pro-melanin-concentrating hormone (PMCH), eotaxin-3/CCL26, arachidonate-15 lipoxigenase (ALOX15), periostin (POSTN) and Charcot-Leyden crystals (CLC) were highly up-regulated (> 10 fold) genes compared with in the control subjects.

Comparison of gene expression profiles in EoE between Japan and the US

We compared our list of unique up- and down-regulated genes to that of Blanchard et al. (2006),¹⁴ using the same fold difference (≥ 2 fold difference). After removing duplicate genes, expressed sequence tags and hypothetical genes, both the up- and down-regulated genes showed correlations between these datasets (Fig. 2). Fifty-six dysregulated genes were found to overlap between this study and the US data. Those genes are listed in [Table 2](#).

Discussion

Because the prevalence rates of EoE in Japan and Western countries are quite different, we compared the gene expression profiles in EoE patients between them. Our microarray data obtained from Japanese EoE patients substantially overlapped with earlier data from EoE patients in the US,¹⁴ suggesting that, even in different regions and countries with distinct dietary habits, EoE is the same disease entity.

Our results confirm that an EoE-specific-transcript signature is composed of dysregulated genes involved in type-2 inflammation, which plays a central role in the pathogenesis of EoE. Notably, IL-13, a type-2 cytokine that plays a pivotal role in allergic inflammation and eosinophil-associated tissue remodeling, is known to be responsible for various types of immune dysregulation.¹⁸ Importantly, our dataset confirmed that IL-13 transcript was significantly—although the level was quite low—upregulated in patients with EoE. In agreement with Blanchard et al.'s report,¹⁴ we identified eotaxin-3/CCL26, an IL-13-inducible chemokine that plays a crucial role in inducing selective recruitment of eosinophils into the esophageal epithelium, as one of the most highly induced genes in our Japanese EoE tissue biopsy specimens. A number of other IL-13-inducible genes also exhibited dysregulated expression in our Japanese patients with EoE. Interestingly, PMCH mRNA, an appetite-stimulating peptide, was significantly upregulated in both

Table 1
 Characteristics of the subjects.

Age (y)	M/F	Diagnosis	Response to PPI therapy	Clinical symptoms			Endoscopic findings				Eosinophils		Atopic history (BA) and/or allergic rhinitis (RA)	
				Dysphagia	Heartburn	Other esophageal symptom	Furrow	Concentric ring	White plaque	Stricture	Histology eosinophils/HPF	Peripheral blood eosinophils (μl)		
83	F	EoE	—	+	—	—	+	—	—	—	—	20	122	—
46	F	EoE	—	+	—	—	+	—	—	—	—	>20	NA	—
60	M	EoE	—	+	—	—	+	+	—	—	—	>20	169	AR
43	M	EoE	—	+	—	—	+	+	+	—	—	>50	308	BA, AR
59	M	control	NA	—	—	—	—	—	—	—	—	<1	NA	—
42	M	control	NA	—	—	—	—	—	—	—	—	<1	NA	—
44	M	control	NA	—	—	—	—	—	—	—	—	<1	NA	—
53	F	control	NA	—	—	—	—	—	—	—	—	<1	NA	—

EoE, Eosinophilic Esophagitis; PPI, Proton Pump Inhibitor; NA, Not Applicable

Table 2
Overlapping up- and down-regulated genes in EoE between Japan and the US.

ProbeName	Fold difference :		Gene symbol	Description
	EoE vs control	US [†]		
Japan				
Up-regulated genes				
A_23_P502957	26.4	23.4	CDH26	cadherin 26
A_23_P321223	22.5	19.0	PMCH	pro-melanin-concentrating hormone
A_23_P215484	22.3	53.2	CCL26	chemokine (C-C motif) ligand 26
A_23_P55373	21.5	20.9	ALOX15	arachidonate 15-lipoxygenase
A_24_P605563	19.7	17.6	IGLJ3	immunoglobulin lambda joining 3
A_33_P3511265	13.4	46.3	POSTN	periostin, osteoblast specific factor
A_23_P101683	12.0	12.7	CLC	Charcot-Leyden crystal protein
A_23_P167168	12.0	14.6	IGJ	immunoglobulin J polypeptide, linker protein for immunoglobulin alpha and mu polypeptides
A_24_P406754	11.7	2.6	LOXL4	lysyl oxidase-like 4
A_32_P315178	10.4	15.0	ATP13A5	ATPase type 13A5
A_33_P3304696	10.0	8.3	IGLV3-21	immunoglobulin lambda variable 3-21
A_24_P240259	8.9	10.9	LRR31	leucine rich repeat containing 31
A_23_P81898	8.3	14.9	UBD	ubiquitin D
A_23_P105144	8.2	6.2	SCUBE2	signal peptide, CUB domain, EGF-like 2
A_23_P140384	6.4	3.6	CTSG	cathepsin G
A_23_P321354	5.6	14.0	TMEM71	transmembrane protein 71
A_23_P7212	5.4	5.7	CFI	complement factor I
A_23_P18017	4.9	13.1	CPA3	carboxypeptidase A3
A_23_P1904	4.8	4.2	MS4A2	membrane-spanning 4-domains, subfamily A, member 2
A_23_P117662	4.7	3.1	HDC	histidine decarboxylase
A_24_P263786	4.1	6.1	IGKC	immunoglobulin kappa constant
A_23_P330070	4.1	5.1	TFPI	tissue factor pathway inhibitor
A_23_P1552	3.9	9.0	CTSC	cathepsin C
A_23_P169092	3.7	4.3	CYP7B1	cytochrome P450, family 7, subfamily B, polypeptide 1
A_33_P3405728	3.3	5.3	PKP2	plakophilin 2
A_33_P3322804	3.2	7.4	NTRK2	neurotrophic tyrosine kinase, receptor, type 2
A_24_P3249	3.2	2.2	RARB	retinoic acid receptor, beta
A_23_P135548	3.1	3.4	DPYD	dihydropyrimidine dehydrogenase
A_33_P3373775	3.1	9.7	BCL2L15	BCL2-like 15
A_24_P157370	3.0	3.4	IL17RB	interleukin 17 receptor B
A_24_P419300	3.0	8.1	PP7080	uncharacterized LOC25845
A_33_P3224819	3.0	3.1	SLC45A4	solute carrier family 45, member 4
A_33_P3364268	2.9	3.1	LBH	limb bud and heart development homolog
A_24_P161018	2.8	2.3	PARP14	poly (ADP-ribose) polymerase family, member 14
A_23_P10506	2.7	5.3	HPGDS	hematopoietic prostaglandin D synthase
A_23_P27606	2.6	3.3	IL27RA	interleukin 27 receptor, alpha
A_24_P397489	2.6	2.9	GCNT2	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme
A_24_P133253	2.5	3.9	KITLG	KIT ligand
A_23_P3532	2.5	3.6	LITAF	lipopolysaccharide-induced TNF factor
A_23_P121527	2.5	2.5	KLHL5	kelch-like 5
A_23_P144096	2.4	3.2	CISH	cytokine inducible SH2-containing protein
A_33_P3245908	2.4	2.2	C10orf128	chromosome 10 open reading frame 128
A_32_P53524	2.3	2.6	NTN1	netrin 1
A_23_P42718	2.2	2.9	NFE2L3	nuclear factor (erythroid-derived 2)-like 3
A_24_P408736	2.2	2.4	GALNT5	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 5
A_33_P3410093	2.2	2.2	LTA4H	leukotriene A4 hydrolase
A_33_P3342528	2.1	2.4	P2RY1	purinergic receptor P2Y, G-protein coupled, 1
A_23_P30655	2.0	2.1	NFKBIE	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon
Down-regulated genes				
A_24_P206776	-4.1	-6.3	CRYAB	crystallin, alpha B
A_33_P3293958	-3.8	-2.8	TUBB2A	tubulin, beta 2A class IIa
A_23_P69810	-3.0	-4.7	AGPAT9	1-acylglycerol-3-phosphate O-acyltransferase 9
A_23_P4536	-2.4	-14.6	EPB41L3	erythrocyte membrane protein band 4.1-like 3
A_24_P46130	-2.3	-5.0	ACPP	acid phosphatase, prostate
A_24_P152968	-2.1	-3.9	AKR1C1	aldo-keto reductase family 1, member C1
A_33_P3265030	-2.1	-2.4	GP1BB	glycoprotein Ib (platelet), beta polypeptide
A_33_P3231252	-2.1	-3.0	NHLH2	nescient helix loop helix 2

[†] US data: Previously reported by Blanchard et al.¹⁴

datasets, although its pathophysiological role in EoE is not yet known. We previously reported that PMCH was the most strongly induced gene (over 1200-fold up-regulation) in the human vascular endothelial cell transcriptome in response to such type-2 cytokines as IL-4 and IL-13, in a STAT6-dependent manner.¹⁹ Since PMCH is an endothelial, but not an epithelial, cell-specific transcript, over-expression of PMCH may imply that an IL-13-mediated inflammatory response might extend to esophageal endothelial cells, in part

because of esophageal epithelial barrier dysfunction. Greater understanding of the pathophysiological roles of MCH in EoE patients may lead to serum MCH becoming a useful surrogate biomarker for IL-13 in EoE patients.

Our microarray data also showed that a number of eosinophil-specific genes were dramatically upregulated in EoE patients, including CLC (12 fold), ALOX15 (21 fold) and C-C chemokine receptor type 3 (CCR3) (3.5 fold). CLC likely promotes

proinflammatory changes, including epithelial hyperplasia, and it correlates with mucosal inflammation in EoE.²⁰ Matoso et al. recently reported that ALOX15 may be a useful diagnostic marker for EoE.²¹ CCR3 is known to contribute to accumulation and activation of eosinophils, and it binds and responds to eotaxin-3/CCL26.¹⁴ Furthermore, Transforming growth factor (TGF)- β 1 produced by eosinophils is responsible for overexpression of various molecules involved in the tissue remodeling process. Notably, periostin, a matricellular protein, is known to be critically involved in the pathogenesis of allergic diseases,²² not only as a component in subepithelial fibrosis but also by playing a key role in the amplification and chronic nature of allergic skin inflammation.²³ We previously reported that both IL-13 and TGF- β 1 play a role in inducing periostin production by subepithelial cells, including endothelial cells, in a corticosteroid-insensitive manner.²⁴ Indeed, periostin expression remains increased in corticosteroid-treated patients with EoE.²⁵ The genes identified in this study were also identified by Wen et al.,²⁶ using an EoE diagnostic panel based on a 96-gene expression profile. These observations further support our present findings suggesting that EoE in both Japan and Western countries should be considered to be the same disease entity.

The findings of our dataset confirmed almost exactly the findings of the earlier EoE dataset from the US. There were small discrepancies, probably due in part to the use of different microarray platforms in the studies. For instance, a recent genome-wide association study by Kottyan et al. showed that calpain 14 (CAPN14) is closely associated with EoE.²⁷ It was also identified as a significantly increased gene in our Japanese EoE patients, but not in Blanchard's study. On the other hand, flaggrin (FLG), which might disrupt normal esophageal barrier function,²⁸ was identified as a significantly decreased gene in Blanchard's dataset, but not in our study. Despite recent evidence showing that thymic stromal lymphopoietin (TSLP) plays a crucial role in the pathogenesis of EoE,¹⁸ TSLP expression was not significantly increased in our EoE patients when compared with the healthy control subjects, as in Blanchard et al.'s study.¹⁴

Several mechanisms might explain the regional differences in prevalence of EoE. The reported low prevalence of EoE in Japan might be due not only to a lack of awareness of this illness but also different genetic and environmental factors between Japan and Western countries. EoE was recently recognized as a form of food allergy.² Although Japanese dietary habits, which have been different from those of Westerners for a long time, might be associated with the low prevalence of EoE in Japan, our data suggest that the mechanism underlying the pathogenesis of EoE is similar. In that scenario, once disease develops, dietary habits would no longer play any role in the progression of EoE. Currently, EGE is more prevalent than EoE in Japan, from children to adults.^{5,29} Application of our microarray analysis to the entire GI tract might lead to elucidation of the precise mechanisms of EGID, and more studies are clearly warranted.

In conclusion, this transcriptome study suggests that the pathogenetic mechanisms of EoE in Japan and Western countries are similar. Our findings may contribute to a better understanding of the pathogenesis of EoE and to more accurate diagnosis of this disease in Japanese individuals.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.alit.2015.03.002>.

Conflict of interest

The authors have no conflicts of interest to declare.

Authors' contributions

TS and HM contributed equally to this work. TS designed the study, analyzed the data and wrote the manuscript. HM designed the study, performed the experiments and analyzed the data. IN designed the study and received grant support. NI and SI contributed to sample collection. AM interpreted the results and critically revised the manuscript. KM designed the study and critically revised the manuscript. YK designed the study, provided overall supervision and edited the manuscript. All authors read and approved the final manuscript.

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