Comment on: 'Metagenome-wide association study of gut microbiome revealed novel aetiology of rheumatoid arthritis in the Japanese population' by Kishikawa *et al*

The recent paper from Kishikawa *et al*[1] provides an extremely important new insight on the concept of oral-gut microbiome axis and rheumatoid arthritis (RA) pathogenesis.

Alterations in the gut microbiome at mucosal sites have been implicated in the pathogenesis of RA.[2–4] Increasing evidence suggests a link between RA and periodontal infections caused by *Porphyromonas gingivalis (Pg)*.[5] Oral infection by *Pg* in an animal model revealed increased serum levels of lipopolysaccharide (LPS), dysbiosis and aggravation of arthritis.[6 7] Therefore, the pathogenesis of RA is considered to be associated with the immunomodulatory activity of oral and gut microbiome.[8] However, few studies have explored the relationship between the oral-gut microbiome axis and RA pathogenesis.

We previously investigated the relationship between RA disease activity using activity indices and biomarkers; the total bacterial counts and the counts of five well-known gut bacteria species; LPS-related biomarkers and IgG and IgA anti-*Pg*-LPS antibodies in 87 patients with established RA showing inadequate responses to conventional synthetic disease-modifying antirheumatic drugs or exhibiting severe complications.[9] (online supplementary table 1)

Little significant relationship was observed between the counts of the total bacteria, five species of bacteria and activity indices and biomarkers.(table 1) The levels of LPS-related biomarkers, particularly serum LPS-binding protein (LBP), were positively correlated with activity indices and biomarkers, suggesting that bacterial LPS-LBP complexes from the gut microbiome may activate nuclear factor κB via toll-like receptor 4 and may initiate and perpetuate inflammation[10 11] in established RA.

IgA antibody responses against *Pg*-LPS, were inversely correlated with the counts of intestinal bacteria, affecting the microbiome balance, and showed a positive correlation with serum LPS and LBP levels, suggesting barrier damage due to intestinal infection with *Pg*. These results support previous findings in collagen-induced arthritis models showing that oral administration of *Pg* significantly affected the gut barrier function and the gut microbiota composition, specifically by decreasing the proportion of phylum *Bacteroidetes,* increasing the proportion of phylum *Firmicutes*.[7]

Furthermore, IgG anti-*Pg*-LPS antibody levels, which are indicative of systemic infection, are inversely correlated with RA activity indices; these results are comparable to those of our previous study of destructive RA.[12] Thus, these results demonstrate that the oral-gut microbiome axis relationship may aggravate disease activity in RA.

Kishikawa *et al* recently performed a genome-wide association study to analyse the role of the gut microbiome in RA; they compared 82 Japanese patients with early RA with 42 age-matched and sex-matched normal controls[1] (online supplementary table 1).

In this study, faecal samples were subjected to whole-genome shotgun sequencing. Case–control phylogenetic association analyses, conducted using a generalised linear regression model, showed that multiple species belonging to the *Prevotella* genus increased in the RA gut metagenome. Multiple *Prevotella spp.*, in addition to *Prevotella copri*, which was recently identified by shotgun sequencing, have been identified in the oral cavity. It has been speculated that colonisation of the intestine by oral bacteria is related to the pathogenesis of RA and other diseases suggesting existence of oral-gut microbiome axis relationship.

In Kishikawa's study, a representative finding was decreased expression of the redox reaction-related gene *R6FCZ7* which is involved in oxidative stress in the genus *Bacteroides* in RA than in healthy subjects. It was previously reported that the counts of *Prevotella* and *Bacteroides* in the gut in RA show an inverse relationship.[3] They suggested that *R6FCZ7* and the genus *Prevotella* are inversely associated via the relationship with the genus *Bacteroides*. In our study, levels of serum LPS, a potent generator of reactive oxygen species,[10] as well as IgA anti-*Pg*-LPS antibody levels, which indicate gut infection with *Pg*, are inversely associated with *Bacteroides* counts. Both studies showed that the key gene *R6FCZ7* and LPS are associated with the generation of reactive oxygen species and are regulated by the balance of gut bacteria, including oral bacteria.

From the viewpoint of clinical application, we have questions to authors after comparison with two studies discussing two oral origin microbiome; *Prevotella spp.* and *Pg*.

Prevotella copri was most abundant in patients with new-onset RA[3 4] suggesting its pathogenic role. Moreover, Maeda *et al* reported SKG mice harbouring microbiota dominated by *P. copri* from early RA patients had an increased number of intestinal Th17 cells and developed destructive arthritis when treated with zymosan.[4] Another microbiome from oral cavity, *Pg* is also considered to play a pathogenic role in RA since *Pg* peptidylarginine deiminase is implicated in the autoimmunity of RA by creating mimic antigen, circular citrullinated peptide (CCP), by autocitrullination.[5] Interestingly, anti-*Pg*-LPS antibody associated with RA clinical indices and biomarker in our study with established RA, suggesting the role for continuation of RA inflammation.[9] These data by Maeda and ours might show the possibility that multiple *Prevotella spp.* other than *P. copri* or *Pg* play not only pathogenic but prognostic role. Were there significant differences of values in prognostic factors (rheumatoid factor, anti-CCP antibody, matrix metalloproteinase 3, HLA-*DRB1* gene, bone erosion and so on) between high and low abundant groups in multiple *Prevotella spp.* or *Pg*? ; results from such analyses would have provided crucial information for understanding of RA pathogenesis.

Overall, our findings and those of others suggest that modulation of the oral-gut microbiome axis is a promising strategy for the treatment and management of RA.

Table 1 Relationships between RA activity indices, disease biomarkers, gut bacterial counts, LPS-related biomarkers and IgG and IgA anti-*Pg* LPS antibodies in 87 patients with RA.

		Activity indices											Disease biomarker								LPS-related biomarker			Anti-*Pg* -LPS	
	Top: slope values Bottom: p valu	DAS28-ESR	DAS28-CRP	SJC	TJC	pVAS	dVAS	Pain VAS	mHAQ	SDAI	CDAI	ESR	CRP	RF	Anti-CCP	Hb	MMP-3	TNF	IL-6	Faecal LPS	Serum LPS	LBP	ENC	IgG anti-Pg- LPS	IgA anti-Pg- LPS
Gut bacterial counts	Total bacteria	−0.042 0.698	−0.132 0.224	**−0.180** **0.096**	−0.058 0.591	**−0.223** **0.038**	−0.098 0.365	**−0.260** **0.015**	−0.119 0.271	−0.178 0.100	**−0.187** **0.082**	0.115 0.289	−0.032 0.768	−0.093 0.391	−0.060 0.580	0.088 0.418	**−0.179** **0.097**	−0.033 0.768	**0.230** **0.034**	−0.092 0.396	**−0.492** <0.001	**−0.242** **0.024**	**0.435** <0.001	0.078 0.471	**−0.441** <0.001
	Bifidobacterium	−0.019 0.864	0.073 0.505	0.047 0.668	0.134 0.218	−0.031 0.774	−0.015 0.889	0.025 0.816	−0.024 0.828	0.101 0.353	0.115 0.290	**−0.189** **0.080**	0.018 0.866	0.163 0.131	0.125 0.248	0.153 0.157	−0.068 0.533	0.046 0.687	0.047 0.670	**0.182** **0.092**	**0.258** **0.016**	−0.044 0.688	−0.142 0.188	−0.010 0.930	−0.160 0.139
	Lactobacillus	−0.100 0.356	−0.057 0.597	−0.172 0.112	−0.092 0.398	−0.127 0.241	−0.010 0.924	−0.085 0.433	−0.004 0.974	−0.113 0.297	−0.137 0.205	0.010 0.927	0.050 0.649	0.009 0.933	−0.051 0.640	−0.080 0.464	0.039 0.722	0.088 0.436	0.035 0.752	0.071 0.512	**−0.268** **0.012**	−0.139 0.199	**0.229** **0.033**	0.019 0.859	**−0.224** **0.037**
	Bacteroides	−0.102 0.348	−0.078 0.475	0.001 0.996	−0.047 0.667	−0.044 0.685	−0.036 0.738	0.031 0.773	−0.045 0.682	−0.061 0.573	−0.060 0.582	−0.031 0.775	−0.064 0.556	−0.024 0.829	−0.059 0.591	−0.056 0.609	−0.025 0.820	0.053 0.636	0.034 0.754	0.027 0.806	**−0.230** **0.032**	−0.122 0.259	0.125 0.250	−0.029 0.789	**−0.200** **0.064**
	Escherichia coli	−0.051 0.642	0.048 0.660	−0.070 0.519	0.041 0.709	0.004 0.968	0.163 0.132	0.018 0.869	0.026 0.815	−0.016 0.884	−0.026 0.813	−0.041 0.709	0.063 0.560	0.063 0.564	−0.060 0.583	0.044 0.688	0.000 1.000	−0.031 0.783	0.001 0.997	−0.058 0.593	−0.075 0.490	−0.033 0.759	0.050 0.648	**−0.243** **0.024**	**−0.260** **0.015**
	Staphylococcus	−0.120 0.267	−0.083 0.443	−0.156 0.150	−0.075 0.488	0.049 0.654	0.002 0.988	−0.005 0.961	0.087 0.423	−0.058 0.594	−0.058 0.594	−0.156 0.149	−0.080 0.464	0.067 0.539	−0.130 0.230	0.039 0.721	−0.087 0.424	0.108 0.335	0.020 0.853	0.075 0.492	−0.039 0.723	−0.113 0.298	−0.083 0.447	**0.219** **0.041**	−0.127 0.240
LPS-related biomarker	Faecal LPS	**0.237** **0.027**	**0.245** **0.022**	0.053 0.627	**0.203** **0.059**	0.123 0.256	**0.203** **0.059**	0.065 0.548	0.077 0.482	**0.233** **0.030**	**0.238** **0.027**	0.044 0.688	0.097 0.370	−0.029 0.790	−0.033 0.760	0.063 0.564	−0.009 0.933	0.050 0.656	−0.027 0.808		0.006 0.956	0.125 0.248	−0.029 0.793	0.006 0.953	0.037 0.736
	Serum LPS	0.032 0.766	0.053 0.626	0.112 0.303	0.090 0.405	**0.180** **0.095**	−0.026 0.814	0.155 0.152	0.112 0.303	0.115 0.289	0.159 0.141	−0.163 0.132	−0.177 0.101	−0.022 0.838	0.074 0.494	**0.220** **0.040**	−0.060 0.579	0.010 0.929	**−0.209** **0.053**	0.006 0.956		0.085 0.436	**−0.255** **0.017**	−0.075 0.491	**0.284** **0.008**
	LBP	**0.300** **0.005**	**0.244** **0.023**	0.170 0.116	−0.092 0.395	0.025 0.816	0.021 0.844	0.042 0.703	0.065 0.552	0.117 0.279	0.036 0.739	**0.497** <0.001	**0.697** <0.001	**0.234** **0.029**	**0.273** **0.010**	**−0.271** **0.011**	**0.546** <0.001	0.004 0.969	**0.348** **0.001**	0.125 0.248	0.085 0.436		0.035 0.748	0.046 0.676	**0.247** **0.021**
	ENC	0.151 0.164	0.068 0.534	**−0.229** **0.033**	−0.063 0.564	−0.037 0.732	−0.024 0.827	0.016 0.886	−0.007 0.948	−0.066 0.545	−0.126 0.245	**0.338** **0.001**	**0.289** **0.007**	−0.137 0.207	−0.012 0.910	−0.142 0.189	0.063 0.564	−0.004 0.969	**0.245** **0.023**	−0.029 0.793	**−0.255** **0.017**	0.035 0.748		0.060 0.579	**−0.321** **0.002**
Anti-*Pg* LPS	IgG anti-*Pg*-LPS	−0.148 0.170	**−0.277** **0.009**	−0.122 0.261	**−0.218** **0.043**	**−0.376** <0.001	**−0.315** **0.003**	**−0.433** <0.001	**−0.192** **0.074**	**−0.308** **0.004**	**−0.309** **0.004**	0.096 0.376	0.039 0.721	−0.016 0.885	−0.003 0.981	−0.100 0.355	**−0.199** **0.065**	0.052 0.645	0.129 0.228						
	IgA anti-*Pg*-LPS	−0.021 0.847	−0.049 0.655	−0.189 0.079	−0.170 0.117	0.071 0.511	−0.108 0.321	−0.031 0.778	−0.020 0.854	−0.013 0.907	−0.010 0.924	0.063 0.561	0.042 0.700	**0.238** **0.027**	0.157 0.146	−0.040 0.713	0.133 0.218	0.122 0.277	−0.125 0.253						

Bacterial DNA extracted from individual faecal samples was subjected to real time-PCR using the group-specific or species-specific primers. The methods used to analyse LPS-biomarkers and anti-*Pg*-LPS antibodies are described in references.[5 9 12] Patient age, sex, disease duration, treatment with MTX, drinking habits and smoking habits showed little effect on bacterial counts (data not shown).
Top values are the slopes of the correlation (correlation coefficient "ρ") and bottom values are the p values determined by Spearman's non-parametric rank correlation analysis. dark brown, bold: significant positive correlation ($p<0.05$); light brown, bold: trend to positive correlation ($0.05\leq p<0.1$); dark blue, bold: significant negative correlation ($p<0.05$); light blue, bold: trend to positive correlation ($0.05\leq p<0.1$).
Anti-CCP, anti-cyclic citrullinated peptide antibody; CDAI, Clinical Disease Activity Index; CRP, C reactive protein; DAS28-ESR, disease activity score with 28 joint counts-erythrocyte sedimentation rate; EGA, evaluator's global assessment; ENC, endotoxin neutralising capacity; Hb, haemoglobin; IL-6, interleukin-6; LBP, LPS-binding protein; LPS, lipopolysaccharide; mHAQ, modified Health-Associated Questionnaire; MMP-3, matrix metalloproteinase-3; *Pg*, *Porphyromonas gingivalis*; PGA, patient's global assessment; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; SJC, swollen joint count; TJC, tender joint count; TNF, tumour necrosis factor; VAS, Visual Analogue Scale.

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Contributors HS, KoK and KT designed basic study plans, acquired, analysed and interpreted the data. KoK, KaK, HS, HB and KT drafted this manuscript. KaK, SS conducted most of the analysis in this study including faecal bacteria and LPS-related biomarker analysis. KoK, SU, CA, HT and KN conducted the clinical study with their patients. TW confirmed data by conducting related, but independent experiments, interpreted data and contributed to the preparation of this manuscript. RF conducted the statistical analysis and interpreted the data based on statistical significance. All authors have read and approved of the final manuscript.

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