

Supplementary Material

The antibody response to *Porphyromonas gingivalis* in relation to anti-citrullinated protein antibodies, smoking and *HLA-DRB1* shared epitope in rheumatoid arthritis

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SUPPLEMENTARY METHODS

RgpB IgG ELISA

High-binding Nunc MaxiSorp plates were coated over night (ON) at 4°C, with RgpB protein (2.5µg/ml) in 50mM carbonate buffer; washed in PBS-Tween (0.05%) and blocked (2% BSA in PBS) ON at 4°. Serum, diluted 1:800 in RIA-buffer (1% BSA, 350mM NaCl, 10mM Tris-HCl (pH 7.6), 1% Triton X-100, 0.1% SDS), was incubated for 1.5h at room temperature (RT). Plates were then washed and incubated for 1h at RT with peroxidase-conjugated goat anti-human IgG (diluted 1:10.000 in RIA-buffer). Bound antibodies were detected using tetramethylbenzidine substrate (Sigma). The colour reaction was stopped with 0.5M H₂SO₄. Absorbance was measured at 450nm. Antibody levels were presented as arbitrary units (AU/ml), based on a standard curve made from a serially diluted highly positive serum pool.

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SUPPLEMENTARY TABLE 1

Supplementary Table 1. Associations between anti-RgpB IgG exposure and RA in subgroups of patients based on the presence/absence of ACPA fine-specificities, RF, anti-Carb-fib and anti-CCP antibodies

ACPA; anti-Carb-fib; RF / anti-CCP antibodies	Anti-RgpB IgG		OR*	95%CI
	Negative N (%)	Positive N (%)		
CEP-1/CCP				
Controls	341 (90.45)	36 (9.55)	1.0	ref.
-/-	559 (79.63)	143 (20.37)	2.5	1.6-3.8
-/+	441 (76.43)	136 (23.57)	3.2	2.1-4.9
+/-	16 (66.67)	8 (33.33)	5.6	1.8-17.3
+/+	502 (74.81)	169 (25.19)	3.5	2.3-5.4
-/+	1000 (78.19)	279 (21.81)	2.8	1.8-4.1
+/+	518 (74.53)	177 (25.47)	3.6	2.3-5.4
Cit-vim₆₀₋₇₅/CCP				
Controls	341 (90.45)	36 (9.55)	1.0	ref.
-/-	558 (79.15)	147 (20.85)	2.5	1.7-3.9
-/+	403 (75.89)	128 (24.11)	3.3	2.2-5.2
+/-	17 (80.95)	4 (19.05)	2.6	0.7-10.3
+/+	540 (75.31)	177 (24.69)	3.4	2.2-5.1
-/+	961 (77.75)	275 (22.25)	2.9	1.9-4.3
+/+	557 (75.47)	181 (24.53)	3.3	2.2-5.0
Cit-C1/CCP				
Controls	341 (90.45)	36 (9.55)	1.0	ref.
-/-	518 (80.06)	129 (19.94)	2.4	1.5-3.7
-/+	455 (76.60)	139 (23.40)	3.0	2.0-4.6
+/-	57 (72.15)	22 (27.85)	4.1	2.1-8.1
+/+	488 (74.62)	166 (25.38)	3.6	2.4-5.5
-/+	973 (78.40)	268 (21.60)	2.7	1.8-4.0
+/+	545 (74.35)	188 (25.65)	3.6	2.4-5.5
Cit-fib₃₆₋₅₂/CCP				
Controls	341 (90.45)	36 (9.55)	1.0	ref.
-/-	552 (79.08)	146 (20.92)	2.5	1.7-3.9
-/+	553 (76.81)	167 (23.19)	3.0	2.0-4.6
+/-	23 (82.14)	5 (17.86)	3.4	1.0-11.0
+/+	390 (73.86)	138 (26.14)	3.7	2.4-5.6
-/+	1105 (77.93)	313 (22.07)	2.8	1.9-4.1
+/+	413 (74.28)	143 (25.72)	3.6	2.3-5.6
Anti-Carb-fib/CCP				
Controls	341 (90.45)	36 (9.55)	1.0	ref.
-/-	456 (79.86)	115 (20.14)	2.4	1.6-3.8
-/+	267 (73.96)	94 (26.04)	3.8	2.4-6.1
+/-	119 (76.77)	36 (23.23)	3.3	1.9-5.8
+/+	676 (76.21)	211 (23.79)	3.2	2.1-4.8
-/+	723 (77.58)	209 (22.42)	2.9	1.9-4.4
+/+	795 (76.30)	247 (23.70)	3.1	2.1-4.7
RF/CCP				
Controls	341 (90.45)	36 (9.55)	1.0	ref.
-/-	398 (78.81)	107 (21.19)	2.6	1.6-4.0
-/+	118 (74.68)	40 (25.32)	4.6	2.5-8.3
+/-	177 (80.09)	44 (19.91)	2.7	1.6-4.7
+/+	825 (75.69)	265 (24.31)	3.2	2.1-4.8
-/+	516 (77.83)	147 (22.17)	2.9	1.9-4.4
+/+	1002 (76.43)	309 (23.57)	3.1	2.1-4.6

*: Odds ratios (OR) were adjusted for age, gender and residential area. ACPA= anti-citrullinated peptide/protein antibodies; Carb-fib= carbamylated fibrinogen; CCP= cyclic citrullinated peptide; CI= confidence interval; IgG= immunoglobulin G; RA= rheumatoid arthritis; RF= rheumatoid factor; RgpB= arginine gingipainB. CCP status was not considered in the following subsets: -/+ and +/+