

## SUPPLEMENTAL MATERIAL

### **Oral carriage of *Streptococcus mutans* harboring the *cnm* gene relates to an increased incidence of cerebral microbleeds**

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## Supplemental Tables

**Supplemental Table I. Characteristics of subjects receiving oral bacterial examination**

	<u>All acute stroke subjects (n = 3782)</u>		p value
	Oral bacterial examination (+) (n = 404)	Oral bacterial examination (-) (n = 3378)	
Age, y	73.0 (64.0–81.0)	76.0 (67.0–83.0)	< 0.001
Male, n (%)	253 (63)	2033 (60)	0.343
Hypertension, n (%)	351 (87)	2897 (86)	0.541
SBP (mmHg)†	123 (114–134)	123 (112–133)	0.570
DBP (mmHg)†	70 (63–79)	70 (63–80)	0.691
Diabetes mellitus, n (%)	53 (13)	472 (14)	0.639
Dyslipidemia, n (%)	217 (54)	1740 (52)	0.402
Atrial fibrillation, n (%)	97 (24)	808 (24)	0.968
Smoking, n (%)	186 (46)	1540 (46)	0.864
NIHSS‡	3.0 (1.0–7.0)	4.0 (1.0–14.0)	< 0.001
mRS§	2.0 (1.0–4.0)	2.0 (1.0–4.0)	0.005

Data represent median (interquartile range) or number (percent). †Systolic blood pressure (SBP) and diastolic blood pressure (DBP) at discharge was described in 402 subjects receiving oral bacterial examination and 3244 without. Those who died in hospital were excluded. ‡National Institutes of Health Stroke Scale (NIHSS) was measured on admission. §Lower scores of modified Rankin Scale (mRS) at discharge were observed in subjects receiving oral bacterial examination.

**Supplemental Table II. Clinical profiles in the three groups**

	<i>cnm</i> -positive <i>S. mutans</i> (+) (n = 21)	<i>cnm</i> -negative <i>S. mutans</i> (+) (n = 69)	<i>S. mutans</i> (-) (n = 21)	p value
Age, y	73.0 (63.0–78.0)	69.0 (63.0–81.0)	75.0 (65.5–80.0)	0.531
Male, n (%)	14 (67)	42 (61)	12 (57)	0.822
Hypertension, n (%)	18 (86)	55 (80)	17 (81)	0.940
SBP (mmHg)	126 (116–134)	131 (118–150)	125 (110–144)	0.310
DBP (mmHg)	76 (64–85)	75 (65–85)	70 (62–87)	0.701
Diabetes mellitus, n (%)	3 (14)	17 (25)	5 (24)	0.654
Dyslipidemia, n (%)	13 (62)	40 (58)	12 (57)	0.963
Renal impairment, n (%)	10 (48)	30 (43)	11 (52)	0.798
Atrial fibrillation, n (%)	2 (10)	7 (10)	6 (29)	0.136
ATA use, n (%)	15 (71)	55 (80)	18 (86)	0.564
Antiplatelet agents, n (%)	12 (57)	43 (62)	11 (52)	0.735
Anticoagulants, n (%)	5 (24)	16 (23)	7 (33)	0.670
≥ 2 ATA use, n (%)	2 (10)	12 (17)	3 (14)	0.810
Recent IS, n (%)	7 (33)	25 (36)	6 (29)	0.883
Recent TIA, n (%)	1 (5)	9 (13)	1 (5)	0.475
Recent ICH, n (%)	1 (5)	6 (9)	1 (5)	1.000
Previous IS, n (%)	13 (62)	29 (42)	13 (62)	0.131
Previous TIA, n (%)	0 (0)	4 (6)	1 (5)	0.820
Previous ICH, n (%)	5 (24)	9 (13)	1 (5)	0.206
Smoking, n (%)	10 (48)	33 (48)	9 (43)	0.963
mRS	1.0 (0–3.5)	1.0 (0–3.0)	1.0 (0–1.5)	0.218
CRP (mg/dL)†	0.15 (0.04–0.79)	0.08 (0.04–0.26)	0.08 (0.03–0.18)	0.347
Fibrinogen (mg/dL)‡	344 (274–415)	305 (271–345)	359 (282–433)	0.135
CMB, n (%)	12 (57)	32 (46)	6 (29)	0.171
Lacunar infarcts, n (%)	13 (62)	21 (30)	7 (33)	0.033
PVH = 3, n (%)	8 (38)	8 (12)	5 (24)	0.022
DWMH ≥ 2, n (%)	16 (76)	43 (62)	6 (29)	0.004
Total SVD severity	3.0 (1.0–3.0)	1.0 (1.0–2.0)	1.0 (0–2.0)	0.008

Data represent median (interquartile range) or number (percent). †CRP data was missing in 1 subject with *cnm*-negative *S. mutans*. ‡Fibrinogen was obtained 18 subjects with *cnm*-positive *S. mutans*, 62 with *cnm*-negative *S. mutans*, 8 without *S. mutans*. ATA indicates anti-thrombotic agents; IS, ischemic stroke; TIA, transient ischemic attack; ICH, intracerebral hemorrhage; CRP, C-reactive protein; CMB, cerebral microbleeds; PVH, periventricular hyperintensities; DWMH, deep white matter hyperintensities; SVD, small vessel disease.

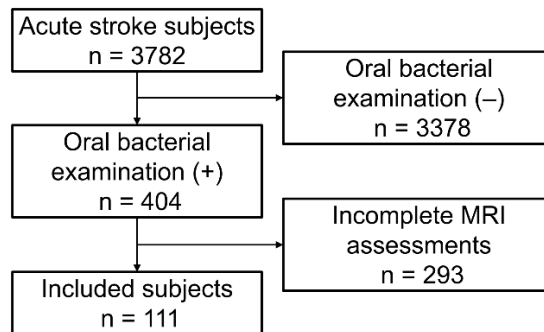
**Supplemental Table III. Incidence rate ratios of newly-developed CMB in the three groups**

	<u>Unadjusted</u>		<u>Adjusted*</u>	
	IRR (95% CI)	p value	IRR (95% CI)	p value
<i>cnm</i> -positive <i>S. mutans</i> (+)	4.1 (1.5–11.0)	0.005	3.9 (1.5–9.9)	0.004
<i>cnm</i> -negative <i>S. mutans</i> (+)	reference		reference	
<i>S. mutans</i> (–)	0.2 (0.0–3.4)	0.275	0.2 (0.0–3.2)	0.278

IRR of newly-developed CMB in any brain region are shown. Subjects with *cnm*-positive *S. mutans* and without *S. mutans* were compared to those with *cnm*-negative *S. mutans*. \* Adjusted for age, sex, hypertension and renal impairment. IRR indicates incidence rate ratios; 95% CI, 95% confidence interval.

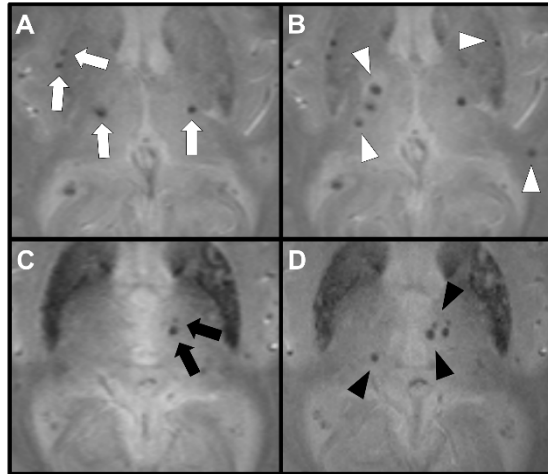
## Supplemental Figures

### Supplemental Figure I. Flow diagram of subject selection



Subjects who satisfied the following criteria were included in the study: (1) subjects who developed acute ischemic stroke, transient ischemic attack or intracerebral hemorrhage from February 15th 2014 to April 8th 2018, (2) subjects who signed an informed consent form for the current research, including receiving oral bacterial assessments from February 15th 2014 to April 30th 2018 and (3) subjects receiving 3 Tesla MRI scans twice with more than a 180-day interval from February 15th 2014 to February 15th 2019.

**Supplemental Figure II. Representative images showing an increase in deep CMB**



Head MRI gradient echo T2\*-weighted images of subjects with *cnm*-positive *S. mutans*. (A and B) 72-year-old woman. (C and D) 82-year-old woman. (A) Baseline MRI. Four CMB: two in the right putamen and two in the bilateral thalamus (white arrows). (B) Follow-up MRI at six months later than A. Four newly developed CMB: two in the right thalamus, one in the left temporal lobe and one in the left putamen (white arrowheads). (C) Baseline MRI. Two CMB in the left thalamus (black arrows). (D) Follow-up MRI at seventeen months later than C. Three newly developed CMB: one in the right thalamus and two in the left thalamus (black arrowheads).