Is oral Streptococcus mutans with collagen-binding protein a risk factor for intracranial aneurysm rupture or formation?

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Article

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Abstract

Objective: *Streptococcus mutans* (*SM*) with the collagen-binding protein Cnm is a unique member of the oral resident flora because it causes hemorrhagic vascular disorders. In the multicenter study, we examined the relationship between Cnm-positive *SM* (CP-*SM*) and intracranial aneurysm (IA) rupture, which remains unknown.

Methods: Between May 2013 and June 2018, we collected whole saliva samples from 431 patients with ruptured IAs (RIAs) and 470 patients with unruptured IAs (UIAs). Data were collected on age, sex, smoking and drinking habits, family history of subarachnoid hemorrhage, aneurysm size, number of teeth, and comorbidities of lifestyle disease.

Results: There was no difference in the positivity rate of patients with CP-*SM* between the patients with RIAs (17.2%) and those with UIAs (19.4%). The rate of positivity for CP-*SM* was significantly higher in all IAs <5 mm than in those ≥10 mm in diameter (P=0.0304). In the entire cohort, the rate of positivity for CP-*SM* was lower in larger aneurysms than in smaller aneurysms (P=0.0393).

Conclusions: The rate of positivity for CP-*SM* was lower among patients with large UIAs. These findings are consistent with the hypothesis that CP-*SM*plays a role in the formation of vulnerable IAs that tend to rupture before becoming larger.

Introduction

Streptococcus mutans (*SM*), which is found in the oral resident flora of > 70% of healthy individuals[1] and 90% of patients with active caries[2], is a well-known cariogenic bacterium that can also cause bacteremia through oral infection. Some *SM* strains that possess the collagen-binding protein Cnm[3] also are associated with hemorrhagic vascular disorders through the inhibition of platelet aggregation and the activation of matrix metalloprotease 9 (MMP-9)[4]. Importantly, Cnm-positive *SM* has been reported to cause cerebral hemorrhage[5] and deep cerebral microbleeds[6,7]. However, only one study reported an association between Cnm-positive *SM* and intracranial aneurysms (IAs)[8]. In a previous study, we compared the rate of positivity for Cnm-positive *SM* among patients with ruptured IAs (RIAs), unruptured IAs (UIAs), and healthy volunteers[8]. We found that patients with Cnm-positive *SM* showed a significantly higher positive rate in those with RIAs than in healthy volunteers. However, the positive rate of patients with Cnm-positive *SM* was not significantly different between those with UIAs and healthy volunteers.

The Unruptured Cerebral Aneurysm Study of Japan (UCAS Japan)[9] reported that the frequency of rupture increased with the increase in IA size. However, no study to date examined small RIAs, which account for 35–50% of all RIAs[9–14] Similar to that reported by the UCAS Japan, the annual rupture rate of small UIAs is low although the actual number of small IAs ruptures is significantly high[7]. These findings suggest that many small aneurysms are likely to bleed immediately after formation and that only aneurysms that grow without rupture are detected as UIAs, which have a low annual rupture rate of < 0.5%[15].

Widely accepted risk factors for subarachnoid hemorrhage due to RIAs include sex, smoking, alcohol, and hypertension. However, other currently unknown risk factors might be associated with aneurysm formation and rupture. The mechanisms underlying the formation, structural weakening, and rupture of aneurysms have been extensively studied[16–18]. However, few studies have examined the relationship between Cnm-positive *SM* and IAs. Therefore, we conducted a multicenter study to investigate the relationship between Cnm-positive *SM* and IAs. We hypothesized that Cnm-positive *SM* might be involved in RIAs, which we suspected to be higher in the RIAs than UIAs.

Methods

Study design and participants

This was a large-scale, multicenter retrospective observational study including patients with IAs who were admitted to one of the study hospitals between May 1, 2013 and June 30, 2018. The study was approved by the ethics committees of Seirei Hamamatsu General Hospital and all other participating institutions. The study was registered with the University Hospital Medical Information Network Clinical Trials Registry (registration no: UMIN000010404).

In the present study, UIA was defined as an unruptured saccular aneurysm, ≥ 3 mm in largest diameter, in the subarachnoid space. Even if one of UIAs ruptured, it was classified as an RIA The diagnosis of UIA and RIA was made by board-certified neurosurgeons or neuroradiologists. The largest aneurysm diameter was measured on images captured using ≥ 1.5-T magnetic resonance images without contrast media or computed tomography angiography with contrast media. The exclusion criteria were antibiotic use within 2 months before the collection of saliva specimens, IA due to bacterial endocarditis, and dissecting IAs. Patients without teeth were also excluded because *SM* can be cultured only from the oral cavity with at least one tooth[19,20].

Among a total of 992 patients who were admitted to one of the study institutions during the study period, 91 patients, including 59 patients without teeth, 34 patients with antibiotic usage within 2 months, 22 patients with extradural aneurysms, and 4 patients with dissecting aneurysms, were excluded. Therefore, the final study cohort included 901 patients with at least one IA, including 431 patients with RIAs and 470 patients with UIAs.

In the present study, data were collected on age, sex, smoking and drinking habits, family history of subarachnoid hemorrhage, number of teeth, and known risk factors, including hypertension, diabetes mellitus (DM), hyperlipidemia, and chronic kidney disease (CKD).

Definitions of known risk factors

In the present study, hypertension in inpatients was defined as (i) the use of antihypertensive medications before stroke or at discharge or (ii) systolic blood pressure (sBP) ≥ 140 mmHg or diastolic blood pressure (dBP) ≥ 90 mmHg at every measurement during the week before discharge. In outpatients, hypertension

was defined as (i) the use of antihypertensive medications, (ii) sBP ≥ 140 mmHg or dBP ≥ 90 mmHg at all visits within 3 months, or (iii) home-measured sBP ≥ 140 mmHg or dBP ≥ 90 mmHg at all measurements.

DM in inpatients was defined as the use of oral hypoglycemic agents or insulin before stroke or at discharge or a fasting blood glucose level ≥ 6.99 mmol/L (126 mg/dL) measured during the week before discharge. DM in outpatients was defined as the use of oral hypoglycemic agents or insulin or a fasting blood glucose level ≥ 6.99 mmol/L (126 mg/dL) measured in the last 3 months.

In all patients, hyperlipidemia was defined as fasting or nonfasting total plasma cholesterol ≥ 5.7 mmol/L (220 mg/dL) or low-density lipoprotein cholesterol ≥ 3.63 mmol/L (140 mg/dL) at admission.

CKD was defined as proteinuria during the 3 months before admission, decline in renal function defined as an estimated glomerular filtration rate < 60 mL/min/1.73 m^2 during the 3 months before admission, or self-reported CDK.

Smoking was defined as current smoking, and alcohol use was defined as consumption of > 150 gram of alcoholethanol per week.

Definition of Cnm-positive *SM*

To determine the presence of *SM* in patients, whole saliva samples were collected using a sterile cotton swab (Seed Swab no. 2; Eiken Chemical, Tokyo, Japan). Samples from patients with RIAs were collected immediately after admission and before the initiation of prophylactic antibiotics. In patients with UIAs, samples were also collected before the use of antibiotics. The collected samples were immediately frozen and cultured within 3 weeks of collection.

For *SM* cultures, the saliva samples were streaked onto mitis Salivarius Agar plates (Difco Laboratories, Detroit, MI, USA) containing bacitracin (0.2 U/mL; Sigma-Aldrich, St. Louis, MO, USA) and 15% (w/v) sucrose and were anaerobically cultured at 37°C for 48 h. After identifying the characteristic *SM*, colony morphology, five colonies were picked for each patient sample and further cultured in brain heart infusion broth (Difco Laboratories) at 37°C for 18 h[21].

The presence of *SM* was confirmed in all colonies using polymerase chain reaction with TaKaRa Ex *Taq* polymerase (Takara, Otsu, Japan) and the following primer pair: MKD-F, GGC ACC ACA ACA TTG GGA AGC TCA GTT; MKD-R, GGA ATG GCC GCT AAG TCA ACA GGA T[22]. Additionally, the detection of *cnm* was performed using polymerase chain reaction with TaKaRa Ex *Taq* polymerase and the following primer pair: Cnm-1F, GAC AAA GAA ATG AAA GAT GT; Cnm-1R, GCA AAG ACT CTT GTC CCT GC[21].

Study outcomes

In the present study, we evaluated four outcomes. First, we evaluated the risk factors for rupture in all IA cases. Second, we determined whether the positive rate of Cnm-positive *SM* was different between the

patients with RIAs and UIAs. Third, we determined whether the rate of Cnm-positive *SM* differed on the basis of IA size. To that end, we categorized IAs into those with diameters of < 5, ≥5 and < 10, and ≥ 10 mm. Finally, we compared the rate of Cnm-positive *SM* between the patients with UIAs and RIAs based on an IA size of < 10 mm versus ≥ 10 mm.

Statistical analysis

We used the χ^2 and the Cochran–Mantel–Haenszel tests to compare binary variables and the Mann–Whitney *U* test to compare continuous variables. Statistical significance was defined as a *P* value of < 0.05. Logistic regression analysis with corresponding odds ratios and 95% confidence intervals were used to adjust covariates. In our previous study including healthy volunteers[8], the rate of positivity for Cnm-positive *SM* was 43.5% in patients with RIAs and 14.9% in healthy volunteers. In the present study including patients with UIAs as controls, we assumed that the rate of positivity for Cnm-positive *SM* in patients would be twice of that for healthy volunteers[5], which would mean a difference of 10% using the χ^2 test with α and β error levels of 0.05 and 0.2, respectively[23]. The ratio of patients with RIAs to those with UIAs would be 1:1; therefore, the total sample size was calculated as 742 patients with a two-tailed test. Assuming a dropout rate of 20%, a target of 891 patients was set for the present study. All statistical analyses were performed using SAS University Edition (SAS Institute, Cary, NC, USA).

Trial Registry

University Hospital Medical Information Network Clinical Trials Registry (registration no: UMIN000010404; https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000012168)

Results

Table 1 summarizes the baseline characteristics of all patients included in the study. A total of 901 patients with IA were included in our study. Of them were 306 males (34.0%) with a mean age 65 years and averaging 6.17 mm of aneurysmal size. Briefly, significant risk factors for rupture were smoking history, alcohol use, and the presence of daughter sac in aneurysm. The positive rate of patients with Cnm-positive *SM* was 17.2% (n = 74) among those with RIAs and 19.4% (n = 91) among those with UIAs, with no statistically significant difference between the two groups (Table 1).

Table 1
Baseline characteristics of patients with ruptured and unruptured aneurysms

	Patients with RIA (n = 431)	Patients with UIA (n = 470)
Age, years, mean		
≤ 50	88 (20.4%)	47 (10%)
51–75	270 (62.6%)	339 (72.1%)
≥ 76	73 (16.9%)	84 (17.9%)
Sex, male	151 (35.0%)	155 (33.0%)
Fewer than 9 teeth	59 (13.7%)	44 (9.4%)
Past and current smoking	192 (44.5%)	218 (46.4%)
Alcohol use (> 150 g/week)	65 (15.1%)	44 (9.4%)
Family history of SAH	67 (15.5%)	75 (16.0%)
HT	236 (54.8%)	271 (57.7%)
DM	28 (6.5%)	37 (7.9%)
HL	121 (28.1%)	159 (33.8%)
CKD	12 (2.8%)	16 (3.4%)
Size of aneurysm		
< 5 mm	133 (30.9%)	228 (48.5%)
≥ 5 and < 10 mm	246 (57.1%)	209 (44.5%)
≥ 10 mm	52 (12.1%)	48 (10.2%)
Daughter sac present	257 (59.6%)	147 (31.3%)
Cnm-positive *SM* using PCR	74 (17.2%)	91 (19.4%)

Data are presented as numbers with percentages.

CKD, chronic kidney disease; DM, diabetes mellitus; HL, hyperlipidemia; HT, hypertension; PCR, polymerase chain reaction; RIA, ruptured intracranial aneurysm; SAH, subarachnoid hemorrhage; *SM*, *Streptococcus mutans*; UIA, unruptured intracranial aneurysm

In the entire cohort, positivity for Cnm-positive *SM* was inversely correlated with IA size, wherein the rate of Cnm-positive *SM* was lower in patients with larger aneurysms than in those with smaller aneurysms ($P = 0.039$). In multivariate analysis, the rate of positivity for Cnm-positive *SM* was higher in patients with RIAs or UIAs that were < 5 mm in diameter than in those with RIAs or UIAs that were ≥ 10 mm in diameter ($P = 0.030$) (Table 2).

Table 2
Multivariate analysis of aneurysmal subarachnoid hemorrhage based on Cnm-positive *Streptococcus mutans* carrier status

	Patients with Cnm-positive *SM* (n = 165)	Patients with Cnm-negative *SM* (n = 736)	Odds ratio	95%CI	*P* value
Age, years, mean					
≤ 50	20 (12.1%)	115 (15.6%)	1 (Ref)		
51–75	118 (71.5%)	491 (66.7%)	1.4323	0.8354–2.4555	0.1915
≥ 76	27 (16.4%)	130 (17.7%)	1.3672	0.6882–2.7161	0.3718
Male sex	58 (35.1%)	248 (33.7%)	1.1405	0.7347–1.7705	0.5579
Patients with fewer than 9 teeth	14 (8.5%)	89 (12.1%)	0.6495	0.3505–1.2037	0.1704
Ever or current smoking history	76 (46.1%)	334 (45.4%)	1.1168	0.7294–1.7098	0.6113
Alcohol use (> 150 g/week)	12 (7.3%)	97 (13.2%)	0.4584	0.2373–0.8853	0.0202*
Family history of SAH	29 (17.6%)	151 (20.5%)	0.6382	0.3071–1.3265	0.229
HT	90 (54.5%)	417 (56.7%)	0.9082	0.6301–1.3092	0.606
DM	17 (10.3%)	48 (6.5%)	1.7448	0.9526–3.1957	0.0714
HL	49 (29.7%)	231 (31.4%)	0.8659	0.5859–1.2796	0.4699
CKD	4 (2.4%)	24 (3.3%)	0.6673	0.2247–1.9819	0.4664
Size of aneurysm					

Data are presented as numbers with percentages.

* $P < 0.05$

CI, confidence interval; CKD, chronic kidney disease; DM, diabetes mellitus; HL, hyperlipidemia; HT, hypertension; SAH, subarachnoid hemorrhage; *SM*, *Streptococcus mutans*

Patients younger than 50 years of age and those with aneurysms smaller than 5 mm in diameter are used as reference groups

	Patients with Cnm-positive *SM* (n = 165)	Patients with Cnm-negative *SM* (n = 736)	Odds ratio	95%CI	*P* value
< 5 mm	74 (44.8%)	282 (38.3%)	1 (Ref)		
≥ 5 and < 10 mm	81 (49.1%)	369 (50.1%)	0.8542	0.5808–1.2562	0.4231
≥ 10 mm	10 (6.1%)	85 (11.5%)	0.4577	0.2256–0.9286	0.0304*
Daughter sac present	65 (39.4%)	398 (54.1%)	0.8145	0.5638–1.1768	0.2745
Data are presented as numbers with percentages.					
* *P* < 0.05					
CI, confidence interval; CKD, chronic kidney disease; DM, diabetes mellitus; HL, hyperlipidemia; HT, hypertension; SAH, subarachnoid hemorrhage; *SM*, *Streptococcus mutans*					
Patients younger than 50 years of age and those with aneurysms smaller than 5 mm in diameter are used as reference groups					

Only 4.7% of the patients with UIAs ≥ 10 mm in diameter were positive for Cnm-positive *SM*, and the rate of positivity for Cnm-positive *SM* was lower in these patients than in the patients in the other three groups in Fig. 1(*P* = 0.036).

Discussion

In the present study including a relatively large cohort of 901 patients with RIAs and UIAs, our analyses indicated that the rate of positivity for Cnm-positive *SM* was inversely correlated with the aneurysm size and that the positive rate of patients with Cnm-positive *SM* was significantly lower among those with large UIAs. However, we did not observe an association between the presence of Cnm-positive *SM* and aneurysm rupture.

UCAS Japan reported that the annual risk of rupture was 0.95% in patients with UIAs and that the annual rupture rate was low for small IAs[9]. Furthermore, other studies on UIAs reported that smaller IA size was associated with lower rupture rate[15]. However, the rupture of aneurysms smaller than 5 mm in diameter has been reported to account for 35–50% of all RIAs[10,24–27]. Albeit the low rupture rate, the actual number of small IAs that rupture is high; this discrepancy may lead to uncertainty regarding the choice of treatment strategies especially in patients with small IAs that are identified during routine evaluation.

In the present study, the positive rate of patients with Cnm-positive *SM* was low among those with UIAs larger than 10 mm. The findings of UCAS Japan and numerous other reports indicate that more severe inflammation is associated with a higher rate of rupture[9]. Therefore, our finding suggests that UIAs in patients with Cnm-positive *SM* may rupture before they become larger.

Oral commensal bacteria can easily cause bacteremia not only through surgical tooth extraction but also during routine oral care such as tooth brushing[28]. These bacteria include species leading to dental caries and periodontal disease. The causative bacteria for periodontal disease are well known for their role in exacerbating atherosclerosis and obstructive vascular disease[29]. In the other hand, *SM* is the most common caries-causing bacterium. *SM* is serologically classified into c, e, f, and k serotypes. In the oral cavity, c and e are the most common serotypes, accounting for > 90% of all *SM* serotypes[30,31]. In healthy subjects, *SM* with collagen-binding proteins accounts for 12.5% of all *SM* strains. Serotypes f and k possess the collagen-binding protein Cnm, which is present in < 5% of all oral *SM* strains[32]. Cnm-positive *SM*, which exhibits low binding capacity to enamel and dental plaques, possesses high binding capacity to denatured dentin containing collagen[33]. Additionally, Cnm-positive *SM* is not easily phagocytosed because of specific glucose side chains and can survive for a long period in peripheral circulation[34–36]. Thus, following its entry to the circulation, *SM* can easily bind to the collagen layer of the arterial wall with damaged endothelium.

Furthermore, Cnm-positive *SM* inhibits platelet aggregation and activates MMP-9, leading to bleeding. High wall shear stress activates NF-kappa B in vascular endothelial cells, leading to aneurysm formation at the apex of the vessel wall[37]. MMP-9 expression has been reported to be increased in studies of patients with IAs[38]. Conversely, the inhibition of NF-kappa B was shown to reduce the expression of a group of NF-kappa B-dependent inflammation-related genes, such as MMP-9, in IA[39], and the administration of an MMP-9 inhibitor was demonstrated to suppress IA formation in rats[40]. The clinical importance of Cnm-positive *SM* as a risk factor for intracranial hemorrhage and cerebral microbleeds has already been reported[5,7,41]. Although we did not directly demonstrated that Cnm-positive *SM* was a direct risk factor for IAs, our analyses in the present study revealed that the rate of positivity for Cnm-positive *SM* was inversely correlated with the IA size and that the rate of positivity for Cnm-positive *SM* was significantly lower in patients with large UIAs. MMP-9 is involved in the NF-kappa B pathway and has been shown to be involved in IA formation[40], supporting the hypothesis that Cnm-positive *SM* is involved in the formation of cerebral aneurysms that are prone to rupture before becoming larger. Future studies should utilize this finding to determine the indications for surgery in patients with small IAs. Additionally, the removal or neutralization of Cnm-positive *SM* might be considered a conservative therapeutic option for UIA.

Cnm-positive *SM* has already been reported to cause infective endocarditis[42], hemorrhagic stroke[5–7], IgA nephropathy[43], ulcerative colitis[44], and nonalcoholic steatohepatitis[45]. Therefore, the eradication of Cnm-positive *SM* can possibly prevent not only UIA rupture but also these clinical conditions.

In the present study, we could not obtain primary expected result that RIAs have the higher rate of Cnm-positive *SM* than UIAs. In future studies, we will directly compare patients with IAs with those without UIA or RIA. In a study investigating the relationship between IgA nephropathy and Cnm-positive *SM*, collagen binding ability was used to demonstrate the relationship between *SM* and IgA nephropathy[43], which we will consider in future studies examining the presence of Cnm-positive *SM* in patients with IAs.

In summary, despite the lack of a significant difference in the rate of positivity for Cnm-positive *SM* between the patients with RIAs and UIAs, the rate of positivity for Cnm-positive *SM* was lower in patients with larger IAs, including UIAs and RIAs. In particular, the rate of positivity for Cnm-positive *SM* was significantly lower in patients with UIAs larger than 10 mm than in those with UIAs smaller than 10 mm and in those with all RIAs regardless of the size.

Abbreviation

CKD, chronic kidney disease

dBP, diastolic blood pressure

DM, diabetes mellitus

IA, intracranial aneurysm

MMP-9, matrix metalloprotease 9

RIA, ruptured intracranial aneurysm

sBP, systolic blood pressure

SM, *Streptococcus mutans*

UCAS Japan, Unruptured Cerebral Aneurysm Study of Japan

UIA, unruptured intracranial aneurysm

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Figures

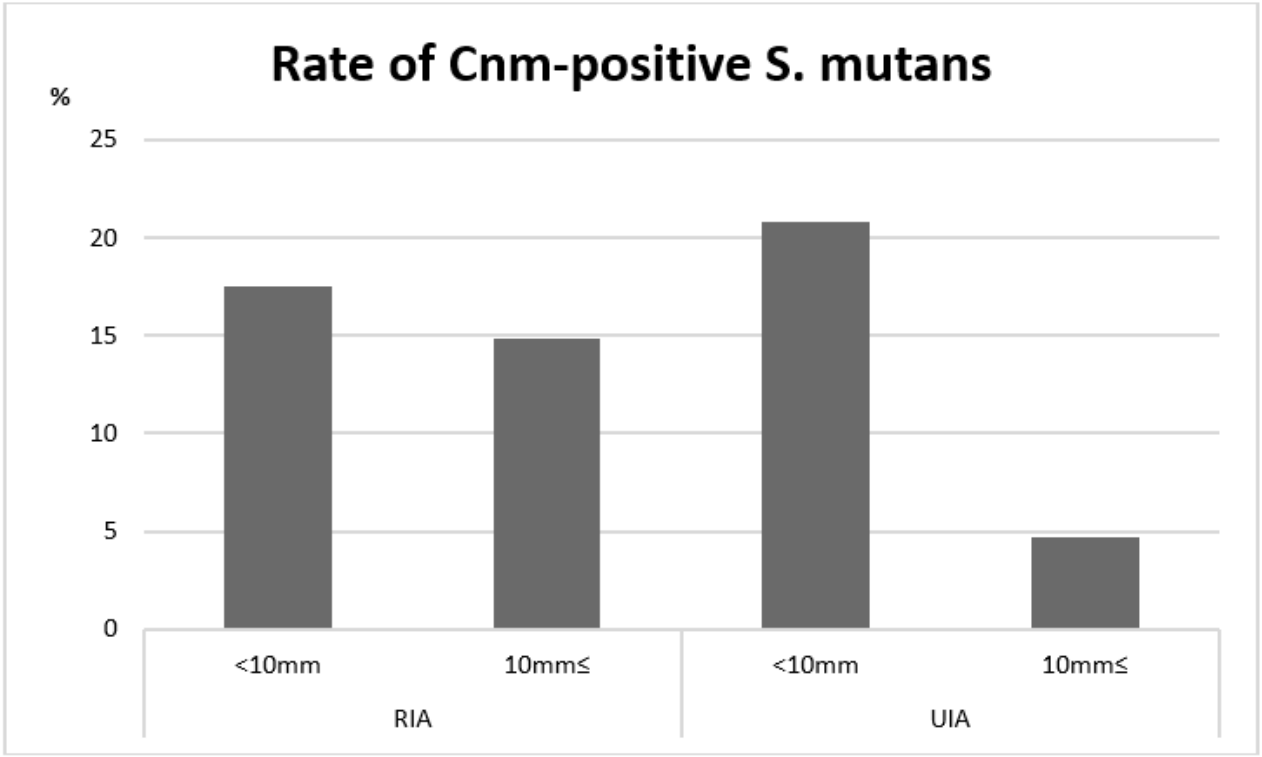

Figure 1

Rate of positivity for Cnm-positive *Streptococcus mutans* among patients with ruptured and unruptured intracranial aneurysms categorized according to size

RIA, ruptured intracranial aneurysm; UIA, unruptured intracranial aneurysm

* $P = 0.036$