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Common and distinct risk profiles of asymptomatic extra- and intracranial atherosclerosis in the Nagahama cohort

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ABSTRACT

Background and purpose: Atherosclerotic burden increases the risk of both extracranial internal carotid artery stenosis (ICS) and intracranial large artery disease (ICAD). However, the differences in risk profiles have not been thoroughly investigated.

Methods: Participants were recruited from the Nagahama study cohort in Japan. Individuals over 60 years old who underwent 1.5-T head and neck magnetic resonance angiography (MRA) between July 2013 and February 2017 were included. ICAD was defined as WASID $\geq$ 50 %, and ICS was defined as NSCET $\geq$ 30 %. The prevalence and association of risk factors, including proatherogenic and proinflammatory factors, and the p.R4810K variant in the *RNF213* gene, were investigated. Multivariable logistic regression analyses were performed.

Results: A total of 3089 individuals participated in the study, with a mean age of 68.1 $\pm$ 5.3 years, and 36.0 % were males. Among them, 52 (1.7 %) had ICS, 119 (3.8 %) had ICAD, and 15 (0.49 %) had both conditions. Alopecia areata was an independent predictor for both ICS (Odds ratio [OR] 3.5; 95 % CI 1.3-8.3) and ICAD (OR 2.1; 95 % CI 1.0-3.9). Diabetes (OR 3.7; 95 % CI 2.0-7.0) and older age (OR 2.4; 95 % CI 1.2-4.5) were associated only with ICS, while the *RNF213* variant was associated with only ICAD (OR 5.7; 95 % CI 1.6-16.0). ICS and ICAD were also independently associated with each other.

Conclusions: In this MRA-based large scale study, alopecia areata, known as a systemic inflammatory disease, was shown to be a common risk factor for ICS and ICAD. While conventional atherosclerotic factors were associated with ICS, non-atherosclerotic factors appear to contribute to ICAD in Japan.

Introduction

Internal carotid artery stenosis (ICS) and intracranial artery disease (ICAD) are both arterial stenotic diseases that directly contribute to ischemic stroke. Considerable interest has been devoted to identifying the risk factors for ICS and ICAD in recent decades with investigations primarily focusing on symptomatic or hospitalized subjects.[1–4] While previous narrative reviews have suggested a higher association of dyslipidemia with ICS and diabetes and hypertension with ICAD,[5] similarities and differences in risk factor between both these conditions still have been discussed until today. The recent case-control study with symptomatic ICS and ICAD patients reported that metabolic syndrome were significantly associated with ICAD, but not with ICS.[6] A large-scale multicenter study compared the risk factors between symptomatic ICS and ICAD, suggesting aging and smoking more related to ICS, while hypertension to ICAD.[7] One-hospital-based retrospective study analyzed 1129 symptomatic or asymptomatic patients and suggested the correlation between elevated triglyceride-glucose and ICAD or combined

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ICAD/ICS.[8] Moreover, studies investigating both conditions simultaneously in the general population are limited. López et al.[9] examined 933 white subjects >50 years with a moderate to high vascular risks by ultrasonography screening followed by magnetic resonance angiography (MRA), and they found the association of hypertension with ICS and further suggested a stronger relationship between diabetes and ICAD compared to ICS. Jin et al.[10] examined 2589 asymptomatic Chinese subjects >30 years by ultrasonography in 2017, and they showed the age and diabetes were common risk factors for ICS and ICAD. However, there have been no large scale MRA-based study that directly compared the risks of ICS and ICAD in the general population irrespective of vascular risks.

Chronic inflammation has recently gained attention as a potential factor in arterial stenotic diseases. Systemic inflammatory diseases, where underling chronic inflammation affects various organs, have been reported to be linked to ischemic stroke[11] and coronary artery diseases.[12] However, evidence on the associations of systemic inflammatory disease with ICS and ICAD is still limited. Specifically, alopecia areata is a systemic inflammatory disease with higher risk of atherosclerosis and cardiovascular diseases including stroke,[13,14] but the association with ICS or ICAD remains unknown. Other systemic inflammatory diseases such as gout, rheumatoid arthritis, asthma, atopic dermatitis, dermatophytosis, psoriasis have also not been fully investigated.

The p.R4810K variant in the Ring finger protein 213 (*RNF213*) gene, initially identified as a susceptibility variant for moyamoya disease,[15] has also been associated with ICAD in East Asian countries.[16,17] Over the past decade, this *RNF213* variant has been found to be an inflammatory factor which is associated with other extracranial arterial stenoses or diseases such as ischemic stroke,[18] hypertension,[19] pulmonary artery hypertension,[20] and coronary artery disease[21,22] in East Asian populations. However, only a few studies have investigated the associations between this variant and ICS. Although they yielded negative results, it is inconclusive due to their small sample size.[16,17,23]

This study aims to determine the prevalence of asymptomatic ICS and ICAD and compare their risk profiles. To achieve these objectives, we assessed both conditions in a large sample of 3089 stroke-free general residents in Nagahama city using MRA examinations. Additionally, we investigated potential risk factors, including systemic inflammatory diseases and the *RNF213* variant, along with conventional atherosclerotic risk factors.

Methods

Study population

We conducted the cross-sectional analysis using the information from the second survey of Nagahama Prospective Genome Cohort for Comprehensive Human Bioscience (The Nagahama Study)[24] between July 2013 and February 2017. The Nagahama Study cohort was recruited from the general population living in Nagahama City, a largely rural city of 125,000 inhabitants in Shiga Prefecture, located in the center of Japan. Among 9,850 individuals aged 30 or above who joined the second survey, we selected 3,184 individuals older than 60 years at the date of the health check visit and performed the MRI/MRA-based brain and cerebrovascular examination. The principal component analysis using genome-scan results confirmed that all of them fell into the Japanese cluster.[25] To assess the prevalence and the risk factors for asymptomatic ICS and ICAD, we selected individuals who do not have a history of stroke and excluded individuals who lacks information of MRA or genotyping data. Therefore, as shown in Fig. 1, exclusions were made for individuals with incomplete questionnaire information, clinical measurement data, or insufficient quality MRI/MRA images, diagnosed with or suspected of having Moyamoya disease, those who with complete ICA occlusions, those with suspected Moyamoya disease by the brain MRI image examination, those with a history of stroke, and those with no genotyping result of *RNF213* p.4810K. The study protocol was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (Registry ID G0278) and the Nagahama Municipal Review Board. Written informed consent was obtained from all the participants.

Assessment of risk factors for atherosclerosis

Various atherosclerotic risk factors were assessed, including hypertension (use of antihypertensive drugs or systolic brachial blood

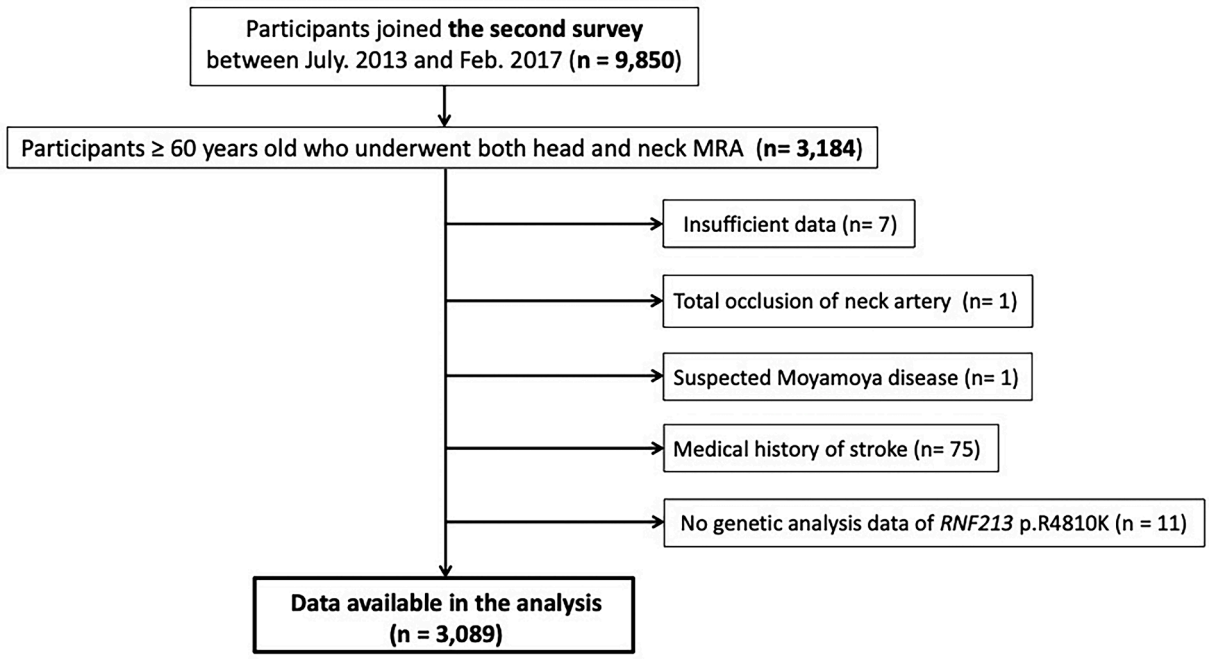

Fig. 1. Sample selection.
The chart shows the sample selection in the present study.

pressure ≥ 140/90 mmHg), dyslipidemia (use of lipid-lowering drugs, plasma total cholesterol level ≥ 200 mg/dL, plasma HDL cholesterol ≤ 40 mg/dL, or plasma LDL cholesterol ≥ 130 mg/dL), diabetes mellitus (use of oral hypoglycemic agents, insulin injections, plasma fasting glucose ≥ 126 mg/dL, or HbA1c (NGSP) ≥ 6.5 %), obesity (body mass index ≥ 30 kg/m^2), asymptomatic peripheral arterial disease (PAD; ankle/brachial index < 0.9), chronic kidney disease (glomerular filtration rate <60 ml/min/1.73m^2). All participants underwent a structured questionnaire survey to assess risk factors as follows; smoking status (current or past smokers), habitual drinking, physical activity (moderate exercise for >30 minutes at least 2 times a week last more than one year), history of stroke (ischemic or hemorrhagic stroke diagnosed by neurologists or neurosurgeons at hospitals), and ischemic heart disease, and family history of stroke and ischemic heart disease.

Inflammatory biomarkers, including C-reactive protein (CRP) and the neutrophil lymphocyte ratio (NLR; calculated as the neutrophil count divided by the lymphocyte count),[26] were also measured. Systemic inflammatory diseases, such as gout, rheumatoid arthritis, pediatric asthma, allergic rhinitis, atopic dermatitis, tinea pedis, alopecia areata, psoriasis, chronic sinusitis, aspiration pneumonia, and tuberculosis, were assessed based on self-reports of physicians' diagnosis. Periodontal disease was also assessed through dental examinations by trained dental hygienists and was defined based on the Community Periodontal Index.[27] We selected variables related to infection and inflammation from those collected in the Nagahama study. We did not adopt the criterion of whether they had been previously reported to be associated with stroke. Although there are infectious diseases, such as HIV infection, that have been reported to be associated with stroke, we did not conduct additional inquiries about variables that were not asked at baseline. Therefore, not all items related to stroke or vascular stenosis are comprehensively covered. To minimize the selection bias, we also included other past medical history including chronic kidney disease, chronic obstructive pulmonary disease, liver disease, anemia, osteoporosis, epilepsy, and malignancy, were included in the univariate analysis.

Statistical analyses

The participants were grouped in 4 age categories in 5-year increments, and the trend of the prevalence of ICS or ICAD across different age categories was assessed using the Cochran-Armitage test. Univariate analysis was done by chi-square test or Fisher's exact test as appropriate to find a possible risk factors for ICS and ICAD. Multivariate logistic regression analyses were conducted to identify predictors of the presence of ICS or ICAD, with the non-ICS or non-ICAD group serving as the reference. The risk factors were all the categorical variables and presented as a number of cases and percentages. Only variables that meet $p < 0.05$ in univariate analysis are included in the multivariate analysis. For ICS, multivariable regression analysis was done by adjusting for sex, the age group (≥ 75 vs < 75), smoker (never vs former or current), hypertension (yes vs no), dyslipidemia (yes vs no), diabetes (yes vs no), peripheral artery disease (yes vs no), ischemic heart disease (yes vs no), gout (yes vs no), atopic dermatitis (yes vs no), allergic rhinitis (yes vs no), alopecia areata (yes vs no), ICAD (yes vs no), and NLR (≥ 3 vs < 3) were included, while sex, the older age group, alcohol drinking (never vs habitual), hypertension, dyslipidemia, diabetes, peripheral artery disease, gout, alopecia areata, ICS (yes vs no), *RNF213* variant (yes vs no), and CRP (≥ 5 vs < 5) were included for ICAD. With regard to specific covariates, alopecia areata and *RNF213* variant, we conducted further analyses as follows. Because the prevalence of alopecia areata is known to differ between male and female, the significance of multiplicative interactions was evaluated by adding a corresponding interaction term i.e., sex*alopecia, in each multivariate regression model for ICS or ICAD. Additionally, we conducted the sex-stratified subgroup analysis on alopecia related to ICS or ICAD. Lastly, to analyze the factors confounded by the *RNF213* mutation, we examined whether there were differences in each explanatory variable based on the presence or absence of the mutation. All *p*-values were two-sided, and a significance level of $p < 0.05$ was considered statistically significant. The calculations were performed using R version 4.2.2.

The diagnostic criteria for ICS and ICAD and the method for *RNF213* genotyping are described in supplemental file 1.

Results

Prevalence and distribution of ICS and ICAD

Out of the initial total of 3,184 participants, the following 95 individuals were excluded from the analysis (Fig. 1). Among the 3089 eligible participants, 52 patients were diagnosed with ICS (1.7 %) and 119 patients were diagnosed with ICAD (3.8 %) (Table 1). Both ICS and ICAD were significantly more common in men compared to women (3.2 % in men vs. 0.76 % in women, chi-square test $p < 0.01$ for ICS; 5.4 % in men vs. 3.0 % in women, $p < 0.01$ for ICAD). The prevalence of both of ICS and ICAD were higher in older individuals (≥75 years) compared to younger individuals (40.4 % vs 13.6 %, $p < 0.01$ for ICS; 21.8z % vs 13.8 %, $p = 0.02$ for ICAD), showing an increase with advanced age (p for Cochran-Armitage trend test < 0.001 for ICS and p for trend = 0.002 for ICAD). When stratified by sex, this trend persisted for ICS in both males (p for trend < 0.001) and females (p for trend = 0.007), while such trend was not observed in male patients with ICAD and less significant in female patients with ICAD (p for trend = 0.039) (Fig. 2). Furthermore, the prevalence of ICAD was consistently higher than that of ICS across all age groups and for both sexes. Among the 119 ICAD patients, there were a total of 128 affected vessels, and ICAD was significantly more frequently located in the anterior circulation compared to the posterior circulation (89.9 % vs 10.2 %) (Supplemental file 2).

Univariate analysis

As shown in Table 1, we estimated an association of 34 variables with ICS and ICAD, including 13 inflammatory diseases and one serum marker of NLR, and a genomic variant in *RNF213*. Inflammatory diseases except periodontal disease were assessed through a structured questionnaire. The proportions of elderly individuals and males were higher in both ICS and ICAD groups (Table 1). The only risk factor that showed significant association with both ICS and ICAD was alopecia areata, with an odds ratio (OR) of 3.2 (95 % confidence interval [CI], 1.2-7.5; $p = 0.011$) for ICS and 2.1 (95 %CI, 1.0-3.9; $p = 0.029$) for ICAD after adjusted for covariates (Table 2). The additional multivariable models to test if the effect of alopecia deferred between sexes did not show significant interactions between sex and the presence of alopecia in relation to ICS or ICAD (p for interaction = 0.58 for ICS and 0.57 for ICAD). However, when further stratified by sex, a significant association was observed in female patients with ICS ($p = 0.043$) (Supplemental file 3). We further tested the association of inflammatory biomarkers, C-reactive protein (CRP) and the neutrophil lymphocyte ratio (NLR; calculated as the neutrophil count divided by the lymphocyte count),[26] with ICS and ICAD. Although NLR was positively associated with ICS ($p = 0.010$) and CRP was associated with ICAD ($p = 0.012$) in univariate analyses (Table 1), no association was confirmed after adjusting for covariates (Table 2).

Multivariable analysis

As shown in Table 2, multivariable analyses showed that age (OR 2.4; 95 % CI 1.2-4.5), diabetes (OR 3.7; 95 % CI 2.0-7.0) and atopic dermatitis (OR 4.6; 95 % CI 1.6-11.7) were independently associated with ICS, but not with ICAD. Concomitant PAD (OR 6.7; 95 % CI 2.1-18.1) and hypertension (OR 1.8; 95 % CI 1.2-2.8) were independent risk factors for ICAD, but not for ICS. The *RNF213* variant was independently associated with ICAD (OR 5.7; 95 % CI 1.6-16.0), while

Table 1
Characteristics of the study participants (n = 3,089).

	Total	ICS			ICAD		
		Present	Absent	*P* value	Present	Absent	*P* value
Number	3089	52	3037	-	119	2970	-
Male sex	1113 (36.0)	37 (71.2)	1076 (35.4)	**< 0.01**	60 (50 .4)	1053 (35.5)	**< 0.01**
Age ≥ 75 years	435 (14.1)	21 (40.4)	414 (13.6)	**< 0.01**	26 (21.8)	409 (13.8)	**0.019**
Smoker	908 (29.4)	31 (59.6)	877 (28.9)	**< 0.01**	45 (37.8)	863 (29.1)	0.051
Alcohol drinker	1537 (49.8)	31 (59.6)	1506 (49.6)	0.20	71 (59.7)	1466 (49.4)	**0.035**
Obesity	54 (1.8)	1 (1.9)	53 (1.7)	0.60	0 (0)	54 (1.8)	0.27
Hypertension	1657 (53.6)	40 (76.9)	1617 (53.2)	**< 0.01**	86 (72.3)	1571 (52.9)	**< 0.01**
Dyslipidemia	1743 (56.4)	39 (75.0)	1704 (56.2)	**< 0.01**	79 (66.4)	1664 (56.0)	**0.032**
Anemia	589 (19.1)	4 (7.7)	585 (19.3)	0.05	14 (11.8)	575 (19.4)	0.05
Diabetes	318 (10.3)	20 (38.5)	298 (9.8)	**< 0.01**	21 (17.6)	297 (10.0)	**0.011**
Peripheral arterial disease	23 (0.7)	3 (5.8)	20 (0.66)	**< 0.01**	6 (5.0)	17 (0.6)	**< 0.01**
Ischemic heart disease	131 (4.2)	9 (17.3)	122 (4.0)	**< 0.01**	8 (6.7)	123 (4.1)	0.26
Chronic kidney disease	16 (0.5)	0	16 (0.5)	1	0	16 (0.5)	1
Chronic obstructive pulmonary disease	49 (1.6)	0	49 (1.6)	1	4 (3.4)	45 (1.5)	0.12
Liver disease	106 (3.5)	0	106 (3.5)	0.26	4 (3.4)	102 (3.4)	1
Osteoporosis	497 (16.1)	5 (9.6)	492 (16.2)	0.28	21 (17.7)	476 (16.0)	0.73
Chronic sinusitis	312 (10.1)	9 (17.3)	303 (10.0)	0.13	14 11.8)	298 (10.0)	0.65
Aspiration pneumonia	12 (0.4)	0	12 (0.4)	1	1 (0.8)	11 (0.4)	0.38
Tuberculosis	35 (1.1)	0	35 (1.2)	1	0	35 (1.2)	0.64
Epilepsy	8 (0.3)	0	8 (0.3)	1	1 (0.8)	7 (0.2)	0.27
Malignancy	288 (9.3)	3 (5.8)	285 (9.4)	0.47	9 (7.6)	279 (9.4)	0.61
Gout	128 (4.1)	7 (13.5)	121 (4.0)	**< 0.01**	11 (9.2)	117 (3.9)	**0.015**
Rheumatoid	71 (2.3)	1 (1.9)	70 (2.3)	1	4 (3.4)	67 (2.3)	0.35
Pediatric asthma	150 (4.9)	1 (1.9)	149 (4.9)	0.52	8 (6.7)	142 (4.8)	0.45
Atopic dermatitis	122 (3.9)	6 (11.5)	116 (3.8)	**0.015**	5 (4.2)	117 (3.9)	0.81
Allergic rhinitis	914 (29.6)	8 (15.4)	906 (29.8)	**0.035**	35 (29.4)	879 (29.6)	1
Alopecia areata	150 (4.9)	7 (13.5)	143 (4.7)	**0.012**	12 (10.1)	138 (4.6)	**0.013**
Psoriasis	166 (5.4)	3 (5.8)	163 (5.4)	0.76	8 (6.7)	158 (5.3)	0.65
Periodontal disease	1298 (42.0)	24(46.2)	1274 (41.9)	0.64	51 (42.9)	1247 (42.0)	0.93
Tinea pedis	1684 (54.5)	31 (59.6)	1653 (54.4)	0.55	71 (59.7)	1613 (54.3)	0.29
ICAD	119 (3.9)	15 (28.8)	104 (3.4)	**< 0.01**	-	-	-
ICS	55 (1.8)	-	-	-	15 (12.6)	37 (1.2)	**< 0.01**
RNF213 variant	26 (0.8)	1 (1.9)	25 (0.8)	0.36	4 (3.4)	22 (0.7)	**0.016**
NLR ≥ 3	222 (7.2)	9 (17.3)	213 (7.0)	**0.010**	11 (9.2)	211 (7.1)	0.48
CRP ≥ 5	1328 (43.0)	29 (55.8)	1299 (42.8)	0.083	65 (54.4)	1263 (42.5)	**0.012**

ICS, internal carotid artery stenosis; ICAD, intracranial artery disease; NLR, neutrophil lymphocyte ratio; CRP, C-reactive protein.

significant associations were not observed with ICS. When comparing the risk factors between *RNF213* variant carriers and non-carriers, participants with the variant had a lower prevalence of diabetes (3.8 % in the carrier group vs 10.3 % in the non-carrier group), although this difference was not statistically significant (Table 3). Regarding the factors previously reported to be inversely associated with *RNF213* variant,[18,21,22,28] our multivariable analysis showed neither older age, male sex, nor diabetes were independently associated with ICAD (Table 2).

Discussion

We analyzed the prospectively collected large data of asymptomatic Japanese residents and found the prevalence of 1.8 % for ICS and 3.9 % for ICAD. The prevalence of ICS in our study was slightly lower compared to previous reports ranging from 2.2 % to 8.0 %[9,10,29–31]. The lower prevalence observed in our study would mainly attributable to the characteristics of the study population. Most of the previous studies targeted individuals who had high vascular risk burdens including history of stroke. In fact, the prevalence in our study (1.8 %) was close to that of the community-based Chinese study (2.2 %)[10] which did not account for the presence of vascular risks, while the prevalence of another community-based study with vascular burdens was 3.1 %.[9] The different examination modality and threshold of ICS might also affect the prevalence. Previous studies used ultrasonography to detect ICS and threshold was varies among studies (35 %, 50 % stenosis, or any degree of stenosis), while we used MRA with a threshold of ≥30 %. Another reason could be the higher proportion of females (64.5 %) in our population, where the prevalence is lower in women than men. The prevalence of asymptomatic ICAD was 3.9 %, which was consistent with previous large community-based studies using MRA among stroke-free general residents (1.5- 7.6 %).[32–34]

Alopecia areata is an autoimmune disease where hair follicles are attacked by cytotoxic T cells. It is known to be associated with conditions like atopic dermatitis and thyroid diseases.[35] Beyond scalp samples, serum biomarkers and vascular/atherosclerosis-related biomarkers have been reported to significantly increase in patients with alopecia areata.[36] In such patients, the odds ratio for cardiovascular diseases such as hypertension and arterial disease are higher,[13] with a reported 1.6-fold increased risk of stroke.[14] However, conflicting reports exist, with some studies suggesting no association or even an inverse correlation.[37,38] Importantly, it has been noted that the risk decreases in the short term but continues to rise in the long term,[39] offering an explanation for these contradictory results. After adjusting for cardiovascular risk factors, the risk of acute myocardial infarction was initially lower in patients with alopecia areata compared to controls (adjusted hazard ratio of 0.17 [95 % CI, 0.12-0.25] between 0-2 years). However, by 8 years post-diagnosis, the risk increased in those with alopecia areata (adjusted hazard ratio of 1.37 [95 % CI, 1.11-1.70] between 8-10 years), with a further increase thereafter (adjusted HR, 4.51 [95 % CI, 3.65-5.58] between 10-12 years). Considering the increased incidence of ICS and ICAD with age, it is estimated that alopecia areata elevates the risk of cerebrovascular disorders in the long term. However, there are no large-scale reports on asymptomatic carotid artery stenosis or intracranial arterial stenosis, and our data suggested that alopecia areata is an independent risk factor for both ICS and ICAD. Together with the association of atopic dermatitis with ICS and *RNF213* mutation with ICAD, association of alopecia areata suggest that inflammation plays a crucial role in the progression of cervical and intracranial vascular stenosis.

Alopecia areata is recognized as a systemic inflammatory disease[36].

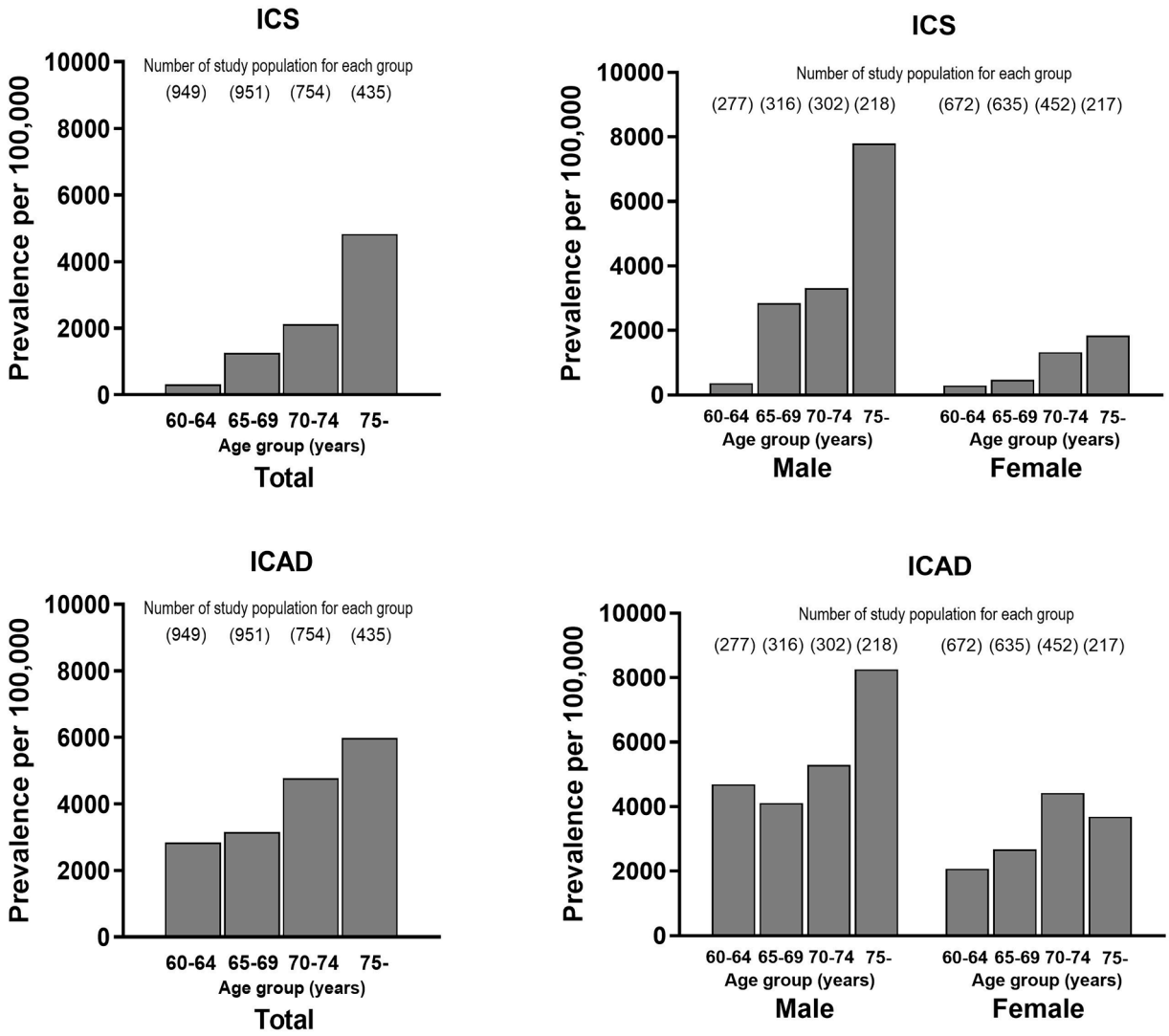

Fig. 2. Age- and sex-stratified prevalence of ICS and ICAD.
The prevalence of stenosis increased with advanced age both in male and female group for ICS, while such linear trend was not observed in ICAD. The prevalence of ICAD was constantly higher than that of ICS in all age groups for both sexes. Each number in the bracket indicates the number of participants in each age group for both sexes. ICS represents internal carotid artery stenosis; ICAD, intracranial artery disease.

Similarly, atopic dermatitis, another systemic inflammatory disease, showed an association with ICS in our study. Atopic dermatitis is known to increase the risk of ischemic stroke,[11,12] myocardial infarction, and the presence of coronary calcifications.[12] Our study suggests that systemic inflammatory diseases may serve as common risk factors for both ICS and ICAD, contributing to the development of atherosclerosis. We did not show significant associations between inflammatory biomarkers, such as CRP or NLR,[26] and either ICS or ICAD in the multivariable analysis, which may be because our cohort was consisted of asymptomatic general participants,[41–43] which might underestimate the association. Further investigations are required to test whether other inflammatory biomarkers, such as high-sensitivity CRP, can properly estimate the risk of ICS and ICAD.

We found that *RNF213* variant was independently associated with ICAD, but not with ICS. Risk allele frequency in our whole cohort (0.84 %) falls within the range previously reported among selected general populations (0.4-2.7 %).[18,44] *RNF213* is a known genetic factor of moyamoya disease that shows specific pathological changes in stenotic lesions, which is distinct from atherosclerotic changes.[45] Also clinically, individuals carrying *RNF213* variants have been found to exhibit certain characteristics different from those belonging to arterial atherosclerosis; higher prevalence among females,[18] younger onset of ICAD,[18,28] and lower prevalence of concomitant diabetes.[21,22] In accordance with these characteristics, the variant carriers had a low prevalence of diabetes, compared to non-carriers albeit statistically not significant. Likewise, age was not associated with ICAD, while it was significantly associated with ICS. These findings suggest that non-atherosclerotic mechanisms may be involved in the pathogenesis of ICAD. The reasons behind the differential effects of the *RNF213* variant on ICAD and ICS remain unclear, highlighting the need for further investigation to elucidate the mechanisms by which *RNF213* variants increase the risk of ICAD.

The study has several limitations. First, the population consisted only of participants aged 60 years and older, with a significantly higher proportion of females (64.0 %) compared to males. Secondly, the participants in this study were volunteers who joined the health promotion project of the Nagahama Cohort Study, rather than being randomly selected from the general population. Although the sample size was large and the data were prospectively collected, these may introduce selection biases and limit the representativeness of the findings. Third, past medical history relies on self-reports of physician diagnoses, which may be limited in accuracy due to recall bias. Fourth, inflammatory diseases that have been reported to be associated with vascular stenosis

Table 2
Multivariate analysis for factors associated with ICS or ICAD.

	ICS		ICAD	
	OR (95 %CI)	*P* value	OR (95 %CI)	*P* value
Male sex	1.8 (0.7-4.4)	0.19	1.3 (0.8-2.0)	0.33
Age ≥ 75 years	2.4 (1.2-4.5)	**< 0.01**	1.1 (0.7-1.8)	0.62
Smoker	1.6 (0.7-3.9)	0.24	-	-
Alcohol drinker	-	-	1.3 (0.9-2.1)	0.20
Hypertension	1.4 (0.7-3.0)	0.33	1.8 (1.2-2.8)	**< 0.01**
Dyslipidemia	1.3 (0.6-2.0)	0.43	1.5 (1.0-2.2)	0.069
Diabetes	3.7 (2.0-7.0)	**< 0.01**	1.2 (0.7-2.0)	0.52
Peripheral artery disease	1.7 (0.3-7.8)	0.50	6.7 (2.1-18.1)	**< 0.01**
Ischemic heart disease	2.8 (1.2-6.3)	0.15	-	-
Gout	1.4 (0.5-3.4)	0.45	1.4 (0.6-2.7)	0.38
Atopic dermatitis	4.6 (1.6-11.7)	**< 0.01**	-	-
Allergic rhinitis	0.5 (0.2-1.1)	0.08	-	-
Alopecia areata	3.5 (1.3-8.3)	**< 0.01**	2.1 (1.0-3.9)	**0.029**
ICAD	7.4 (3.5-14.9)	**< 0.01**	-	-
ICS	-	-	6.5 (3.1-12.7)	**< 0.01**
RNF213 variant	-	-	5.7 (1.6-16.0)	**< 0.01**
NLR ≥ 3	1.8 (0.7-3.9)	0.18	-	-
CRP ≥ 5	-	-	1.4 (0.9-2.1)	0.091

Only variables that meet $p < 0.05$ in univariate analysis are included in the multivariate analysis; sex, age, smoker, alcohol hypertension, dyslipidemia, diabetes, peripheral artery disease, ischemic heart disease, gout, allergic rhinitis, atopic dermatitis, alopecia areata, ICAD, and NLR for ICS; sex, age, alcohol drinking, hypertension, dyslipidemia, diabetes, peripheral artery disease, gout, alopecia, ICS, *RNF213* variant, and CRP for ICAD. ICS, internal carotid artery stenosis; ICAD, intracranial artery disease; NLR, neutrophil lymphocyte ratio; CRP, C-reactive protein.

Table 3
Basic characteristics of *RNF213* variant carriers and non-carriers.

	Total	*RNF213* variant		*P* value
		Carrier	Non-carrier	
Number	3089	26	3063	-
Male sex	1113 (36.0)	5 (19.2)	1108 (36.2)	0.11
Age ≥ 75 years	435 (14.1)	5 (19.2)	430 (14.0)	0.40
Smoker	908 (29.4)	4 (15.4)	904 (29.5)	0.17
Alcohol drinker	1537 (49.8)	10 (38.5)	1527 (50.0)	0.35
Obesity	54 (1.7)	1 (3.8)	53 (1.7)	0.37
Hypertension	1657 (53.6)	16 (61.5)	1641 (53.6)	0.54
Dyslipidemia	1743 (56.4)	14 (53.8)	1729 (56.4)	0.95
Diabetes	318 (10.3)	1 (3.8)	317 (10.3)	0.51
Metabolic syndrome	246 (8.0)	1 (3.8)	245(8.0)	0.71
Peripheral artery disease	23 (0.7)	0	23 (0.7)	1
Ischemic heart disease	131 (4.2)	0	131 (4.3)	0.63
ICAD	119 (3.9)	4 (15.4)	115 (3.8)	**0.02**
ICS	52 (1.7)	1 (3.8)	51 (1.7)	0.36

ICAD, intracranial artery disease; ICS, internal carotid artery stenosis.

are not comprehensively assessed. HIV infection, for example, was not included in the Nagahama study due to its low prevalence among Japanese individuals, and thus it was not included as an explanatory variable in this study. Additionally, the possibility of bias cannot be ruled out due to the selection bias of the variables. Lastly, there were slight variations in the MRA sequences used among the institutions in the study. These limitations should be taken into consideration when interpreting the findings of the study and may warrant further investigations with a more diverse and randomly selected population to validate the results.

Future directions

Taken together, our data indicate that inflammatory conditions are significant risk factors for both ICS and ICAD, as evidenced by the associations with alopecia areata, atopic dermatitis, and the *RNF213* mutation. Therefore, in patients with lifestyle habits conducive to atherosclerosis and a history of alopecia areata, atopic dermatitis, or genetic susceptibility, it may be necessary to consider the potential increase in the risk of vascular stenosis over time. In this study, we were unable to demonstrate significant associations with inflammatory biomarkers such as CRP and NLR. However, there may be potential associations that can be captured by high-sensitivity CRP or other measures. Indeed, genotyping of *RNF213* serves as a form of inflammatory biomarker, and further research is warranted to explore more accurate ones.

Conclusions

This was a community-based prospective registry study examining asymptomatic ICS and ICAD both based on MRA among the large general population, revealing the prevalence of these conditions in Japan.. Alopecia areata was shown to be involved in asymptomatic ICS and ICAD. The results suggested that systemic inflammatory diseases may play a shared role in the development of ICS and ICAD. Additionally, diabetes and age were associated with ICS, while *RNF213* variants and hypertension were linked to ICAD, underscoring distinct risk profiles for these arterial stenotic diseases.

CRediT authorship contribution statement

Megumu Suzuki: Conceptualization, Methodology, Investigation, Writing – original draft. **Yohei Mineharu:** Writing – review & editing, Validation, Methodology, Conceptualization. **Masakazu Okawa:** Writing – review & editing. **Kazumichi Yoshida:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Manabu Nagata:** Investigation. **Tao Yang:** Investigation. **Keita Suzuki:** Investigation. **Naoki Takayama:** Investigation. **Yu Yamamoto:** Investigation. **Yasuharu Tabara:** Resources. **Susumu Miyamoto:** Supervision. **Yoshiki Arakawa:** Supervision. **Fumihiko Matsuda:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

We declare no competing interests.

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Supplementary materials

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