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TPO-Ab plays a role in arterial remodeling in patients with intracranial stenosis

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HIGHLIGHTS

- Intracranial stenosis (ICS) had negative remodeling (NR) and positive remodeling pattern.
- Elevated thyroid peroxidase antibody (TPO-Ab) was correlated with NR.
- TPO-Ab increases vascular smooth muscle cells (VSMCs) migration.
- VSMCs migration may be involved in the process of NR.

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ABSTRACT

Background and aims: Intracranial stenosis (ICS), the common cause of ischemic stroke worldwide, is associated with a high risk of recurrent stroke. We aimed to investigate the relationship between arterial remodeling and antithyroid peroxidase-antibody (TPO-Ab) level in ICS and the effect of TPO-Ab level on the migration of vascular smooth muscle cells (VSMCs).

Methods: We analyzed data of mild-to-severe ICS patients with normal thyroid function who underwent high-resolution magnetic resonance imaging in our center. Vessel area (VA), lumen area, wall area and plaque size were assessed at the most narrowed lumen (MNL) and reference site, respectively. The remodeling index (RI) was defined as VAMNL/VAreference. Negative remodeling (NR) or non-NR was defined as RI $\leq$ 0.95 or $>$ 0.95. A scratch-wound healing assay was also designed to analyze the impact of TPO-Ab level on migration of VSMCs, which were isolated from thoracic aorta segments of Sprague Dawley rats.

Results: A total of 88 patients were included. Patients with elevated TPO-Ab had smaller VA, wall area, plaque size and RI than those with normal level ($p < 0.05$). Elevated TPO-Ab was significantly associated with NR after adjusting for demographic and vascular risks (odds ratio 10.629, 95% confidence interval, 1.842–61.327, $p = 0.008$). The rate of VSMCs migration was significantly increased after culture with TPO-Ab (TPO-Ab 1 μg/ml *vs.* Mock, 29.8% *vs.* 12.0%, $p < 0.01$).

Conclusions: Elevated TPO-Ab in ICS patients was related to NR. TPO-Ab could promote VSMCs migration, which might be involved in the NR of intracranial artery.

1. Introduction

Intracranial stenosis (ICS), the common cause of ischemic stroke worldwide, is associated with a high risk of recurrent stroke [1]. Although ICS is typically associated with intracranial atherosclerosis, which is characterized by atherosclerotic lesions, a substantial minority of cases show other different pathological changes without traditional risk factors, and some advanced atherosclerosis also presents in the absence of distinct stenosis. Our previous study demonstrated that thyroid autoimmunity, especially the level of antithyroid peroxidase antibody (TPO-Ab), was associated with the development of ICS in young stroke patients with apparent euthyroid state [2]. In addition, a

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high prevalence of thyroid autoantibodies was also observed in patients with adult-type Moyamoya disease (MMD), which is characterized by progressive stenosis of intracranial internal carotid arteries and their proximal branches, even with an apparent euthyroid state [3,4]. However, the underlying mechanism of TPO-Ab in the pathogenesis of ICS remains unclear.

Recently, Kaku et al. showed that arterial constrictive remodeling was a major pathogenesis in MMD since they revealed outer-diameter narrowing of the internal carotid and middle cerebral arteries (MCAs) in steady-state MRI [5]. Constriction of vascular lumen may be due to the negative remodeling (NR) of the vascular wall, while positive remodeling (PR) causes enlargement of vessels and alleviates vascular stenosis to some extent [6]. Novel high-resolution MRI (HR-MRI) could precisely assess these two remodeling patterns of vessel wall in ICS [6–8]. Recently, researchers have suggested that HR-MRI was a promising tool to detect the characteristics of middle cerebral artery (MCA) and could reveal the relationship between remodeling patterns and ischemic stroke [7]. Ma and colleagues showed that PR was more commonly seen in advanced basilar artery atherosclerosis and PR lesions had greater lumen area and wall area with significantly larger plaque than non-positive remodeling lesions [8]. Indeed, vascular remodeling depends on the balance between matrix protein synthesis and enzymatic breakdown [9]. Previous studies demonstrated that the migration of vascular smooth muscle cells (VSMCs), which mediated the synthesis of matrix protein, could promote arterial constriction and the development of inward remodeling, through upregulating urokinase plasminogen activator (uPA) and its receptor (uPAR) [10–12]. VSMCs migration is a fundamental process that leads to the thickening and progression of intima media thickness. Through the uPA/uPAR system, VSMCs may migrate from media to intima to promote neointima, which could lead to overall increase of carotid intima media thickness (cIMT). Interestingly, compared with TPO-Ab negative patients, TPO-Ab positive patients were found to have larger progression of cIMT, and the progression rate was ten-fold bigger than that of placebo patients in large statin trials [13].

Therefore, taken together, we hypothesized that elevated levels of TPO-Ab might be involved in the arterial constrictive remodeling in ICS patients and this process might be related to its effect on VSMCs migration of vessel walls. We thus aimed to investigate the relationship between TPO-Ab level and remodeling patterns of the vessel wall using HR-MRI in ICS patients and then designed a scratch-wound healing assay to visualize the effect of TPO-Ab on the migration of VSMCs.

2. Materials and methods

2.1. Patient selection

We retrospectively reviewed our prospectively collected database for ischemic stroke or transient ischemic attack (TIA) patients in a euthyroid state who received HR-MRI between June 2011 and December 2017. Patients who had HR-MRI of M1 segment of MCA (MCA-M1) or BA were enrolled if they met all of the following inclusion and none of the exclusion criteria. Inclusion criteria were: (1) MCA-M1 or BA stenosis > 30% detected on magnetic resonance angiography (MRA); (2) had TPO-Ab test the day after the admission and normal thyroid function. Exclusion criteria were: (1) HR MRI defined arterial stenosis, caused by arteritis and dissection; (2) Other secondary causes of arterial stenosis, such as vasospasm and radiation-induced vasculopathy, evaluated by clinical presentation, lab work and imaging; (3) poor image quality for analysis.

2.2. Ethics statement

The study was approved by our local human ethics committee. All clinical investigation had been conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained for all patients.

2.3. MR acquisition

A 3.0 T GE scanner (GE Discovery MR750) with an 8-channel head coil was used. Conventional 3 dimensional time-of-flight (3D-TOF) MRA, Fast Spin Echo (FSE)-T2 weighted imaging (T2WI) and double inversion recovery T1 weighted imaging (T1WI) sequence of HR MRI were set according to previous published protocol [8,14].

2.4. Image analysis

All parameters were measured using Osirix Lite, a free DICOM viewer. We scanned the stenotic MCA-M1/BA using HR-MRI and the scanned area included the proximal and distal non-stenotic part of vessel or BA was chosen as the reference site. We used the neighboring distal site if the proximal reference site was not available. According to the previous report [15], we defined the percent stenosis of MCA/BA on MRA as follows: percent stenosis = [(1 − (Dstenosis/Dnormal))] × 100%, where Dstenosis = the diameter of the artery at the site of the most severe stenosis and Dnormal = the diameter of the proximal normal artery. Based on previous studies [7,8], the vessel area (VA) and lumen area (LA) of MCA-M1 or BA were measured both at the most narrowed lumen (MNL) and the reference site.

The wall area (WA) = VA − LA, the plaque size (PS) $= {}^{WA}MNL - {}^{WA}reference$. The percent plaque burden = (PS/VAMNL) × 100%. The remodeling index (RI) = VAMNL/VAreference (Fig. 1). Then NR, PR, and intermediate remodeling (IR) were defined as RI ≤ 0.95 (Fig. 2), RI ≥ 1.05 (Fig. 3), RI between 0.95 and 1.05, respectively. Non-NR included PR and IR. The measurement work was done by two neuroradiologists (XZ and YZ) who were blinded to clinical details, and their average values were then calculated and applied.

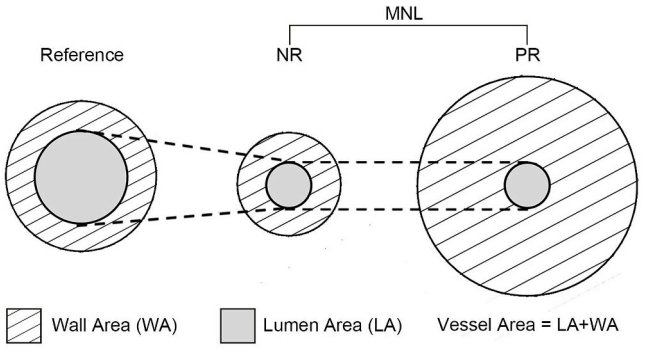

Figure 1. Schematic overview of different types of arterial remodeling.
The figures from left to right shows the schematic illustration of the reference site, negative remodeling (NR) and positive remodeling (PR). Remodeling index (RI) is defined as Vessel Area (VA) of MNL/VAreference. NR and PR are defined as RI ≤ 0.95 and RI ≥ 1.05.

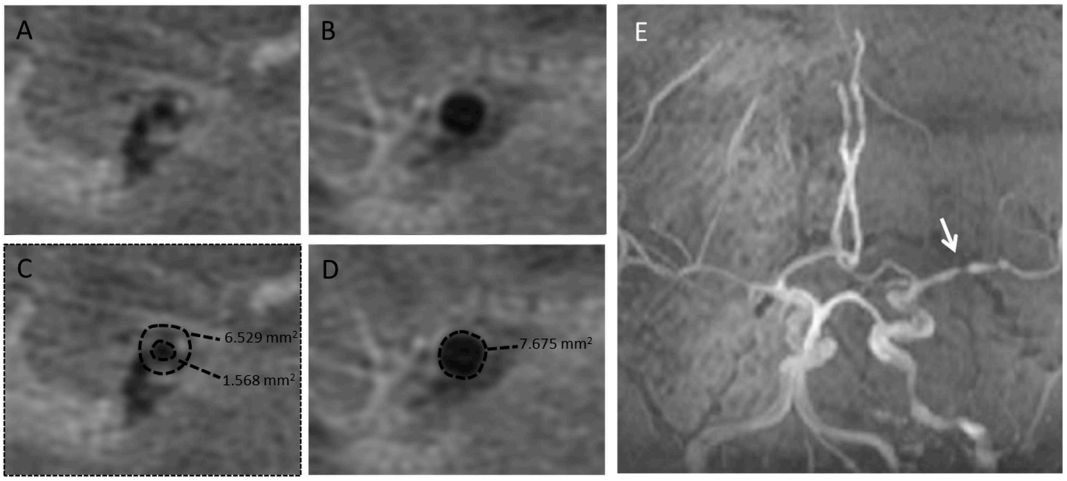

Fig. 2. Negative remodeling (NR) in a case with stenosis of the M1 segment of the middle cerebral artery (MCA-M1).
An 80-year-old woman with hypertension, diabetes, and TPO-Ab + presented with left limb weakness for 2 weeks. On T2-weighted image of MCA-M1 at the maximal lumen narrowing (MLN) (A) and proximal (B) sites, the MCA-M1-cerebrospinal fluid border or the blood-intima border was manually traced to measure the vessel area (VA) or lumen area (LA), respectively. Reference VA is the proximal VA (D; 7.675 mm^2) and the remodeling index of the vessel at MLN is the ratio of VA at MLN (C; 6.529 mm^2) to reference VA (D; 7.675 mm^2). Negative remodeling was then defined as the remodeling index was 0.85 (< 0.95).

2.5. Assessment of clinical courses

We collected baseline demographic data and risk factors for ischemic stroke including prior stroke or TIA, hypertension, diabetes mellitus, hyperlipidemia, and smoking history. Laboratory tests included blood lipid profile and thyroid function studies. Elevated TPO-Ab (marked as TPO-Ab+) was defined as TPO-Ab level above the normal limit (≥5.61 IU/ml), while normal TPO-Ab (marked as TPO-Ab-) was defined as TPO-Ab level within the normal range (< 5.61 IU/ml).

2.6. Cell isolation

VSMCs were isolated from thoracic aorta segments of Sprague Dawley rat. Briefly, the aorta segments were cut and washed with phosphate buffered solution (PBS) in sterile condition. After removing off the endothelial and adventitial layers, the vascular media was subjected to digestion. The medial layer was cut into 1 mm^2 and transferred to a culture flask with 0.25% parenzyme. The fragments were digested in the incubator at 37 °C containing 5% CO2 for 3.5 h. After digestion, the cells were collected with centrifugation and cultured in a new flask. VSMCs were identified by immunofluorescence using alpha-smooth muscle actin (α-SMA) antibody.

2.7. Scratch-wound healing assay

After the cell fusion got 90%, the cell culture medium was changed with corresponding serum free medium. VSMCs were cultured with TPO-Ab with different concentration, 1 μg/ml, 2 μg/ml, 4 μg/ml and 8 μg/ml. TPO-Ab groups, positive control (platelet derived growth factor, PDGF 15 ng/ml), or neither of the above (Mock) were subjected to scratch-wound healing analysis. PDGF has been implicated as a major potent factor that stimulates the migration of VSMCs during the development of intimal thickening of the artery [16]. 110-μl sterile pipette tip was used to generate scratch wound. Wound areas were

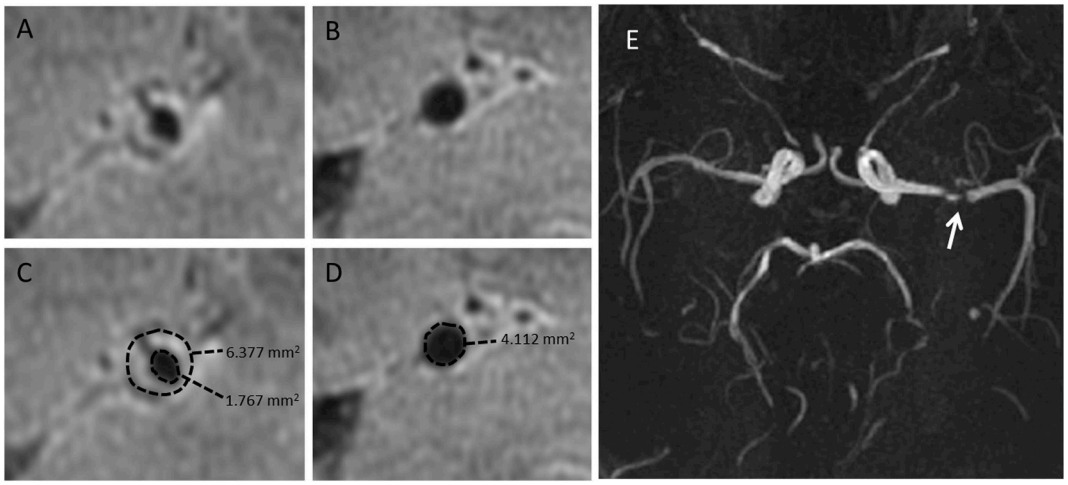

Fig. 3. Positive remodeling (PR) in a case with stenosis of the M1 segment of the middle cerebral artery (MCA-M1).
A 63-year-old woman with hypertension and TPO-Ab- presented with right limb weakness for 2 days. Figures of the upper row are T2-weighted MCA-M1 images at the maximal lumen narrowing (MLN) (A) and proximal (B) sites. The MCA-M1-cerebrospinal fluid border or the blood-intima border was manually traced to measure the vessel area (VA) or lumen area (LA) at the MLN (C; VA 6.377 mm^2, LA 1.767 mm^2) and proximal (D; VA 4.112 mm^2). Reference VA is 4.112 mm^2; remodeling index of the vessel at MLN is 1.55, which is the ratio of VA at MLN to reference VA, > 1.05 (PR).

marked and photographed at 0 h, 24 h, 36 h and 72 h, respectively. We used Leica Application Suite Software to photograph and quantify the migrated distance of cells moving from the wound edge toward the center.

The distance of cellular movement from the wound edge toward the center at 72 h after scratching was measured and the rate of migration was then calculated as (distance0h - distance72 h)/distance0h. All experiments were repeated three times.

2.8. Statistical analysis

Statistical analysis was performed using SPSS 17.0 (SPSS, Inc, Chicago, USA). All non-normally distributed variables were presented as median (25th-75th percentile). Categorical variables were presented as frequency (percentage). Comparison between groups was assessed with the Mann-Whitney *U* test for data that did not follow normal distribution and Fisher's exact test for categorical data. Binary logistic regression analysis was used in analysis of remodeling patterns. In binary logistic regression analysis, TPO-Ab was used as an independent variable, and NR as a dependent variable. A p value of < 0.05 was considered to be statistically significant.

3. Results

3.1. Subject characteristics

Between June 2011 and December 2017, 102 patients met the inclusion criteria. Patients were excluded due to arteritis (n = 2), dissection (n = 1), and poor quality of MRI image due to motion artifacts (n = 11). Finally, 88 patients were included. There were 4 cases whose proximal reference site was not available and then the neighboring distal site was used instead. The interobserver intraclass correlation coefficients (ICCs) were 0.98, 0.97, 0.97, 0.95 for VA of reference artery, LA of reference artery, VA of the MNL artery, LA of the MNL artery, respectively.

The mean age was 61.9 ± 14.3 years, and 31 (35.2%) were female. TPO-Ab+ was found in 12 (13.6%) patients. The percent stenosis of MCA/BA presented no difference in TPO-Ab+ and TPO-Ab- groups (TPO-Ab + *vs.* TPO-Ab-, 62.1% *vs.* 60.7%, $p = 0.877$). Compared with the TPO-Ab- group, patients with TPO-Ab+ had smaller vessel area and wall area at MNL, and smaller plaque size, percent plaque burden and remodeling index (Table 1). At the reference site, compared with the TPO-Ab- group, patients with TPO-Ab + had smaller vessel area [median (25th-75th percentile), 12.9 (10.8–16.0) *vs.* 10.7 (8.9–11.6), $p = 0.003$] and lumen area [6.7 (5.2–7.7) *vs.* 5.1 (4.3–5.9), $p = 0.005$], but had similar wall area [6.6 (4.7–8.1) *vs.* 5.3 (4.7–6.4), $p = 0.108$] (Table 1).

3.2. Comparison among the NR group and the non-NR group

PR, NR, and IR were found in 54 (61.4%), 17 (19.3%), and 17 (19.3%) patients, respectively. As Table 2 shows, patients in the NR group had higher rate of TPO-Ab+ (35.3% *vs.* 8.5%, $p < 0.010$), smaller VA and WA at MNL, and smaller PS, percent plaque burden and remodeling index than those in the non-NR group. After adjusting for age, sex, and history of prior stroke or TIA, hypertension, diabetes, hyperlipidemia and smoking, TPO-Ab+ was independently associated with the presence of NR (odds ratio 10.629, 95% confidence interval, 1.842–61.327, $p = 0.008$).

3.3. The scratch-wound healing assay

As Fig. 4A shows, the scratch-wound healing assay revealed that migration of VSMCs toward the wound area was significantly increased when cells were cultured with TPO-Ab. As Fig. 4B shows, migration rate of the TPO-Ab 1 μg/ml group was significantly higher than the mock group (29.8% *vs.* 12.0%, $p < 0.01$). Furthermore, the migration rate showed no difference among groups with various concentrations of TPO-Ab (29.8% *vs.* 33.8% *vs.* 31.7% *vs.* 31.9%, $p = 0.886$).

Table 1
Demographic and clinical characteristics of the TPO-Ab- and TPO-Ab + groups.

	TPO-Ab- (n = 76)	TPO-Ab+ (n = 12)	*p*
Age, y, mean ± SD	62.3 ± 13.9	59.7 ± 17.2	0.561
Female, n (%)	25 (32.9)	6 (50.0)	0.331
Risk factors			
Prior stroke or TIA, n (%)	15 (19.7)	5 (41.7)	0.134
Hypertension, n (%)	51 (67.1)	7 (58.3)	0.534
Diabetes, n (%)	19 (25.0)	2 (16.7)	0.723
Hyperlipidemia, n (%)	13 (17.1)	1 (8.3)	0.682
Smoking, n (%)	30 (39.5)	1 (8.3)	0.050
Laboratory examinations			
Total cholesterol, mmol/L, median (IQR)	4.5 (3.8–5.3)	4.1 (3.7–4.9)	0.443
Triglycerides, mmol/L, median (IQR)	1.4 (0.9–1.8)	1.5 (1.0–2.1)	0.518
Low density lipoprotein cholesterol, mmol/L, median (IQR)	2.5 (2.0–3.2)	2.3 (1.8–2.9)	0.534
High density lipoprotein cholesterol, mmol/L, median (IQR)	1.1 (0.9–1.2)	1.1 (1.0–1.3)	0.295
Imaging data			
At reference site, mm^2, median (IQR)			
Vessel area	12.9 (10.8–16.0)	10.7 (8.9–11.6)	0.003
Lumen area	6.7 (5.2–7.7)	5.1 (4.3–5.9)	0.005
Wall area	6.6 (4.7–8.1)	5.3 (4.7–6.4)	0.108
At maximal lumen narrowing site, median (IQR)			
Vessel area, mm^2	15.8 (13.1–18.8)	9.8 (5.9–11.8)	< 0.001
Lumen area, mm^2	2.9 (1.5–4.4)	2.0 (1.1–3.5)	0.117
Wall area, mm^2	12.6 (10.5–15.2)	6.8 (4.6–8.6)	< 0.001
Plaque size, mm^2	6.4 (4.0–8.5)	1.0 (0.1–3.0)	< 0.001
Percent plaque burden	0.4 (0.3–0.5)	0.1 (0.0–0.3)	< 0.001
Remodeling index	1.2 (1.0–1.3)	0.9 (0.6–1.0)	< 0.001

The *p*-value < 0.01 indicates significance.
TPO-Ab: antithyroid peroxidase-antibody; TIA: transient ischemic attack.
Data are presented as number (%) or mean ± SD or median (25th-75th percentile).

Table 2
Demographic and clinical characteristics of the NR non-NR groups.

	NR (n = 17)	Non-NR (n = 71)	*p*
Age, y, mean ± SD	64.9 ± 16.5	61.2 ± 13.8	0.346
Female, n (%)	3 (17.6)	28 (39.4)	0.156
Risk factors			
Prior stroke or TIA, n (%)	8 (47.1)	12 (16.9)	0.020
Hypertension, n (%)	12 (70.6)	46 (64.8)	0.780
Diabetes, n (%)	2 (11.8)	19 (26.8)	0.341
Hyperlipidemia, n (%)	4 (23.5)	10 (14.1)	0.459
Smoking, n (%)	5 (29.4)	26 (36.6)	0.778
Laboratory examinations			
Total cholesterol, mmol/L, median (IQR)	4.0 (3.6–5.3)	4.5 (3.8–5.3)	0.507
Triglycerides, mmol/L, median (IQR)	1.0 (0.8–1.6)	1.4 (1.0–1.9)	0.110
Low density lipoprotein cholesterol, mmol/L, median (IQR)	2.5 (1.8–3.2)	2.6 (2.0–3.1)	0.500
High density lipoprotein cholesterol, mmol/L, median (IQR)	1.3 (1.0–1.4)	1.1 (0.9–1.2)	0.061
TPO-Ab+, n (%)	6 (35.3)	6 (8.5)	0.010
Imaging data			
At reference site, mm^2, median (IQR)			
Vessel area	14.0 (11.5–18.9)	12.4 (10.4–15.1)	0.124
Lumen area	7.3 (5.4–9.5)	6.2 (5.0–7.3)	0.199
Wall area	7.2 (5.1–8.5)	5.9 (4.7–7.4)	0.071
At maximal lumen narrowing site, median (IQR)			
Vessel area, mm^2	12.0 (8.6–16.6)	15.7 (12.7–18.9)	0.003
Lumen area, mm^2	2.9 (1.6–5.1)	2.8 (1.3–4.0)	0.167
Wall area, mm^2	7.7 (5.0–11.5)	12.6 (10.7–15.6)	< 0.001
Plaque size, mm^2	0.8 (0–2.4)	6.5 (4.4–8.6)	< 0.001
Percent plaque burden	0.1 (0–0.2)	0.4 (0.3–0.5)	< 0.001
Remodeling index	0.9 (0.7–0.9)	1.2 (1.1–1.4)	< 0.001

The *p*-value < 0.01 indicates significance.
TPO-Ab: antithyroid peroxidase-antibody; TIA: transient ischemic attack; PR: positive remodeling; NR: negative remodeling; Non-NR includes PR and IR.
Data are presented as number (%) or mean ± SD or median (25th-75th percentile).

4. Discussion

Our study delineated two major findings: (1) in stroke or TIA patients with mild-to-severe MCA-M1 or BA stenosis, elevated TPO-Ab was associated with NR of vascular wall. (2) TPO-Ab could promote VSMCs migration. Therefore, we assume that elevated TPO-Ab might enhance the NR of the intracranial artery via promotion of VSMCs migration. To our knowledge, this is the first study investigating the underlying mechanism of TPO-Ab on intracranial vascular remodeling. We found TPO-Ab could directly promote VSMCs migration, which has not been reported before.

In a previous autopsy study of MMD, pathological examination revealed that the outer diameters of the affected internal carotid artery terminations were markedly diminished, and a microscope study showed attenuated media and thickened intima [17–19]. It is noteworthy that the thickened intima was found mainly composed of VSMCs, and the number of VSMCs in the medium was decreased [18], indicating that VSMCs might migrate from media to intima. The migration of VSMCs from media to intima causes neointima, leading to the fibroproliferative-predominance process [20]. The histology of PR is known to include lipid-rich plaque, intraplaque hemorrhage, fibrin cap, and infiltration of inflammatory cells, detected from the atherosclerotic arteries in ICAS, including MCAs, BAs, posterior cerebral arteries, anterior cerebral arteries, vertebral arteries and intracranial carotid arteries [21–23]. However, detailed histological findings of NR in ICS have been rarely reported. Using integrated backscatter-intravascular ultrasound, the investigation of *in vivo* tissue characterization demonstrated that coronary artery with NR contained a more fibrous component in intima and less lipid content compared to PR [24], we thus inferred that the promoted migration of VSMCs by elevated TPO-Ab might be involved in the cause of NR in intracranial artery.

Previous studies also indicated that T-cell dysfunction was involved in the development of MMD [25]. T-cell dysfunction was found to induce pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) [26], moreover, patients with high TPO-Ab titer had significantly higher percentages of T cells producing interferon (IFN)-γ [27]. These cytokines could promote the de-differentiation of VSMCs from a quiescent contractile phenotype to a proliferating synthetic phenotype. Proliferating VSMCs subsequently migrate into the intima and enhance the production of extracellular matrix proteins, participating in the formation of the fibrous cap [28]. A high prevalence of TPO-Ab was observed in MMD patients. In a previous study of Moyamoya disease, authors found smooth muscle cell migration from media to intima, and aberrant expression of IgG and S100A4 protein in vascular smooth muscle cells [29]. S100A4 protein is one of the S100 family of calcium binding proteins, and plays an important role in tumor progression and metastasis. We speculate that calcium binding proteins such as S100A4 may be involved in the process of increased VSMC migration by TPO-Ab. Based on the above findings, we thus assume that TPO-Ab may promote NR by increasing the VSMCs migration directly through calcium binding proteins, or mediated by T-cell dysfunction indirectly.

Additionally, in the scratch-wound healing assay of vascular smooth muscle cells, we found no obvious relationship between TPO-Ab titers and the migration rate of VSMCs. This is in accordance with the clinical findings of other studies, which demonstrated the TPO-Ab titers had no relationship with the severity of stenosis [30].

Excitingly, in a previous case report, the stenosis of the major intracerebral arteries was reversible after high-dose methylprednisolone therapy and plasmapheresis in a patient with autoimmune thyroid disease [31]. Plasmapheresis has also been suggested for the stabilization of MMD with hyperthyroidism [32]. These findings need to be confirmed in prospective studies with repeated measures. Using more refined methodologies to study the vessel wall, TPO-Ab might be a potential prophylactic and therapeutic target of ICS.

Limitations of the study included a single stroke center and a

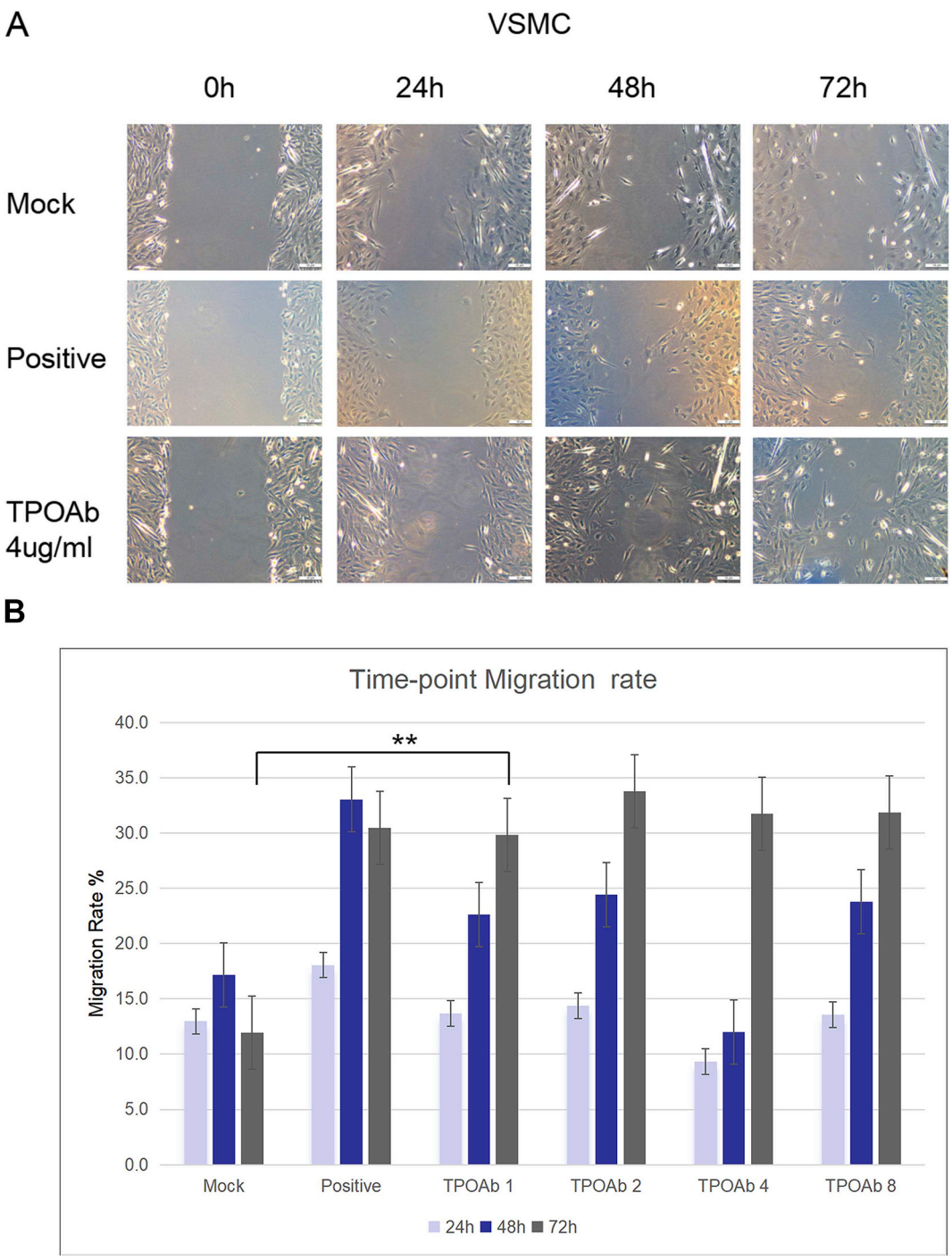

Fig. 4. Antithyroid peroxidase-antibody (TPO-Ab) promotes the migration of smooth muscle cells (VSMCs).
VSMCs were cultured with different concentrations of TPO-Ab, 1 μg/ml, 2 μg/ml, 4 μg/ml and 8 μg/ml. TPO-Ab groups, positive control (platelet derived growth factor, PDGF 15 ng/ml), or Mock (neither of the above) were subject to scratch-wound healing analysis. (A) The cell migration to the wounded area was photographed by microscopy at 0 h, 24 h, 36 h and 72 h post-wounding. (B) The rate of migration was examined by measuring the distance of cells moved from the wound edge toward the center at 72h after scratching and was calculated by (distance0h-distance72 h)/distance0h. Data are presented as mean ± standard deviation of at least three independent experiments. $^{**}p < 0.01$.

relatively modest sample size. Due to the limited numbers of patients in this study, it is possible that the power to detect the association of remodeling with vascular risk factors was not enough. Besides, we only focused on TPO-Ab due to the lacking data of other thyroid autoantibodies. Moreover, we only did a cytology experiment to verify the effect of TPO-Ab on VSMCs which cannot provide a mechanistic angle to explain the phenomenon. Animal experiments are needed to validate our assumptions such as the roles of S100A4 and T cells played in the mechanism. Thirdly, the vascular RI depended on the reference vessel area.

Because there is a natural tapering of intracranial arteries, underestimation or overestimation of the RI might have occurred when we chose proximal or distal segments as a reference site. But if we calculate with the average of distal and proximal sites to the stenosis, measurement error may be increased.

In conclusion, elevated level of TPO-Ab may play a role in arterial negative remodeling in patients with intracranial stenosis, which is involved with the migration of VSMCs. This finding may provide a potential therapeutic target for intracranial stenosis with immune-mediated therapy targeting TPO-Ab in future.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

Xuting Zhang conceived the hypothesis, did the experiments and analyzed the data. Ying zhou analyzed the images. Ruiting Zhang, Shengqiang Yan and Feng gao contributed to recruitment of patients and discussions. Yujie Deng gave advice on experiment and analysis. Wenhong Ding provided technical advice on MRI parameter setting and scanning. Min Lou directed the project and revised the manuscript.

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