Lysyl oxidase family gene polymorphisms and risk of aneurysmal subarachnoid hemorrhage: a case-control study

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Background: Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating disease caused by intracranial aneurysm (IA) rupture. Lysyl oxidase (*LOX*) family genes (*LOX-like [LOXL] 1-4*) have roles in collagen cross-linking in the extracellular matrix (ECM) and may be associated with IA rupture. We aimed to explore the association between *LOX* polymorphisms and the risk of aSAH.

Methods: This case-control study included 2 cohorts: 133 single ruptured and 115 unruptured IA patients, and 65 multiple ruptured and 71 unruptured IA patients. Genotyping of 27 single nucleotide polymorphisms (SNPs) in *LOX* was performed. Logistic regression analysis was performed to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) of the SNPs of *LOX* and the risk of aSAH.

Results: *LOX* rs1800449 and *LOXL4* rs3793692 were positively associated with the risk of single IA rupture in the recessive model (OR =5.66, 2.06; 95% CI =1.22–26.24, 1.11–3.82, respectively) and *LOX* rs10519694 demonstrated a protective effect on single IA rupture (dominant model: OR =0.42, 95% CI =0.21–0.83; recessive model: OR =0.16, 95% CI =0.04–0.65; additive model: OR =0.46, 95% CI =0.28–0.78). *LOXL1* rs2165241, *LOXL2* rs1063582, and *LOXL3* rs17010021 showed risk effects on multiple IAs rupture. *LOXL3* rs17010022 showed a protective effect on multiple IAs ruptures (dominant model: OR =0.41, 95% CI =0.21–0.82; additive model: OR =0.51, 95% CI =0.30–0.85).

Conclusions: *LOX* and *LOXL4* may be susceptibility genes for single IA rupture, whereas *LOXL1-3* may have a role in susceptibility to multiple IAs ruptures in the Chinese population, suggesting that *LOX* family genes may be associated with aSAH.

Keywords: Intracranial aneurysms (IAs); subarachnoid hemorrhage; aneurysmal subarachnoid hemorrhage (aSAH); *LOXL2*; *LOX*

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Introduction

Intracranial aneurysm (IA) is a complex disease characterized by incomplete integrity of the artery wall, which is typically induced by pathological expansion and swelling of the weak artery wall prone to rupture (1). IA affects 3–5% of the global population and approximately 7% of the Chinese population aged 35–75 years (2-4). IA can be classified as single (i.e., 1 aneurysm) or multiple (i.e., equal to or more than 2 aneurysms) events, which accounts for approximately 20–30% of cases (5). Most IAs are asymptomatic before rupture; however, rupture can result in an aneurysmal subarachnoid hemorrhage (aSAH), which is a devastating condition with a poor prognosis (6). Thus, to manage IA more effectively, it is necessary to identify the risk factors associated with aSAH as early as possible.

Although the etiology of IA rupture is not entirely clear, both environmental and genetic factors have been recognized to possibly lead to IA rupture (7,8). For instance, a study has revealed that smoking and hypertension are predictors of IA rupture (9). Furthermore, first-degree relatives of aSAH patients are more likely to be diagnosed with IA or aSAH, which indicates a familial tendency (10). Histopathologically, extracellular matrix (ECM)plays an important role in the structural support of cerebral artery blood vessels (11). Some IA remain stable over time, and the walls of unruptured IAs exhibit with ECM defects and tissue thrombosis, but in others mural cell die, the ECM degenerates too fragile to resist intravascular hemodynamic pressure, the IA will rupture, causing aSAH (1,12). Elastin and collagens are abundant matrix proteins in the ECM, while the lysyl oxidase (*LOX*) family of genes in the extracellular copper-containing enzyme family initiate cross-linking of collagen and elastin by oxidative deamination of lysine residues (13). Therefore, *LOX* family genes may play roles in aSAH, and the alteration of matrix proteins may cause vasculature-related pathological changes in aSAH.

Although numerous studies have explored the genetic susceptibility of aSAH, there are few studies on the association between *LOX* family gene polymorphisms and aSAH. Our previous study found that *LOX* was associated with the risk of single IA, while *LOX*-like 2 (*LOXL2*) was associated with the risk of multiple IAs (14). *LOXL2*, which belongs to the *LOX* family of genes, was found to be associated with susceptibility to familial IA (FIA) in Chinese and Japanese populations (15,16); In Korea, Hong *et al.* discovered that *LOX* was associated with IA formation and rupture using candidate gene association analysis (17). However, since the pathophysiology of IA rupture is somewhat different from the process of IA formation (12), whether *LOX* family genes are also associated with aSAH in Chinese population remains unclear. Since there is high homology among the subtypes of the *LOX* family genes, other members of this family may also be associated with aSAH. Therefore, we aimed to explore whether *LOX* family gene polymorphisms are associated with aSAH by comparing ruptured and unruptured IA in a Chinese sample and provide a reference for the etiological study of aSAH. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3484/rc).

Methods

Study population

A total of 384 patients with IA were collected from 2 third-class hospitals in Hunan province (Xiangya Hospital of Central South University and Hunan People's Hospital) from July 2018 to December 2020. This case-control study included 248 single IA (133 ruptured and 115 unruptured) and 136 multiple IA (65 ruptured, 158 aneurysms; 71 unruptured, 183 aneurysms) patients. IA patients were confirmed by cerebral angiography (computed tomography, magnetic resonance angiography, and digital subtraction angiography) or detected during surgery. IA patients with autosomal dominant polycystic nephropathy, Marfan's syndrome, other autosomal dominant hereditary diseases, other cerebrovascular diseases, and first- or second-degree relatives diagnosed with IA or aSAH disease were excluded. Moreover, patients with IA who had ruptured aneurysms were classified into the ruptured group. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The current project was approved by the Ethics Committee of Central South University (permit No. CTXY–150002–1), and the other hospital (Hunan People's Hospital) was informed and agreed the study. All patients provided informed consent.

Single nucleotide polymorphism (SNP) selection and genotyping

SNPs were selected based on tag SNPs and the Genome Variation Server 150 (http://*gvs*.gs.washington.edu/

Table 1 General demographic and clinical characteristics of the subjects

Variables	Single IA			Multiple IAs		
	Ruptured (n=133)	Unruptured (n=115)	P	Ruptured (n=65)	Unruptured (n=71)	P
Age (years), mean ± SD	58.31±10.8	56.18±9.8	0.109	55.42±11.23	57.74±10.64	0.218
Female, n (%)	95 (71.4)	71 (61.7)	0.106	52 (80.0)	49 (69.0)	0.143
Smoking, n (%)	23 (17.3)	19 (16.5)	0.872	7 (10.8)	10 (14.1)	0.559
Drinking, n (%)	16 (12.0)	8 (7.0)	0.178	5 (7.7)	7 (9.9)	0.656
Hypertension, n (%)	64 (48.1)	63 (54.8)	0.295	39 (60.0)	42 (59.2)	0.920
Diabetes, n (%)	6 (4.5)	9 (7.8)	0.275	3 (4.6)	6 (8.5)	0.580
Hyperlipidemia, n (%)	4 (3.0)	9 (7.8)	0.090	3 (4.6)	6 (8.5)	0.580
Intracranial aneurysm, n	133	115		158	183	
Shape of the aneurysm, n (%)			<0.001*			<0.001*
Regular	112 (84.2)	112 (97.4)		128 (81.0)	175 (95.6)	
Irregular	21 (15.8)	3 (2.6)		30 (19.0)	8 (4.4)	
Location, n (%)			0.001*			0.055
Internal carotid artery	53 (39.8)	60 (52.2)		78 (49.4)	96 (52.5)	
Anterior cerebral artery	9 (6.8)	6 (5.2)		14 (8.9)	10 (5.5)	
Middle cerebral artery	21 (15.8)	24 (20.9)		34 (21.5)	35 (19.1)	
Posterior cerebral artery	1 (0.8)	1 (0.9)		1 (0.6)	12 (6.6)	
Anterior communicating artery	34 (25.6)	7 (6.1)		11 (7.0)	9 (4.9)	
Posterior communicating artery	11 (8.3)	7 (6.1)		9 (5.7)	7 (3.8)	
Vertebral basilar artery	4 (3.0)	10 (8.7)		5 (3.2)	11 (6.0)	
Others	0	0		6 (3.8)	3 (1.6)	

*, P<0.05. SD, standard deviation.

GVS150/index.jsp). After screening SNPs by name, preference was given to mutations associated with IA, SNPs located in the functional exon region, or those covering many other loci. Finally, we selected 27 SNPs from the *LOX* family genes to be included (Table S1).

Fasting blood samples (5–10 mL) of each participant were obtained in the morning before the treatment, placed in EDTAK2 anticoagulant tubes (10 mL), and refrigerated at 4 ℃. A blood genomic DNA extraction kit (Tiangen Biochemical Technology Co. Ltd., Beijing, China) was used for DNA extraction and refrigerated at −80 ℃. Genotyping was conducted using the MassARRAY iPlex platform (Agena Bioscience Inc., San Diego, CA, USA). The primers were designed using Assay Design 3.1 software (Sequenom, San Diego, CA, USA), as detailed in *Table 1*. The mixture for the polymerase chain reaction (PCR) amplification reaction included $dddH_2O$ (1.8 μL), 10× PCR buffer solution (0.5 μL), $MgCl_2$ (0.4 μL), deoxynucleoside triphosphate (0.1 μL), Taq polymerase (0.2 μL), PCR primer (1 μL), and DNA sample (1 μL). The PCR amplification was conducted in the following steps: pre-denaturation for 2 min at 95 ℃, followed by 45 cycles of 30 s at 9 ℃, 30 s at 56 ℃, 60 s at 72 ℃, and finally extension for 5 min at 72 ℃. The final products were stored at 25 ℃ until further use. After shrimp alkaline phosphatase, single nucleotide extension, resin desalination steps, and the matrix assisted laser desorption ionization time-of flight mass spectrometry reaction, MassArray TYPER 4.0 software (Sequenom, San Diego,

CA, USA) was used to interpret the genotype of each sample target site.

Statistical analysis

Statistical analysis was performed using SPSS (version 23.0; IBM, Armonk, NY, USA). Data following a normal distribution were described using mean ± standard deviation. Normally distributed continuous variables were compared using *t*-tests, and non-normally distributed variables were compared using Mann-Whitney U tests. Categorical variables were compared between the two groups using chi-square or Fisher's exact tests. The association between *LOX* family gene polymorphisms and the risk of IA rupture was evaluated by odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression in additive, recessive, and dominant models. Differences were considered statistically significant at P<0.05.

Results

Characteristics of the participants

The basic characteristics of the participants are listed in *Table 1*. We included 248 single IA patients (133 ruptured and 115 unruptured) and 136 patients with multiple IAs (65 ruptured, 158 aneurysms; 71 unruptured, 183 aneurysms). Among the single and multiple IAs patients, there were no differences in age, sex, smoking status, drinking status, hypertension, diabetes, and hyperlipidemia between the ruptured and unruptured groups (P>0.05), but there were differences in the morphological distribution of IA (P<0.05). The distribution of aneurysm location was different in the single IA ruptured and unruptured groups (P<0.05), but there was no difference in multiple IAs patients (P>0.05).

Associations between LOX family gene polymorphisms and the risk of single IA rupture

Univariate analysis revealed that *LOX* rs1800449, *LOX* rs10519694, and *LOXL4* rs3793692 were associated with single IA rupture, which remained significant after adjusting for the shape and location of IA (Table S2). *LOX* rs1800449 was associated with the risk of a single IA rupture (recessive model: OR =5.66, 95% CI =1.22–26.24, P=0.027). Nevertheless, *LOX* rs10519694 demonstrated a protective effect on single IA rupture under all 3 genetic models (dominant: OR =0.42, 95% CI =0.21–0.83, P=0.013; recessive: OR =0.16, 95% CI =0.04–0.65, P=0.010; additive: OR =0.46, 95% CI =0.28–0.78, P=0.004). *LOXL4* rs3793692 was associated with single IA rupture in the recessive model (OR =2.06, 95% CI =1.11–3.82, P=0.022). These results are shown in *Table 2*.

Associations between LOX family gene polymorphisms and the risk of multiple IAs ruptures

Since every patient with multiple IAs had 2 or more IAs, we were unable to adjust for morphological confounders in the multivariate analysis. The univariate analysis results indicated that *LOXL1* rs2165241 was associated with multiple IAs ruptures (dominant model: OR =2.99, 95% CI =1.32–6.78, P=0.009; additive model: OR =2.53, 95% CI =1.16–5.56, P=0.020). Moreover, *LOXL2* rs1063582 was associated with the risk of multiple IAs ruptures in the recessive model (OR =4.12, 95% CI =1.08–15.71, P=0.038). We found that 2 sites in *LOXL3* were significantly associated with multiple IAs ruptures, but the directionality of the function was different. Furthermore, rs17010021 was associated with the risk of multiple IAs ruptures (additive model: OR =1.72, 95% CI =1.03–2.89, P=0.039), but rs17010022 may be an effective factor for reducing the risk of multiple IA ruptures (dominant model: OR =0.41, 95% CI =0.21–0.82, P=0.011; additive model: OR =0.51, 95% CI =0.30–0.85, P=0.010; *Table 3*).

Discussion

The present study extensively explored the associations between *LOX* family gene polymorphisms and the risk of aSAH. We demonstrated that *LOX* and *LOXL4* polymorphisms were associated with single IA rupture, whereas *LOXL1-3* polymorphisms were associated with multiple IAs ruptures, suggesting that members of the *LOX* family may have roles in aSAH.

The *LOX* family can be classified into two groups based on the structure of their N-terminal domains: *LOX* and *LOXL1* have a propeptide at their N-terminal, whereas *LOXL2*, *LOXL3*, and *LOXL4* have 4 scavenger receptor cysteine-rich domains (18). The *LOX* family gene subtypes (*LOX*, *LOXL1-4*) are all amine oxidases and contain a highly conserved C-terminal binding domain that forms a special lysine tyrosylquinone cofactor-moiety after binding to the copper ion cofactor (19). These family genes are critical enzymes that regulate the crosslinking of elastin and collagen and have a regulatory role in ECM assembly

Table 2 Multivariate analysis of the association between *LOX* family gene polymorphisms and single IA rupture

Gene	SNP	Genotype†		Dominant model		Recessive model		Additive model	
		Ruptured (n)	Unruptured (n)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
LOX	rs1800449(C>T)	86/36/11	77/36/2	1.11 (0.65–1.91)	0.706	5.66 (1.22–26.24)	0.027*	1.33 (0.85–2.06)	0.212
	rs2956540(G>C)	75/41/17	62/46/7	0.87 (0.52–1.47)	0.610	2.22 (0.87–5.70)	0.096	1.08 (0.73–1.59)	0.713
	rs10519694(C>T)	115/15/3	86/17/12	0.42 (0.21–0.83)	0.013*	0.16 (0.04–0.65)	0.010*	0.46 (0.28–0.78)	0.004*
	rs2303656(G>T)	122/11/0	111/4/0	2.38 (0.71–7.99)	0.160	–	–	2.38 (0.71–7.99)	0.160
	rs763497(A>G)	100/32/1	77/32/6	0.66 (0.37–1.16)	0.148	0.19 (0.02–1.63)	0.130	0.63 (0.38–1.05)	0.076
	rs3900446(A>G)	110/19/4	93/21/1	0.78 (0.40–1.54)	0.475	3.44 (0.36–32.79)	0.283	0.93 (0.52–1.66)	0.793
LOXL1	rs2165241(C>T)	108/23/2	93/20/2	0.87 (0.44–1.70)	0.674	0.92 (0.13–6.72)	0.933	0.89 (0.49–1.60)	0.694
	rs3825942(G>A)	103/26/4	83/30/2	0.76 (0.42–1.38)	0.367	1.90 (0.32–11.37)	0.482	0.86 (0.51–1.45)	0.564
	rs2304721(C>A)	77/45/11	64/39/12	1.09 (0.65–1.84)	0.749	0.78 (0.32–1.89)	0.588	1.00 (0.68–1.48)	0.998
	rs12441130(T>C)	63/54/16	53/43/19	1.03 (0.61–1.73)	0.919	0.73 (0.35–1.54)	0.412	0.94 (0.65–1.35)	0.738
LOXL2	rs2294128(C>T)	105/26/2	93/2/20	1.24 (0.65–2.35)	0.510	0.34 (0.03–3.51)	0.366	1.12 (0.62–2.01)	0.708
	rs7818494(A>G)	84/40/9	74/34/7	0.99 (0.58–1.69)	0.961	1.14 (0.40–3.30)	0.805	1.01 (0.66–1.55)	0.951
	rs4323477(A>G)	32/67/34	26/64/25	1.00 (0.54–1.86)	0.989	1.21 (0.66–2.23)	0.543	1.08 (0.74–1.57)	0.699
	rs7818416(G>A)	44/66/23	33/59/23	0.79 (0.45–1.39)	0.418	0.90 (0.47–1.74)	0.761	0.87 (0.60–1.27)	0.479
	rs1063582(G>T)	82/47/4	72/38/5	1.10 (0.64–1.86)	0.738	0.57 (0.13–2.49)	0.455	1.01 (0.64–1.61)	0.961
	rs2280936(C>G)	86/43/4	67/42/6	0.75 (0.44–1.27)	0.286	0.39 (0.10–1.59)	0.188	0.73 (0.46–1.15)	0.171
	rs2294133(C>T)	81/39/13	67/35/13	0.86 (0.51–1.46)	0.579	0.86 (0.37–2.00)	0.725	0.90 (0.61–1.31)	0.575
	rs2280935(A>C)	53/57/23	41/61/13	0.81 (0.48–1.38)	0.445	1.87 (0.88–3.96)	0.102	1.06 (0.73–1.54)	0.754
	rs1010156(T>C)	45/63/25	30/55/30	0.66 (0.38–1.17)	0.154	0.64 (0.35–1.20)	0.166	0.73 (0.51–1.05)	0.088
	rs142252012(G>A)	129/4/0	112/3/0	1.04 (0.21–5.14)	0.958	–	–	1.04 (0.21–5.14)	0.958
LOXL3	rs715407(T>G)	90/41/2	71/38/6	0.85 (0.49–1.45)	0.540	0.31 (0.06–1.58)	0.158	0.79 (0.49–1.25)	0.310
	rs6707302(C>T)	94/38/1	74/35/6	0.81 (0.47–1.40)	0.446	0.16 (0.02–1.34)	0.091	0.73 (0.45–1.19)	0.205
	rs17010021(T>A)	57/63/13	54/49/12	1.00 (0.60–1.68)	0.999	0.70 (0.29–1.69)	0.422	0.93 (0.62–1.39)	0.717
	rs17010022(C>G)	57/63/13	53/49/13	1.25 (0.74–2.10)	0.406	0.89 (0.39–2.05)	0.780	1.10 (0.75–1.64)	0.621
LOXL4	rs3793692(G>A)	25/69/39	26/68/21	1.29 (0.68–2.46)	0.432	2.06 (1.11–3.82)	0.022*	1.48 (1.00–2.19)	0.051
	rs1983864(G>T)	40/73/20	32/55/28	0.93 (0.53–1.65)	0.806	0.58 (0.30–1.13)	0.108	0.81 (0.56–1.18)	0.274
	rs7077266(G>T)	95/37/1	75/36/4	0.70 (0.40–1.22)	0.208	0.26 (0.03–2.38)	0.233	0.68 (0.41–1.13)	0.137

†, genotype presented as wild type/heterozygous/homozygous; *, P<0.05. IA, intracranial aneurysm; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; –, not available.

(20,21), while the dysregulation of ECM may disrupt the function or structure of the arterial wall, and may be a risk factor in the pathogenesis of aSAH (22,23). Therefore, they are plausible functional candidates for exploring the associations with aSAH.

The *LOX* gene is located on chromosome 5q23.3-31.2. Being a copper amine oxidase, *LOX* initiates the covalent cross-linking of collagen and elastin by condensing the oxidized peptidyl α-aminoadipic-δ-semialdehyde with neighboring peptidyl aldehydes, thereby consolidating the collagen and elastin fibers of the ECM (13,24). Genetic mouse models for *LOX* have also demonstrated its

Table 3 Univariate analysis of the association between *LOX* family gene polymorphisms and multiple IAs ruptures

Gene	SNP	Genotype†		Dominant model		Recessive model		Additive model	
		Ruptured (n)	Unruptured (n)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
LOX	rs1800449(C>T)	35/25/5	39/28/4	1.05 (0.53–2.05)	0.899	1.40 (0.36–5.44)	0.631	1.09 (0.63–1.87)	0.767
	rs2956540(G>C)	27/27/11	31/33/7	1.09 (0.55–2.15)	0.803	1.86 (0.68–5.14)	0.23	1.22 (0.74–1.99)	0.437
	rs10519694(C>T)	53/9/3	59/9/3	1.11 (0.46–2.69)	0.812	1.10 (0.21–5.64)	0.912	1.08 (0.56–2.08)	0.824
	rs2303656(G>T)	55/10/0	60/11/0	0.99 (0.39–2.52)	0.986	–	–	0.99 (0.39–2.52)	0.986
	rs763497(A>G)	45/16/4	51/17/3	1.13 (0.54–2.37)	0.740	1.49 (0.32–6.91)	0.613	1.15 (0.64–2.06)	0.646
	rs3900446(A>G)	51/14/0	51/17/2	0.70 (0.32–1.54)	0.374	–	–	0.65 (0.31–1.35)	0.248
LOXL1	rs2165241(C>T)	43/23/0	60/10/1	2.99 (1.32–6.78)	0.009*	–	–	2.53 (1.16–5.56)	0.020*
	rs3825942(G>A)	46/18/1	52/16/3	1.13 (0.53–2.39)	0.749	0.35 (0.04–3.49)	0.374	0.99 (0.52–1.89)	0.981
	rs2304721(C>A)	43/21/1	39/28/4	0.62 (0.31–1.25)	0.183	0.26 (0.03–2.41)	0.236	0.61 (0.33–1.13)	0.117
	rs12441130(T>C)	28/31/6	36/26/9	1.36 (0.69–2.67)	0.374	0.70 (2.24–2.09)	0.523	1.10 (0.67–1.81)	0.717
LOXL2	rs2294128(C>T)	47/17/1	53/17/1	1.13 (0.53–2.42)	0.757	1.09 (0.07–17.85)	0.950	1.11 (0.55–2.24)	0.765
	rs7818494(A>G)	44/19/2	39/28/4	0.58 (0.29–1.17)	0.129	0.53 (0.09–3.01)	0.475	0.63 (0.34–1.14)	0.126
	rs4323477(A>G)	21/30/14	18/36/17	0.71 (0.34–1.50)	0.371	0.87 (0.39–1.95)	0.738	0.83 (0.52–1.34)	0.446
	rs7818416(G>A)	21/28/16	21/37/13	0.88 (0.43–1.82)	0.731	1.46 (0.64–3.32)	0.371	1.07 (0.67–1.72)	0.771
	rs1063582(G>T)	39/16/10	43/25/3	1.02 (0.52–2.04)	0.947	4.12 (1.08–15.71)	0.038*	1.31 (0.78–2.18)	0.306
	rs2280936(C>G)	40/20/5	48/20/3	1.30 (0.65–2.64)	0.460	1.89 (0.43–8.24)	0.397	1.30 (0.74–2.29)	0.356
	rs2294133(C>T)	46/17/2	42/25/4	0.60 (0.29–1.22)	0.158	0.53 (0.09–3.01)	0.475	0.64 (0.35–1.18)	0.153
	rs2280935(A>C)	33/27/5	28/32/11	0.63 (0.32–1.25)	0.185	0.46 (1.15–1.39)	0.166	0.65 (0.39–1.09)	0.102
	rs1010156(T>C)	11/41/13	17/38/16	1.55 (0.66–3.61)	0.314	0.86 (0.38–1.96)	0.719	1.11 (0.66–1.87)	0.687
	rs142252012(G>A)	64/1/0	68/3/0	0.35 (0.04–3.49)	0.374	–	–	0.35 (0.04–3.49)	0.374
LOXL3	rs715407(T>G)	44/20/1	47/22/2	0.94 (0.46–1.91)	0.853	0.54 (0.05–6.09)	0.617	0.90 (0.47–1.72)	0.757
	rs6707302(C>T)	46/18/1	51/18/2	1.05 (0.50–2.22)	0.891	0.54 (0.05–6.09)	0.617	0.99 (0.51–1.92)	0.980
	rs17010021(T>A)	22/31/12	33/33/5	1.70 (0.85–3.40)	0.135	2.99 (0.99–9.02)	0.052	1.72 (1.03–2.89)	0.039*
	rs17010022(C>G)	38/22/5	26/33/12	0.41 (0.21–0.82)	0.011*	0.41 (0.14–1.24)	0.113	0.51 (0.30–0.85)	0.010*
LOXL4	rs3793692(G>A)	18/31/16	17/38/16	0.82 (0.38–1.78)	0.618	1.12 (0.51–2.48)	0.775	0.97 (0.60–1.56)	0.890
	rs1983864(G>T)	27/31/7	27/33/11	0.86 (0.43–1.72)	0.676	0.66 (0.24–1.82)	0.419	0.84 (0.51–1.38)	0.480
	rs7077266(G>T)	48/14/3	53/17/1	1.04 (0.48–2.25)	0.915	3.39 (0.34–33.41)	0.296	1.16 (0.60–2.24)	0.649

†, genotype presented as wild type/heterozygous/homozygous; *, P<0.05. IA, intracranial aneurysm; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; –, not available.

significant contribution to the cardiovascular system (25,26). In the present study, significant associations between *LOX* (rs1800449 and rs10519694) and single IA rupture were detected. Similar to our previous study, it was found that *LOX* was associated with IA susceptibility (14), but these results are inconsistent with those of a previous study by Hong *et al.*, who conducted a case-control study with 41 ruptured and 39 unruptured IA patients in a Korean population and showed that *LOX* may not be a susceptibility gene for IA rupture (17). We found that population heterogeneity may be the reason for the discordance between these 2 countries, and minor allele frequency in the

2 sites was discrepant between these 2 populations.

The *LOXL1* gene is located on chromosome 15q24.1. The homogeneity of *LOX* and *LOXL1* has been found to be as high as 88%, so their functions are similar (27). The pro-sequence contained by *LOX* and *LOXL1* can directly interact with the ECM to direct these enzyme deposits on the elastic tissues (28). The distinction of *LOXL1* from *LOX* is that *LOXL1* specifically locates at the elastic formation site and interacts with fibulin-5. Mice deficient in *LOXL1* did not deposit normal elastic fibers postpartum, thus demonstrating their specific role in elastogenesis (29). Recent studies have indicated that *LOXL1* may also have a role in type II collagen formation and suppression, as well as the promotion of tumorigenesis (30,31). *LOXL1* deficiency has been associated with pseudoexfoliation syndrome, idiopathic pulmonary fibrosis, and aneurysms (28,32). Our present study demonstrated that *LOXL1* rs2165241 was associated with multiple IAs ruptures. This is different from our previous study, which did not find an association between *LOXL1* and IAs susceptibility (14). This may be due to the fact that the pathobiology leading to IAs formation and its rupture are not exactly the same, causing the existing IA rupture (aSAH) is a separate process from an IA formation (12). Therefore, the association between *LOXL1* polymorphisms and aSAH and its mechanism needs to be further explored.

LOXL2 is located on chromosome 8p21.3 and its protein products are helpful in maintaining the integrity and stability of the vascular wall. Thus, *LOXL2* may play a role in susceptibility to IA rupture (33). The unbiased proteomic analysis demonstrated that LOXL2 could accelerate vascular sclerosis by promoting matrix stiffness and vascular smooth muscle stiffness and contractility (34), and an additional study has identified that *LOXL2* polymorphisms are associated with blood pressure (33). Increased vascular stiffness and high blood pressure are independent risk factors for cardiovascular diseases, such as stroke and subarachnoid hemorrhage (35). Akagawa *et al.* conducted an association study to systematically screen the *LOX* family genes in 402 IA patients and 462 controls from a Japanese population and found that *LOXL2* rs1010156 was associated with FIA (15). Using whole-exome sequencing, a significant association was also found with *LOXL2* in FIA patients from a Chinese population (16). Our previous research also found that *LOXL2* is associated with IA (14). Similarly, our present results also demonstrated that *LOXL2* is associated with IA rupture but with multiple IAs, not total IA or single IA rupture. If the same gene has different roles in the process of single and multiple IAs ruptures, this may be due to the higher rupture risk in patients with multiple IAs than in patients with a single IA (36); however, the mechanism of *LOXL2* in IA rupture is unclear, and further studies are required.

The *LOXL3* gene is located on chromosome 2p13.1 and its expression level has been found to be high in the heart, spleen, lung, aorta, and coronary arteries (37). *LOXL3* showed beta-aminopropionitrile inhibition of amine oxidase activity towards elastin and collagen. The highest activity was observed for type VIII collagen, which is a network collagen mainly expressed in vascular endothelial cells and smooth muscle cells, possibly having a role in the maintenance of vessel wall integrity (38). Mouse models have also described the oxidative effect of *LOXL3* on ECM fibronectin (39). In the present study, we found that *LOXL3* was associated with multiple IAs ruptures, suggesting that a variant of *LOXL3* may have a role in aSAH, but the mechanism of function needs to be further studied.

The *LOXL4* gene is located on chromosome 10q24.2, and contains an additional 13 amino acid inserts that differ from *LOXL2* and *LOXL3*. *LOXL4* is present in multiple human tissues, including the lung, liver, heart, brain, and colon (40). It has been found to be abnormally expressed in several tumors, and the potential biological function of *LOXL4* has been extended to the remodeling of the vascular ECM (41). Although our current results suggest that *LOXL4* may have a role in single IA rupture, whether it leads to IA rupture by affecting the remodeling of ECM or other methodologies is unclear; therefore, future studies are needed.

Our study had several limitations. First, the sample size was relatively small, which may have contributed to false associations due to limited statistical power; therefore, it is important to use larger studies to further verify the association between *LOX* family genes and aSAH. Second, we could not modify the morphological factors for multivariate analysis due to patients with 2 or more aneurysms in the multiple IA group; however, irregular aneurysms are more likely to rupture than regular aneurysms, and irregular aneurysms were more common in the ruptured group. Hence, we suggest that the univariate analysis results of multiple IA ruptures may provide a reference for multiple IA etiological research. Third, functional studies on susceptibility genes of IA rupture were not conducted, and we did not explore the specific mechanisms of aSAH; therefore, further research is needed to clarify the mechanism of function in the future. Despite

the above limitations, our present work provides evidence of the association between *LOX* family gene polymorphisms and aSAH. This may provide a basis for management and treatment of aSAH.

In summary, after exploring the association between *LOX* family genes and single and multiple IAs ruptures, we found that *LOX* and *LOXL4* may be associated with single IA rupture, while *LOXL1-3* were associated with multiple IAs ruptures in this Chinese sample. This suggests that the expression of *LOX* family genes may be associated with aSAH, which should be further studied and explored.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3484/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3484/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3484/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was reviewed and approved by The Ethics Committee at Central South University (permit No. CTXY–150002–1), and the other hospital (Hunan People's Hospital) was informed and agreed the study. The patients/participants provided their written informed consent to participate in this study, and the study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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Supplementary

Table S1 PCR primer information of SNPs site

Gene	SNPs	primer sequences (5'-3')		bp
		Upstream primer	Downstream primer	
LOX	rs1800449	AGAAGTTCCTGCGCTCAGTA	TGGGCCTTTCATAAGTATCG	134
	rs2956540	TTCACCTGTGAAACCATTCC	GAAATGGTGTCCTTCTGCTC	152
	rs10519694	ATGCCACATCACTCCACTTG	CTGAGGAAACTTCTCTAGAC	135
	rs2303656	CTGGGCAACACAAAGAGTTC	TTTCCATAACGTCTCCAGAG	141
	rs763497	ACATCTAGGCCTACATCGAG	TAAATGGCCCCCAACACAAG	129
	rs3900446	AGGAAGCAAAGCTCAGGTGG	CTTGAAGTTTCCCAGTAAGG	120
LOXL1	rs2165241	AAACTGAGCTCTCAAATGCC	CTCTCAATCAACTGGCTTCC	131
	rs3825942	ACCTCCGTCTCCCAGCAAC	TAGTTCTCGTACTGGCTGAC	143
	rs2304721	TGTTCATGTCCAATGTCCCC	CTGAGACCTAAATCTTCGGC	140
	rs12441130	AGCTTACATCTCGAGCTCTG	TTCATGCTGTTTTCCCTGCC	143
LOXL2	rs2294128	TGCCAAGTGGCCACACCTC	CATGAAGAATGTCACCTGCG	146
	rs7818494	GTTGGAAGGGAGGATAACAG	AGAATAGCGCAGACCTCAAC	140
	rs4323477	ATAGACGTTCAGCCACAAGG	AGCCAACTTAAGAGCCTAGC	126
	rs7818416	CAAGAGATCCTCCTACTCAG	ACCTTTGGCAATTCATTGGC	148
	rs1063582	TCTCTTGCCTTGTTGACCAG	AGTTCTCCTCCATGGCACAC	136
	rs2280936	AGCAGCTCTGTGGACAAACC	CTACAGCTGTGTCTAAGCTC	119
	rs2294133	CATTACCCCGAGTACTTCCA	CATCATAGTACACCTCCACC	139
	rs2280935	GGAGGGTTTCATTGGAAGAG	TGACACGTGGACAAATGCGG	127
	rs1010156	CATGAAGAATGTCACCTGCG	GTCCTCACCTCTGGCTTGTA	120
	rs142252012	CATCATAGTACACCTCCACC	ATTACCCCGAGTACTTCCAG	138
LOXL3	rs715407	GTCCCCTTTGGAACCTTTAC	AAGCTTCCCACTTCGAGTTC	133
	rs6707302	CACTATGATATCCTCACCCC	AAGGCTGTGCAATGGATACC	136
	rs17010021	ACTCAGTGTCTTCGAGACAG	CTTCTCCCCACAGGCATTAC	120
	rs17010022	ACTTCCTGATCTTTGCCATC	GTGAACAATCCTCAGCTGTG	128
LOXL4	rs3793692	GGATGACTGGGTTTCCTTAC	GATGGCAAGATCACCAATCC	140
	rs1983864	GATATGAGCGGACCCTCAG	ATGCTCAGACCCAAACTCAC	136
	rs7077266	TGGCATGAAGGGCCTCTATC	GGAGTTCTTATTCGTCAGGC	151

PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

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Table S2 Univariate analysis of the association between *LOX* family gene polymorphisms and single IA rupture

Gene	SNP	Genotype†		Dominant model		Recessive model		Additive model	
		Ruptured (n)	Unruptured (n)	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
LOX	rs1800449(C>T)	86/36/11	77/36/2	1.11 (0.65–1.88)	0.704	5.09 (1.11–23.48)	0.037*	1.30 (0.84–2.00)	0.239
	rs2956540(G>C)	75/41/17	62/46/7	0.91 (0.55–1.49)	0.696	2.26 (0.90–5.67)	0.082	1.10 (0.76–1.60)	0.618
	rs10519694(C>T)	115/15/3	86/17/12	0.46 (0.24–0.89)	0.021*	0.20 (0.05–0.72)	0.014*	0.51 (0.31–0.83)	0.007*
	rs2303656(G>T)	122/11/0	111/4/0	2.50 (0.77–8.09)	0.125	–	–	2.50 (0.77–8.09)	0.125
	rs763497(A>G)	100/32/1	77/32/6	0.67 (0.39–1.16)	0.154	0.14 (0.02–1.16)	0.068	0.63 (0.38–1.02)	0.058
	rs3900446(A>G)	110/19/4	93/21/1	0.88 (0.46–1.69)	0.708	3.54 (0.39–32.09)	0.262	1.02 (0.58–1.77)	0.958
LOXL1	rs2165241(C>T)	108/23/2	93/20/2	0.98 (0.51–1.85)	0.947	0.86 (0.12–6.22)	0.883	0.97 (0.55–1.71)	0.920
	rs3825942(G>A)	103/26/4	83/30/2	0.76 (0.43–1.34)	0.340	1.75 (0.32–9.75)	0.522	0.85 (0.52–1.41)	0.528
	rs2304721(C>A)	77/45/11	64/39/12	0.91 (0.55–1.51)	0.722	0.77 (0.33–1.83)	0.559	0.90 (0.62–1.32)	0.600
	rs12441130(T>C)	63/54/16	53/43/19	0.95 (0.58–1.57)	0.840	0.69 (0.34–1.42)	0.313	0.89 (0.63–1.27)	0.523
LOXL2	rs2294128(C>T)	105/26/2	93/2/20	1.13 (0.60–2.10)	0.707	0.86 (0.12–6.22)	0.883	1.09 (0.62–1.90)	0.769
	rs7818494(A>G)	84/40/9	74/34/7	1.05 (0.63–1.77)	0.846	1.12 (0.40–3.11)	0.828	1.05 (0.70–1.58)	0.810
	rs4323477(A>G)	32/67/34	26/64/25	0.92 (0.51–1.67)	0.788	1.24 (0.69–2.23)	0.481	1.05 (0.73–1.51)	0.786
	rs7818416(G>A)	44/66/23	33/59/23	0.81 (0.47–1.40)	0.457	0.84 (0.44–1.59)	0.585	0.86 (0.60–1.24)	0.422
	rs1063582(G>T)	82/47/4	72/38/5	1.04 (0.62–1.74)	0.877	0.68 (0.18–2.60)	0.576	0.99 (0.63–1.54)	0.957
	rs2280936(C>G)	86/43/4	67/42/6	1.76 (0.46–1.28)	0.302	0.56 (0.16–2.05)	0.383	0.77 (0.49–1.19)	0.237
	rs2294133(C>T)	81/39/13	67/35/13	0.90 (0.54–1.49)	0.672	0.85 (0.38–1.92)	0.695	0.91 (0.63–1.32)	0.629
	rs2280935(A>C)	53/57/23	41/61/13	0.84 (0.50–1.40)	0.497	1.64 (0.79–3.41)	0.185	1.04 (0.72–1.50)	0.837
	rs1010156(T>C)	45/63/25	30/55/30	0.69 (0.40–1.20)	0.186	0.66 (0.36–1.20)	0.170	0.75 (0.53–1.06)	0.102
	rs142252012(G>A)	129/4/0	112/3/0	1.16 (0.24–5.28)	0.850	–	–	1.16 (0.25–5.28)	0.850
LOXL3	rs715407(T>G)	90/41/2	71/38/6	0.77 (0.46–1.30)	0.330	0.28 (0.06–1.40)	0.121	0.73 (0.46–1.15)	0.169
	rs6707302(C>T)	94/38/1	74/35/6	0.75 (0.44–1.28)	0.288	0.14 (0.02–1.16)	0.068	0.68 (0.43–1.10)	0.114
	rs17010021(T>A)	57/63/13	54/49/12	1.18 (0.71–1.95)	0.517	0.93 (0.41–2.13)	0.863	1.08 (0.74–1.59)	0.680
	rs17010022(C>G)	57/63/13	53/49/13	1.14 (0.69–1.88)	0.610	0.85 (0.38–1.92)	0.695	1.04 (0.71–1.52)	0.839
LOXL4	rs3793692(G>A)	25/69/39	26/68/21	1.26 (0.68–2.34)	0.459	1.86 (1.02–3.39)	0.044*	1.40 (0.96–2.04)	0.082
	rs1983864(G>T)	40/73/20	32/55/28	0.90 (0.52–1.56)	0.697	0.55 (0.29–1.04)	0.066	0.78 (0.54–1.13)	0.189
	rs7077266(G>T)	95/37/1	75/36/4	0.75 (0.44–1.28)	0.294	0.21 (0.02–1.91)	0.166	0.71 (0.44–1.16)	0.173

†, genotype presented as wild type/heterozygous/homozygous; *, P<0.05; SNP, single nucleotide polymorphism; IA, intracranial aneurysm; OR, odds ratio; CI, confidence interval; –, not available.

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