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Lysyl oxidase family genes polymorphisms and risk of aneurysmal subarachnoid hemorrhage: A case control study

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Abstract

Introduction:

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 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 two cohorts: 133 and 115 single ruptured and unruptured IA patients, and 65 and 71 multiple ruptured and unruptured IAs patients, respectively. Genotyping of 27 single nucleotide polymorphisms (SNPs) in *LOX* was performed. Logistic regression analysis was performed to calculate the odds ratios (OR) and 95% confidence intervals (CI) of the SNPs of *LOX* and risk of aSAH.

Results

LOX rs180044 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 demonstrated risk effects on multiple IAs rupture. *LOXL3* rs17010022 showed a protective effect on multiple IAs rupture (dominant model: OR = 0.41, 95% CI = 0.21–0.82; additive model: OR = 0.51, 95% CI = 0.30–0.85).

Discussion

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

Introduction

Intracranial aneurysms (IA) are a complex disease characterized by incomplete integrity of the artery wall, which is typically induced by pathologic expansion and swelling of the weak artery wall [1]. It 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., one aneurysm) or multiple (i.e., equal or more than two aneurysms) events, which accounts for approximately 20–30% of cases [5]. Most IAs are asymptomatic before rupture; however, rupture can result in an aSAH, which is one of the most devastating conditions with 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 [7–9]. For instance, studies have revealed that smoking and hypertension are predictors of IA rupture [10]; first-degree relatives of aSAH patients are more likely to be diagnosed with IA or aSAH, which indicates a familial tendency [11]. Studies have reported that dysregulation of the extracellular matrix (ECM) in the vascular wall is a significant cause of aSAH [12]. Vascular remodeling is a complex pathophysiological alteration, such as intimal hyperplasia and adventitial fibrosis, that may subsequently lead to a variety of vascular diseases such as hypertension, aneurysm formation, and rupture [13, 14]. ECM dynamics have been recognized as a significant part of controlling vascular remodeling [13]. Elastin and collagens are abundant matrix proteins in the ECM, while the LOX family of genes in the extracellular copper-containing enzyme family that initiates cross-linking of collagen and elastin by oxidative deamination of lysine residues [15]. Therefore, the LOX family genes may have 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. *LOX-like* (*LOXL*) *2*, which belongs to the *LOX* family of genes, was found to be associated with susceptibility to familial IA (FIA) in Chinese and Japanese populations [16, 17]; however, whether *LOXL2* is also associated with aSAH remains unclear. In Korea, Hong et al. discovered that *LOX* was associated with IA formation and rupture using candidate gene association analysis [18]. 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 the associations between *LOX* family gene polymorphisms and aSAH in a Chinese sample and provide a reference for the etiological study of aSAH.

Materials And Methods

Study population

A total 384 patients of IA were collected from two third-class hospitals in Hunan province 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 current project was approved by the Ethics Committee of Central South University (permit No: CTXY−150002−1). All patients provided informed consent.

Snp Selection And Genotyping

Single nucleotide polymorphisms (SNP) were selected based on tag SNPs and the Genome Variation Server 150 (http://gvs.gs.washington.edu/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, placed in EDTAK2 anticoagulant tubes (10 mL), and refrigerated at 4°C. A blood genomic DNA extraction kit (Tiangen Biochemical Technology Co. Ltd., Beijing, China) was used for DNA extraction and refrigerated at -80°C. 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 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°C, followed by 45 cycles of 30 s at 9°C, 30 s at 56°C, 60 s at 72°C, and finally extension for 5 min at 72°C. The final products were stored at 25°C until further use. After shrimp alkaline phosphatase, single nucleotide extension, resin desalination steps, and matrix assisted laser desorption ionization time-of flight mass spectrometry reaction, MassArray TYPER 4.0 software (Sequenom) was used to interpret the genotype of each sample target site.

Table 1
General demographic and clinical characteristics of the subjects

Variable	**single IA ruptured (n = 133)**	**single IA unruptured (n = 115)**	***P***	**multiple IAs ruptured (n = 65)**	**multiple IAs unruptured (n = 71)**	***P***
Age (year)	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)	

SD, standard deviation; Bold font indicates $p < 0.05$.

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. Continuous variables distributed normally were compared using t-tests, and non-normally distributed variables were compared using Mann-Whitney U tests. Categorical variables were compared using the chi-square or Fisher's exact tests between the two groups. The association between *LOX* family gene polymorphisms and risk of IA

rupture was evaluated by odds ratios (OR) and 95% confidence intervals (CI) using logistic regression in additive, recessive, and dominant models. Differences were considered statistically significant at $P < 0.05$.

Results

Characteristics of 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). Regardless of single or 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 ($P > 0.05$).

Associations between LOX family genes polymorphisms and risk of single IA rupture

Univariate analysis revealed that *LOX* rs1800449, *LOX* rs10519694, and *LOXL4* rs3793692 were associated with a 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, $P = 0.037$). Nevertheless, *LOX* rs10519694 demonstrated a protective effect on single IA rupture under all three 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 a 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.

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

Gene	SNP	Genotype[a]		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

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; –, not available. [a]Genotype presented as wild type/heterozygous/homozygous; Bold font indicates p < 0.05.

Gene	SNP	Genotype[a]		dominant model		recessive model		additive model	
		ruptured (n)	unruptured (n)	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P
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

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; –, not available. [a]Genotype presented as wild type/heterozygous/homozygous; Bold font indicates p < 0.05.

Associations between LOX family genes polymorphisms and risk of multiple IAs rupture

Since every patient with multiple IAs had two or more IA, we were unable to adjust for morphological confounders for multivariate analysis. The univariate analysis results indicated that *LOXL1* rs2165241 was associated with multiple IA rupture (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 rupture in the recessive model (OR = 4.12, 95% CI = 1.08–15.71, *P* = 0.038). We found that two sites in *LOXL3* were significantly associated with multiple IAs ruptures, but the directionality of the function was different. rs17010021 was associated with the risk of multiple IAs rupture (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 IAs rupture (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).

Table 3
Univariate analysis of the association between LOX family genes polymorphisms and multiple IAs rupture

Gene	SNP	Genotypea		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

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; –, not available. [a]Genotype presented as wild type/heterozygous/homozygous; Bold font indicates p < 0.05.

Gene	SNP	Genotypea		dominant model		recessive model		additive model	
		ruptured (n)	unruptured (n)	OR(95%CI)	P	OR(95%CI)	P	OR(95%CI)	P
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

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; –, not available. [a]Genotype presented as wild type/heterozygous/homozygous; Bold font indicates p < 0.05.

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 rupture, suggesting that members of the *LOX* family genes 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 four scavenger receptor cysteine-rich domains [19]. 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 [20]. These family genes are critical enzymes that regulate the crosslinking of elastin and collagen to have a regulatory role in ECM assembly [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 [14, 22]. 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 [15, 23]. Genetic mouse models for *LOX* have also demonstrated its significant contribution to the cardiovascular system [24, 25]. In the present study, significant associations between *LOX* (rs1800449 and rs10519694) and single IA rupture were detected. Our results are inconsistent 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, suggesting that *LOX* may not be a susceptible gene for IA rupture [18]. We found that population heterogeneity may be the reason for this discordance between these two countries, and minor allele frequency in the two sites was discrepant between these two populations [18].

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 similarly expressed [26]. The pro-sequence contained by *LOX* and *LOXL1* can directly interact with the ECM to direct these enzyme deposits on the elastic tissues [27]. The distinction from *LOX* was that *LOXL1* specifically locates at the elastic formation site and interacts with fibuin-5. Mice deficient in *LOXL1* did not deposit normal elastic fibers postpartum, thus demonstrating their specific role in elastogenesis [28]. Recent studies have indicated that *LOXL1* may also have a role in type II

collagen formation and suppression, and promotion of tumor tumorigenesis [29, 30]. *LOXL1* deficiency has been associated with pseudoexfoliation syndrome, idiopathic pulmonary fibrosis, and aneurysms [27]. Our present study demonstrated that *LOXL1* rs2165241 was associated with multiple IAs rupture, which has not previously been found in other studies. 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 have a susceptible role in IA rupture [44, 32]. The unbiased proteomic analysis demonstrated that LOXL2 could accelerate vascular sclerosis by promoting matrix stiffness and vascular smooth muscle stiffness and contractility [31], and additional studies have identified that *LOXL2* polymorphisms are associated with blood pressure [32]. Increased vascular stiffness and high blood pressure are independent risk factors for cardiovascular diseases, such as stroke and subarachnoid hemorrhage [33]. 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 [16]. Using whole-exome sequencing, a significant association was also found with *LOXL2* in FIA patients from a Chinese population [17]. 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 IA ruptures, this may due to the higher rupture risk in patients with multiple IAs than patients with a single IA [34]; 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 [35]. *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 [36]. Mouse models have also described the oxidative effect of *LOXL3* on ECM fibronectin [37]. Recently, *a LOXL3* mutation was identified in a family with a father and son, with Stickler syndrome characterized by myopia and retinopathy [38]. In the present study, we found that *LOXL3* was associated with multiple IAs rupture, 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 insert that differs from *LOXL2* and *LOXL3*. *LOXL4* is present in multiple human tissues, including the lung, liver, heart, brain, and colon [39]. Detected to be abnormally expressed in several tumors, the potential biological function of *LOXL4* has been extended to the remodeling of the vascular ECM [40]. Although our current results suggest tha*t 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 the *LOX* family genes and aSAH. Second, we could not modify the morphological factors for multivariate analysis due to patients with two or more aneurysms in the multiple IA group; however, considering irregular aneurysms are more likely to rupture than regular aneurysms, and irregular aneurysms were more common in the ruptured group [41, 42]. Hence, we suggest 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 further 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, which can be used in future studies.

Conclusions

In summary, after exploring the association between *LOX* family genes and single and multiple IA ruptures, we found that *LOX* and *LOXL4* may be associated with single IA rupture, while *LOXL1-3* were associated with multiple IA ruptures in this Chinese sample. This suggests that although there is existing homology among *LOX* family genes, there are still some differences in the pathogenesis of single and multiple IA ruptures, which should be further studied and explored.

Declarations

Ethics approval and consent to participate

The studies involving human participants were reviewed and approved by The Ethics Committee at Central South University (Permit No: CTXY−150002−1). The patients/participants provided their written informed consent to participate in this study and abides to the Helsinki Declaration ethical principles for medical research involving human participants.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors have no conflicts of interest to declare.

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Authors' contributions

Conceptualization: RD, CL, and JY. Data curation: RD, CL, and JL. Formal analysis: CL and RD. Funding acquisition: JY. Investigation: CL, RD, CH, BL, JL, LH, XL, JZ, LX, SL, YL, DY, and WJ. Methodology: RD, CL, and JY. Project administration: CL, RD, and JY. Resources: CH, JL, WJ, and JY. Supervision: CL, CH, and JY. Writing—original draft preparation: CL. Writing— review and editing: JY. All authors contributed to the article and approved the submitted version.

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