Association between *AXIN1* gene polymorphisms and epithelial ovarian cancer in Chinese population

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Aim: The study was aimed to explore the association between *AXIN1* gene polymorphism and epithelial ovarian cancer (EOC) susceptibility as well as prognosis. **Methods:** A total of 165 EOC cases and 327 healthy controls were recruited to participate in this study. In total three tag SNPs in *AXIN1* gene were genotyped using PCR-restriction fragment length polymorphism. **Results:** Significantly increased EOC risk was found associated with the A/C heterozygous genotype of rs12921862, the C allele and C/T genotype in the rs1805105 and the T/T genotype of rs370681. Meanwhile, Kaplan–Meier survival curves showed that patients with rs12921862 C/C genotype improved overall survival in the EOC group. **Conclusion:** Our results suggest that the *AXIN1* gene may be related to susceptibility and overall survival in EOC.

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Keywords: *AXIN1* gene • epithelial ovarian cancer • morphisms • PCR-RFLP • single nucleotide polymorphisms • Wnt/ β -catenin signaling pathway

Epithelial ovarian cancer (EOC) is the most common type of invasive ovarian cancer and is a leading cause of cancer-associated mortality from gynecological tumors globally [1]. Although a number of factors are involved in the molecular mechanism regulating EOC, these risk factors are not well defined and so disease pathogenesis remains unclear. Even with treatment, patient outcomes for EOC are not optimistic, as prognosis and relative 5-year survival rates have remained disappointing [2]. For this reason, the search for specific biomarkers, which can be used to screen for EOC, its development and prognosis have become a focus in the molecular oncology field. Current studies have shown that many biomarkers, such as *TP53* and *BRCA1/2*, are associated with the development of EOC [3,4]. However, further research is needed to find additional unique markers that represent the development and prognosis of EOC.

AXIN1 encodes 65-kb gene region on chromosome 16p13.3 and plays a vital role during embryonic development [5]. *AXIN1* is a multidomain scaffolding protein that interacts with multiple proteins and serves as a key negative regulator of Wnt signaling pathway [6]. This pathway is critical in human tumorigenesis by regulating cellular proliferation and differentiation in vertebrates as well as invertebrates via the β -catenin destruction complex [5]. Impaired regulation of the Wnt-dependent target genes has been shown to be involved in the occurrence of several other human cancers and its abnormal expression has been associated with tumorigenesis, for example, breast cancer, endometrial cancer [7], colon carcinoma [8], medulloblastoma [9], melanoma [10], hepatocellular carcinoma [11] and gastric cancer [12]. In addition, studies have shown that complete inactivation of *AXIN1* function led

Table 1. Characteristics of the studied population.

Characters	Patients (%)	Controls
Sample size, N	165	327
Age $\pm$ SD, years	49.5 $\pm$ 10.9	51.0 $\pm$ 12.2
Clinical histology, n:		–
– Serous	96 (58)	–
– Mucinous	19 (12)	–
– Endometrioid	7 (4)	–
– Clear cell	12 (7)	–
– Other types	31 (19)	–
Tumor stage, n:		
– I	39 (24)	–
– II	17 (10)	–
– III	101 (61)	–
– IV	8 (5)	–
Tumor grade, n:		
– G1–G2	37 (22)	–
– G3	107 (65)	–
– NA	21 (13)	–

G: Grade; NA: Not available; SD: Standard deviation.

to early embryonic lethality in mice. In other words, the *AXIN1* gene may participate in embryonic and postnatal development as well as pathogenesis [13].

SNPs are the most common type of genetic variation in human genome. A SNP can alter the expression of a gene, which leads to a specific cellular phenotype. For example, polymorphisms within *AXIN1* gene have been shown to be associated with the development of diseases and cancers [5,14]. While previous studies have investigated the role of Wnt/ β -catenin pathway in the development of EOC. Here, we aimed to analyze the association between *AXIN1* polymorphisms and EOC prevalence as well as survival.

Materials & methods

Subjects

Our study was performed with the approval of the ethics review board of the West China Second University Hospital of Sichuan University, and all the participants gave written informed consent. A total of 165 unrelated EOC patients ranging in age from 15 to 77 years old (mean $\pm$ standard deviation; 49.5 $\pm$ 10.9) between 2007 and 2016 at the West China Second University Hospital of Sichuan University were enrolled in the hospital-based case–control study. Clinical characteristics and follow-up data of patients were abstracted from telephone calls every 6 months for 5 years. All the diagnosis of patients was confirmed by histological examination of tissue from resected specimens. Patients with borderline ovarian tumors, two or more different malignancies, autoimmune and infectious diseases, or metastasized cancer from other origins, which might affect the final results, were excluded. Tumor stage was classified based on the International Federation of Gynecology and Obstetrics ovarian cancer staging criteria (FIGO, 2014) [15]. The control subjects including 327 participants ranging in age from 22 to 83 years old (mean $\pm$ standard deviation; 51.0 $\pm$ 12.2) had no genetic relationship with each other from a routine health survey in the same hospital, excluding the one with personal or family history of EOC or other severe diseases. All clinical information include age, histological type, tumor grades and FIGO stages were showed in Table 1. All subjects were Han population living in southwest of China.

Tissue collection & DNA extraction

Genomic DNA of all participants was extracted from 200 μ l EDTA-anticoagulated peripheral blood sample by a whole-blood DNA isolation kit (Bioteke, Peking, China). The procedure was performed according to the instructions of the manufacturer, and the products were stored at -20°C until needed.

Table 2. Primer sequences and enzymes.

SNPs	Primer (5'-3')	Annealing temperature (°C)	Enzyme	Product length (bp)
rs12921862	F: ctcacgccagtgccctact R: atgcatccatgtggaact	59	ScrFI	A:216 C:110 + 106
rs1805105	F: ctggatacctgccgacctta R: acctttcctggcttgttct	65	FokI	C:245 T:186 + 59
rs370681	F: gaggcctaagctccaggcact R: aaggaaagtggtttctccacca	66	Bts ⁹ I	T:166 C:150 + 16

bp: Base pair; SNP: Single nucleotide polymorphism.

Figure 1. Genotypes of AXIN1 rs12921862, rs1805105 and rs370681. (A) Genotypes of rs12921862. 1-2: A/C genotype; 3-4: C/C genotype; 5: A/A genotype; M: marker. **(B)** Genotypes of rs1805105. 1,4: C/T genotype; 2: C/C genotype; 3: T/T genotype. **(C)** Genotypes of rs370681. 1: C/T genotype; 2,4: T/T genotype; 3: C/C genotype. M: Marker.

SNP & genotyping

A tag SNP is a representative SNP in a region of the genome with high linkage disequilibrium (the nonrandom association of alleles at two or more loci). Three tag SNPs were picked out according to data from tag SNPs genotyped in the CHB population sample of the HapMap Project (Data Release 24/Phase II, National Center for Biotechnology Information [NCBI] build 36 assembly, dpSNPb126; www.hapmap.org/index.html.zh) [5]. Primers were selected by utilizing online software (<http://frodo.wi.mit.edu/primer3/>). The three-tag SNPs (rs12921862, rs1805105 and rs370681) were genotyped by a PCR-restriction fragment length polymorphism assay (PCR-RFLP), which is a sensitive, accurate and cost-effective approach to genotype the SNPs. The reaction procedure referred to our group's previous research [16]. Table 2 presented the primer sequences (TsingKe, Chengdu, China), PCR conditions, restriction enzymes (Biolabs, UK) and length of digested products. Digested products were distinguished on 6% polyacrylamide gels and stained with 1.0 g/l silver nitrate (Figure 1). In addition, about 10% of the samples were randomly selected to perform repeated assays (DNA sequencing) and the results were 100% concordant.

Table 3. Allele frequencies of tag single nucleotide polymorphisms in *AX* among patients and controls, and their association with epithelial ovarian cancer risk.

SNP	Allele	Patients, n (%) [‡]	Controls, n (%) [§]	OR (95% CI)	p-value
rs12921862	C	268 (81%)	631 (96%)	1.00	1e⁰⁴
	A	62 (19%)	23 (4%)	6.35 (3.85–10.46) [†]	
rs1805105	T	230 (70%)	501 (77%)	1.00	0.019
	C	100 (30%)	153 (23%)	1.42 (1.06–1.92) [†]	
rs370681	C	228 (69%)	472 (72%)	1.00	0.314
	T	102 (31%)	182 (28%)	0.86 (0.65–1.15)	

[†]Significant difference at the 5% level.

[‡]N = 165.

[§]N = 327.

OR: Odds ratio.

Patients' clinical characteristics & follow-up

The information of tumor stage (FIGO stages I + II and III + IV), tumor grade (G1 + G2 and grade G3), the histological type (serous and others) and ages (<45 and ≥45 years old) was collected from the medical records of the 165 patients. Based on the records, 116 patients were randomly recruited in the follow-up study for the survival outcomes and tumor recurrence analysis. We collected the patient clinical outcomes from the primary date of the diagnosis to the cancer recurrence or death. The clinical follow-up study was performed in a blind manner with regard to patient status.

Statistical analysis

Relevant methodologies for data statistics can refer to relevant literature [17] and our team's research [5]. Differences of genotype and allele frequency between the cases and controls were evaluated using χ^2 test, with odds ratios (ORs) and 95% CI. Pearson's χ^2 test was used to test Hardy–Weinberg equilibrium among the cases and controls. OR > 1 indicates the exposure factor is positively correlated with the disease, and that the exposure factor increases the risk of disease occurrence; OR < 1 indicates the exposure factor is negatively correlated with the disease, and the exposure factor has protective effect on the prevention of disease occurrence; if OR is close or equal to 1, it indicates that the exposure has no association with the disease. Genotypic association test in a case–control pattern assuming co-dominant, dominant, recessive and overdominant genetic models was performed by SNPStats (www.snpsstats.net/start.htm; take rs1292862 as an example, C/C is a wild homozygous genotype, A/C is a heterozygous mutant genotype and A/A is a homozygous mutant genotype. There are co-dominant genetic model: A/A vs C/C and A/C vs C/C; dominant genetic model: A/C-A/A vs C/C; recessive genetic model: A/A vs A/C-C/C; and overdominant genetic model: A/A-C/C vs A/C. Survival rates were calculated with the Kaplan–Meier method, with the association between *AXIN1* SNPs genotypes and patients, outcome from the date of primary diagnosis until recurrence or death evaluated by the log-rank test. We also used multivariate survival analysis carried out by Cox regression analysis to determine the independent effects on recurrence and survival. A level of $p < 0.05$ was considered to be statistically significant. SPSS for Windows™22.0 (SPSS, Inc., IL, USA) was used for the statistical analyses.

Results

Association between *AXIN1* gene polymorphisms & EOC

The SNPs rs12921862, rs1805105 and rs370681 in the *AXIN1* gene were successfully genotyped in 165 EOC patients and 327 healthy control subjects. Genotype distribution of these three polymorphisms in control subjects was consistent with the Hardy–Weinberg equilibrium. Allele frequencies of these three polymorphisms for all subjects are shown in Table 3. The frequency of A allele at the forrs12921862 locus was significantly higher in patients with EOC (OR: 6.35; 95% CI: 3.85–10.46; $p < 0.0001$). For rs1805105, the C allele carried an increased risk of EOC (OR: 1.42; 95% CI: 1.06–1.92; $p = 0.019$), but allele frequencies for rs370681 polymorphisms had no difference between EOC patients and control subjects (OR: 0.86; 95% CI: 0.65–1.15; $p = 0.314$).

As shown in Table 4, for SNP rs12921862, a significantly increased EOC risk was associated with the A/C genotype compared with the C/C genotype in a co-dominant model (OR: 6.45; 95% CI: 3.47–11.96; $p < 0.0001$). Compared with C/C carriers, A/C-A/A genotypes carriers had a 6.24-fold greater EOC risk (OR: 6.24; 95% CI: 3.45–11.27; $p < 0.0001$) in the dominant model. Moreover, compared with the patients carrying C/C-A/A

Table 4. Genotype frequencies of tag single nucleotide polymorphisms in *AXIN1* among patients and controls, and their association with epithelial ovarian cancer risk.

Genetic model	Genotype	Patients, n (%)	Controls, n (%)	Logistic regression	
				OR (95% CI)	p-value
rs12921862					
Co-dominant	C/C	108 (65.5%)	306 (93.6%)	1.00	1e ⁰⁴ †
	A/C	52 (31.5%)	19 (5.8%)	6.45 (3.47–11.96)†	
	A/A	5 (3%)	2 (0.6%)	4.54 (0.80-25.62)	
Dominant	C/C	108 (65.5%)	306 (93.6%)	1.00	1e ⁰⁴ †
	A/C-A/A	57 (34.5%)	21 (6.4%)	6.24 (3.45–11.27)†	
Recessive	C/C-A/C	160 (97%)	325 (99.4%)	1.00	0.160
	A/A	5 (3%)	2 (0.6%)	3.30 (0.58–18.65)	
Overdominant	C/C-A/A	113 (68.5%)	308 (94.2%)	1.00	1e ⁰⁴ †
	A/C	52 (31.5%)	19 (5.8%)	6.25 (3.37–11.59)†	
Log additive				4.96 (2.84–8.65)†	1e ⁰⁴ †
rs1805105					
Co-dominant	T/T	74 (44.9%)	196 (59.5%)	1.00	0.0072†
	C/T	82 (49.7%)	109 (33.3%)	2.00 (1.29–3.10)†	
	C/C	9 (5.5%)	22 (6.7%)	1.47 (0.60–3.58)	
Dominant	T/T	74 (44.9%)	196 (59.9%)	1.00	0.0022†
	C/T-C/C	91 (55.1%)	131 (40.1%)	1.92 (1.26–2.93)†	
Recessive	T/T-C/T	156 (94.5%)	305 (93.3%)	1.00	0.870
	C/C	9 (5.5%)	22 (6.7%)	1.08 (0.45–2.57)	
Overdominant	T/T-C/C	83 (50.3%)	218 (66.7%)	1.00	0.0025†
	C/T	82 (49.7%)	109 (33.3%)	1.92 (1.26–2.94)†	
Log additive				1.55 (1.11–2.18)†	0.011†
rs370681					
Co-dominant	C/C	81 (49.1%)	163 (49.9%)	1.00	0.095
	C/T	66 (40%)	146 (44.6%)	0.91 (0.61–1.35)	
	T/T	18 (10.9%)	18 (5.5%)	2.01 (0.99–4.08)	
Dominant	C/C	81 (49.1%)	163 (49.9%)	1.00	0.87
	C/T-T/T	84 (50.9%)	164 (50.1%)	1.03 (0.71–1.50)	
Recessive	C/C-C/T	147 (89.1%)	309 (94.5%)	1.00	0.034†
	T/T	18 (10.9%)	18 (5.5%)	2.10 (1.06–4.16)†	
Overdominant	C/C-T/T	99 (60%)	181 (55.4%)	1.00	0.32
	C/T	66 (40%)	146 (44.6%)	0.83 (0.57–1.21)	
Log additive				1.17 (0.87–1.57)	0.30
†Significant difference at the 5% level. OR: Odds ratio.					

† Significant difference at the 5% level.

OR: Odds ratio.

genotypes, A/C genotype carriers had a 6.25-fold greater EOC risk (OR: 6.25; 95% CI: 3.37–11.59; $p < 0.0001$). Distributions of rs1805105 polymorphisms were also significantly different between patients and control subjects. Compared with the T/T genotype carriers, the C/T genotype carriers had a twofold increased risk of EOC (OR: 2.00; 95% CI: 1.29–3.10; $p = 0.0072$). C/T-C/C genotypes had a 1.92-fold increased EOC risk (OR: 1.92; 95% CI: 1.26–2.93; $p = 0.0022$) compared with the T/T genotype in the dominant model. Compared with C/C-T/T homozygotes, a significantly increased EOC risk was observed to be associated with C/T heterozygotes (OR: 1.92; 95% CI: 1.26–2.94; $p = 0.0025$). As for rs370681, the carriers of the T/T genotype had increased EOC susceptibility in a recessive model, compared with C/C-C/T genotypes (OR: 2.10; 95% CI: 1.06–4.16; $p = 0.034$).

AXIN1 SNPs & EOC patient characteristics

Some studies have found that the prognosis of EOC is related to some factors, such as age, stage, histological type and grade – among others [18,19]. In order to further evaluate whether specific genotypes and allele polymorphisms were associated with certain clinical features of EOC patients, we performed a stratification analyses of genotypes and allele distributions in EOC patients with different FIGO stages (I + II and III + IV), tumor grades (G1 + G2 and G3), histological types (serous and others) and ages (<45 and ≥ 45 years old). However, no significant association was observed among three tag SNPs and stratifications of EOC patient characteristics (Tables 5–7, $p > 0.05$).

Table 5. Association between the genotype distribution of rs12921862 polymorphism of *AXIN* gene and clinical features of epithelial ovarian cancer.

Clinical features	rs12921862														
	Genotype			Genetic model											
	C/C	A/C	A/A	Co-dominant (C/C vs A/C vs A/A)	OR (95% CI)	p-value	Dominant (C/C vs A/C-A/A)	OR (95% CI)	p-value	Recessive (C/C-A/C vs A/A)	OR (95% CI)	p-value	Overdominant (C/C-A/A vs A/C)	OR (95% CI)	p-value
Tumor stage:															
– I–II	24	11	2	1.07 (0.46–2.51)	0.10	0.91 (0.40–2.06)	0.81	NA (0.00–NA)	0.03*	1.16 (0.50–2.70)	0.73				
– III–IV	53	26	0	NA (0.00–NA)											
Tumor grade:															
– G1–G2	52	26	0	0.88 (0.38–2.06)	0.99	1.04 (0.46–2.36)	0.93	NA (0.00–NA)	0.03*	0.81 (0.35–1.90)	0.63				
– G3	25	11	2	NA (0.00–NA)											
Histology:															
– Serous	41	24	1	0.62 (0.27–1.39)	0.49	0.64 (0.29–1.41)	0.26	1.33 (0.08– 21.74)	0.84	0.61 (0.27–1.38)	0.23				
– Others	36	13	1	1.14 (0.07– 18.87)											
Age:															
– <45	26	14	1	0.84 (0.37–1.89)	0.83	0.82 (0.37–1.81)	0.62	0.54 (0.03–8.87)	0.67	0.85 (0.38–1.92)	0.70				
– ≥45	51	23	1	0.51 (0.03–8.48)											
*Significant difference at the 5% level. It is not analyzed here because of the null values. G: Grade; NA: Not applicable; OR: Odds ratio.

Figure 2. Kaplan–Meier survival curves. Showed that patients with rs12921862 A/A-A/C genotype had a higher risk for death than patients with C/C genotype ($p = 0.028$).

Analysis of association between *AXIN1* gene polymorphisms & patient prognosis

One hundred and sixteen EOC patients who had provided contact information were contacted for follow-up. A total of 25 patients had died and 21 patients experienced recurrence. These findings were used to evaluate recurrence-free survival and overall survival in the univariate analysis by Kaplan–Meier survival curves. We found that patients with the rs12921862 C/C genotype had a longer overall survival than patients with the A/C-A/A genotype ($p = 0.028$) (Figure 2). However, rs1805105 and rs370681 of *AXIN1* were not associated with the prognosis of EOC. In addition, Cox regression model, adjusted for age, histology type, FIGO stage and tumor

Table 6. Association between the genotype distribution of rs1805105 polymorphism of *AXIN1* gene and clinical features of epithelial ovarian cancer.

Clinical features	rs1805105										
	Genotype			Genetic model							
	C/C	C/T	T/T	Co-dominant (T/T vs C/T vs C/C)	Dominant (T/T vs C/T-C/C)	Recessive (T/T-C/T vs C/C)		Overdominant (T/T-C/C vs C/T)			
						OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Tumor stage:											
– I–II	2	20	15	0.90 (0.40–2.01)	0.72	0.86 (0.39–1.89)	0.70	0.45 (0.06–3.36)	0.44	0.96 (0.44–2.11)	0.93
– III–IV	2	42	35	0.43 (0.06–3.33)							
Tumor grade:											
– G1–G2	4	41	33	0.99 (0.45–2.18)	0.20	0.91 (0.41–1.98)	0.80	NA (0.00–NA)	0.08*	1.11 (0.51–2.43)	0.78
– G3	0	21	17	NA (0.00–NA)							
Histology:											
– Serous	4	33	28	1.12 (0.53–2.36)	0.09	1.00 (0.48–2.09)	0.99	NA (0.00–NA)	0.03*	1.28 (0.61–2.67)	0.51
– Others	0	29	22	NA (0.00–NA)							
Age:											
– <45	0	23	18	0.95 (0.44–2.07)	0.17	1.05 (0.49–2.27)	0.90	NA (0.00–NA)	0.06*	0.85 (0.39–1.82)	0.67
– ≥45	4	39	32	NA (0.00–NA)							
*Significant difference at the 5% level. It is not analyzed here because of the null values. G: Grade; NA: Not applicable; OR: Odds ratio.											

*Significant difference at the 5% level. It is not analyzed here because of the null values.
G: Grade; NA: Not applicable; OR: Odds ratio.

grade, was used to estimate the prognosis of EOC. The results revealed that there was no significant association between *AXIN1* SNPs and prognosis of EOC.

Discussion

EOC is the most common type of invasive ovarian cancer and arises from the ovarian surface epithelium or the secondary müllerian system [20]. In the last two decades, taxane/platinum-based chemotherapy, intraperitoneal delivery of chemotherapy and dose-dense chemotherapy – among others, have largely improved the overall survival of patients with advanced ovarian cancer. However, the overall survival for EOC still remains poor so that new therapeutic paradigms were required. In addition, more studies are needed to understand the molecular pathways in EOC [21]. Hence, we aimed to provide a clearer understanding of pathogenesis, metastasis, recurrence and drug resistance in EOC with a molecular genetic study. Involvement of Wnt pathway in embryonic development of the ovary was established in the late 1990s [7]. The frequent defects of Wnt signaling pathway played an important role in ovarian endometrioid adenocarcinomas [22,23]. EOC often harbors mutations in the *β-catenin* gene, but mutations in the Wnt/*β-catenin* pathway were rare in serous, clear cell and mucinous ovarian carcinomas [24]. Despite do not have mutations, the Wnt/*β-catenin* pathway plays a role in carcinogenesis of all ovarian cancer subtypes [25–27]. However, gene mutations in the Wnt/*β-catenin* pathway are relatively uncommon in ovarian cancer, especially in serous ovarian cancer, components of the pathway are still important in molecular events that lead to EOC development [27].

AXIN, together with APC proteins, is thought to function in the assembly of *β-catenin* destruction complex because interactions between AXIN–APC and AXIN–*β-catenin* are essential for promotion of *β-catenin* phosphorylation and degradation. As one regulates components of the Wnt/*β-catenin* pathway, playing a crucial role in controlling axis formation during embryonic development [28]. Subsequent research of *AXIN* in some human hepatocellular carcinomas and colorectal cancer (CRC), indicated that *AXIN* is a tumor suppressor gene [29,30].

Table 7. Association between the genotype distribution of rs370681 polymorphism of *AXIN1* gene and clinical features of epithelial ovarian cancer.

Clinical features	rs370681										
	Genotype			Genetic model							
	C/C	C/T	T/T	Co-dominant (C/C vs C/T vs T/T)		Dominant (C/C vs C/T-T/T)		Recessive (C/C-C/T vs T/T)		Overdominant (C/C-T/T vs C/T)	
				OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Tumor stage:											
– I–II	14	19	4	0.50 (0.21–1.17)	0.23	0.49 (0.22–1.12)	0.09	0.64 (0.15–2.68)	0.55	0.56 (0.25–1.27)	0.16
– III–IV	42	32	5	0.46 (0.10–2.06)							
Tumor grade:											
– G1–G2	40	32	6	1.57 (0.69–3.57)	0.56	1.50 (0.68–3.30)	0.31	0.94 (0.22–4.07)	0.94	1.54 (0.69–3.39)	0.29
– G3	16	19	3	1.17 (0.25–5.33)							
Histology:											
– Serous	31	29	5	0.97 (0.45–2.09)	1.00	0.97 (0.46–2.02)	0.93	0.97 (0.24–3.86)	0.96	0.97 (0.46–2.05)	0.94
– Others	25	22	4	0.95 (0.23–3.97)							
Age:											
– <45	21	16	4	1.26 (0.56–2.83)	0.76	1.17 (0.54–2.52)	0.69	0.71 (0.18–2.87)	0.64	1.30 (0.60–2.85)	0.51
– ≥45	35	35	5	0.79 (0.19–3.33)							

G: Grade; OR: Odds ratio.

AXIN1, instead of its homolog *AXIN2*, was demonstrated to be responsible for a series of dominant ‘kinky’ tail mouse phenotypes resulting from spontaneous transposon insertions into an *Axin1* exon [31].

AXIN1 gene polymorphisms were associated with various diseases, such as clear cell renal cell carcinoma [14], gastrointestinal cancers [30], esophageal cancer [32], breast cancer as well as colon cancer [33,34] and so on. It is plausible that SNPs in *AXIN1* affect an individual’s susceptibility to EOC. *AXIN1* gene polymorphisms might become useful prognostic biomarkers for EOC patients and novel genetic therapeutic interventions involving *AXIN1* can be envisaged.

In this study, we investigated the association between three SNPs in the *AXIN1* gene (rs12921862A/C, rs1805105C/T and rs370681C/T) and the susceptibility as well as prognosis in EOC patients for the first time. Among the three tag SNPs in *AXIN1*, two of them are located in the intron region, and the other is located in the synonymous codon region. Introns and other nonprotein coding RNAs may have evolved to function as network control molecules in complex organisms [35]. Therefore, we suspected that the SNPs may work as controls influencing the conduct of other genes, instead of affecting protein structure by influencing amino acid coding. Our results show that in EOC population, patients with the A allele and A/C genotype of rs12921862 polymorphism in *AXIN1* gene are more common. Additionally, A allele carriers in rs12921862 had a worse overall survival, which was consistent with earlier discussion of A allele as a risk factor for EOC. These results are in line with previous observations from Yoshida *et al.* that showed molecular changes of Wnt pathways are a major contributor to a poor prognosis in EOC [36]. ‘Power and Sample Size Calculation’ software (version 3.0.43) was used to calculate the sample power, the results indicated that the present study has >80% power to certify the association between *AXIN1* polymorphisms and EOC susceptibility. C/T genotype carriers of rs1805105 and T/T genotype carriers of rs370681 increased the risk of cryptorchidism [5]. This important discovery was consistent with our findings. Pu *et al.* indicated the rs1805105 C/T-C/C genotypes, and rs12921862 A/A genotype may increase clear cell renal cell carcinoma susceptibility. In addition, patients carrying the rs1805105 C/T genotype had an increased risk of developing terminal cancer [14]. In the current study, the C allele and C/T genotype of rs1805105 polymorphisms

also increased the risk of EOC. In another study, increased atrial septal defect (ASD) risk was found to be associated with rs12921862 polymorphism as well as rs370681 [37].

In brief, the *AXIN1* gene affected disease susceptibility and prognosis in diverse diseases and could be used for risk factor analysis. We also performed stratification analysis; however, the genetic polymorphisms of *AXIN1* gene were not significant even among different clinical stratifications. Contrastingly, research by Dubeau shows that endometrioid ovarian carcinomas harbor mutations in the β -catenin gene, but mutations in the Wnt/ β -catenin pathway are more rare in other ovarian carcinomas [38]. Some limitations of our study should be noted. For example, the number of endometrioid ovarian carcinomas patients was too small to compare with other groups, with only seven patients. More recent studies have shown that gene mutations in the Wnt/ β -catenin pathway are relatively uncommon in ovarian cancer, especially in serous ovarian cancer [27]. Remarkably, no significant association was found between groups of serous ovarian cancer and other types of ovarian cancer in our results. The precise mechanism of *AXIN1* in the development of EOC remains unclear, so further studies are necessary to confirm these findings.

Conclusion

In conclusion, our results indicated that SNPs within *AXIN1* may affect individual susceptibility and prognosis in EOC. The *AXIN1* may be a potential prognostic biomarker for EOC, while novel genetic therapeutic interventions involving *AXIN1* can also be envisaged.

Summary points

- *AXIN1* has been involved in the development of epithelial ovarian cancer (EOC).
- A total of 165 EOC cases and 327 healthy controls were enrolled in the hospital-based case-control study.
- The three tag SNPs were genotyped by a PCR-RFLP assay.
- Significantly increased EOC risk was found associated with the A/C heterozygous genotype of rs12921862, the C allele and C/T genotype in the rs1805105 and the T/T genotype of rs370681.
- Kaplan–Meier curves revealed a correlation between the C/C homozygote of rs12921862 and an improved prognosis in the EOC group.
- Our results suggested that the *AXIN1* gene may be related to susceptibility and overall survival in EOC.

Financial & competing interests disclosure

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Ethical conduct of research

Our study was performed with the approval of the ethics review board of the West China Second University Hospital of Sichuan University and all the participants gave written informed consent. The number of the ethical approval is 2012016.

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