

Original Article

MicroRNA-155 as a potential plasma non-invasive biomarker for the diagnosis and prognosis of abdominal aortic aneurysm

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Received January 20, 2016; Accepted July 14, 2016; Epub September 1, 2016; Published September 15, 2016

Abstract: Objective: To study the relationships of microRNA-155 (miR-155) expression with the clinicopathological features and prognosis in patients with abdominal aortic aneurysm (AAA). Methods: One hundred and sixteen cases with AAA were selected for the case group, and one hundred and thirty elderly individuals who had undergone physical examination during the same period at The First Affiliated Hospital of Wenzhou Medical University were selected for the control group. Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to detect the expression of miR-155. Results: The plasma miR-155 expression in the case group increased significantly compared to that in the control group ($P < 0.05$). According to the AAA classification, the miR-155 expression in patients with type I was higher than that in type IIA; the expression in type I and IIA was higher than that in type IIB; and the expression in type I and IIA was higher than that in type IIC (all $P < 0.05$). The miR-155 expression was positively related to age and tumor size ($r = 0.643, P < 0.001$ and $r = 0.240, P = 0.010$, respectively). Also, the miR-155 expression increased in patients with smoking history, hypertension, and renal dysfunction. The higher miR-155 expression after treatment indicated an increased incidence of complication. Conclusion: These findings suggested that the miR-155 expression is related to the clinicopathological features and prognosis of AAA, indicating that miR-155 serves as a potential plasma non-invasive biomarker for the diagnosis and prognosis of AAA.

Keywords: MicroRNA-155, abdominal aortic aneurysm, prognosis, diagnosis, clinicopathological features, biomarker

Introduction

Abdominal aortic aneurysm (AAA) is a fatal disease clinically known as a localized enlargement of the infra-renal aorta with a diameter of ≥ 3 cm or larger than normal by more than 50% [1]. The incidence of AAA ranks first in all kinds of aneurysm; if untreated, it is common to develop aneurysm rupture. Upon aneurysm ruptures, the mortality rate could be as high as 80% within 12 h [2]. Some risk factors are associated with the increased incidence of AAA, including old age, male gender, smoking, hypertension, atherosclerosis and gene-related factors [3]. Evidence indicates that AAA is a result of an interaction of genetics, oxidative stress, inflammation and other factors [4]. A variety of micro-RNAs (microRNA and miRNA) are involved in mediating the development and

progression of AAA, including miRNA-29 and miRNA-21 [5, 6]. Currently, miRNA provides a new therapeutic approach for the treatment of AAA [7].

MiRNA is a class of endogenous non-coding RNA with regulatory functions in eukaryotic cells and with a size of approximately 20-24 nucleotides [8]. MiRNA is highly conserved, cell-specific, phase-specific and time-specific, with an important role in the regulation of immune response, and its expression can be considered an ideal tumor diagnostic indicator because it is related to the tumor pathological process. Some scholars have speculated that serum miRNA comes from tumor cells and has certain functions and thus can be used as direct evidence for the existence of tumor cells [9, 10]. MicroRNA-155 (miR-155) is a typical

miRNA located on human chromosome 21 [11]. MiR-155 is widely expressed in multicellular organisms and viruses mainly via matching nucleic acid complementary sequences and binding to a specific target miRNA to inhibit miRNA target translation or to modulate the degradation of the target miRNA. Interestingly, miR-155 is also a product of B-cell integration cluster (BIC) oncogene expression [12]. It is found that miR-155 showed high expression in a variety of human solid-state malignant tissues, including pancreatic cancer, renal cell carcinoma, nasopharyngeal carcinoma, cervical cancer, colon cancer and other malignancies [13-17]. The data also indicated that plasma miR-155 expression in patients with pancreatic ductal adenocarcinoma significantly increased, indicating that blood biomarkers may be considered prognostic indicators [18].

MiR-155 is highly expressed in a variety of malignant tumors, suggesting that miR-155 may be associated with the occurrence of AAA. The current study was conducted to explore the association of miR-155 with clinicopathological features of AAA by detecting plasma miR-155 expression in patients with AAA.

Material and methods

Ethics statement

This study was approved by the ethics committee of The First Affiliated Hospital of Wenzhou Medical University, and all of the patients provided signed informed consent.

Research subjects

Between January 2010 and March 2014, 116 patients with AAA admitted to The First Affiliated Hospital of Wenzhou Medical University were selected for the case group. The clinical diagnosis of AAA was confirmed by Doppler Ultrasound, Computed Tomography and angiography with a tumor diameter greater than normal by more than 50%. Among 116 cases, there were 59 males and 57 females, with a mean tumor diameter of 6.4 ± 1.3 cm and a mean age of 57.0 ± 14.1 years (age range 37~87). The inclusion criteria were as follows: Patients meeting diagnostic criteria for AAA [19]; Patients with clinical symptoms of AAA; Patients without any clinical manifestations but with a tumor diameter of > 5 cm or tumor growth of > 1 cm/year

and with a tendency to rupture; and Patients with a tumor diameter of > 4.5 cm with treatment required. The exclusion criteria were as follows: Patients not meeting the inclusion criteria; Patients with thoracic AAA; Patients diagnosed with infectious AAA; Patients diagnosed with secondary AAA; Patients with serious autoimmune diseases; Patients with severe mental illness, making it difficult for investigation and physical examination; and Patients pregnant or having cancer. Based on the study of Schumacher *et al.* [20], the classification for AAA was defined as follows: 43 cases of type I with a proximal aneurysm neck greater than 1.5 cm and a distal aneurysm neck greater than 1.0 cm; 28 cases of type IIA with a proximal aneurysm neck greater than 1.5 cm and AAA implicated in the aortic bifurcation; 26 cases of type IIB with a proximal aneurysm neck greater than 1.5 cm and AAA involved in the distal iliac artery; 19 cases of type IIC with a proximal aneurysm neck greater than 1.5 cm and abdominal aorta distal tumors involved in the distal iliac artery bifurcation; and 0 cases of type III with a proximal aneurysm neck less than 1.5 cm. All of the patients received endovascular surgery. Alternatively, 130 individuals without AAA and severe chronic diseases were selected for the control group during the same period at The First Affiliated Hospital of Wenzhou Medical University, among which there were 82 males and 48 females with ages ranging from 35~80 years and a mean age of 59.1 ± 10.8 years.

Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Fasting venous blood was collected before treatment and 15 days after surgery in the case group and placed in special tubes. Precipitate samples of red blood cells were collected after centrifugation at 4°C and frozen at -80°C for total RNA extraction. Blood specimens from the control group were extracted and preserved based on the same method used in the case group.

A red blood cell total RNA extraction kit was purchased from Beijing Tianenze Gene Technology Co. Ltd., and a reverse transcriptase kit was purchased from Hangzhou Bori Technology Co., Ltd. All of the procedures described above were performed in accordance with the manu-

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Table 1. Clinical features in the case and control groups

Features	Case group (n = 116)	Control group (n = 130)	P value
Average age	57.0 ± 14.1	59.1 ± 10.8	0.195
Male/Female	59/57	82/48	0.070
Smoking history	60	90	0.006
Diabetes mellitus	12	18	0.440
Hypertension	51	34	0.005
Coronary heart disease	26	12	0.005
Chronic renal dysfunction	8	4	0.236

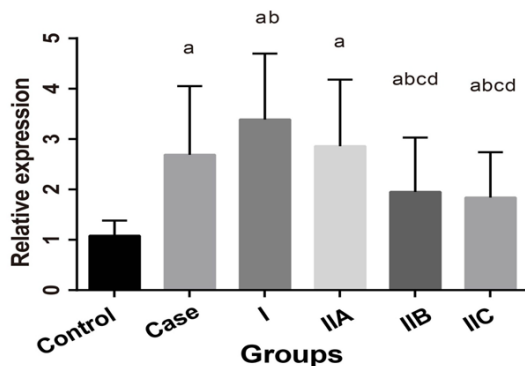


Figure 1. Difference in the plasma miR-155 expression between the case and control groups, and among the AAA patients with type I, type IIA, type IIB and type IIC. Note: ^a*P* < 0.05 compared to the control group; ^b*P* < 0.05 compared to the case group; ^c*P* < 0.05 compared to the Type I group; ^d*P* < 0.05 compared to the type IIA group. (AAA, abdominal aortic aneurysm).

facturer's instructions. qRT-PCR was used to detect target mRNA in the samples. The primer sequences were as follows: microRNA-155, forward: 5'-ACACTCCAGCTGGGTAGCTTATCAGACT-3', reverse: 5'-CTCAACTGGTGTCTGGAGTCGGCAA-3'. U6 was used as an internal reference: upstream: 5'-CTCGCTTCGGCAGCACAA-3', downstream: 5'-AACGCTTCACGAATTTGCGT-3' [21]. qRT-PCR kits were purchased from Bio-Rad, and the RT-PCR instrument was the 7500 PCR System from ABI Germany. The PCR reaction procedure was performed as follows: pre-denaturation for 5 min at 94°C, denaturation for 30 s at 94°C, annealing for 30 s at 58°C, and extension for 1 min at 72°C, with 40 cycles of denaturation-annealing-extension. Differences between the case group and the control group in mRNA gene expression are expressed using the following formula: $N = (1 + E) \cdot \Delta\Delta C_T$ [22], where E represents the amplification efficiency of the target gene primers, E =

$10^{-1/\text{standard curve slope}}$ [23], $\Delta\Delta C_T = (C_{T \text{ miR-155-1}} - C_{T \text{ U6-1}}) - (C_{T \text{ miR-155-2}} - C_{T \text{ U6-2}})$, 1 represents the case group, 2 represents the control group, and U6 is the internal reference.

Follow-up

All of the patients that were discharged from the hospital were followed up with eight months to two years through hospital review or telephone interview, starting from the time of AAA diagnosis until March 2015, with a follow-up interval of two months. No cases were lost to follow-up.

Statistical methods

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses, with the expression of mean ± standard deviation (SD) for measurement data. A one-Way ANOVA was used to analyze the measurement data among multiple groups, a *t* test was used to compare the two groups, and count data were compared using a χ^2 test. A *t* test was used in the comparison of age and miR-155 expression between the case group and control group, and Receiver operating characteristic (ROC) curve in the analysis of power of miR-155 expressions used to detect abdominal aortic aneurysm. The relationship between miR-155 expression and clinicopathologic features of patients was analyzed by one-way analysis of variance (ANOVA) and *t* test, and the change of miR-155 expression after treatment was also examined by *t* test. Chi-square test was employed to compare the general information including gender, diabetes history, chronic renal insufficiency history, smoking history, and comorbidity with hypertension between the case group and control group, and to analyze complication condition of the patients with different miR-155 expression. *P* < 0.05 was considered statistically significant.

Results

General condition of the study subjects

The clinical features of the two groups are shown in **Table 1**. In the case group, there were 116 cases in total, including 59 males and 57 females with a mean age of 57.0 ± 14.1 years. In the control group, there were 130 individuals in total, including 82 males and 48 females

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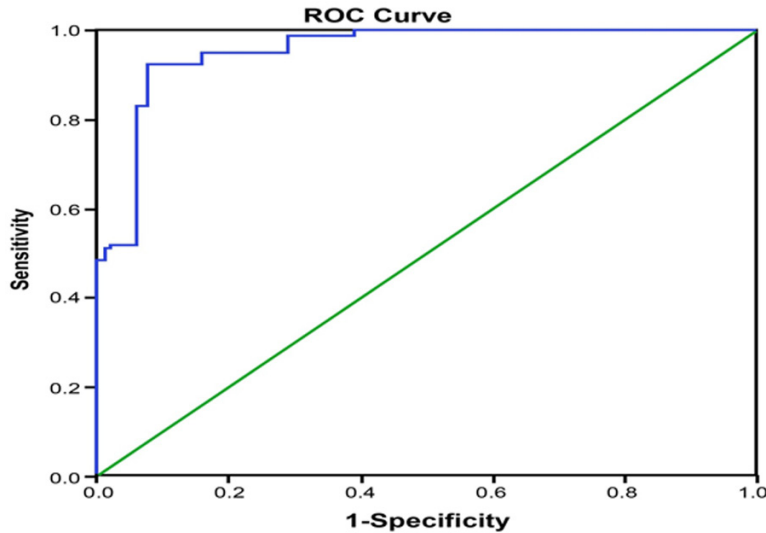


Figure 2. ROC curve analysis for the plasma miR-155 expression used to diagnose AAA. Note: the ROC curve for miR-155 expression in the AAA tissue and adjacent normal tissue. AUC for miR-155 is 0.952, indicating statistical significance. (CI: confidence interval; AUC: area under the curve; ROC curve: receiver operating characteristic curve; AAA, abdominal aortic aneurysm).

with a mean age of 59.1 ± 10.8 years. The differences between the two groups in age, sex ratio, diabetes history and history of chronic renal dysfunction were not statistically significant (all $P > 0.05$). In the case group, there were 60 cases with a history of smoking, 51 cases with hypertension and 26 cases with coronary heart disease. In the control group, there were 90 cases with a history of smoking, 34 cases with hypertension and 12 cases with coronary heart disease. The ratios of patients with a history of smoking, hypertension or coronary heart disease in the case group were significantly higher than those in the control group (all $P < 0.05$).

Comparison of plasma miR-155 expression between the case group and control group

Plasma miR-155 expression was compared between the case group and control group, which were shown in **Figure 1**. The results showed that plasma miR-155 expression in the case group significantly increased compared to that in the control group in terms of age, tumor diameter, smoking history and a history of hypertension (all $P < 0.05$). The difference in miR-155 expression in the case group was shown here: type I group was higher than type IIA; type I and type IIA were higher than type IIB; and type I and type IIA were higher than type IIC (all $P < 0.05$).

Diagnostic value for miR-155 expression in abdominal aortic aneurysm

A receiver operating characteristic (ROC) curve (**Figure 2**) showed that the area under the curve (AUC) of plasma miR-155 of AAA was 0.952 [95% CI (0.927, 0.977)], and the optimal cutoff value was 1.306 (sensitivity 92.2%, specificity 83.8%) with the maximum Youden index as an alternative basis.

Relationship between plasma miR-155 expression and clinicopathological features in patients with AAA

Our results showed that miR-155 expression in patients aged > 50 years were higher

than those in patients aged ≤ 50 years ($P < 0.05$). Also, the larger tumor diameter was, the higher miR-155 expression was (all $P < 0.05$). In the case group, the miR-155 expression in patients with a history of smoking and hypertension was higher than that in patients without the corresponding symptoms or complications (all $P < 0.05$). The miR-155 expression was positively correlated with age and tumor size ($r = 0.643$, $P < 0.001$ and $r = 0.240$, $P = 0.010$, respectively, **Table 2**).

Relationship between miR-155 expression and prognosis of abdominal aortic aneurysm

The difference in the expression of miR-155 between patients with AAA before and after treatment was shown in **Table 3**. The results demonstrated that the expression of miR-155 in all patients after treatment was lower than that before treatment ($P < 0.05$). Four types of AAA patients were assigned into the low miR-155 expression group and the high miR-155 expression group, in accordance with the optimal cutoff value from the ROC curve. The follow-up conducted within 12 months after surgery showed that among the 116 cases, there were 22 death cases within one month with a mortality rate of 18.97%. The causes of death were as follows: (1) 5 cases of sudden cardiopulmonary arrest and death and (2) 17 cases died of multiple organ failure, of which 6 cases

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Table 2. Relationship between plasma miR-155 expression in patients with abdominal aortic aneurysm and clinicopathological features

Features	MiR-155 expression			
	I (n = 43)	IIA (n = 28)	IIB (n = 26)	IIC (n = 19)
Age				
> 50	4.10 ± 0.91 ^a	3.78 ± 0.78 ^a	3.01 ± 1.68 ^a	2.70 ± 1.55 ^a
≤ 50	2.58 ± 1.25	2.06 ± 1.16	1.56 ± 0.29	1.54 ± 0.03
Gender				
Male	3.60 ± 1.28	3.14 ± 1.22	1.99 ± 1.05	1.90 ± 0.90
Female	3.19 ± 1.35	2.53 ± 1.39	1.89 ± 1.18	1.81 ± 0.94
Tumor diameter				
> 7 cm	3.86 ± 0.74 ^c	3.68 ± 1.20 ^{b,c}	3.44 ± 1.78 ^{b,c}	4.36 ± 0.59 ^{b,c}
5-7 cm	3.57 ± 1.35 ^c	2.76 ± 1.30	1.74 ± 0.72	1.54 ± 0.02
≤ 5 cm	2.73 ± 1.38	2.04 ± 1.10	1.43 ± 0.20	1.57 ± 0.10
Smoking history				
Yes	3.74 ± 1.14 ^d	3.35 ± 1.11 ^d	2.70 ± 1.48 ^d	2.67 ± 1.57 ^d
No	2.81 ± 1.42	1.98 ± 1.24	1.49 ± 0.15	1.55 ± 0.05
Diabetes mellitus				
Yes	3.28 ± 1.42	3.12 ± 1.11	1.73 ± 0.53	1.56 ± 0.00
No	3.41 ± 1.32	2.84 ± 1.35	1.98 ± 1.14	1.88 ± 0.95
Hypertension				
Yes	3.84 ± 1.01 ^e	3.45 ± 1.19 ^e	2.52 ± 1.30 ^e	2.50 ± 1.47 ^e
No	2.92 ± 1.11 ± 1.45	2.18 ± 1.14	1.70 ± 0.90	1.54 ± 0.03
Coronary heart disease				
Yes	3.35 ± 1.11 ± 1.78	2.91 ± 1.20	1.92 ± 1.24	1.62 ± 0.10
No	3.40 ± 1.24	2.84 ± 1.39	1.97 ± 1.03	1.87 ± 0.95
Chronic renal dysfunction				
Yes	3.29 ± 2.49	2.64 ± 1.97	1.70 ± 0.21	1.51 ± 0.01
No	3.40 ± 1.29	2.87 ± 1.31	1.97 ± 1.12	1.88 ± 0.95

Note: ^a*P* < 0.05 compared to age ≤ 50 years; ^b*P* < 0.05 compared to a tumor diameter of 5-7 cm; ^c*P* < 0.05 compared to a tumor diameter of ≤ 5 cm; ^d*P* < 0.05, compared to patients without a smoking history; ^e*P* < 0.05 compared to patients without hypertension.

Table 3. Difference in the miR-155 expression before and after treatment for abdominal aortic aneurysm

Types	Before treatment (n = 116)	After treatment (n = 94)	<i>P</i> value
I	3.39 ± 1.31	1.72 ± 0.86	< 0.001
IIA	2.86 ± 1.32	1.56 ± 0.81	< 0.001
IIB	1.95 ± 1.08	1.56 ± 0.49	0.001
IIC	1.84 ± 0.90	1.62 ± 0.61	0.037

developed into gastrointestinal bleeding in postoperative time and hemorrhagic shock leading to organ failure; 8 cases developed into severe pulmonary infection and respiratory failure, leading to organ failure; and 3 cases developed into acute renal failure, leading to multiple

organ failure. The incidence probability of complication for patients with high miR-155 expression was greater than patients with low miR-155 expression (*P* < 0.05) (Table 4). Based on the results above, it can be inferred that the miR-155 expression was related to the prognosis of AAA. Moreover, the higher the expression of miR-155 was before treatment, the higher expression of miR-155 was at prognosis, leading to a greater chance of developing complications.

Discussion

AAA is a life-threatening arterial degenerative process [24] and can lead to aneurysm rupture under the worst conditions with a high mortality rate. With the difficulty of the self-discovery of

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Table 4. Complications of AAA patients with low- and high-expression miR-155 within the 12-month follow-up after treatment

	I (n = 43)		IIA (n = 19)		IIB (n = 19)		IIC (n = 13)	
	Low (n = 28)	High (n = 15)	Low (n = 13)	High (n = 6)	Low (n = 12)	High (n = 7)	Low (n = 8)	High (n = 5)
Retroperitoneal abscess	0	0	1	0	0	0	0	0
Infections around stents	0	0	0	1	1	1	0	0
Lower limb ischemia	1	3	1	1	0	1	0	1
Brachial artery pseudoaneurysms	0	1	0	1	0	0	0	0
Inner leakage	0	3	0	1	0	2	0	1
In-stent restenosis	1	1	0	0	0	0	0	0
Iliac artery occlusion	0	1	0	0	0	0	0	1
Total case number	2	9	2	4	1	4	0	3
Complication probability (%)	7.14	60.00 ^a	15.38	66.67 ^a	8.33	57.14 ^a	0	60.00 ^a

Note: AAA, abdominal aortic aneurysm; ^a*P* < 0, compared with patients with low miR-155 expression.

AAA, it is urgent to develop new diagnostic markers. Also, miRNA is an important factor for tumorigenesis and development, including up-regulating certain cancers and down-regulating other factors [25, 26], with a similar role in suppressing tumor genes. This study focused on the systematic study of plasma miR-155 expression in patients with AAA to explore the relationship between the clinicopathological features of AAA and its prognosis.

This study found that plasma miR-155 significantly increased in the case group (different age, different tumor diameter, smoking history and hypertension) compared to the control group. In addition, some differences in the miR-155 expression within the case group were shown: Type I was higher than type IIB; Type IIA was higher than type IIB; and type IIA was higher than type IIC. The results indicated that the expression of miR-155 was related to the occurrence and type of AAA. AAA is an inflammatory disease and is related to the activity and expression of inflammatory transcription factors in tissue [4]. In patients with AAA at active stages of inflammation, the serum levels of the Th1-type cytokines INF- γ and TNF- α in the culture supernatant of T lymphocytes and peripheral blood increased and were positively correlated with the abdominal aortic diameter [27]. Moreover, a study found that INF- γ could induce the up-regulation of miR-155 expression [28] and that elevated INF- γ expression could result in increased miR-155 and TNF- α expressions.

In this study, miR-155 expression was positively correlated with age and tumor size, and

patients with smoking history, coronary heart disease, renal failure and chronic obstructive pulmonary disease presented an increased miR-155 expression, suggesting that increased age may contribute to the enlargement of the tumor diameter. The miR-155 expression increased, especially in patients with complications, indicating that miR-155 may be involved in the occurrence of AAA through a joint action with a certain substance or mechanism. The occurrence of AAA is a complex process involving inflammatory reactions, biochemical factors, hemodynamic factors and other factors. Among them, MMPs play an important role in the development of aneurysms [29]. Increased MMPs can degrade elastin, causing changes in the aortic wall tension and elasticity, resulting in the formation of aneurysms [30]. Correspondingly, a previous study found that MMP-2 and MMP-9 can inhibit the formation of AAA [31]. On the other hand, miR-155 expression specifically down-regulates MMPs [32]. When miR-155 increased, MMPs expression decreased, reducing the inhibition of aneurysms and eventually leading to AAA formation.

Our study also found that the increased postoperative miR-155 expression indicates increased chances of complications, suggesting that the miR-155 expression may be used as a predictor of AAA for diagnosis and prognosis. MiR-155 may be involved in cell proliferation by down-regulating target genes and plays a role in anti-apoptosis by blocking Caspase 3 activities or the inhibition of pro-apoptotic genes [33]. It can also target or regulate the TGF- β /

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Smad4 pathway, which is related to cell invasion [34], indicating that increased miR-155 expression enhances inflammation in patients with AAA, changing apoptosis and invasion and resulting in an increased complication incidence. This was supported by a study that showed that higher miR-155 expression in the uterus leads to a worse prognosis of endometrial cancer tissue for patients [35].

This study investigated the relationship between miR-155 and AAA and confirmed that miR-155 serves as a potential non-invasive biomarker for AAA diagnosis and prognosis. However, our results have certain limitations. For instance, our study also discussed the prognosis of patients with AAA receiving endovascular treatment, and no involvement was mentioned regarding traditional surgical methods. To determine whether the results of endovascular treatment were consistent with those of traditional surgery, additional case data are needed for validation. Moreover, due to the limitation of the small sample size, our results can only represent research trends; many more samples are needed for more accurate results.

Acknowledgements

We are particularly grateful to all the people who have assisted with our article, without whose support, this study would not have been possible. No foundation project supported this study.

Disclosure of conflict of interest

None.

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