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Effect of remote ischemic preconditioning on perioperative neurocognitive disorder in elderly patients undergoing major surgery and associated genetic variant analysis: a randomized clinical trial

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Abstract

Objective To investigate whether remote ischemic preconditioning (RIPC) could reduce the incidence of perioperative neurocognitive disorder (PND) in elderly patients undergoing major surgery (> 2 h), to assess the potential of myeloid differentiation factor 2 (MD2) and cystatin C as biomarkers and to identify key genetic variants associated with PND.

Methods From August 2020, 250 patients scheduled for major surgeries under general anesthesia were screened and 120 patients were randomly assigned to the control group or the RIPC group. After anesthesia induction, patients in the RIPC group received a blood pressure cuff around their right upper limb, which was pressurized to 200 mmHg to induce ischemia, whereas the cuff in the control group was pressurized to only 60 mmHg. A total of five cycles were repeated with ischemia for five minutes and reperfusion for five minutes. Six neurological tests were performed before and after the surgery to assess the incidence of PND. Serum levels of myeloid differentiation factor 2 (MD2) and Cystatin C and PND-associated single nucleotide polymorphisms were analyzed by ELISA and whole genome sequencing, respectively. This study adhered to CONSORT research guidelines.

Results In the RIPC group, the incidence of PND (44%) was comparable to that in the control group (44%, $P = 0.982$). There was no significant difference in the concentrations of MD2 or cystatin C between the NPND and PND groups. A total of 3877 mutated genes were exclusively identified in PND patients. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that these mutated genes are enriched in synapse function. Notably, a *Shank3* variant (SNP rs4824145) was included.

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Conclusions RIPC had little effect on the incidence of PND in elderly patients who underwent major surgery (> 2 h). MD2 and cystatin C were unable to predict the occurrence of PND. Patients harboring rs4824145 in the *Shank3* gene may be more susceptible to PND.

Trial registration.

Chinese Clinical Trial Registry (ChiCTR2000035020(07/28/2020)).

Keywords Remote ischemic preconditioning, PND, Elderly patients, Whole genome sequencing

Background

Perioperative neurocognitive disorder (PND) is a common complication induced by major surgery and manifests as acute impairments in memory, attention, information processing and execution [Xin et al. 2022]. It is associated with productivity losses and increased disability and mortality. The incidence of PND varies from 9 to 54%, affected by multiple predisposing risk factors, including old age, surgical type and protocols used to diagnose PND [Bhushan et al. 2021]. Currently, PND has been recognized as a major clinical problem, identifying patients at high risk of PND and attempts to reduce the occurrence of PND through perioperative period are highly important.

In a variety of clinical observations, remote ischemic preconditioning (RIPC), a transient sublethal episode of ischemia–reperfusion applied in limbs, has been shown to increase the ischemia tolerance of remote vital organs, including the brain[Pico et al. 2020], heart[Hausenloy et al. 2019], liver[Rakic et al. 2018] and kidney [Zarbock et al. 2015]. However, the therapeutic potential of RIPC in preventing the occurrence of PND is still under debate. A pilot study involving 70 patients aged below 40 years or above 80 years who underwent off-pump coronary artery bypass graft surgery demonstrated that RIPC could not reduce the incidence of PND[Joung et al. 2013], this finding is supported by another pilot study in which 180 adult patients (≥ 18 years) who underwent elective cardiac surgery with cardiopulmonary bypass were enrolled [Meybohm et al. 2013]. However, these studies were challenged by a follow-up pilot investigation suggesting that patients (≥ 55 years) treated with RIPC exhibited better cognitive performance after cardiac surgery [Hudetz et al. 2015]. Notably, all the studies mentioned above were limited to cardiac surgery, the potential effect of RIPC on PND remains to be elucidated in patients undergoing other major surgery. Although it has been reported that RIPC could reduce the incidence of PND in elderly patients (≥ 65 years) after laparoscopic cholecystectomy [Han et al. 2023].

Myeloid differentiation factor 2 (MD2), an essential secreted glycoprotein for toll-like receptor 4 activation, is critical for the activation of the immune system and the release of inflammatory cytokines [Fang et al. 2021]. We previously reported that MD2 silencing or administration of the MD2-degrading peptide Tat-CIRP-CMA could attenuate cognitive decline after surgery [Zuo et al. 2021]. Additionally, lower serum cystatin C, a cysteine-protease inhibitor, is associated with higher risk of Alzheimer's disease independently of age [Sundelof et al. 2008]. Our previous work demonstrated that Cystatin C mediated the neuroprotective autophagic flux induced by hyperbaric oxygen preconditioning [Fang et al. 2019]. However, whether serum MD2 or cystatin C functions as a peripheral biomarker for PND and mediates the neuroprotection of RIPC therapy remains to be elucidated.

This study aimed to assess the effect of RIPC on the incidence of PND in elderly patients who underwent major surgeries for at least two hours and to evaluate the potential role(s) of MD2 and cystatin C, two molecules we reported previously and focused on [Fang et al. 2019; Zuo et al. 2021], in the pathogenesis of PND and the RIPC therapeutic strategy. Additionally, preliminary studies suggest that genetic factors are likely to influence the occurrence of PND [Heinrich et al. 2021; Westphal et al. 2019], but the evidence is extremely poor. Therefore, the additional objective of this study was to screen for susceptibility gene mutations associated with PND via whole-genome sequencing.

Methods

Patients and randomization

This single-center, parallel-group, randomized controlled trial was approved by the Internal Review Board of Xijing Hospital (No. KY20192064-F-1(04/29/2020)), registered at Chinese Clinical Trial Registry (No. ChiCTR2000035020(07/28/2020)) and conducted after receiving approval from the local ethics committee according to guidelines from the Helsinki Declaration. After providing informed consent, patients at Xijing Hospital who were scheduled for surgery under general anesthesia were screened beginning on 04, August 2020 and finishing on 09, February 2022. The inclusion criteria included age ≥ 60 years, duration of surgery ≥ two hours and American Society of Anesthesiologists (ASA) status II–III. The exclusion criteria included patients

suffering from degenerative diseases of the central nervous system, such as dementia, Alzheimer's disease, Parkinson's disease or other mental illness; patients who received neurological assessment within three months; Mini-Mental State Examination (MMSE) scores < 24 when first evaluated [Creavin et al. 2016; Salis et al. 2023; *X* Xu et al. 2015]; patients with previous symptomatic cerebrovascular disease; patients with previous cardiac or central nervous system surgery; patients with previous cardiopulmonary resuscitation; patients with kidney or severe liver dysfunction; those unable to comply or unwilling to cooperate; patients with hearing, visual or language impairments; those with alcohol or drug abuse; patients with uncontrolled hypertension (more than 180/100 mmHg) and those who participated in other clinical studies [Li et al. 2021; Zhao et al. 2017]. Enrolled participants were randomly assigned at a 1:1 ratio to receive RIPC or a controlled intervention via a computer-generated random allocation sequence (PASS V.15; NCSS, Kaysville, UT, USA) with a random block size. The randomization code was placed in a sealed envelope that was blinded to the patients and anesthetists and was opened by an investigator until the patients arrived at the operating room. The investigator who was blind to surgeons, anesthetists, outcome assessors and the independent statistician administrated the RIPC/sham intervention. The blood pressure cuffs are visibly identical. Outcome assessors, other clinical staff (including surgeons, anesthetists and hospital ward staff), the independent statistician and patients were blinded. We followed the CONSORT reporting guideline.

Intervention

All surgeries were performed under general anesthesia with continuous monitoring, including an electrocardiogram, oxygenation saturation (SpO2), blood pressure and the bispectral index (BIS). Every patient was given a crystalloid fluid infusion on the basis of individual physiological requirements prior to anesthesia. During anesthesia induction, midazolam (0.05 mg·kg − 1), sufentanil (0.1–0.3 μg·kg − 1), etomidate (0.1–0.2 mg·kg − 1) and rocuronium bromide (0.6–0.9 mg·kg − 1) were used. After three minutes once the BIS reached 40–60, tracheal intubation was performed and then ventilation mode was adopted. The respiratory parameters were adjusted to maintain the O2 partial pressure at 100–300 mmHg and the CO2 partial pressure at 35–45 mmHg. During anesthesia maintenance, remifentanil (0.1–0.3 μg·kg − 1·min − 1) and propofol (4–12 mg·kg − 1·h − 1) were used to maintain the BIS values at 40–60 and relatively stable hemodynamics.

Intervention was performed after anesthesia induction. Patients in the RIPC group received a blood pressure cuff around their right upper limb and were pressurized to 200 mmHg to induce ischemia for five minutes and then vented to 0 mmHg followed by five minutes of reperfusion [Gorog et al. 2021; Zhao et al. 2017]; a total of five cycles were repeated. In the control group, the blood pressure cuff was placed in the same position and was pressurized to only 60 mmHg for five minutes and then vented to 0 mmHg for five minutes; a total of five cycles were repeated.

For postoperative analgesia, the whole incision was infiltrated with 0.3–0.35% ropivacaine, then, 0.5 ml of PCA containing sufentanil (1 μg·ml − 1) and butorphanol (0.08 mg·ml − 1) was administered at an interval of 10 min. The analgesia was adjusted when necessary.

Outcomes

The primary outcome measure was the incidence of PND in patients with or without remote ischemia preconditioning. Other outcomes included the serum levels of MD2 and cystatin C at T0 (preoperative), T1 (1-day postoperative), or T3 (3-day postoperative) and single nucleotide polymorphisms in PND patients and NPND patients. Blood samples were collected and allowed to clot at room temperature, followed by immediate centrifugation for 20 min at 1000 × g. The supernatants were stored at − 80 °C until analysis via enzyme-linked immunosorbent assay (ELISA) kits or whole-genome sequencing. Neurocognitive assessment: Six neurological tests, including the Auditory Word Memory Test (from the Wechsler Memory Scale), Stroop Test, Digit Span Test, Digit Symbol Substitution Test, Verbal Fluency Test and Word Recall from the Wechsler, were performed by a researcher who was blinded to the groupings. The assessment was performed three days after the surgery. If a patient refused the assessment due to physical discomfort or being in the ICU, the evaluation was postponed until the patients could cooperate. Subjects whose postoperative scores declined by one standard deviation or more as compared to their preoperative scores were defined as having PND according to previous studies [Borchers et al. 2021; O'Gara et al. 2020]. Genotyping: A magnetic universal genomic DNA kit (Tiangen Biotech Co., Ltd., Beijing) was used to extract genomic DNA from whole blood samples collected from patients before surgery. Then, sequencing was performed with the MGISEQ-2000 (MGI Tech Co., Ltd.) platform. The raw data were filtered with SOAPnuke (v2.3.0) and the clean reads were subsequently mapped to the human reference genome (GRCh38) via the Burrows–Wheeler Aligner (BWA, v0.7.17). GATK software (v4.4.0.0) was subsequently used to exclude duplicate reads and to call the SNPs. The SNPs were annotated via SnpEff (v4.3). Gene

Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of genes harboring SNPs were implemented via the R package (4.1.2). A p value < 0.05 was considered statistically significant.

Statistical analysis

A pilot study showed that the incidence of PND in patients was 53%. At an alpha error of 0.05, we estimated that a sample size of 80 patients (40 patients per group) would provide the trial with 80% power to detect a reduction in the frequency of PND from 53% in the control group to 23% in the RIPC group, calculated using PASS 15.0 software (NCSS, Kaysville, UT, USA). At least 88 samples should be included for a 10% loss-to-follow rate. Thus, 90 patients were ultimately enrolled in this study. This sample size is close to that reported in a previous study [Han et al. 2023]. The data were sorted and analyzed with SPSS 23.0 software (SPSS Inc., Chicago, IL). Categorical variables were expressed as means ± percentages and analyzed by the $\chi 2$ test. Continuous variables were expressed as the means ± SDs and analyzed by t tests (two groups) or one-way ANOVA followed by Tukey's test (multiple groups). $P < 0.05$ was considered statistically significant.

Results

Patient characteristics and perioperative data

As shown in Fig. 1, a total of 250 patients were screened in this study; 130 patients were excluded according to the exclusion criteria and the remaining 120 patients were randomly divided into the RIPC group and the control group. Finally, 59 patients in the RIPC group were analyzed since one patient withdrew from the operation and one patient died after the operation; 57 patients in the control group were analyzed since one patient changed the operation type and one changed the anesthesia management. The demographic characteristics of the patients at baseline are presented in Table 1, suggesting that there were no significant differences between the two groups. In particular, the age of patients in the RIPC group (66.45 ± 5.57 years) was not significantly different from that in the control group (66.26 ± 4.38 years) (95% confidence interval [CI], −1.6611–2.0331; $P = 0.842$). Additionally, the perioperative data shown in Table 1 revealed that there were no obvious differences in the durations of anesthesia/operation, intraoperative bleeding, postoperative hospital stay or total liquid intake.

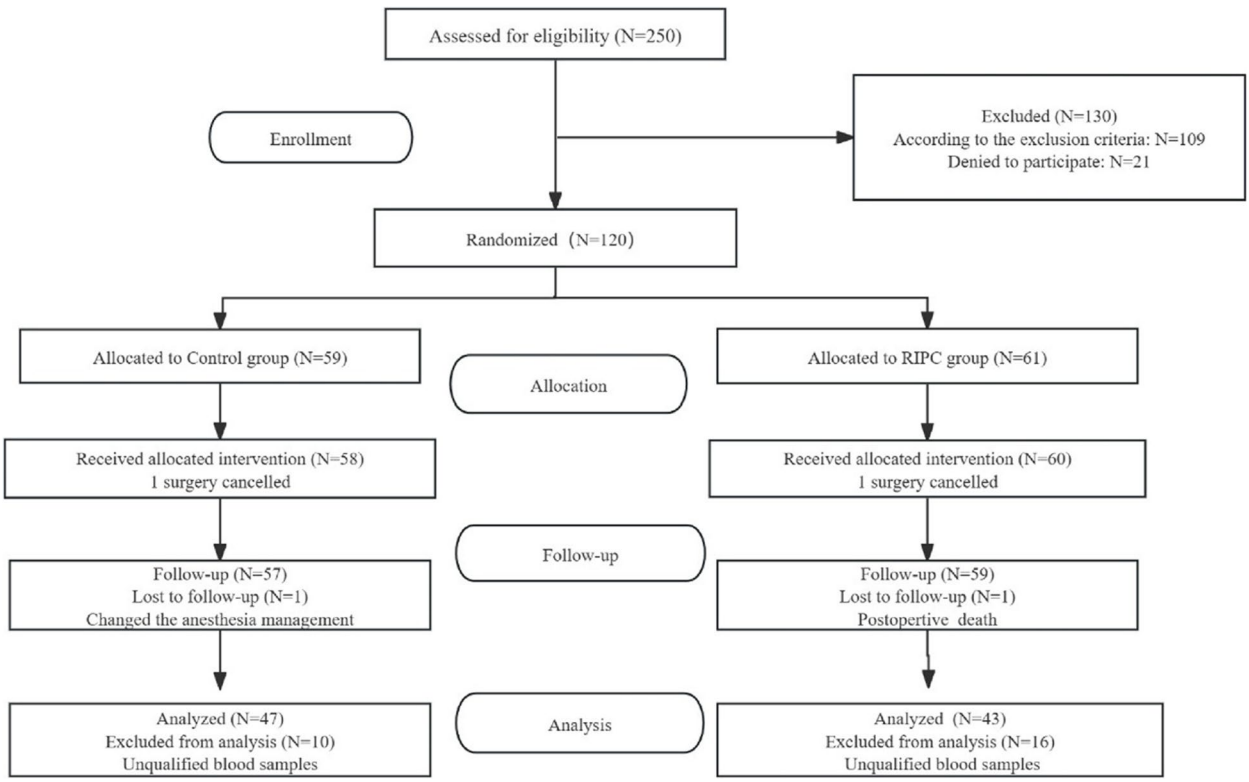

Fig. 1 Randomization and follow-up

Table 1 Patient characteristics and perioperative data

Variable	Control Group ($n=57$)	RIPC Group ($n=59$)	*P*
Age (years)	66.26 ± 4.38	66.45 ± 5.57	0.842
Male, n (%)	34(60)	40(68)	0.000
BMI (kg/m^2)	25.63 ± 4.39	24.63 ± 3.28	0.599
ASA, II/III	57/0	59/0	
Educational level, n (%)			0.087
Primary	7(12)	3 (5)	
Junior high school	13 (23)	19 (32)	
Senior high school	30 (53)	21 (36)	
College degree	7 (12)	16 (27)	
Smoking, n (%)	10 (18)	14 (24)	0.000
Drinking, n (%)	14 (25)	12 (20)	0.000
Hypertension, n (%)	27 (47)	28(48)	0.000
Diabetes, n (%)	6(11)	9 (15)	0.000
Mild liver disease, n (%)	5 (9)	10 (17)	0.000
MMSE	26 ± 1.3	26.23 ± 1.29	0.44
PHQ-9	2.65 ± 2.68	2.70 ± 2.47	0.924
Total liquid intake (mL)	2829.35 ± 1001.28	2700.78 ± 934.87	0.476
Intraoperative bleeding(mL)	315.71 ± 261.51	310.34 ± 306.86	0.920
Anesthesia duration (minute)	244.07 ± 75.85	248.31 ± 87.59	0.782
Surgery duration (minute)	206.04 ± 66.43	209.63 ± 82.73	0.80
Length of stay (day)	6.94 ± 4.40	6.95 ± 3.54	0.998

Note: Chronic hepatitis and cirrhosis without portal hypertension were defined as mild liver disease (NCI Thesaurus Code: C188393)

Table 2 Numbers (proportion) at each postoperative assessment time-points

Postoperative Assessment Time-points	Control Group ($n=57$)	RIPC Group ($n=59$)	$P=0.958$
Day 3	28 (49.1%)	28 (47.5%)	
Day 4	10 (17.5%)	10(16.9%)	
Day 5	10 (17.5%)	8(13.6%)	
Day 6	5 (8.8%)	5 (8.5%)	
Day 7	1 (1.8%)	4(6.8%)	
Day 8	2 (3.5%)	3(5.1%)	
Day 14	1 (1.75%)	1 (1.69%)	

Assessment of cognitive function and the incidence of PND

All the participants underwent neurological assessment before and after surgery. The number of subjects at each postoperative assessment time-points are shown in Table 2, suggesting that there is no significant difference between the control and RIPC group. We analyzed the differences between preoperative and postoperative scores for each test, subjects whose postoperative scores declined by one standard deviation or more as compared to their preoperative scores were defined as having PND. As shown in Table 3, forty-four percent of

Table 3 The incidence of PND is expressed as a number (proportion)

Variable	Control Group ($n=57$)	RIPC Group ($n=59$)	*P* value
Auditory Word Memory Test	9 (16)	13 (22)	0.391
Stroop test			
Reading Time	8 (14)	6 (10)	0.523
Error number	4 (7)	7 (12)	0.373
Digit span test			
Positive	10 (18)	12 (20)	0.701
Reverse	4 (7)	4 (7)	0.960
Digit Symbol Substitution Test			
Total number	17 (30)	16 (27)	0.747
Number of Errors	2 (4)	4 (7)	0.426
Number of Corrects	10 (18)	17(29)	0.151
Verbal Fluency Test	8 (14)	6(10)	0.523
Word Recall Test	7 (12)	4 (7)	0.312
PND, n (%)	**25 (44)**	**26((44)**	**0.982**

Note: Subjects whose postoperative scores declined by one standard deviation or more as compared to their preoperative scores were defined as having PND

Table 4 Changes in Cystatin C and MD2 (ng/ml, mean ± SD) between the control group and the RIPC group

		Control Group ($n=47$)	RIPC Group ($n=43$)	*P* value
Cystatin C	T0	677.91 ± 699.15	519.19 ± 453.1	0.209
	T1	985.39 ± 587.39*	970.94 ± 390.9****	0.892
	T3	874.32 ± 583.06	764.2 ± 406.27*	0.306
MD2	T0	7.31 ± 7.02	6.72 ± 6.79	0.690
	T1	11.75 ± 7.53**	11.58 ± 6.79**	0.908
	T3	9.82 ± 6.22	9.41 ± 6.36	0.755

* $P<0.05$

** $P<0.01$

**** $P<0.0001$, compared with the preoperative group (T0)

Table 5 Changes in Cystatin C and MD2 (ng/ml, mean ± SD) between the NPND group and the PND group

		NPND Group (n = 45)	PND Group (n = 45)	*P* value
Cystatin C	T0	572.6 ± 484.76	631.56 ± 695.04	0.642
	T1	962.56 ± 474.51***	994.41 ± 530.08*	0.765
	T3	820.71 ± 421.25*	822.71 ± 584.55	0.985
MD2	T0	7.28 ± 6.44	6.78 ± 7.35	0.735
	T1	11.43 ± 6.37**	11.91 ± 7.91**	0.748
	T3	9.84 ± 5.78	9.41 ± 6.76	0.749

* $P<0.05$

** $P<0.01$

*** $P<0.001$, compared with the preoperative group (T0)

patients were defined as PND patients in control group, which was not significantly different from that in the RIPC group (44%) ($P=0.982$).

Serum concentrations of cystatin C and MD2

As shown in Fig. 1, 43 patients in the RIPC group and 47 patients in the control group were analyzed due to the failure with blood sampling. As shown in Table 4, the serum concentrations of Cystatin C and MD2 were not significantly different between the control group and the RIPC group at T0 (preoperative) (Cystatin C: CI, −408.0616–90.6186, $P=0.209$; MD2: CI, −3.4818–2.3130, $P=0.690$), T1 (1-day postoperative) (Cystatin C: CI, −225.5026–196.6128, $P=0.892$; MD2: CI, −3.1892–2.8374, $P=0.908$), or T3 (3-day postoperative) (Cystatin C: CI, −322.4993–102.2751, $P=0.306$; MD2: CI, −3.0534–2.2230, $P=0.755$), suggesting that RIPC has little effect on the serum concentrations of Cystatin C and MD2. The serum concentrations of both Cystatin C and MD2 were significantly greater at T1 than at T0, which is consistent with the findings of previous studies[Yoo et al. 2014; Zuo et al. 2021]. The serum concentrations of neither Cystatin C nor MD2 in the PND group were obviously different from those in the NPND group at T0 (preoperative) (Cystatin C: CI, −192.0780–309.9979, $P=0.642$; MD2: CI, −3.3895–2.4011, $P=0.735$), T1 (1-day postoperative) (Cystatin C: CI, −178.9112–242.6155, $P=0.765$; MD2: CI, −2.5209–3.4966, $P=0.748$) or T3 (3-day postoperative) (Cystatin C: CI, −211.4570–215.4487, $P=0.985$; MD2: CI, −3.0620–2.2090, $P=0.749$) (Table 5). Additionally, there were no significant differences between the two groups, neither in demographic characteristics nor in the incidence of PND (Supplementary Tables. 1–3). These results indicate that neither Cystatin C nor MD2 can be applied in the prediction of PND.

Identification of potential PND-associated SNPs

A total of 13,948,949 SNPs were identified in the 90 patients included in this study, including 6,435,809 common SNPs (MAF > 0.05) and 7,513,140 rare SNPs (MAF < = 0.05). As shown in Fig. 2, the SNPs were located mainly on chromosomes 1—7 and the majority of the SNPs were located in introns (46.66%) or intergenic regions (44.97%). In 45 PND patients and 45 NPND patients, 11,350,233 SNPs and 11,307,700 SNPs were identified, respectively. Among these SNPs, 8,708,984 were filtered out since they were in both PND patients and NPND patients and unlikely to participate in the occurrence of PND; 2,641,249 SNPs in PND patients and 2,598,716 SNPs in NPND patients were retained, including 25,349 common SNPs (MAF > 0.05) in PND patients and 20,599 common SNPs in NPND patients, which were annotated to 5682 genes and 4989 genes, respectively, via SnpEff software (version 4.3). Among these genes, 1805 genes were filtered out because they were identified in both PND patients and NPND patients and the remaining 3877 genes in PND patients were subjected to follow-up analysis (Supplementary Table 4).

GO and KEGG analyses were performed on the genes identified in the PND patients above. Our results revealed that 126 GO terms were significantly enriched, including calcium ion binding, neurotransmitter receptor activity, folic acid binding and toll-like receptor binding (Supplementary Table 5) and 21 KEGG terms were significantly enriched, including axon guidance, dopaminergic synapses, serotonergic synapses and glutamatergic synapses, among which 287 genes were involved (Supplementary Table 6). We then examined the SNP locations and removed SNPs located in the upstream/downstream/introns/noncoding transcript exon region as well as the synonymous SNPs; only SNPs located in the 5' UTR/3'UTR of the genes and the missense SNPs were retained, which were annotated to 14 genes, as shown

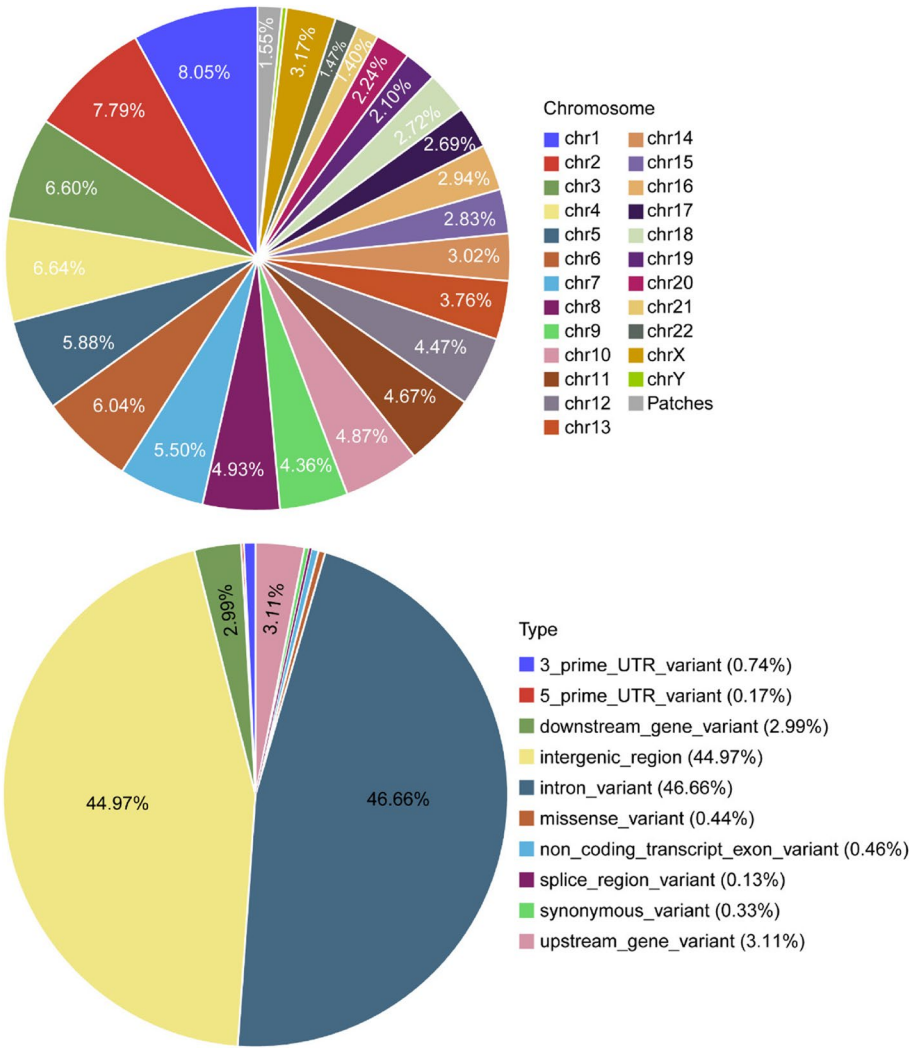

Fig. 2 Genome-wide distribution of the SNPs and identification of mutation types

in Table 6, including rs4824145 located in the 5'UTR of the Shank3 (SH3 and multiple ankyrin repeat domains 3) gene.

Discussion

In this randomized clinical trial, the occurrence of PND in patients who received RIPC was comparable to that in patients who received a control procedure, suggesting that RIPC has little effect on the occurrence of PND in elderly patients who underwent scheduled major surgery lasting more than two hours. Moreover, the concentrations of serum MD2 and Cystatin C in PND patients were not significantly different from those in non-PND patients, indicating an infeasibility of them as molecular markers. Finally, but more importantly, our results indicated that patients harboring rs4824145, which is located in the Shank3 gene, may be more vulnerable to PND.

As a safe, convenient and nonintrusive method, remote ischemia preconditioning has long been shown to be neuroprotective against stroke in animal experiments and clinical trials [Nizari et al. 2021; Pico et al. 2020]. However, there is currently no consensus on the preconditioning stimulation parameters (number of cycles and ischemia–reperfusion time), which may lead to diametrically different results. For example, a previous clinical trial suggested that patients who received RIPC (five cycles, ischemia for five minutes during which cuffs were inflated to a pressure of 200 mm Hg and reperfusion for another five minutes) exhibited decreased ischemic brain injury compared with patients who received sham RIPC, during which the cuffs were inflated to a pressure of 60 mm Hg [Zhao et al. 2017], which is inconsistent with another study showing that patients who received RIPC (four cycles, ischemia for five minutes/110 mm Hg and

Table 6 List of potential PND-related SNPs

Chr	SNP	REF	ALT	Gene symbol
chr1	rs142996143	T	C	RAB4A
chr1	rs7695	T	C	SEMA4A
chr2	rs149117087	T	A	COL4A4
chr3	rs118116962	A	G	MYLK
chr3	rs75604544	C	G	WNT5A
chr4	rs374451575	G	A	TLR2
chr6	rs41553715	T	A	HLA-B
chr10	rs75414481	C	T	NRP1
chr11	rs79201182	C	T	CFL1
chr11	rs191847213	G	C	HTR3B
chr12	rs138200728	G	A	VPS37B
chr12	rs148485462	G	A	HSD17B6
chr17	rs75981990	A	G	AP2B1
chr22	rs4824145	G	A	SHANK3

reperfusion for another five minutes) did not exhibit any improvements in brain infarction growth compared with patients who received sham RIPC without any inflations [Pico et al. 2020]. For PND, a recent study demonstrated that patients who received RIPC (three cycles of ischemia for five minutes during which the cuffs were inflated to a pressure of 120 mm Hg and reperfusion for another five minutes) performed better than patients who received sham RIPC without any inflations [Han et al. 2023]; such different stimulation parameters may have led to the seemingly contradictory results in this study.

To date, the diagnosis of PND relies on a battery of neurological tests, which are time-consuming and difficult to administer and looking for objective biomarkers is vital for early detection and intervention in the clinic. We previously reported that the level of MD2 increased significantly after surgery in aged mice [Zuo et al. 2021]; thus, the serum of patients was subjected to ELISA to evaluate the potential of MD2 as a molecular marker for PND. We found that there was no significant difference in the levels of MD2 in PND patients compared with NPND patients, although the serum MD2 clearly increased after surgery (Tables 4, 5), which is consistent with our previous study [Zuo et al. 2021]. In addition, cystatin C is a small secreted cysteine protease inhibitor and has long been recognized as an independent predictor of perioperative complications, including kidney injury [Turino Miranda et al. 2024] and cardiovascular diseases [Costa et al. 2024]. Our group previously reported that cystatin C acts as a pivotal endogenous mediator of hyperbaric oxygen preconditioning-induced neuroprotection against stroke [Fang et al. 2019]; however, whether serum cystatin C is associated with PND or the neuroprotection of RIPC is unclear. Here, we showed that the concentrations of serum cystatin C in PND patients are comparable to those in NPND patients. These results suggest that neither MD2 nor cystatin C could predict the occurrence of PND. Further proteomics or metabolomics may help us identify biomarker candidates that are susceptible to PND.

Previous studies demonstrated that patients harboring genetic variants are more vulnerable to the development of perioperative complications such as acute kidney injury, myocardial infarction, acute stroke and delirium [Westphal et al. 2019]. We performed whole-genome sequencing to explore the risk of single nucleotide polymorphisms in PND and identified 14 potential PND-susceptible SNPs, including rs4824145 in the 5' UTR of the *Shank3* gene. *Shank3* is located at chromosome 22q13.3 in humans and dramatically influences brain function through its intragene promoters and variable splicing exon-dependent isoforms. Shank3 is intolerant to heterozygous loss-of-function and the loss or pathogenic variants of *Shank3* are major contributors to autism spectrum disorder (ASD) in infants and young children, which is characterized mainly by social behavior disorders and stereotyped behavior [Bidinosti et al. 2016]. However, little attention has been given to the effect of *Shank3* on the elderly population. In the central nervous system of rodents, Shank3 is enriched in cognition-associated regions, including the hippocampus, cortex, thalamus and striatum [Monteiro and Feng 2017], highlighting its potential importance in regulating PND. Further studies in rodents are necessary to elucidate whether transgenic mice harboring rs4824145 are more vulnerable to PND and to elucidate the underlying mechanism.

As a key excitatory postsynaptic scaffold protein, Shank3 interacts with PSD-95 and glutamate receptors to promote the formation of synapses [Monteiro and Feng 2017]. We previously reported that glutamate receptor NR2B signaling is impaired after surgery in the hippocampus of aged mice [*F* Xu et al. 2023]. The potential role of the Shank3-NR2B interaction in the pathogenesis of PND should be investigated. Moreover, given that the effect of Shank3 on behavior is brain region-dependent, for example, Shank3 in the anterior cingulate cortex is key in social behavior, whereas striatonigral/striatopallidal dysfunction underlies repetitive behavior [Guo et al. 2019; Wang et al. 2017], whether Shank3 participates in different manifestations of PND through modulating the activity of different brain regions is worth exploring.

There are several limitations in this study. First, bias may arise because of the scarcity of patients enrolled in a single center, the relatively homogenous patients with lots of exclusion criteria and the use of 200 mmHg which may affect patients differently since the blood pressure varies

among participants, multicenter trials with more participants are necessary to elucidate whether adaptable RIPC (e.g. 15 to 20 mmHg above the arterial pressure [Gorjipour et al. 2022]) is beneficial for PND in the future. Second, multiple assessments were used to test different domains of PND, including word learning, word recall, cognitive flexibility, distractibility and working memory [Li et al. 2021; Monk et al. 2008]. However, we didn't assess the individual domains or combine any results into composite outcome and the PND was assessed 3 to 14 days after the surgery to detect the delayed neurocognitive recovery after surgery as described in 2018 nomenclature recommendations [Evered et al. 2018; Li et al. 2021], further work conducted over multiple time points with control subjects who are not exposed to surgery would help mitigate the potential bias by calculating a combined *Z* score to diagnose PND [Li et al. 2021]. Lastly, GWASs are generally conducted with a large sample size; although a previous study performed GWASs with 452 patients, the authors failed to obtain PND-associated genetic variants with genome-wide significance [Heinrich et al. 2021], further GWASs with larger sample sizes are necessary.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
Hailong Dong, Haopeng Zhang and Zhihong Lu designed and supervised the research. Feifei Xu and Tingting Liu conducted the clinical trial and wrote the manuscript. Huiqing Liu and Jiao Deng helped revise the manuscript. All the authors reviewed the results and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study involved human participants and was approved by the Ethics Committee of Xijing Hospital (No. KY20192064-F-1(04/29/2020)). The participants provided informed consent to participate in the study before taking part.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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