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RESEARCH

# Investigating the role of the Pon1-rs854560 (L55M) SNP in colorectal Cancer susceptibility

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## Abstract

**Background** Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide, with both genetic and environmental risk factors. The PON1 rs854560 (L55M) polymorphism has been implicated in cancer susceptibility through its role in oxidative stress regulation, but its association with CRC remains unclear, particularly in Asian populations.

**Aim** This study aimed to investigate the association between the PON1 rs854560 polymorphism and CRC susceptibility in a Chinese cohort, while assessing its impact on PON1 expression and enzymatic activity.

**Method** A case-control study was conducted on 1,003 CRC patients and 1,303 healthy controls. The impact of the Pon1-rs854560 SNP was assessed by comparing the genotypes of individuals diagnosed with CRC to those of controls without the disease.

**Results** Genotype distribution showed slight differences between the case and control groups. The frequency of the AA genotype was slightly lower in the case group (91.72%) than in the control group (93.71%). The AT genotype was observed at similar frequencies in both groups (8.28% in the case group and 6.14% in the control group). Notably, the TT genotype was absent in the case group but present in 0.15% of the control group. Genotype combination analysis suggested that individuals carrying the AT+TT genotype (8.28%) had a higher susceptibility to CRC compared to those with the AA+AT genotype (100%). Allele frequency analysis revealed a slightly higher frequency of allele T in the case group (8.28%) than in the control group (6.45%). Additionally, lower PON1 mRNA and protein expression were associated with CRC progression, including features such as poorer differentiation, deeper tumor invasion, and vascular, nerve, and lymphatic metastasis.

**Conclusion** The *PON1* rs854560 polymorphism influences CRC risk in Chinese individuals, likely through reduced PON1 expression and detoxification capacity. These findings highlight its potential as a genetic biomarker for CRC susceptibility and suggest *PON1*'s role in tumor progression. Further studies should validate these associations in diverse populations and explore therapeutic strategies targeting PON1 activity.

**Keywords** Colorectal cancer · Single nucleotide polymorphism · PON1 gene · Oxidative stress · Genetic susceptibility · Biomarker · Tumor progression

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## Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed malignancies and a leading cause of cancer-related mortality worldwide. In 2020, CRC accounted for over 1.9 million new cases and approximately 935,000 deaths, highlighting its global health burden (Sung et al. 2021). While modifiable risk factors such as smoking, alcohol consumption, poor diet, and obesity are well-documented contributors to CRC development (Kuipers et al. 2015; Araghi et al. 2019), inherited genetic factors also play a substantial role, with heritability estimates ranging from 12 to 35% (Huyghe et al. 2019).

Advances in genome-wide association studies (GWAS) have identified numerous single nucleotide polymorphisms (SNPs) associated with CRC susceptibility (Schmit et al. 2019). Although most individual SNPs confer modest risk, their cumulative effect can support personalized screening and prevention strategies. Among the genes of interest, paraoxonase 1 (PON1), a high-density lipoprotein-associated enzyme has emerged due to its role in modulating oxidative stress and detoxifying lipid peroxides and organophosphates (Furlong et al. 2016).

The PON1 rs854560 (L55M) polymorphism is a missense variant that results in a leucine-to-methionine substitution at codon 55. This substitution has been linked to altered enzyme activity, mRNA stability, and protein expression, with the T allele generally associated with reduced PON1 levels and detoxification capacity (Aviram et al. 1998). Importantly, this polymorphism has been investigated in several cancer types, yielding conflicting results depending on the cancer type and ethnic population. For instance, Akkız et al. reported no significant association between PON1 rs854560 and hepatocellular carcinoma risk in a Turkish population, contrasting with findings in prostate and breast cancers, where the variant has been linked to increased susceptibility(Akkız et al. 2013). These inconsistencies suggest that the functional impact of PON1 polymorphisms may vary depending on tumor biology and genetic background. Despite this, few studies have explored the association between the rs854560 variant and CRC, particularly in Asian populations. Given the known role of oxidative stress in CRC pathogenesis and PON1 detoxifying function, further investigation is warranted.

Therefore, the aim of this study was to evaluate the association between the PON1 rs854560 polymorphism and CRC susceptibility in a Chinese population. Additionally, we examined the relationship between this SNP and PON1 expression as well as enzymatic activity, to assess its functional relevance and potential as a biomarker for CRC risk.

## Materials and methods

### Study population

This case-control study was conducted at the Affiliated People's Hospital of Jiangsu University in Zhenjiang City, Jiangsu Province, China, encompassing 1003 CRC cases and 1303 cancer-free individuals recruited consecutively between August 2023 and September 2024. For molecular analyses (qRT-PCR, Western blot, and IHC), a representative subset of 50 CRC cases with paired tumor and adjacent normal tissues was selected, comprising 32 CC and 18 RC samples. The genotype distribution in this subset mirrored the full cohort, with 92% (46/50) exhibiting the AA genotype and 8% (4/50) the AT genotype; no TT cases were observed. The SNP-based risk estimation utilized 1003 cases and 1303 controls who underwent genome-wide testing via the Illumina Human1M v1 or Illumina Human1M-Duo v3.0 platform as part of a GWAS focused on identifying new CRC susceptibility genes. To achieve this objective, cases were selectively chosen to be either younger or older than 61 years at the time of diagnosis, with 10% sampling from other age groups at diagnoses, while controls were primarily selected without a family history of CRC. Cases underwent testing for germline mutations in DNA mismatch repair genes and MUTYH, with exclusion of all mutation carriers. Informed consent was obtained from all participants (Table 1).

### Ethics statement

The study was conducted in accordance with the 1964 Helsinki Declaration and its later amendments. The IRB granted a waiver of informed consent, as the study involved the use of anonymized data and posed minimal risk to participants.

### Genotyping

Genomic DNA was isolated from venous blood samples using the High Pure PCR Template Preparation Kit and stored at 4 °C. Genotyping procedures were carried out on a real-time PCR platform (Light Cycler 480, Roche, Germany) utilizing the Light Cycler FastStart DNA Master HybProbe and Roche LightSNP assay probes as per the manufacturer's protocols. A 20 µL reaction mix per sample included 1X FastStart DNA Master Mix, 2 mM MgCl 2, 0.2 µM LightSNP HybProbe, PCR grade water, and 500 ng DNA sample. Negative and positive controls, consisting of sterile water and a known genotyped sample, respectively, were included in each run to ensure 100% agreement. Further information

**Table 1** Distribution of demographic variables and risk factors in cases and controls

| Variable | Case($n=1,003$) | | Control ($n=1,303$) | | $P$ |
|---|---|---|---|---|---|
| | n | % | n | % | |
| Age(years) | 61.10 (±12.17) | | 61.40 (±9.61) | | 0.496 |
| Age(years) | | | | | 0.605 |
| <61 | 451 | 44.97 | 600 | 46.05 | |
| ≥61 | 552 | 55.03 | 703 | 53.95 | |
| Sex | | | | | 0.867 |
| Male | 620 | 61.81 | 801 | 61.47 | |
| Female | 383 | 38.19 | 502 | 38.53 | |
| Smoking status | | | | | 0.002* |
| Yes | 744 | 74.18 | 1038 | 79.66 | |
| No | 259 | 25.82 | 265 | 20.34 | |
| Alcohol use | | | | | <0.001* |
| Yes | 829 | 82.65 | 1,167 | 89.56 | |
| No | 174 | 17.35 | 136 | 10.44 | |
| BMI(Kg/m2) | | | | | |
| <24 | 670 | 66.80 | 688 | 52.80 | <0.001* |
| ≥24 | 333 | 33.20 | 615 | 47.20 | |
| Site of tumor | | | | | |
| Colon cancer | 431 | 42.97 | | | |
| Rectum cancer | 572 | 57.03 | | | |

Note *: Statistically significant, $P<0.05$

**Table 2** Primary information for PON-1 rs854560 A>T polymorphisms

| Genotyped SNPs | rs854560 A>T |
|---|---|
| Chromosome | 7 |
| Function | missense |
| Chr Pos (GRCh38) | 95,316,772 |
| MAF for Chinese in database | 0.03 |
| MAF in our controls ($n=1,303$) | 0.03 |
| $P$ value for HWE test in our controls | 0.498 |
| Genotyping method | SNP scan |
| % Genotyping value | 98.87% |

MAF: minor allele frequency;

HWE: Hardy–Weinberg equilibrium;

**Table 3** Primer sequences and amplification conditions for PON1 and β-actin genes

| Gene | Forward Primer | Reverse Primer | Size (bp) |
|---|---|---|---|
| PON1 | 5'GGTGAACCATCCAGATGCCA3' | 5'CTTCT-GCCAC-CACTC-GAACT3' | 263 |
| β-actin | 5' CTTCGCGGGCGACGAT 3' | 5'CCACAT AGGAATC CTTCTGAC C 3' | 104 |

Bp: Base pairs

regarding the custom-designed LightSNiP assay probes can be found in Table 2. To explore the functional relevance of the rs854560 polymorphism, the genotype data were correlated with PON1 mRNA and protein expression, as well as enzymatic activity, in the subset of 50 CRC cases and their matched controls with available molecular data.

## Methods

### Tissue RNA was extracted by trizol reagent

Total RNA was extracted from 50 paired tumor/adjacent normal tissues (32 CC, 18 RC) using TRIzol reagent. Reverse transcription reaction was conducted by applying the standard conditions provided by reverse transcription reagent, and the obtained cDNA served as PCR reactive template. Primers were designed from the target sequences retrieved from the RefSeq Sequence Database: PON1 forward primer, 5'GGTGAACCATCCAGATGCCA3' and reverse primer 5'CTTCTGCCACCACTCGAACT3'; amplified fragment was 263 bp. Serving as the internal reference, β-actin forward primer, 5'CTTCGCGGGCGACGAT 3' and reverse primer 5' CCACATAGGAATCCTTCTGACC 3'; amplified fragment was 104 bp (Table 3). The reaction conditions were as follows: 95˚C for 5 min, 40 cycles at 95˚C for 10 s and 60˚C for 30 s, extension was performed at 72˚C for 10 min. $C_T$ values were processed using $2^{-\Delta\Delta Ct}$ method, and relative expression level of PON1 was normalized to endogenous control β-actin.

### Detection of the expression of PON1 protein by Western blot analysis

Total protein was extracted from the same 50 paired tissue samples (32 CC, 18 RC) using RIPA lysis solutions were used to extract total protein, and protein samples were quantitated by BCA assay. After that, 10% SDS/PAGE gel electrophoresis was carried out using 20 µg protein from each sample, followed by gel transfer to PVDF membranes. After blocking with 5% skimmed milk at room temperature for 1 h, PDVF membranes were washed and incubated with primary antibodies including rabbit anti-PON1 antibody (1:5000, ab92466, Abcam), rabbit anti-β-actin antibody (1:5000, ab8227, Abcam) overnight at 4◦C. The next day, membranes were washed with TBST and incubated with Goat Anti-Rabbit IgG -HRP secondary antibody (1:1000, ab7090, Abcam) at room temperature for 2 h. Then membranes were washed again with TBST, and signals were detected by ECL and Gel Imaging. The protein expression level of PON1 was normalized to endogenous control β-actin using ImageJ. PON1 protein levels were compared

across rs854560 genotypes to examine genotype-dependent differences in protein expression.

## Immunohistochemistry

The 50 paired tissue samples (32 CC, 18 RC) were analyzed using EnVision two-step method to detect the expression level of PON1 in CRC tumor tissues and adjacent tissues. This experiment uses the Phosphate buffer saline (PBS) solution as the negative control. The tissue samples treated with 10% neutral formalin were embedded in paraffin, and sectioned into thin slices of 4 μm. Heat the slices at 60°C for 45 min, then use xylene to deparaffinize the slices, changing the xylene every 10 min for a total of three repetitions. After that, the slices were reacted in 3% $H_2O_2$ (SML0790, Sigma-Aldrich) at 37°C for 30 min to block endogenous peroxidase. Then the slices were boiled at 95°C for 20 min with 0.01 mol/L citrate. After cooling, the sections were blocked in PBS BSA 1% goat serum 5% at room temperature for 1 h, and incubated with primary rabbit antibodies to PON1 (1:250, ab92466, Abcam) at 4°C overnight. On the next day, the slices were probed with Goat Anti-Rabbit IgG -HRP secondary antibody (1:300, ab7090, Abcam) for 45 min at 37°C. Finally, the slices were developed with diaminobenzidine, counterstained by hematoxylin and then mounted. PON1 positivity was localized in the cytoplasm. Five middle-power fields(200×) were randomly chosen from each slice with 200 cells per field. The slices were evaluated according to the percentage of positive cells in total cells.

## Detection of PON1 activity

The organophosphorase activity (OPase) of serum PON1 was measured using organophosphorus esters as substrates: P-nitrophenol was formed with paraoxon as the substrate under the catalysis of PON1. The absorption peak at 405 nm was measured and the increase of absorbance at 405 nm per unit time after enzymatic reaction was measured and calculated. The aromatase activity (AEase) of serum PON1 was measured using aromatic esters as substrates: Phenol and acetic acid were generated by the catalysis of PON1 with phenyl acetate as substrate. Phenol had an absorption peak at 270 nm, and the increase in absorbance at 270 nm per unit time after enzymatic reaction was measured and calculated. Serum PON1 lactonase activity was measured using lactonase as substrates: Homocysteine was formed from homocysteine thiolactone catalyzed by PON1. After coupling with 5,5 '-Dithiobis (2-nitrobenzoicacid) and DTNB, an absorption peak at 412 nm was observed. The increase in the absorbance of the enzymatic reaction at 412 nm per unit time was measured and calculated. Genotype-stratified analysis was performed for all enzymatic activities, with samples grouped as: AA (n=920 cases/1,221 controls), AT (n=83 cases/80 controls). The two TT control samples were included in initial measurements but excluded from comparative statistical analysis due to insufficient sample size. All assays were performed blinded to genotype status.

### Statistical analysis

Statistical analyses were performed using SPSS software (version 28). Chi-square ($\chi^2$) tests were used to compare allele and genotype frequencies, as well as demographic factors, between groups. Linear regression was applied to adjust for confounding variables such as age and gender, and to calculate odds ratios (OR) with 95% confidence intervals (95% CI) for associations between genetic variants and clinicopathological features. For genotype analysis, the allele with the lowest frequency was designated as the "risk allele," and the dominant (0 for no risk allele, 1 for at least one risk allele) and recessive (0 for at least one wild-type allele, 1 for two copies of the risk allele) models were used. Statistical significance was set at $p<0.05$ for all comparisons.

### Result

We have collected several clinical features of 1003 CRC cases and 1303 cancer-free subjects, including age, gender, social history (smoking and alcohol use), site of tumor and BMI (Table 1). According to P values, the levels of Pon1-rs854560 were not obviously correlated with age, and sex in CRC patients. There could be a significant association between BMI and social history and the levels of PON1 ($P=0.002$, $<0.001$ and $<0.002$ respectively).

In the case group, the AA+AT genotype was significantly more prevalent (100%) compared to the TT genotype. Similarly, in the control group, the AA+AT genotype was predominantly found (99.85%) with a rare occurrence of the TT genotype (0.15%). Furthermore, when comparing the AA genotype to the combined AT+TT genotypes, the AA genotype was significantly more frequent (91.72%) in the case group, with a relatively low frequency of AT+TT genotypes (8.28%). In the control group, the AA genotype was also more prevalent (93.71%) compared to the AT+TT genotypes (6.29%). The AT genotype has a frequency of 8.28% in the case group and 6.14% in the control group. However, there were no occurrences of the TT genotype in the case group, while there were 2 (0.15%) occurrences in the control group. It is worth noting that patients carrying the AT+TT genotype (8.28%) were found to be more susceptible to CRC compared to patients carrying the AA+AT

**Table 4** Logistic regression analyses of associations between PON-1 rs854560 A>T polymorphisms in colorectal cancer

| Genotype (rs854560 A>T) | CRC case ($n$=1,003) | | Controls ($n$=1,303) | | Crude OR (95%CI) | $P$ | OR [a] (95%CI) |
|---|---|---|---|---|---|---|---|
| | $n$ | % | $n$ | % | | | |
| AA | 920 | 91.72 | 1,221 | 93.71 | 0.76(0.23–122) | 0.74 | 1.00 |
| AT | 83 | 8.28 | 80 | 6.14 | 1.20 (0.71–1.43) | 0.54 | 1.00(0.71–1.43) |
| TT | 0 | 0 | 2 | 0.15 | 1.02(0.89–1.35) | 0.510 | 0.998(0.99-1.00) |
| AT+TT | 83 | 8.28 | 82 | 6.29 | 1.17(0.69–1.39) | 0.47 | 0.98(0.69–1.39) |
| AA+AT | 1003 | 100 | 1,301 | 99.85 | 0.19(0.08–0.76) | 0.25 | 1.00 |
| T allele | 83 | 8.28 | 84 | 6.45 | | - | - |

[a] Adjusted for age, sex, smoking status, alcohol use and BMI status

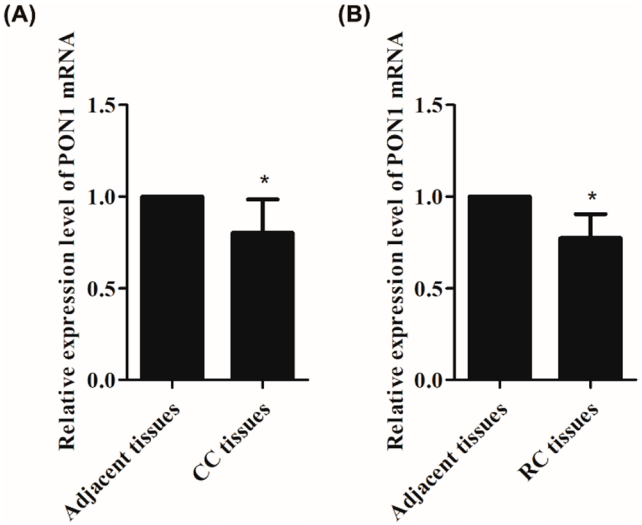


**Fig. 1** Relative expression of PON1 mRNA in tumor and adjacent normal tissues (n=50: 32 CC, 18 RC). Note: (**A**) CC tissues vs. Adjacent normal tissues; (**B**) RC tissues vs. Adjacent normal tissues; *: Statistically significant, $P<0.05$

genotype (100%). In terms of allele frequency, the frequency of allele T in the case group (8.28%) is slightly higher than in the control group (6.45%) Table 4.

## Expression of PON1 mRNA was decreased in tumor tissues compared with adjacent healthy tissues

Expression of PON1 mRNA in tumor tissues and adjacent healthy tissues of 32 CC patients and 18 rectal carcinoma (RC) patients was detected by qRT-PCR. As shown in Fig. 1, analysis of 50 paired samples (32 CC, 18 RC) revealed expression level of PON1 mRNA was significantly decreased in tumor tissues compared with adjacent healthy tissues in 27 out of 32 CC and 15 out of 18 RC patients. Those data suggest that low expression of PON1 may be associated with the progression of CRC.

## Comparison of PON1 protein levels between colorectal tumor tissues and adjacent healthy tissues

Western blot was used to measure levels of PON1 protein in 50 paired samples CRC tissues and adjacent healthy tissues. As shown in Fig. 2, the level of PON1 protein was

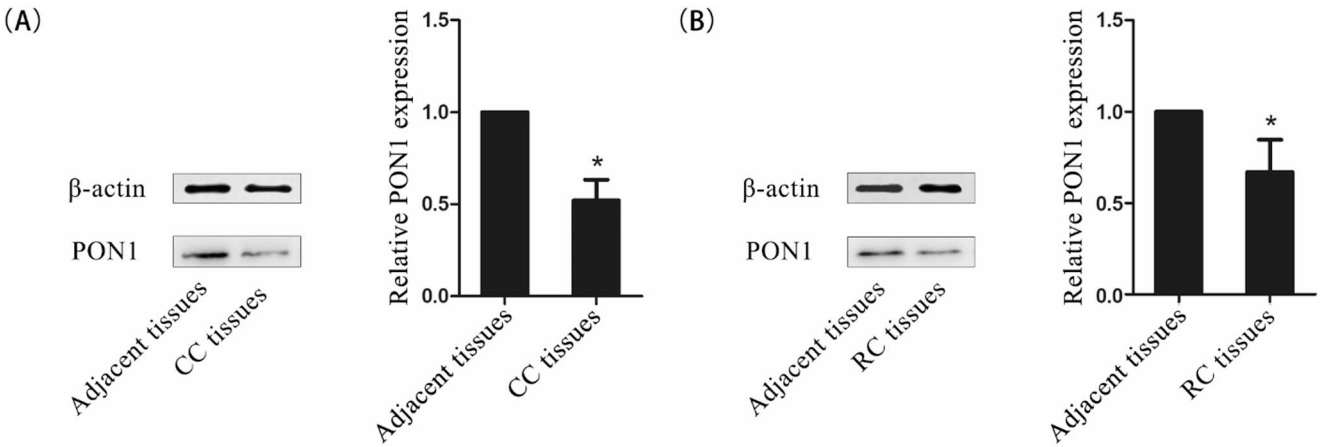


**Fig. 2** Protein expression of PON1 in tumor and adjacent normal tissues (n=50: 32 CC, 18 RC). Note: (**A**) Panels of Western blotting of CC tissues vs. Adjacent normal tissues; (**B**) Panels of Western blotting of RC tissues vs. Adjacent normal tissues; the level of PON1 protein was significantly higher in healthy tissues than that in tumor tissues. *: Statistically significant, $P<0.05$

significantly higher in healthy tissues than that in tumor tissues. In order to further verify the results, the expression of PON1 in the 50 paired pathological tissues of intestinal adenocarcinoma stored in the First People's Hospital of Zhenjiang City was analyzed by immunohistochemistry. The results showed that the expression level of PON1 in CC tissues was significantly lower than that in adjacent tissues ($P<0.05$) (Fig. 3). Based on these results, we speculate that PON1 may play a protective role in the progression of CC. However, there was no significant difference in PON1 expression between rectal cancer tissues and adjacent normal tissues. ($P>0.05$)(Fig. 3).

## Correlation between serum paraoxonase (PON1) activity and clinicopathological features of colorectal carcinoma

There were significant correlations between PON1 enzyme activity and certain clinicopathological features of CRC. Patients with well-differentiated tumors showed significantly higher PON1 activity toward OPase, AEase, and Lactonase compared to those with moderate or poor/undifferentiated tumors ($P<0.05$). Tumors confined to the submucosa and above had higher PON1 activity than those invading the muscularis or beyond ($P<0.05$). Additionally, patients without vascular/nerve invasion or lymphatic metastasis exhibited significantly higher PON1 activity than those with invasion or metastasis ($P<0.05$) (Table 4).

No significant differences in PON1 activity were observed regarding sex, age, tumor type, tumor location, or tumor size ($P>0.05$). Overall, lower PON1 activity may

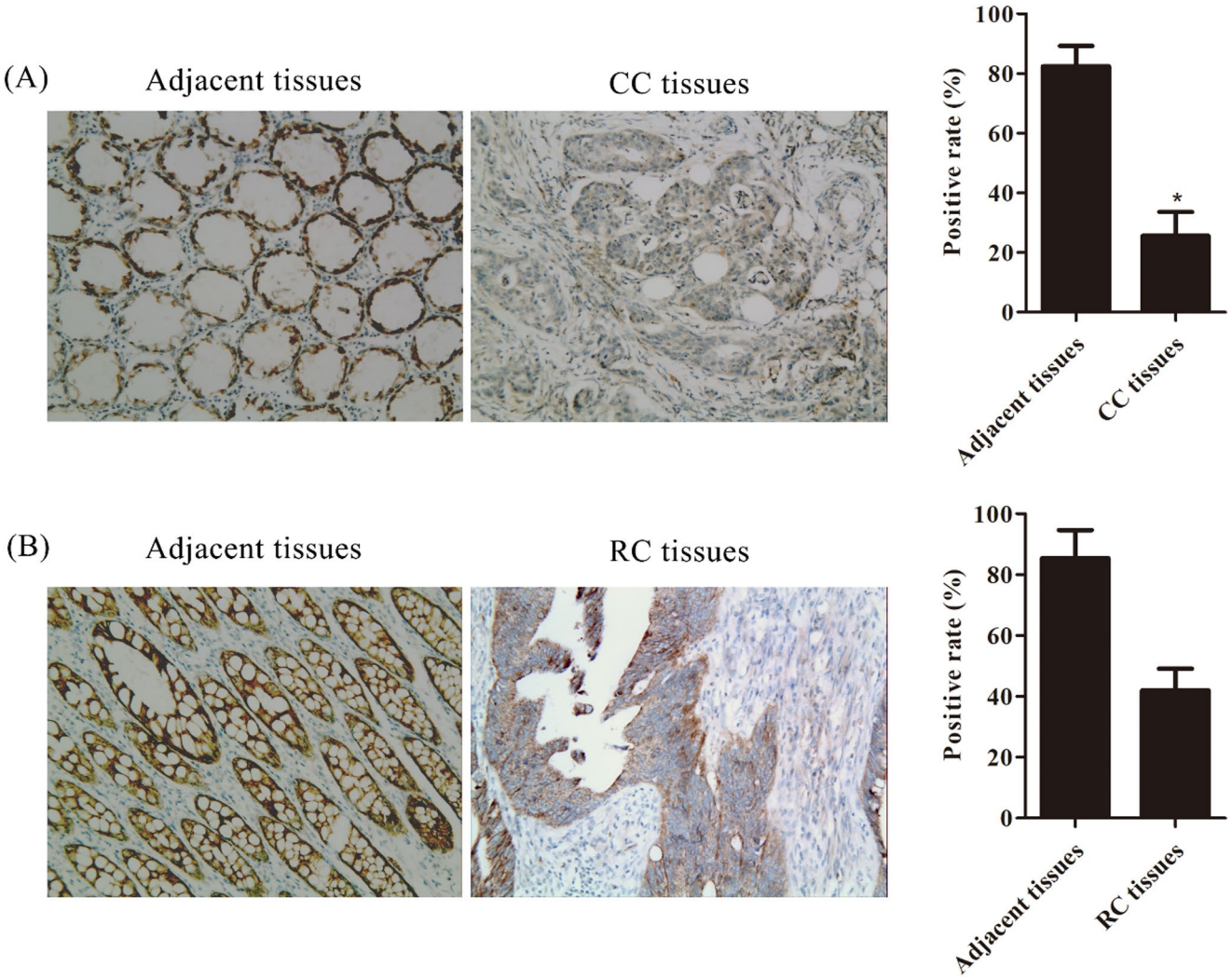


**Fig. 3** PON1 expression in CRC tissues, (n=50: 32 CC, 18 RC). envision two-step method. Note: (**A**) CC tissues vs. Adjacent normal tissues; (**B**) RC tissues vs. Adjacent normal tissues; *: Statistically significant, $P<0.05$

**Table 5** Correlation between serum paraoxonase activity and the clinicopathological features of colorectal carcinoma( $\bar{x}\pm s$)

| Clinicopathologic features | *n* | PON1 activity toward OPase (U /mL) | *P* | PON1 activity toward AEase (U / mL) | *P* | PON1 activity toward Lactonase (U /mL) | *P* |
|---|---|---|---|---|---|---|---|
| Sex | | | | | | | |
| male | 29 | 101.42±6.38 | 0.984 | 72.64±9.64 | 0.734 | 43.36±3.58 | 0.654 |
| female | 21 | 102.65±4.70 | | 73.42±3.86 | | 45.83±6.37 | |
| Age (years) | | | | | | | |
| <61 | 23 | 102.88±8.25 | 0.335 | 72.85±6.01 | 0.128 | 45.38±5.45 | 0.372 |
| ≥61 | 27 | 98.15±5.94 | | 69.52±4.29 | | 43.95±3.69 | |
| Gross type | | | | | | | |
| Polypoid type | 18 | 103.79±6.98 | 0.142 | 71.86±6.40 | 0.254 | 44.42±2.77 | 0.210 |
| Ulcerative type | 32 | 97.43±9.21 | | 68.61±3.25 | | 43.65±3.68 | |
| Site of tumor | | | | | | | |
| Left semicolon | 9 | 96.27±5.30 | 0.073 | 66.45±8.43 | 0.857 | 42.29±2.54 | 0.239 |
| Right semicolon | 23 | 99.53±6.88 | | 68.52±6.57 | | 44.73±4.22 | |
| Rectum | 18 | 100.38±7.42 | | 69.44±8.96 | | 44.69±3.82 | |
| Maximum diameter | | | | | | | |
| <5 cm | 14 | 102.95±9.33 | 0.071 | 71.46±4.37 | 0.152 | 43.74±2.65 | 0.330 |
| ≥5 cm | 36 | 96.67±5.43 | | 68.53±7.49 | | 41.52±4.39 | |
| Differentiation degree | | | | | | | |
| High | 6 | 105.27±9.36 | 0.018* | 72.36±3.97 | 0.035* | 44.38±3.54 | 0.020* |
| Moderate | 41 | 95.54±4.37 | | 66.51±3.68 | | 39.46±3.69 | |
| Low+undifferentiated | 3 | 93.49±6.21 | | 64.84±5.81 | | 37.58±5.42 | |
| Depth of infiltration | | | | | | | |
| Submucosa and above | 8 | 107.59±4.58 | 0.037* | 71.64±4.75 | 0.041* | 45.68±5.19 | 0.036* |
| Muscularis | 32 | 96.73±8.60 | | 66.85±6.46 | | 40.59±4.54 | |
| Beyond muscularis | 10 | 94.35±7.63 | | 65.63±5.74 | | 39.41±5.35 | |
| Vascular/Nerve invasion | | | | | | | |
| No | 39 | 102.79±6.72 | 0.042* | 72.65±5.39 | 0.034* | 44.69±5.63 | 0.047* |
| Yes | 11 | 95.36±3.87 | | 66.54±4.96 | | 39.47±7.48 | |
| Lymphatic metastasis | | | | | | | |
| No | 36 | 101.93±4.02 | 0.039* | 71.53±4.55 | 0.025* | 45.43±6.52 | 0.038* |
| Yes | 14 | 95.69±8.26 | | 65.74±3.87 | | 40.52±2.39 | |

Note *: Statistically significant, $P<0.05$

be associated with more aggressive tumor characteristics, including poorer differentiation, deeper infiltration, vascular/nerve invasion, and lymphatic metastasis (Table 5).

## Discussion

Recent GWAS have identified multiple genetic variants linked to an increased risk of CRC (Peters et al. 2015), yet their predictive value for individuals within Chinese populations remains uncertain. The present study establishes a significant association between PON1 gene variants and CRC susceptibility. Notably, these associations appear population-specific, contrasting with null findings for the same polymorphism in hepatocellular carcinoma patients (Akkız et al. 2013), underscoring how ethnic genetic architecture may modify PON1's role in carcinogenesis. We observed that the AA+AT genotype was prevalent in both the case (100%) and control (99.85%) groups, with the rare TT genotype. The AA genotype appeared more frequently in both groups, suggesting a potential protective effect, while the AT+TT genotypes were associated with an increased CRC risk. These findings gain further significance when considering that altered expression of stress-response genes like PON1 similar to dysregulated miRNAs such as miR-211 has been consistently associated with poor prognosis in CRC, suggesting shared pathways in disease progression(Sümbül et al. 2015).Specifically, individuals with the AT+TT genotype (8.28%) had a higher likelihood of developing CRC compared to those with the AA+AT genotype (100%). Interestingly, allele frequency analysis indicated a slightly higher frequency of allele T in the case group (8.28%) than in the control group (6.45%), reinforcing the notion that the AA+AT genotype may confer protection against CRC.

Furthermore, lower PON1 mRNA and protein expression correlated with CRC progression, including poorer

differentiation and greater tumor invasiveness. This tissue-specific pattern diverges from observations in other gastrointestinal malignancies, where PON1 variants show weaker or inconsistent associations (Akkız et al. 2013), likely reflecting organ-specific detoxification demands. This pattern mirrors observations in other biomarker studies where decreased expression of protective genes or increased expression of oncogenic miRNAs independently predicted adverse clinical outcomes, highlighting the translational potential of our findings (Sümbül et al. 2015).The role of PON1 in combating oxidative stress complicates this relationship; it detoxifies reactive oxygen species (ROS), but its activity levels can fluctuate during cancer development due to genetic and environmental factors. Variants in the PON1 gene have been linked to reduced enzymatic activity and an increased risk of various cancers (Stevens et al. 2008; Antognelli et al. 2005), polymorphisms may enhance cancer susceptibility.

Contrarily, some studies report decreased PON activity among cancer patients, particularly in AA carriers, although these findings were statistically insignificant due to limited sample sizes(Demirel et al. 2021).These discrepancies, along with the ethnic variability demonstrated in studies like Akkız et al. (Akkız et al. 2013), highlight the need for population-specific investigations of PON1's cancer associations. Additional research, such as that by Ahmed et al. (Ahmed et al. 2015), found the QQ genotype equivalent to AA to be predominant in CRC patients, further indicating its potential significance in CRC risk. Inconsistent results from studies on PON1 polymorphisms regarding other cancers suggest that further investigation into their relationship with CRC is warranted (Saadat 2012).

Although some studies propose that variations in PON1, specifically rs662 and rs854560, have minimal impact on CRC development, exploring how these variants influence lipid peroxidation scavenging activities within the context of CRC progression could yield valuable insights (Logt et al. 2005). In summary, the findings imply that genetic variations in the PON1 gene, particularly the AA and TT genotypes, significantly influence CRC susceptibility by affecting enzyme activity or protein expression. These insights could have practical clinical implications, suggesting that genetic screening for PON1 variants may help identify individuals at higher risk for CRC. Such information could facilitate personalized risk assessments, enabling targeted prevention strategies, lifestyle modifications, and earlier surveillance for high-risk individuals. Furthermore, understanding PON1's role in CRC may lead to the development of novel therapeutic approaches aimed at modulating enzyme activity or enhancing oxidative stress defenses, thereby mitigating CRC risk or improving treatment outcomes. This underscores the urgent need for further research to elucidate the biological mechanisms underlying this association and translate these findings into effective screening and prevention strategies for CRC.

Our study has several limitations that should be considered when interpreting the results. First, the sample size for analyzing PON1 activity was relatively small, which may have limited the statistical power and precision of our findings. A larger sample size would provide more robust conclusions and reduce the risk of type I and type II errors. Additionally, while we focused on the Pon1-rs854560 SNP, other genetic variants, environmental factors, and lifestyle factors could confound the observed associations. Factors such as oxidative stress, diet, smoking, alcohol consumption, and physical activity may interact with PON1 genotypes to influence serum PON1 enzyme levels and activity, potentially modifying CRC risk(Frampton et al. 2016; Tenesa and Dunlop 2009). Furthermore, numerous other independent SNPs, beyond Pon1-rs854560, likely contribute to CRC susceptibility, many of which remain unidentified. These SNPs may interact with each other or other genes, complicating the genetic architecture of CRC risk. Enhancements in SNP-based risk prediction models are expected with the discovery of additional SNPs through large-scale studies and advanced genomic techniques, such as DEPTH, and can be further refined by incorporating machine learning approaches that account for all SNP variations and their interactions, offering more robust and accurate risk assessments(Wei et al. 2013; MacInnis et al. 2016). Finally, we acknowledge that the tumor microenvironment may influence adjacent normal tissue samples, potentially altering gene expression and introducing a confounding factor in our interpretation of PON1 expression. Future studies with larger, more diverse cohorts are needed to identify additional risk SNPs and clarify gene-gene and gene-environment interactions.

## Conclusion

Our study demonstrates a significant association between PON1 gene variants and CRC susceptibility. The AA+AT genotype may offer protection against CRC, while the AT+TT genotypes could increase risk. These findings highlight the potential for personalized medicine, enabling targeted prevention and treatment strategies based on PON1 genotype. Further research is needed to fully understand the relationship between PON1 variants and CRC risk, but this study adds to the growing evidence of genetic factors in CRC susceptibility

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

## Declarations

**Ethics approval and consent to participate** The study was conducted in accordance with the 1964 Helsinki Declaration and its later amendments. Ethical approval was obtained from the Institutional Review Board (IRB) of Jiangsu University Affiliated People's Hospital (K-20210038-W). The IRB granted a waiver of informed consent, as the study involved the use of anonymized data and posed minimal risk to participants.

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.



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