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Colorectal cancer screening using a multi-locus blood-based assay targeting circulating tumor DNA methylation: a cross-sectional study in an average-risk population

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Abstract

Background Effective screening for colorectal cancer (CRC) enables earlier diagnosis and intervention to improve patient survival.

Methods In this study, we prospectively conducted a blood-based CRC screening program for community residents in Hanjiang District, Yangzhou City, and evaluated the screening efficacy of a blood-based multi-locus DNA methylation assay (ColonAiQ). The ColonAiQ-positive rate and colonoscopy participation rate of the population, detection rate of intestinal lesions, and positive predictive value (PPV) of CRC and advanced adenoma (AA) were calculated, and the associated factors were explored.

Results A total of 105,285 participants were enrolled from January 2021 to December 2022, all of whom completed the ColonAiQ assay, yielding a positive rate of 6.42% (6759/105,285). The colonoscopy compliance rate was 48.56% (3282/6759). Intestinal lesions were detected in 1773 individuals (54.02%), including 63 cases of CRCs (predominately early-stage), 1195 adenomas (441 cases of AAs), 327 polyps, and 188 other benign lesions. CRC patients exhibited higher ColonAiQ scores and more positive loci compared to healthy individuals. The PPVs were 1.92% for CRC and 13.44% for AA. Among participants, 66,121 (62.8%) completed questionnaires graded by the Asia–Pacific Colorectal Screening score, with 12,139 (18.36%) classified in the high-risk tier. High-risk participants had a higher

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ColonAiQ-positive rate (11.07%) and PPVs for CRC (3.46%) and AA (22.18%). Factors associated with increased detection rates for CRC and AA included male gender, older age, a history of alcohol consumption, and prior polyps.

Conclusions Our study demonstrated that ColonAiQ assay effectively identifies high-risk population. These findings strongly suggest that the ColonAiQ assay represents a promising strategy for the early detection of CRC and AA in individuals at average risk.

Trial registration Registered at ClinicalTrials.gov (NCT05336539).

Keywords Colorectal cancer, Population-based screening, Blood-based ctDNA detection, Risk factor questionnaire assessment

Background

According to the Global Cancer Statistics 2020 (GLOBOCAN), colorectal cancer (CRC) is the second most commonly diagnosed cancer and the fifth most common cause of death by cancer in China [1]. Since 2000, increasing trends in the incidence and mortality of CRC have been observed in the Chinese population, mainly due to changes in lifestyle and diet structure, which brings great challenges to the prevention, diagnosis, and treatment of CRC [2].

Most CRCs are developed from colorectal adenomas via the conventional adenoma-carcinoma pathway together with mutations in critical oncogenes or tumor suppressor genes [3, 4]. This process can take up to 10 years, so early diagnosis and treatment for early-stage CRC could significantly improve cancer prognosis. According to the Surveillance, Epidemiology, and End Results (SEER) program, from 2012 to 2018, the 5-year survival rates of patients with early-stage and late-stage CRC were 90.9 and 15.1%, respectively. In China, the proportion of late-stage CRC is as high as 84.8%, while only 15.2% were detected in the early stage of the disease [5]. Therefore, early screening of CRC and pre-cancerous lesions in the Chinese population is urgently needed for better prevention and treatment.

Population-based screenings of CRC have been implemented worldwide during the past decades [6]. Although colonoscopy is the gold standard for CRC diagnosis, its invasive nature, difficulty in bowel preparation, and limited public awareness significantly reduce compliance rates. Additionally, the requirements of high expense and expertise make colonoscopy unsuitable for the first-line strategy in countries with limited resources or for the initial stage of screening in average-risk populations. Other strategies such as fecal occult blood test (FOBT), fecal immunochemical test (FIT), stool DNA testing, liquid biopsy, and multiple visualization tests are also widely used in different screening programs [7]. However, hemoglobin degradation caused by inappropriate sample handling and missing detection of non-hemorrhagic lesions compromise the accuracy of stool-based assays. Questionnaire-based risk assessment can expand the screening population due to its low cost but may be affected by subjective factors of participants. Although combining risk assessment is a common approach to improve cost-effectiveness [8], low compliance rate remains a significant hurdle. Therefore, there is a pressing need to explore novel, non-invasive, unbiased, and easily standardized screening methods.

In recent years, blood-based non-invasive detection, especially circulating tumor DNA (ctDNA) in blood plasma, has made great progress and application [9, 10]. For average-risk populations, peripheral blood tests are more likely to be accepted than colonoscopy or stool tests. Additionally, peripheral blood samples can be easily collected, stored, and transported in a standardized manner, thereby improving the efficacy and participation of CRC screening. ColonAiQ, a blood-based multi-locus DNA methylation assay, was firstly reported in 2021 that could effectively detect CRC, advanced adenoma (AA), and predict CRC early recurrence [11, 12]. In a case–control study [11], the sensitivity of the ColonAiQ test was 86% for CRC, 42% for AA, and 92% specificity for healthy individuals. However, validation of ColonAiQ in large cohorts, especially in average-risk populations, remains to be assessed.

Since 2021, the Hanjiang District government and Health Commission, Yangzhou City, in collaboration with the National Center for Chronic and Non-Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, have launched a CRC screening program for community residents using a risk factor assessment questionnaire and a blood-based test, ColonAiQ. This study presents findings from the program between January 2021 and December 2022. We analyzed data from 105,285 participants in 2 years to evaluate the performance of ColonAiQ in CRC early screening for average-risk population, and assess risk factors associated with intestinal lesions detection and colonoscopy compliance, aiming to improve CRC screening strategies.

Methods

Study population

The community residents aged 40–74 years old in Hanjiang District, Yangzhou City, were invited from 2021 to 2022 to engage in the CRC screening program. A small number of participants under 40 or over 74 years old were also enrolled considering their voluntary needs. All participants have signed the informed consent form and finished the ColonAiQ assay detection. This study was approved by the Medical Ethics Committee of National Center for Chronic and Non-Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention and has been registered on ClinicalTrials.gov (NCT05336539).

The inclusion criteria were as follows: (a) permanent resident population of Yangzhou City; (b) people with full capacity for behavior; (c) those who voluntarily accept the telephone follow-up after enrollment; (d) those who are willing to provide timely feedback to the researcher on information related to tumor diagnosis during outpatient visits during the study; (e) those who are willing and able to sign the informed consent form.

The exclusion criteria were as follows: (a) previous colorectal resection; (b) undergoing any cancer-related treatment; (c) those who have received major surgical treatment such as blood transfusion or transplantation within 3 months; (d) those who are participating in other interventional clinical studies within 3 months; (e) women who are pregnant or breastfeeding; (f) those with autoimmune diseases, genetic diseases, mental illness/disability, and other diseases that the investigator considers unsuitable for participation in this study; (g) poor compliance, who in the judgment of the investigator are unable to complete the study.

Risk factor assessment questionnaire

Participants were asked to undertake a risk factor assessment questionnaire for information collection. The risk assessment questionnaire was conducted face-to-face by investigators who have received uniform training. The investigated items included gender, age, body mass index (BMI), marital status, reproductive history, education level, occupation, smoking history, alcohol drinking history, and medical histories of chronic diarrhea, chronic constipation, mucus or blood in the stool, chronic appendicitis or appendectomy, chronic cholecystitis or cholecystectomy, chronic enteritis, polyp, cancer, diabetes, serious unhappy life events within the last 20 years, and CRC in a first-degree relative.

We utilized the Asia–Pacific Colorectal Screening score (APCS) to assess CRC risk by questionnaire [13]. The APCS score incorporates four risk factors (age, gender, family history of CRC in a first-degree relative, and smoking history) and is divided into average risk (AR, score: 0–1), moderate risk (MR, score: 2–3), and high risk (HR, score: 4–7) tiers according to the cumulative score [13].

Blood collection and ColonAiQ assay detection

About 10 mL peripheral blood was collected from each participant into EDTA-containing tubes or cell-free DNA BCT® tubes (Streck Inc., Omaha, USA) and shipped on dry ice to Xiangya Medical Laboratory, Central South University (Changsha, China). All personnel involved in experimental work were blinded to the identities of samples. Plasma was isolated using a double centrifugation protocol by centrifugation at 1600 *g* for 10 min, followed by 16,000 *g* for 10 min, and stored at − 80 °C until subsequent analysis. Circulating free DNA (cfDNA) was extracted from plasma specimens using the QIAamp Circulating Nucleic Acid kit (Qiagen, 55,114) according to the manufacturer's instructions. The yield of the extracted DNA was quantified using the Qubit fluorometric method (Thermo Fisher, Q33226). Then, a total of 10–20 ng DNA was bisulfite converted using the Methylcode Bisulfite Conversion Kit (Thermo Fisher, MECOV50) according to the manufacturer's protocol. After purification, bisulfite-converted DNA was eluted in 15 μL and ready for use in a multiplex qPCR assay.

Cf DNA methylation was measured with the ColonAiQ assay as previously described [11, 14]. In brief, the PCR process involved pre-amplification PCR and fluorescence PCR and was performed using the ColonAiQ detection kit [IVD approved, Human *Septin9*, *BCAT1*, *IKZF1*, *BCAN*, *VAV3* gene methylation combined detection kit (PCR-fluorescence probe method), Singlera Health Technologies (Shanghai) Ltd.]. The test results were determined according to the kit's protocol, ensuring that the internal control *ACTB* had a Ct value of ≤ 23 for all samples and controls. The quality control was rigorously performed for each testing batch to ensure accuracy and reliability. Each batch included both positive and negative controls, as well as internal amplification controls, which were strategically incorporated to monitor assay performance throughout the run. After each run, a thorough post-run analysis was conducted, during which amplification curves were examined for proper morphology and consistency.

Colonoscopy screening

Participants with positive ColonAiQ results were recommended to undergo colonoscopy. For patients with positive colonoscopy results, biopsies were performed, and all removed tissues should be evaluated by gastrointestinal pathologists. CRC stage was defined based on the American Joint Committee on Cancer TNM system [15].

AA was defined as adenoma measuring ≥ 10 mm, and/or containing substantial villous component (≥ 25%), or exhibiting high-grade dysplasia. If more than one lesion was discovered, the most advanced lesion was used to classify the participant. Moreover, in the colonoscopy-accepted population, carcinoembryonic antigen (CEA) and FIT tests were simultaneously recommended and optional.

Patient and public involvement

Patients and/or the public were not involved in the design, conduct, reporting, or dissemination plans of this research.

Statistical analysis

Chi-square test and Fisher's exact test were conducted using the SPSS software (version 29.0) to analyze the factors associated with CRC and AA detection rates, ColonAiQ-positive rate, and colonoscopy compliance. Mann–Whitney *U* test was performed by SPSS software (version 29.0) to compare the ColonAiQ score distribution and the number of positive sites detected by colonoscopy in different categories. Odds ratio and multifactor binary logistic regression analyses were conducted using R project (version 4.2.2) and R studio (version 2022.12.0) to identify the key characteristics influencing colonoscopy compliance rates. All *p* values were considered two-sided, with values less than 0.05 deemed statistically significant.

Results

Characteristics of the study population

A total of 105,285 eligible residents participated in the CRC screening program from 2021 to 2022 (Fig. 1). All participants provided blood samples for the ColonAiQ test, while 66,121 (62.8%) completed CRC risk assessment questionnaires. The characteristics of the study population, including gender, age, other baseline information, and medical history are presented in Table 1 and Additional file 1: Table S1. Overall, more women ($N=65,439$, 62.15%) engaged in the program, and the majority of participants were between 40 and 69 years old ($N=80,284$, 76.25%). The distribution of body mass index (BMI) of participants was standard (46.97%), underweight (0.97%), overweight (13.77%), and obesity (1.24%).

ColonAiQ assay performance and outcomes in colonoscopy

The ColonAiQ assay yielded a positive rate of 6.42% ($N=6759$) among participants (Fig. 1). By March 2023, 3282 people had undergone colonoscopy, achieving a colonoscopy compliance rate of 48.56% (Fig. 1). Of the ColonAiQ-positive participants, 5273 completed the questionnaire, and 3018 underwent a colonoscopy.

In 3282 cases of colonoscopy in the ColonAiQ-positive population, the overall detection rate of intestinal lesions was 54.02% ($N=1773$), including 63 CRCs, 1195 adenomas (with 441 cases of AAs), 327 polyps, and 188 other benign lesions (Fig. 1 and Additional file 1: Table S2). The positive predictive value (PPV) of CRC and AA was

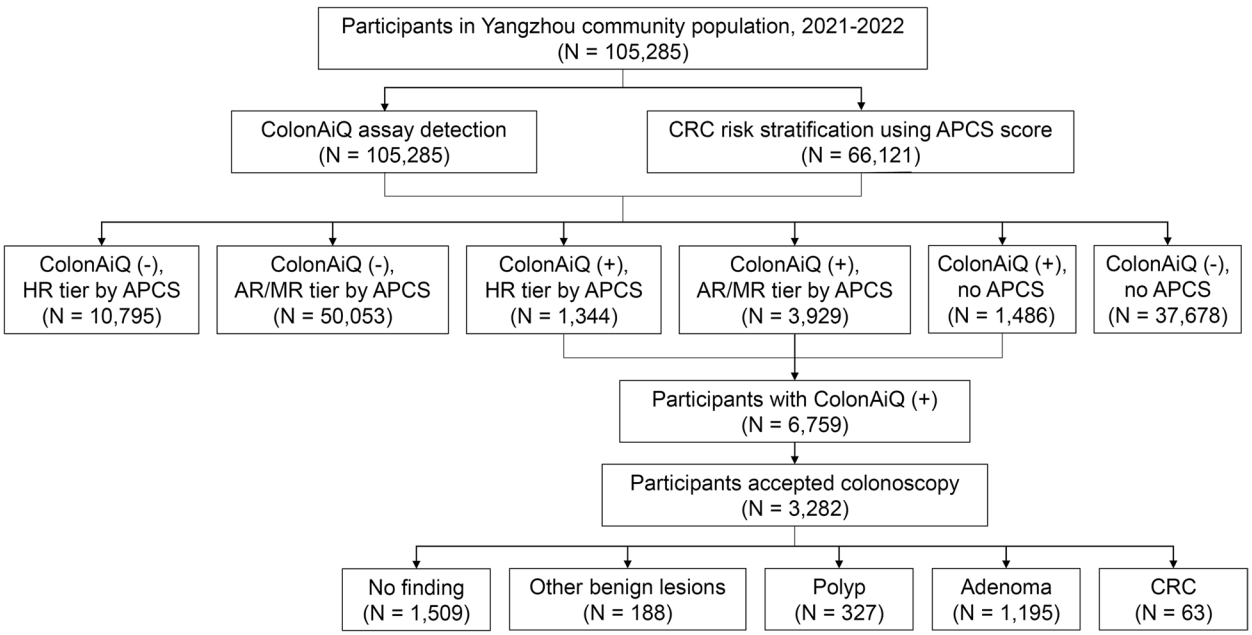

Fig. 1 Flow chart of Yangzhou program for CRC screening, 2021–2022

Table 1 Baseline information of the population who finished the questionnaire

	Group	Number of participants[a] (%)
Gender	Men	24,111 (36.46)
	Women	41,011 (62.02)
Age	< 40	1283 (1.94)
	40–49	8506 (12.86)
	50–59	21,696 (32.81)
	60–69	21,454 (32.45)
	≥ 70	13,182 (19.94)
BMI	< 18.5	640 (0.97)
	18.5–25	31,058 (46.97)
	25–30	9104 (13.77)
	≥ 30	818 (1.24)
Marital status	Single	93 (0.14)
	Married	56,369 (85.25)
Reproductive history	Yes	14,131 (35.11)
	No	14 (0.03)
Education	Low[b]	47,881 (72.41)
	High[c]	3806 (5.76)
Occupation	Government agencies	158 (0.24)
	Public institution	940 (1.42)
	State-owned enterprise	306 (0.46)
	Private enterprise	7407 (11.2)
	Farmer	2283 (3.45)
	Freelance work	7748 (11.72)
	Unemployed	9674 (14.63)
	Others	22,959 (34.72)
Smoking (current or past)	Yes	9140 (13.82)
	No	49,925 (75.51)
Drinking	Yes	9129 (13.81)
	No	49,820 (75.35)
Chronic diarrhea	Yes	1860 (2.81)
	No	56,742 (85.82)
Chronic constipation	Yes	2561 (3.87)
	No	56,577 (85.57)
Mucus or blood in the stool	Yes	133 (0.2)
	No	58,918 (89.11)
Chronic appendicitis or appendectomy	Yes	4012 (6.07)
	No	54,957 (83.12)
Chronic cholecystitis or cholecystectomy	Yes	4188 (6.33)
	No	54,700 (82.73)
Chronic enteritis	Yes	408 (0.62)
	No	58,536 (88.53)
Polyp	Yes	2579 (3.9)
	No	56,190 (84.98)
Cancer	Yes	788 (1.19)
	No	58,420 (88.35)
Diabetes	Yes	5147 (7.78)
	No	53,708 (81.23)
Serious unhappy life events within the last 20 years	Yes	34 (0.05)

Table 1 (continued)

	Group	Number of participants[a] (%)
	No	58,828 (88.97)
CRC in a first-degree relative	Yes	587 (0.89)
	No	58,380 (88.29)

Abbreviation: *BMI* Body mass index

[a] A total of 66,121 participants completed the questionnaire, among which some participants had missing answers. This was mainly due to the length of the questionnaire, which may cause some participants to skip questions during the answering process. For reproductive history, only women were included in the calculation

[b] Low: No formal education/Primary/Middle school

[c] High: College or above

1.92 and 13.44%, respectively. Of the 63 detected CRC cases, 24 cases (38.10%) occurred in the rectum, 22 cases (34.92%) in the left colon, 15 cases (23.81%) in the right colon, and two cases (3.17%) had multiple or other locations (Fig. 2a). About 46% of CRC patients were over 70 years of age (Additional file 1: Fig. S1). Of the 57 CRC patients with definitive stage, patients in stage 0–I, stage II, stage III, and stage IV were 26.32, 43.86, 24.56, and 5.26%, respectively (Fig. 2b). In China, according to a multicenter hospital-based study, 15.2, 32.9, 33.5, and 18.3% of CRC patients are in stage I, stage II, stage III, and stage IV, respectively [5]. Thus, our results suggested a significant stage shift ($\chi^2 = 13.137$, $P = 0.004$) in CRC detection in the Yangzhou program.

We further compared the ColonAiQ scores among participants with different colonoscopy outcomes. The ColonAiQ scores of CRC patients were significantly higher than those of healthy individuals (Fig. 2c), while CRC patients had more positive loci of ColonAiQ than healthy individuals (Additional file 1: Fig. S2, $P < 0.001$, Mann–Whitney *U* test). The ColonAiQ scores of CRC were also significantly higher than those of AA, and there was no significant difference in the ColonAiQ scores between AA and non-AA (Fig. 2c). In addition, the ColonAiQ scores were significantly lower in patients with stage 0–I CRC compared to those with stage II CRC (Fig. 2d, $P = 0.005$). No significant difference in ColonAiQ scores was observed among patients with stage II–IV CRC (Fig. 2d). The distribution of test markers in 3282 participants with a positive ColonAiQ test is shown in Additional file 1: Fig. S3.

Risk stratification using the Chinese population colorectal risk score

Although only 62.8% of participants completed the questionnaire, the distribution of gender and age in this subset of the population shows almost no difference from that of the total population and is thus representative (Additional file 1: Table S1). According to the APCS score system, among the 66,121 participants who completed the questionnaire, 8490 (12.84%) were categorized as AR, 45,492 (68.80%) as MR, and 12,139 (18.36%) as HR (Table 2). The ColonAiQ-positive rate was significantly higher in MR (7.73%) and HR (11.07%) participants compared to AR participants (4.86%), suggesting a unanimous judgment of CRC risk. However, colonoscopy compliance rates did not significantly differ across the various risk tiers. In addition, there was no significant difference in APCS scores between CRC patients of all stages (Additional file 1: Fig. S4).

Next, among 3018 participants who both completed questionnaires and underwent colonoscopy, we further analyzed whether advanced intestinal lesions, including AA and CRC, were detected more frequently in the HR tier compared to AR or MR tiers (Table 2). The PPVs for AA and CRC in this subset of the population were 13.25 and 1.89%, respectively, which are close to the PPVs in the entire population (13.44% and 1.92%). As expected, the PPVs for AA were significantly higher in MR participants (10.81%) and HR participants (22.18%) compared to AR participants (4.07%). Since no CRC cases were detected in AR participants, we compared the PPV for CRC between MR and HR tiers, finding a significantly higher PPV for CRC in HR participants (3.46%) than in MR participants (1.49%). Given that colonoscopy was recommended based on the positive results of the ColonAiQ assay, these findings suggest the effective risk assessment capabilities of both the ColonAiQ and the APCS score.

Factors associated with CRC, AA, ColonAiQ positivity, and coloscopy compliance

We further analyzed associated factors with the detection rate of CRC or AA, the ColonAiQ positive rate, and colonoscopy compliance among participants. Higher detection rates for CRC and AA were associated with male

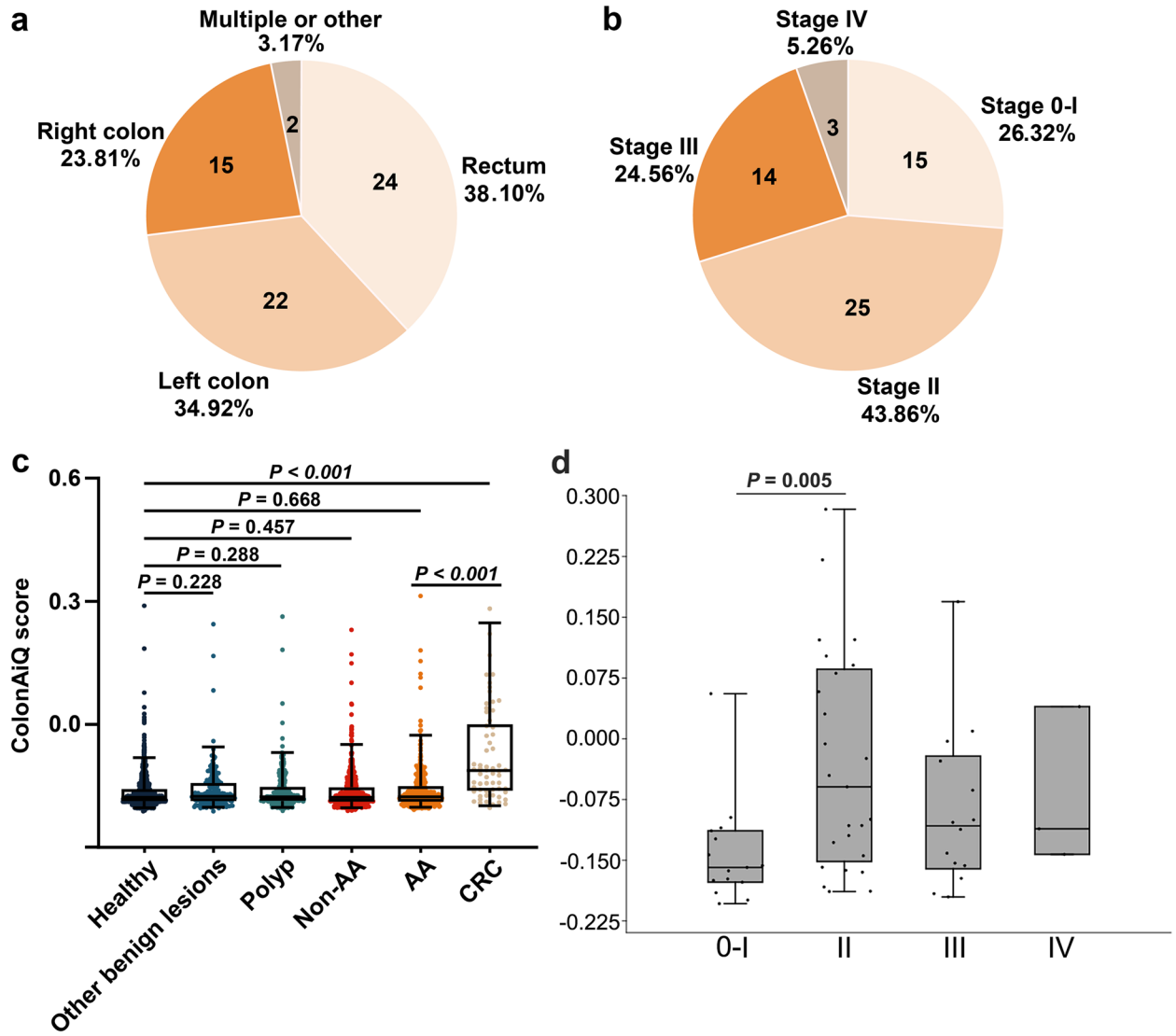

Fig. 2 Results of colonoscopy and ColonAiQ in colonoscopy-accepted participants. **a** The location distribution of 63 detected CRC. **b** The TNM stage distribution of 57 CRC patients. **c** The ColonAiQ score distribution of samples in different categories was detected by colonoscopy. The box extends from the 25th to 75th percentiles, the whiskers are drawn from the 2.5th to 97.5th percentile, and all data points are shown as circles. *P* value was calculated by Mann–Whitney *U* test. Abbreviation: Non-AA, Non-advanced adenoma; AA, advanced adenoma; CRC, colorectal cancer. **d** The ColonAiQ score distribution in different stages. Mann–Whitney *U* test

gender, older age, history of alcohol drinking, polyps, and CRC in a first-degree relative (Fig. 3a–e). Additionally, chronic diarrhea, mucus or blood in the stool, and a history of cancer were linked to increased CRC detection, while smoking, chronic cholecystitis, or cholecystectomy were associated with higher AA detection (Additional file 1: Table S2 and S3).

The ColonAiQ-positive rate was significantly higher in men, older age, and those with abnormal BMI, higher education level, specific occupations, and history of smoking or alcohol drinking (Table 3). Individuals with medical histories, e.g., polyps, cancer, chronic cholecystitis or cholecystectomy, diabetes, and chronic appendicitis or appendectomy also showed increased ColonAiQ positive rate (Additional file 1: Table S4).

Colonoscopy compliance, a determinant factor of the effectiveness of CRC screening, was influenced by various characteristics. We utilized chi-square tests to explore associate factors with colonoscopy compliance rates (Additional file 1: Table S5 and S6). Except for the gender factor ($P=0.044$), we incorporated the factors with P value <0.01 to establish the multifactor binary logistic

Table 2 CRC risk stratification based on APCS score, the ColonAiQ-positive rate, colonoscopy compliance rate, and AA and CRC detection rates of participants in different tiers

		Questionnaire		ColonAiQ				Colonoscopy				AA				CRC			
	Tier	N	Percent (%)	n	Positive rate (%)	OR (95% CI)	*P* value	n	Compliance rate (%)	OR (95% CI)	*P* value	n	Detection rate (%)	OR (95% CI)	*P* value	n	Detection rate (%)	OR (95% CI)	*P* value
All		66,121		5273				3018				400	13.25			57	1.89		
APCS	AR	8490	12.84	413	4.86	*Reference*		221	53.51	*Reference*		9	4.07	*Reference*		0	0		
	MR	45,492	68.80	3516	7.73	1.64 (1.48–1.82)	< 0.0001	2017	57.37	1.17 (0.95–1.43)	0.14	218	10.81	2.85 (1.44–5.64)	0.0026	30	1.49	*Reference*	
	HR	12,139	18.36	1344	11.07	2.44 (2.17–2.73)	< 0.0001	780	58.04	1.20 (0.96–1.50)	0.11	173	22.18	6.71 (3.37–13.36)	< 0.0001	27	3.46	2.38 (1.40–4.02)	0.0013

66,121 participants who finished the questionnaire were included in the statistics. 3018 participants who both underwent colonoscopy and finished the questionnaire were included in the statistics of AA and CRC detection rates

Abbreviation: *APCS* Asia–Pacific Colorectal Screening, *AR* Average risk, *MR* Moderate risk, *HR* High risk

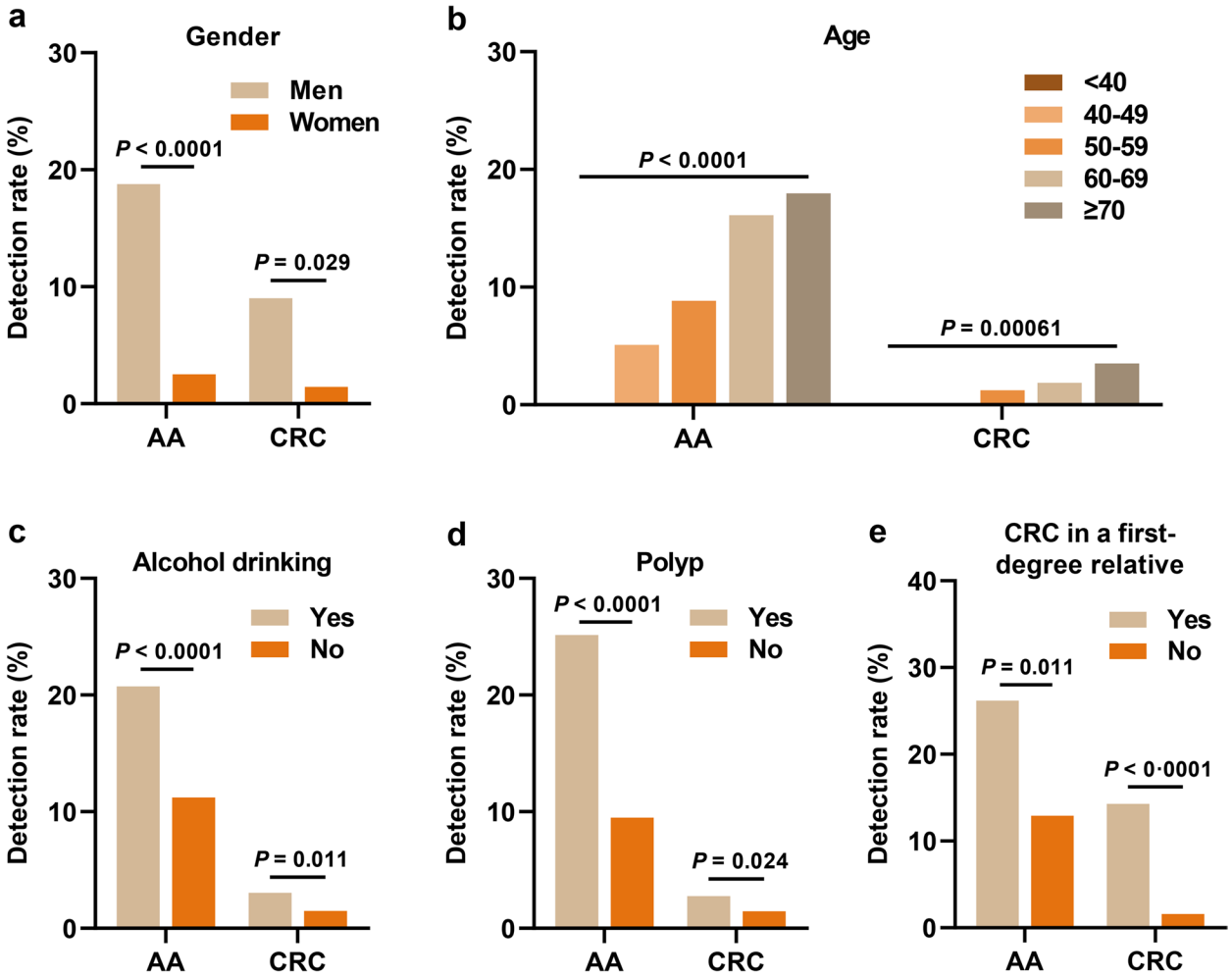

Fig. 3 Characteristics associated with detection rates of both AA and CRC. AA and CRC detection rates in participants with different gender (**a**), age (**b**), history of alcohol drinking (**c**), history of polyp (**d**), and history of CRC in a first-degree relative (**e**). *P* value was calculated by the chi-square test or Fisher's exact test

regression model, which ultimately identified the key characteristics affecting colonoscopy compliance rates in ColonAiQ-positive participants (Additional file 1: Fig. S5). Generally, women, older adults, overweight or obese individuals, those with higher education, farmers, freelance workers, and individuals with a history of chronic constipation or polyps showed a greater willingness to undergo colonoscopy than their counterparts in different groups.

Comparison of CEA and FIT test with ColonAiQ assay

In addition, CEA and FIT tests were completed in a limited number of ColonAiQ-positive participants, with 155 and 141 valid results received, respectively (Additional file 1: Table S7). Among the 17 CEA-positive participants, one case of CRC and six cases of AA were identified, while five cases of CRC and 27 cases of AA were missing in the 138 CEA-negative participants. Similarly, one case of CRC and 1 case of AA were identified in the eight FIT-positive participants, while ten cases of AA were missing in the 133 FIT-negative participants.

Discussion

The Yangzhou program recruited a large-scale, representative, and average-risk population for CRC screening. The main findings include (1) an overall positive rate of 6.42% for ColonAiQ assay and a colonoscopy compliance rate of 48.56% in ColonAiQ-positive population; (2) an overall detection rate of 54.02% for intestinal lesions with PPVs of 1.92 and 13.44% for CRC and AA, respectively; (3) 70.18% of CRC cases were detected at early, curable stages (stage 0–II), demonstrating a significant stage shift in CRC detection; and (4) ColonAiQ assay combined

Table 3 Characteristics associated with ColonAiQ-positive rates

	Group	ColonAiQ assay		
		Number of positive participants (%)	Positive rate (%)	χ^2 value, *P* value
Gender[a]	Men	3112 (46.04)	8.13	278.142, < 0.0001
	Women	3594 (53.17)	5.49	
Age	< 40	67 (0.99)	3.99	364.436, < 0.0001
	40–49	562 (8.31)	4.29	
	50–59	1856 (27.46)	5.46	
	60–69	2344 (34.68)	7.06	
	≥ 70	1877 (27.77)	8.63	
BMI	< 18.5	64 (1.21)	10	51.793, < 0.0001
	18.5–25	2518 (47.75)	8.11	
	25–30	946 (17.94)	10.39	
	≥ 30	87 (1.65)	10.64	
Education	Low	3953 (74.97)	8.26	4.55, 0.033
	High	352 (6.68)	9.25	
Occupation	Government agencies	26 (0.49)	16.46	736.467, < 0.0001
	Public institution	76 (1.44)	8.09	
	State-owned enterprise	65 (1.23)	21.24	
	Private enterprise	537 (10.18)	7.25	
	Farmer	515 (9.77)	22.56	
	Freelance work	593 (11.25)	7.65	
	Unemployed	656 (12.44)	6.78	
	Others	1822 (34.55)	7.94	
Smoking (current or past)	Yes	950 (18.02)	10.39	76.088, < 0.0001
	No	3837 (72.77)	7.69	
Drinking	Yes	930 (17.64)	10.19	64.246, < 0.0001
	No	3836 (72.75)	7.7	
Chronic appendicitis or appendectomy	Yes	358 (6.79)	8.92	4.064, 0.044
	No	4410 (83.63)	8.02	
Chronic cholecystitis or cholecystectomy	Yes	460 (8.72)	10.98	50.848, < 0.0001
	No	4303 (81.6)	7.87	
Polyp	Yes	767 (14.55)	29.74	1694.141, < 0.0001
	No	3998 (75.82)	7.12	
Cancer	Yes	101 (1.92)	12.82	24.088, < 0.0001
	No	4685 (88.85)	8.02	
Diabetes	Yes	501 (9.5)	9.73	20.009, < 0.0001
	No	4271 (81)	7.95	

For other factors, 66,121 participants who finished the questionnaire were included, among which 5,273 participants achieved positive ColonAiQ results

[a] For gender and age statistics, 103,710 participants who accepted the ColonAiQ test were included, among which 6,759 participants achieved positive ColonAiQ results

with APCS score resulted in higher PPVs of CRC (3.46%) and AA (22.18%) in HR participants.

Although colonoscopy is the gold standard for CRC detection, low compliance limits its utility in screening [16]. To improve this, non-invasive strategies such as FOBT, FIT, and risk assessment questionnaires (i.e., Harvard Risk Index [17] and APCS score [13]) have been commonly used [18]. Yangzhou program is notable as the first large-scale study to use novel blood-based technology for CRC screening in an average-risk population. Compared to canSPUC (CRC Screening Program in Urban China) [19] and Coloclear (stool DNA assay-based screening program) [20], Yangzhou program had a significantly higher colonoscopy compliance rate of 48.56% (14% in canSPUC, 29.38% in Coloclear) and a better detection for intestinal lesions. While Coloclear

program resulted PPV of CRC was 1.56%, canSPUC program yielded PPVs of 0.25 and 3.07% for CRC and AA, respectively. In addition, as a blood-based test, ColonAiQ is more acceptable and easily standardized in sample collection, storage, and processing, effectively improving the efficacy and participation in CRC screening.

While CRC patients face serious impacts on their quality of life and a great financial burden [21], the cost-effectiveness analysis of CRC screening is still at an early stage in China [22, 23]. The low compliance has been proven as one of the most important influencing factors of cost-effectiveness. Yangzhou program's high colonoscopy compliance and full participation in the blood-based ColonAiQ assay suggest strong potential for cost-effectiveness. In addition, effective CRC screening theoretically leads to a stage shift of tumor and improvement of 5-year survival [24, 25]. Yangzhou program demonstrated a significant stage shift in detected CRC cases, likely leading to greater prevention, reduced medical costs, and improved social benefits.

Several challenges are present in lower colonoscopy compliances especially in men despite higher lesions detection rates, in young people despite an increasing incidence [26], and in people over 70 years old with complicate chronic diseases [27]. Awareness of the necessity for CRC screening targeting those groups could improve to obtain better benefits and outcomes. With a similar concern of compliance, we chose the ColonAiQ test over the CRC risk assessment questionnaire to recommend colonoscopy to the target population in this study, mainly because that near 40% of participants had not finished the questionnaire at the time point that we collected the data. Nevertheless, the subset of the population who completed the questionnaire displayed no difference in age or gender distribution with the whole population. We noticed that the PPVs of CRC and AA in HR participants were significantly higher, which indicated a strong unanimous judgment and effectiveness of CRC prediction. A combination of the ColonAiQ assay and risk assessment questionnaire would likely optimize future screening programs.

A limitation of this study is the lack of colonoscopy in the ColonAiQ-negative participants, a common issue in real-world CRC screening for average-risk population. Participants defined as HR using the APCS score warrant closer attention in future studies. Moreover, the relatively low percentage (1%) of participants with first-degree relatives diagnosed with CRC in this study may reflect region-specific population characteristics or reporting accuracy [19, 28]. Thirdly, recruitment was conducted across a large number of sites simultaneously that posed great challenges in tracking the initial number of participated individuals. However, we implemented the inclusion and exclusion criteria on site to ensure eligible participants with accurate identification and record at each location. Building on the past 2 years of experience, Yangzhou program aims to improve the colonoscopy compliance and data collection in future recruitment efforts.

Conclusions

In summary, ColonAiQ significantly stratified high-risk individuals, improved colonoscopy compliance, and achieved a high detection rate of CRC and AA in CRC screening for average-risk population. Our findings highlight the crucial benefits of ColonAiQ for early-stage CRC detection, making it an effective strategy for CRC screening.

Abbreviations

AA	Advanced adenoma
APCS	Asia-Pacific Colorectal Screening score
AR	Average risk
BMI	Body mass index
canSPUC	CRC Screening Program in Urban China
CEA	Carcinoembryonic antigen
cfDNA	Circulating free DNA
CRC	Colorectal cancer
ctDNA	Circulating tumor DNA
FIT	Fecal immunochemical test
FOBT	Fecal occult blood test
HR	High risk
MR	Moderate risk
PPV	Positive predictive value

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-024-03777-2.

Additional file 1: Table S1. Overview of the study population by gender and age. Table S2. Colonoscopy results and correlation analysis between CRC and AA detection rates with clinical characteristics. Table S3. Colonoscopy results and correlation analysis between CRC and AA detection rates with different medical histories. Table S4. The positive rate and correlation analysis of ColonAiQ assay in populations with different characteristics. Table S5. The colonoscopy compliance rate and correlation analysis of populations with different characteristics. Table S6. The colonoscopy compliance rate and correlation analysis of populations with different medical histories. Table S7. Results of colonoscopy of ColonAiQ positive participants with different outcomes of CEA test and FIT test. Fig. S1. The distribution of colorectal cancer (CRC) cases detected in different age groups. Fig. S2. Comparison of the number of positive loci between colorectal cancer (CRC) patients and healthy individuals. Fig. S3. The distribution of test markers in 3,282 participants with a positive ColonAiQ test. Fig. S4. The APCS score distribution in different stages. Fig. S5. OR of risk factors associated with colonoscopy compliance rate.

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Authors' contributions

BW, YZ, JW, JYF, and RL developed the study concept and drafted the analysis plan. BW, JL, BD, YL, HJ, QY, CW, and WP collected the data. QL, HL, YS, NW, QX, WL, YC, FL, HY, and YW analyzed the data and prepared the results. BW, YD, and

DX verified the data and results. BW, HL, and YS drafted the initial manuscript. JW, JYF, RL, and YZ revised the manuscript. All authors read 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
Human samples and participation in the study have been done according to the Declaration of Helsinki. Written consent was obtained from all participants and this study was approved by the Medical Ethics Committee of National Center for Chronic and Non-Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention (No. 202322).

Consent for publication
Not applicable.

Competing interests
YZ, HL, YS, YL, HJ, QY, CW, WP, FL, HY, and RL report them as employees of Singlera Genomics. RL reports stock ownership in Singlera Genomics and is an employee of Singlera Genomics.

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