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Research Paper

A Propensity Score-adjusted Analysis of the Effects of Ubiquitin E3 Ligase Copy Number Variation in Peripheral Blood Leukocytes on Colorectal Cancer Risk

Haoran Bi, Yupeng Liu, Tian Tian, Tingting Xia, Rui Pu, Yiwei Zhang, Fulan Hu✉, and Yashuang Zhao✉

Department of Epidemiology, Public Health College, Harbin Medical University, 157 Baojian Street, Harbin, Heilongjiang, People's Republic of China.

✉ Corresponding authors: Yashuang Zhao, Ph.D., Department of Epidemiology, Public Health College, Harbin Medical University, 157 Baojian Street, Nangang District, 150081 Harbin, People's Republic of China. Tel: 86-(0)451-87502823; Fax: 86-(0)451-87502885; E-mail: zhao_yashuang@263.net and Fulan Hu, Ph.D., Department of Epidemiology, Public Health College, Harbin Medical University, 157 BaojianStreet, Nangang District, 150081 Harbin, People's Republic of China. Tel: 86-(0)451-87502823; Fax: 86-(0)451-87502885; E-mail: hufulan@ems.hrbmu.edu.cn

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Abstract

Background: The ubiquitin ligases E3 (E3s) plays a key role in the specific protein degradation in many carcinogenic biological processes. Colorectal cancer (CRC) development may be affected by the copy number variation (CNV) of E3s. Prior studies may have underestimated the impact of potential confounding factors' effects on the association between gene CNV and CRC risk, and CRC risk predictive model integrating gene CNV patterns is lacking. Our research sought to assess the genes CNVs of *MDM2*, *SKP2*, *FBXW7*, *β-TRCP*, and *NEDD4-1* and CRC risk by using propensity score (PS) adjustment and developing models that integrate CNV patterns for CRC risk predictions.

Methods: This study comprising 1036 participants used traditional regression and different PS techniques to adjust the confounding factors to evaluate the relationships between five gene CNVs and CRC risk, and to establish a CRC risk predictive model. The AUC was applied to evaluate the effect of the model. The categorical net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) were analyzed to evaluate the discriminatory accuracy improvement among the models.

Results: Compared to variable adjustment, the odds ratios (ORs) tended to be conservative and accurate with narrow confidence intervals (CIs) after PS adjustment. After PS adjustment, *MDM2* amplification was related to increased CRC risk (Amp-pattern: OR = 8.684, 95% CI: 1.213-62.155, $P = 0.031$), whereas *SKP2* deletion and the (del+amp) genotype were associated with reduced CRC risk (Del-pattern: OR = 0.323, 95% CI: 0.106-0.979, $P = 0.046$; Var-pattern: OR = 0.339, 95% CI: 0.135-0.854, $P = 0.024$). The predictive model integrating the gene CNV pattern could correctly reclassify 1.7% of the subjects.

Conclusions: *MDM2* amplification and *SKP2* CNVs are associated with increased and decreased CRC risk, respectively; abnormal CNV-integrated model is more precise for predicting CRC risk. Further studies are needed to verify these encouraging outcomes.

Key words: Colorectal cancer; Copy number variation; E3 ligase; Propensity score; Predictive model

Introduction

Colorectal cancer (CRC) remains an influential public health threat in most countries. In the United States alone, there are approximately 140,250 new CRC cases and 50,630 deaths owing to CRC are projected to occur in 2018 [1]. In China, CRC is still the fifth leading threat to men and the fourth leading threat to women [2]. Genetic susceptibility was shown to have a significant role in the etiology of CRC [3, 4]. Recently, copy number variation (CNV) has been identified as an important genomic molecular

biomarker of CRC predisposition [5, 6]. CNV can increase or decrease relapsing chromosomes, leading to abnormal gene expression that affects cancer-related biological processes [7].

E3 ubiquitin ligase (E3) plays a key role in the specific protein degradation of the ubiquitin-proteasome system, which participates in cell proliferation, differentiation, apoptosis, angiogenesis, and cell signaling [8]. Studies suggested that the abnormal expression of several key E3 members (*MDM2* [9], *SKP2* [10], *FBXW7* [11], *β-TRCP* [12], and *NEDD4-1* [13]) caused by CNV was associated with many malignancies, including CRC. *MDM2* both negatively regulates p53 and targets p53 for degradation [14]. Moreover, *MDM2* also interacts with pRb [15], E2F1 [16] and Numb [17] to participate in cellular processes. *SKP2* is involved in cell cycle progression, signal transduction, and transcription by mediating the ubiquitination and degradation of some key proteins, such as cyclin E, p57, p21, and E2F1 [18-21]. Specifically, *SKP2* mediates the degradation of p27 from the early S phase [22] and c-Myc during the G1 to S phase [23] to regulate cell cycle transition. *FBXW7* targets several key regulatory proteins involved in cell division and cell fate determination, including cyclin E1, c-Myc, c-Jun and Notch [24-26]. *β-TRCP* regulates cell signaling pathways by degrading key signal transduction factors, such as β-catenin for Wnt/β-catenin signaling and IκBα for NF-κB signaling [27, 28]. *β-TRCP* also ubiquitylates several cell cycle regulators, such as EMI1/2, WEE1A, and CDC25 [29]. *NEDD4-1* not only targets PTEN for proteasomal degradation but also transports PTEN into the nucleus [30]. In addition, *NEDD4-1* targets several important proteins for degradation, such as Ras [31], MDM2 [32], HER3/ErbB3 [33], EGFR [34], and Notch [35].

Currently, CNV in germline DNA is attracting public attention [36, 37], while the relationship between E3s CNV in peripheral blood leukocyte DNA and CRC risk is still poorly explored. CRC risk predictive models mainly incorporate family history, lifestyle and environmental risk factors. Moreover, the predictive effectiveness of models considering single nucleotide polymorphisms (SNPs) and environmental factors are not ideal in that the areas under the curve (AUC) of the receiver operating characteristic (ROC) curve are between 0.57~0.73 [38-40]. CNV as a regional DNA structural variation may provide more powerful evidence for the CRC risk prediction.

Recently, there has been increasing interest in propensity score (PS), with PS being a balancing score, defined as the probability of patients being assigned to an intervention given a set of covariates [41]. Additionally, a comparison of traditional logistic regression using PS to control numerous confounders can be more efficient [42].

The purpose of this second analysis study was to investigate whether the results of our primary study that focused on the associations between gene CNVs of *MDM2*, *SKP2*, *FBXW7*, *β-TRCP*, and *NEDD4-1* and CRC risk analyzed with traditional logistic regression [43] can be attenuated by adjusting the potential confounding factors by PS method. We further developed CRC risk predictive models integrating different CNV patterns and measured their predictive power.

Materials and Methods

Subjects and data collection

After obtaining informed consent from study subjects, and approval from the Institutional Research Board of Harbin Medical University, 518 CRC cases and 518 age- (±2 years) and residence-matched controls were recruited from the Tumor Hospital of Harbin Medical University and the Second Affiliated Hospital, respectively, from November 1st, 2004 to May 1st, 2010 (Figure 1). All participants were interviewed face-to-face with a structured standard questionnaire that was adopted from Shu et al [44], collecting information on demographic characteristics, lifestyle factors (including family history, smoking, alcohol consumption, occupational physical activity), and diet during the 12 months preceding the interview.

DNA extraction and CNV detection

We extracted genomic DNA from 1036 whole blood samples using QIAGEN DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany, Cat#51106). The copy numbers of *MDM2*, *SKP2*, *FBXW7*, *β-TRCP*, and *NEDD4-1* were detected using custom designed TaqMan Copy Number Assays (Table S1) on an Applied Biosystems 7500 Fast real-time PCR system (Thermo Fisher Scientific, America) with a 10 μl reaction volume containing 20 ng DNA, 5 μl TaqMan Universal PCR Master Mix, 0.5 μl of the CNV assay, and 0.5 μl of the reference RNase P assay (Applied Biosystems, Carlsbad, Calif). The PCR conditions were as follows: 95℃ for 15 seconds and 60℃ for 1 minute for 40 cycles. One sample with 2 copies of CNV was used as the quality control in every 96-well assay plate and every sample was repetitively detected three times. Then the CNV detection results were analyzed by Copy Caller version 2.0 software (Applied Biosystems) to estimate the gene copy numbers.

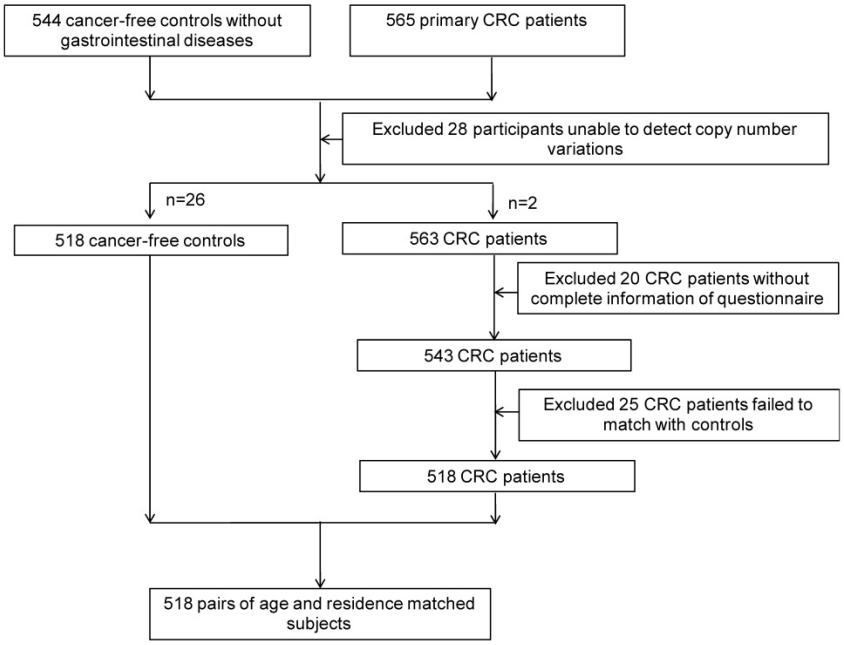

Figure 1. The flow of participants.

Propensity score method

Before PS weighing, the missing values were addressed using multiple imputations. We used the PS strategy to overcome the possible biases in selection and observed differences in baseline characteristics between participants. The estimates of the probability of being in the two groups were derived from a multivariable logistic regression model, including the variables that could potentially affect the CRC risk [45]. We applied stepwise screening to select the independent variables in the regression analysis with an entering significance level of 0.05 and an excluding significance level of 0.2. The model goodness-of-fit test and predictive power were validated with the Hosmer-Lemeshow and C statistic, respectively. The covariates balance after PS matching was checked using statistical significance testing (*P* values < 0.05 in the overall analysis, *P* values < 0.01 in PS stratification by Bonferroni's correction [46]) [47].

After estimating PS, we applied three PS adjusting methods (PS matching, PS stratification and regarding PS as an additional covariate), and the PS matching was performed as a 1:1 nearest neighbor matching analysis with the caliper of 0.2 and without replacement [48]. Five subclasses were stratified based on the quantiles of the score. Additionally, we applied four regression analyses with different covariate adjustments. The first analysis calculated the crude odds ratio (OR); the second analysis was adjusted for the confounding factors that included in the PS in a traditional multiple regression; the third analysis was adjusted by PS as a covariate; and in the last analysis, the cases with the extreme scores were excluded based on the third analysis to exam the authenticity and stability. Finally, we performed subgroup analyses according to tumor location and Duke's Stage to assess CRC risk.

Statistical analyses

We assessed the homogeneity between groups using Student's t-test for continuous variables and a Chi-squared test for categorical variables, and we used a paired t-test or McNemar's test for PS matched paired data. The stratification data were analyzed by the Mantel-Haenszel method [49]. We used the ORs and corresponding 95% confidence intervals (95% CIs) to estimate the associations between *MDM2*, *SKP2*, *FBXW7*, *β-TRCP*, and *NEDD4-1* CNVs and CRC risk via conditional and unconditional logistic regression. We defined two copies as the wild-type (Wt), more than two copies as the amplification-type (Amp) and less than two copies as the deletion-type (Del). Three additive CNV patterns were defined as follows: Del *v.s.* amp+wt (Del-pattern), Amp *v.s.* del+wt (Amp-pattern), and Del+amp *v.s.* wt (Var-pattern). The 95% CIs for the AUC, the categorical net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) were estimated using the MedCalc® version 9.5 (MedCalc Software, Mariakerke, Belgium) and the PredictABEL package in R software version 3.4.0, respectively. Other analyses were performed using SPSS Statistics version 24.0 (IBM, Inc., USA). All statistical tests were two-sided, *P* values < 0.05 were considered significant in the overall analysis, and *P* values < 0.025 were considered significant in subgroup analysis by Bonferroni's correction [46].

CRC risk predictive models with CNV

To explore the predictive effects of CNV patterns on CRC risk, we constructed four integrated predictive models: model 1 comprised age, gender, occupation, marital status, nationality, family history of CRC, and factors of smoking and drinking (BI-model); models 2-4 were based upon model 1 and added five gene Del-pattern, Amp-pattern, and Var-pattern, respectively. The ROC curves and the AUCs were compared with the DeLong method [50]. We applied the risk reclassification table to display the number of subjects predicted to be at consistent or different risk categories by the basic and extended models [51], in which the individuals in the medium-risk category may show more shift in risk category and individuals in the marginal-risk category may be more consistent in the two compared models [52]. We further introduced NRI and IDI to evaluate the improvement in the discriminatory accuracy of each model (taking 0.3 and 0.6 as the cut-off points). NRI assesses the improvement in the classification of subjects into risk categories after adding different CNV pattern into the basic model and IDI reflects the change in the predicted probability between the two models [51]. The predictive models were also evaluated in subgroups based on tumor location and Duke's Stage.

Results

Characteristics of participants

The distribution of patients' characteristics before and after PS matching was shown in Table 1, and after 1:1 PS matching, the covariates were adequately balanced in the PS-matched dataset (Table 1).

Association between gene CNV and CRC risk

The CNV frequencies of the five genes and the relationships between the gene CNVs and CRC risk with unadjusted, variable adjustment, and PS adjustment were shown in Figure 2. Compared to variable adjustment, the ORs tended to be conservative with narrower confidence intervals after PS adjustment. Figure 2 shows the ORs for the associations between *MDM2* amplification and CRC risk were 8.848 (95% CI: 1.231-63.595, P = 0.030) and 8.684 (95% CI: 1.213-62.155, P = 0.031) after PS adjustment for Amp *v.s.* Wt and Amp-pattern, respectively. In the variable adjustment, the ORs were 13.291 (95% CI: 1.179-149.791, P = 0.036 for Amp *v.s.* Wt) and 12.659 (95% CI: 1.137-140.921, P = 0.039 for Amp-pattern), respectively.

The ORs for the relationship between the loss of *SKP2* and CRC risk were 0.314 (95% CI: 0.102-0.967, P = 0.044) and 0.323 (95% CI: 0.106-0.979, P = 0.046) after PS adjustment for Del *v.s.* Wt and Del-pattern, respectively, which became noticeably significant compared with the variable adjusting ORs (Figure 2). The ORs of the relationship between *SKP2* CNVs and CRC risk in Var-pattern were 0.322 (95% CI: 0.111-0.935, P = 0.039) for variable adjustment and 0.339 (95% CI: 0.135-0.854, P = 0.024) for PS adjustment (Figure 2). However, we did not observe any significant associations between the CNVs of *FBXW7*, *β-TRCP*, and *NEDD4-1* and CRC risk (Figure 2).

After stratified on PS, covariates were balanced in each stratification, only drinking alcohol remained significant in the first and fifth quintiles (Table S2), and we observed the similar relations between gene CNV and CRC risk (Figure S1). In the PS matching analysis, we only found the same trend but no significant results (Figure S2).

Sensitivity analyses

As a post hoc sensitivity analysis, we removed the individuals with the extreme score to ensure comparable participants' characteristics between groups. Similar findings to our main analysis were obtained when we only included participants with similar PS (Figure S3).

The predictive effect of CNV models

We first constructed a BI-model, whose AUC for CRC risk was 0.809 (95% CI: 0.784-0.833), and then, we added gene CNVs by different variation patterns and the AUCs for the BI+Del-model, BI+Amp-model and BI+Var-model were 0.814 (95% CI: 0.789-0.838, P < 0.001), 0.816 (95%CI: 0.791-0.839, P < 0.001) and 0.818 (95% CI: 0.793-0.841, P < 0.001), respectively (Table 2). The predictive efficiency of models was compared by delta-AUC and NRI / IDI. Compared with the BI-model, the BI+Var-model increased the AUC by 0.009 (95% CI: 0.002-0.015, P = 0.014), which could more accurately identify 1.7% (95% CI: 0.003-0.052, P < 0.001) of participants as CRC cases or controls (Table 3).

Subgroup analysis

Figure 3A shows, in colon cancer, the ORs of associations between *SKP2* abnormal copy number and cancer risk were 0.235 (95% CI: 0.081-0.684, P = 0.009) and 0.272 (95% CI: 0.115-0.646, P = 0.003) for variable adjustment and PS adjustment, respectively. In rectal carcinoma, *MDM2* amplification was associated with 15.578 (95% CI: 1.520-159.672, P = 0.021) and 14.999 (95% CI: 1.477-152.326, P = 0.022) times CRC risk after PS adjustment for Amp *v.s.* Wt and Amp-pattern, respectively (Figure 3B).

Table 1. Distribution of demographic and environmental information of CRC patients and controls before and after PS matching.

Characteristics	Overall			PS matching		
	CRC (518),(%)	Controls (518),(%)	*P* value[a]	CRC (185),(%)	Controls (185),(%)	*P* value[a]
Age, years			0.687			0.562
Mean ± s.d.	59.8±10.6	60.5±11.2		60.0±11.6	59.6±10.5	
≤ 50	100(19.3%)	100(19.3%)		42(22.7%)	36(19.5%)	
50-60	165(31.9%)	175(33.8%)		55(29.7%)	65(35.1%)	
60-70	143(27.6%)	148(28.6%)		47(25.4%)	50(27.0%)	
> 70	110(21.2%)	95(18.3%)		41(22.2%)	34(18.4%)	
Gender			0.002			0.938
Male	249(48.1%)	299(57.7%)		96(51.9%)	95(51.3%)	
Female	269(51.9%)	219(42.3%)		89(48.1%)	90(48.7%)	
BMI			0.176			0.232
Mean ± s.d.	24.1±4.4	23.8±3.8		23.7±3.6	23.9±4.4	
≤ 24	274(52.9%)	262(50.6%)		97(52.4%)	101(54.6%)	
24-28	173(33.4%)	163(31.5%)		64(34.6%)	51(27.6%)	
> 28	71(13.7%)	93(17.9%)		24(13.0%)	33(17.8%)	
Education			0.089			0.916
Primary school and below	136(26.2%)	113(21.8%)		53(28.6%)	49(26.5%)	
Junior middle school	165(31.9%)	151(29.2%)		59(31.9%)	63(34.0%)	
Senior middle school	113(21.8%)	123(23.8%)		36(19.5%)	34(18.4%)	
University and above	104(20.1%)	131(25.2%)		37(20.0%)	39(21.1%)	
Occupation			0.001			0.723
White collar	92(17.8)	68(13.1%)		26(14.1%)	22(11.9%)	
Blue collar	268(51.7%)	328(63.3%)		97(52.4%)	104(56.2%)	
Both	158(30.5%)	122(23.6%)		62(33.5%)	59(31.9%)	
Marriage			0.001			0.288
Married	496(95.8%)	468(90.4%)		179(96.8%)	175(94.6%)	
Others	22(4.2%)	50(9.6%)		6(3.2%)	10(5.4%)	
Nationality			0.012			0.672
The Han nationality	505(97.5%)	489(94.4%)		178(96.2%)	179(96.8%)	
Others	13(2.5%)	29(5.6%)		7(3.8%)	6(3.2%)	
Family history of colorectal cancer			<0.001			0.472
No	84(16.2%)	222(42.9%)		57(30.8%)	64(34.6%)	
Yes	434(83.8%)	296(57.1%)		128(69.2%)	121(65.4%)	
Appendicitis			0.295			0.565
No	85(16.4%)	98(18.9%)		27(14.6%)	29(15.7%)	
Yes	433(83.6%)	420(81.1%)		158(85.4%)	156(84.3%)	
Refined grains, g/day			<0.001			0.772
≤ 250	274(52.9%)	388(74.9%)		107(57.8%)	109(58.9%)	
> 250	244(47.1%)	130(25.1%)		78(42.2%)	76(41.1%)	
Roughage, g/week			0.012			0.527
< 50	250(48.3%)	210(40.5%)		80(43.2%)	74(40.0%)	
≥ 50	268(51.7%)	308(59.5%)		105(56.8%)	111(60.0%)	
Vegetable, times/week			<0.001			0.674
≤ 2	317(61.2%)	259(50.0%)		108(58.4%)	104(56.2%)	
> 2	201(38.8%)	259(50.0%)		77(41.6%)	81(43.8%)	
Fruit, times/week			0.236			0.979
≤ 2	244(47.1%)	225(43.4%)		87(47.0%)	87(47.0%)	
> 2	274(52.9%)	293(56.6%)		98(53.0%)	98(53.0%)	
Fat meat			<0.001			0.793
No	323(62.4%)	255(49.2%)		108(58.4%)	105(56.8%)	
Yes	195(37.6%)	263(50.8%)		77(41.6%)	80(43.2%)	
Fish, times/week			<0.001			0.597
≤ 1	405(78.2%)	285(55.0%)		138(74.6%)	133(71.9%)	
> 1	113(21.8%)	233(45.0%)		47(25.4%)	52(28.1%)	
Seafood, times/week			0.462			0.800
≤ 1	336(64.9%)	325(62.7%)		127(68.7%)	130(70.3%)	
> 1	182(35.1%)	193(37.3%)		58(31.3%)	55(29.7%)	
Braised fish, times/week			0.004			0.674
≤ 1	328(63.3%)	371(71.6%)		125(67.6%)	129(69.7%)	
> 1	190(36.7%)	147(28.4%)		60(32.4%)	56(30.3%)	
Egg, /week			0.025			1.000
≤ 3	196(37.8%)	232(44.8%)		78(42.2%)	78(42.2%)	
> 3	322(62.2%)	286(55.2%)		107(57.8%)	107(57.8%)	
Tea			0.085			0.952
yes	142(27.4%)	118(22.8%)		45(24.3%)	45(24.3%)	
no	376(72.6%)	400(77.2%)		140(75.7%)	140(75.7%)	
Sausage, times/month			<0.001			0.730
≤ 1	382(73.7%)	448(86.5%)		155(83.8%)	152(82.2%)	
> 1	136(26.3%)	70(13.5%)		30(16.2%)	33(17.8%)	
Spicy food, times/week			0.949			0.855
≤ 3	292(56.4%)	291(56.2%)		97(52.4%)	98(53.0%)	
> 3	226(43.6%)	227(43.8%)		88(47.6%)	87(47.0%)	

Characteristics	Overall			PS matching		
	CRC (518),(%)	Controls (518),(%)	*P* value[a]	CRC (185),(%)	Controls (185),(%)	*P* value[a]
Garlic, times/week			0.595			0.895
≤ 3	304(58.7%)	296(57.1%)		107(57.8%)	105(56.8%)	
> 3	214(41.3%)	222(42.9%)		78(42.2%)	80(43.2%)	
Chinese pickled sour cabbage, times/month			<0.001			0.349
≤ 2	216(41.7%)	320(61.8%)		92(49.7%)	101(54.6%)	
> 2	302(58.3%)	198(38.2%)		93(50.3%)	84(45.4%)	
Canned fruit, times/week			0.557			0.483
≤ 3	464(89.6%)	459(88.6%)		165(89.2%)	169(91.4%)	
> 3	54(10.4%)	59(11.4%)		20(10.8%)	16(8.6%)	
Canned meat, times/week			0.767			0.893
≤ 3	28(5.4%)	30(5.8%)		7(3.8%)	8(4.3%)	
> 3	490(94.6%)	488(94.2%)		178(96.2%)	177(95.7%)	
Tap-water			<0.001			0.772
Yes	418(80.7%)	147(28.4%)		117(63.2%)	108(58.4%)	
No	100(19.3%)	371(71.6%)		68(36.8%)	77(41.6%)	
Leftovers, times/week			<0.001			0.830
≤ 3	301(58.1%)	355(68.5%)		116(62.7%)	114(61.6%)	
> 3	217(41.9%)	163(31.5%)		69(37.3%)	71(38.4%)	
Physical exercise			<0.001			0.853
Yes	455(87.8%)	312(60.2%)		143(77.3%)	142(76.8%)	
No	63(12.2%)	206(39.8%)		42(22.7%)	43(23.2%)	
Smoking			0.344			0.936
No	296(57.1%)	311(60.0%)		116(62.7%)	115(62.2%)	
Yes	222(42.9%)	207(30.0%)		69(37.3%)	70(37.8%)	
Drinking			<0.001			0.514
No	226(43.6%)	376(72.6%)		82(44.3%)	88(47.6%)	
Yes	292(56.4%)	142(27.4%)		103(55.7%)	97(52.4%)	
Tumor location			-			-
Colon	325(62.7%)	-		-	-	
Rectum	193(37.3%)	-		-	-	
Duke's Stage			-			-
A+B	315(60.8%)	-		-	-	
C+D	203(39.2%)	-		-	-	

CRC, Colorectal Cancer; PS, propensity score; s.d., standard deviation; BMI, Body Mass Index.

[a] *P* values calculated using Student's t-test for continuous variables and Pearson's Chi-squared test for categorical variables for overall data; *P* values calculated using paired t-test or McNemar's test for paired data. *P* values < 0.05 were considered statistically significant.

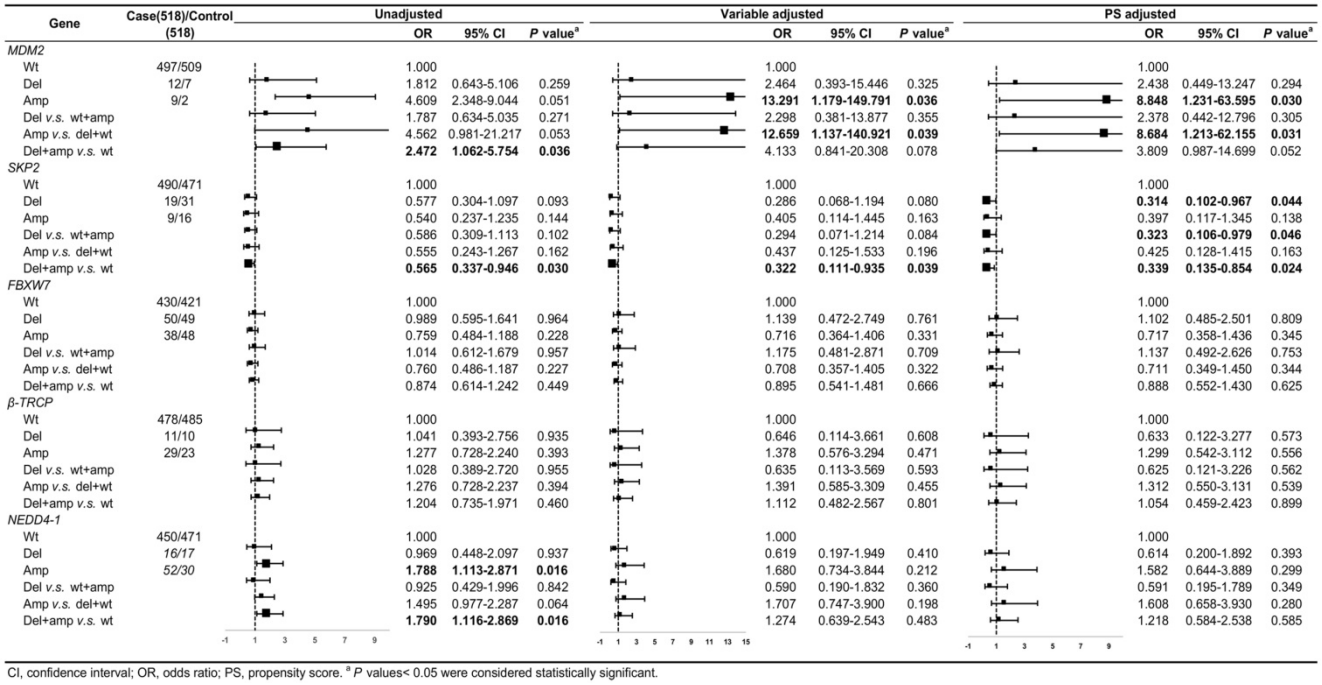

Figure 2. The associations between gene CNVs and CRC risk in different adjusted models for overall participants. The forest plot showed the estimated ORs of the five genes associated with CRC risk and the bold squares indicated statistically significant.

In Duke's Stage A+B patients, the OR of the relationship between *SKP2* abnormal copy number and CRC risk was 0.330 (95% CI: 0.137-0.794, *P* = 0.014) after PS adjustment (Figure 4A). In advanced CRC stage, compared to variable adjustment, the association between *MDM2* amplification and CRC

risk became more conservative after PS adjustment (Figure 4B).

We also evaluated model prediction in each subgroup. The BI+Var-model performed better than the BI-model and the other two CNV pattern models (BI+Del-model and BI+Amp-model) for patients with colon cancer and in tumor Duke's Stage A+B, it could correctly reclassify 7% (95% CI: 0.021-0.119, P = 0.005) and 4.7% (95%CI: 0.001-0.093, P = 0.048) of the subjects, respectively (Table S3-S4).

Table 2. Diagnostic accuracy of models in the prediction of CRC risk.

Models	Cutoff	Sensitivity	Specificity	AUC	95% CI	*P* value[a]
BI-model[b]	0.50	76.83	73.36	0.809	0.784-0.833	<0.001
BI+Del-model[b]	0.51	79.73	70.85	0.814	0.789-0.838	<0.001
BI+Amp-model[b]	0.51	80.89	69.69	0.816	0.791-0.839	<0.001
BI+Var-model[b]	0.51	80.89	70.46	0.818	0.793-0.841	<0.001

CRC, colorectal cancer; AUC, area under the curve; CI, confidence interval.

[a] P values < 0.05 were considered statistically significant.

[b] BI-model, model included age, gender, occupation, marital status, nationality, family history of CRC, and factors of smoking and drinking; BI+Del-model, model included information in BI-model and five gene deletion in Del-pattern (Del *v.s.* wt+amp); BI+Amp-model, model included information in BI-model and five gene amplification in Amp-pattern (Amp *v.s.* wt+del); BI+Var-pattern, model included information in BI-model and five gene variation in Var-pattern (Del+amp *v.s.* wt).

Table 3. The reclassification table and analysis for categorical net reclassification improvement and integrated discrimination improvement for the overall participants.

BI-model	**BI+Del-model**				**BI+Amp-model**				**BI+Var-model**			
	[0, 0.3)	**[0.3, 0.6)**	**[0.6, 1]**	**RC%**	**[0, 0.3)**	**[0.3, 0.6)**	**[0.6, 1]**	**RC%**	**[0, 0.3)**	**[0.3, 0.6)**	**[0.6, 1]**	**RC%**
CRC cases												
[0, 0.3)	70	1	0	1	64	7	0	10	62	9	0	13
[0.3, 0.6)	0	54	0	0	3	49	2	9	1	51	2	6
[0.6, 1]	1	9	383	3	0	9	384	2	0	20	373	5
Controls												
[0, 0.3)	296	3	0	1	288	11	0	4	284	15	0	5
[0.3, 0.6)	8	72	1	11	4	77	0	5	9	71	1	12
[0.6, 1]	0	12	126	9	0	14	124	10	0	26	112	19
Total												
[0, 0.3)	366	4	0	1	352	18	0	5	346	24	0	6
[0.3, 0.6)	8	126	1	7	7	126	2	7	10	122	3	10
[0.6, 1]	1	21	509	4	0	23	508	4	0	46	485	9
NRI (95% CI)[a]	0.014(-0.009-0.036), P=0.232				**0.008(0.001-0.034), P<0.001**				**0.017(0.003-0.052), P<0.001**			
IDI (95% CI)[a]	**0.007(0.002-0.013), P=0.007**				**0.009(0.004-0.014), P=0.001**				**0.014(0.007-0.020), P<0.001**			
Delta-AUC (95% CI)[a]	0.005(-0.001-0.010), P=0.083				**0.007(0.001-0.013), P=0.031**				**0.009(0.002-0.015), P=0.014**			

CRC, colorectal cancer; RC, reclassification percent; CI, confidence interval; NRI, net reclassification improvement; IDI, integrated discrimination improvement; AUC, area under the curve.

[a] P values < 0.05 were considered statistically significant.

Discussion

In this re-analysis case-control study, we applied the PS method to balance all putative influential factors across groups to inspect the more accurate relationships between the germline CNVs of *MDM2*, *SKP2*, *FBXW7*, *β-TRCP*, and *NEDD4-1* and CRC risk. Further adjustment for the PS slightly reduced the point estimates of the associations, showing that *MDM2* amplification significantly increased CRC risk, and deletion and the (Del+Amp) genotype of *SKP2* were associated with reduced CRC risk. While the confidence intervals of the estimate were clearly narrowed, our results became more conservative and accurate by adjusting PS. Additionally, in sub-set analysis, the *MDM2* copy number gain was associated with increased CRC risk in rectal carcinoma and advanced CRC stages, and the *SKP2* abnormal copy number showed a relationship between reduced CRC risk in colon cancer and early Duke's Stages. Moreover, the model-integrated gene abnormal copy number pattern could improve the predictive efficiency of the model in CRC risk prediction compared with the BI-model.

The finding of the infrequent *MDM2* CNVs (21 in 518 CRC cases and 9 in 518 controls, respectively) in peripheral blood was in line with the previous study, in that *MDM2* amplification was observed in only 1 of 88 primary cases [53]. Either as a dual regulator of p53 or being p53-independent, the *MDM2* features in cell cycles progression, apoptosis and DNA damage responses confirmed that amplified *MDM2* had a comprehensive effect on tumorigenesis [54].

In our observations, the frequency of *SKP2* deletion was two times that of the amplification (specifically, 50 and 25 in total participants respectively). *SKP2* down-regulation is critical for cell-cycle arrest, and its deletion restricts oncogenesis and induces apoptosis [55]. Zhu et al. suggested that *SKP2* copy overrepresentation (13%) and loss (35%) were both observed in adenocarcinoma [56]. We first focused on the level of *SKP2* copy in germline DNA, so further research of the copy level of *SKP2* in CRC in peripheral blood is necessary to confirm our results.

The results of PS presented here should be seen as complementary to our earlier results [43] and will tend to be conservative and accurate estimates of the associations between gene CNV and CRC risk. Kerry C. Cho et al. [57] and Isseki Maeda et al. [58] also found that further adjustment for PS slightly modified previous associations. Moreover, study also found that among the four popular PS methods (including matching and stratifying on the basis of the PS, Inverse probability weighting applied to each observation, and simply including the PS as an additional variable in a regression model) covariate adjustment performed better than other three [59], which was consistent with our results. Although we attempted to match participants considering the best possible confounder balance, limited data were available for analyzing the effects of the CNV. Studies by Varlotto J et al. [60] and Shirvani SM et al. [61] also found that PS matching analyses limited the effectiveness of comparisons. Nevertheless, our

multivariate analysis for adjusting PS showed statistically significant associations.

We are the first to introduce CNV patterns into predictive models to forecast the CRC risk. By adding the integrated information of CNVs of *MDM2*, *SKP2*, *FBXW7*, *β-TRCP*, and *NEDD4-1*, the model prediction became more effective. Compared with the BI-model, the BI+Var-model significantly improved the discriminatory performance, as gene CNV information increased the AUC by 1.11%. Recently, a CRC prediction model was developed with the age and family history of CRC together with the gene SNP information, which reported that the inclusion of 8 SNPs could increase the AUC by 0.5% to 4.2% beyond the AUC provided by conventional risk factors [39]. Another CRC predictive model using binary logistic

A.

Colon cancer

Gene	Cases(325)/Controls(518)	Unadjusted OR	95% CI	*P* value[a]	Variable adjusted OR	95% CI	*P* value[a]	PS adjusted OR	95% CI	*P* value[a]
MDM2										
Wt	314/509	1.000			1.000			1.000		
Del	6/7	1.541	0.451-5.268	0.487	2.013	0.216-18.766	0.532	1.784	0.268-11.882	0.547
Amp	5/2	4.053	0.782-21.02	0.096	10.799	0.675-172.719	0.092	8.060	0.762-85.308	0.083
Del *v.s.* wt+amp		1.523	0.446-5.206	0.499	1.921	0.211-17.519	0.556	1.755	0.265-11.611	0.557
Amp *v.s.* del+wt		4.025	0.777-20.863	0.097	10.557	0.658-169.246	0.096	7.985	0.756-84.343	0.084
Del+amp *v.s.* wt		2.140	0.824-5.559	0.118	3.440	0.558-21.223	0.179	2.922	0.626-13.625	0.171
SKP2										
Wt	310/471	1.000			1.000			1.000		
Del	13/31	0.641	0.318-1.289	0.211	0.316	0.094-1.067	0.063	0.355	0.126-0.998	0.050
Amp	2/16	0.159	0.026-0.981	0.048	0.062	0.004-1.038	0.053	0.093	0.010-0.895	0.040
Del *v.s.* wt+amp		0.659	0.328-1.324	0.241	0.334	0.098-1.138	0.078	0.372	0.132-1.046	0.060
Amp *v.s.* del+wt		0.163	0.027-1.002	0.050	0.068	0.004-1.096	0.058	0.100	0.010-0.974	0.048
Del+amp *v.s.* wt		**0.478**	**0.254-0.898**	**0.022**	**0.235**	**0.081-0.684**	**0.009**	**0.272**	**0.115-0.646**	**0.003**
FBXW7										
Wt	275/421	1.000			1.000			1.000		
Del	31/49	0.977	0.559-1.709	0.935	1.096	0.411-2.922	0.848	0.986	0.406-2.394	0.975
Amp	19/48	0.602	0.342-1.059	0.078	0.471	0.21-1.056	0.068	0.515	0.241-1.102	0.087
Del *v.s.* wt+amp		1.019	0.586-1.771	0.946	1.163	0.439-3.077	0.751	1.045	0.429-2.547	0.921
Amp *v.s.* del+wt		0.603	0.345-1.055	0.076	0.468	0.209-1.048	0.065	0.516	0.240-1.110	0.090
Del+amp *v.s.* wt		0.790	0.519-1.204	0.269	0.736	0.392-1.379	0.334	0.725	0.412-1.278	0.266
β-TRCP										
Wt	303/485	1.000			1.000			1.000		
Del	5/10	0.745	0.138-4.016	0.713	0.437	0.056-3.427	0.413	0.358	0.055-2.316	0.269
Amp	17/23	1.181	0.620-2.248	0.613	1.091	0.401-2.968	0.864	1.065	0.408-2.777	0.898
Del *v.s.* wt+amp		0.739	0.137-3.980	0.705	0.435	0.056-3.400	0.409	0.356	0.055-2.307	0.267
Amp *v.s.* del+wt		1.186	0.623-2.257	0.603	1.117	0.413-3.022	0.828	1.088	0.419-2.822	0.863
Del+amp *v.s.* wt		1.055	0.567-1.963	0.865	0.835	0.327-2.132	0.703	0.779	0.323-1.881	0.576
NEDD4-1										
Wt	282/471	1.000			1.000			1.000		
Del	9/17	0.905	0.342,2.398	0.838	0.473	0.135-1.655	0.241	0.619	0.197- 1.949	0.410
Amp	34/30	**1.889**	**1.109,3.218**	**0.019**	2.033	0.853-4.847	0.108	1.680	0.734- 3.844	0.212
Del *v.s.* wt+amp		0.859	0.326,2.261	0.754	0.440	0.127-1.521	0.194	0.590	0.190- 1.832	0.360
Amp *v.s.* del+wt		**1.895**	**1.116,3.216**	**0.018**	2.091	0.877-4.988	0.095	1.707	0.747- 3.900	0.198
Del+amp *v.s.* wt		1.537	0.933,2.533	0.090	1.373	0.662-2.847	0.390	1.274	0.639- 2.543	0.483

CI, confidence interval; OR, odds ratio; PS, propensity score. [a] *P* value calculated using unconditional Logistic regression analysis, *P* values < 0.025 were considered statistically significant.

B.

Rectal cancer

Gene	Cases(93)/Controls(518)	Unadjusted OR	95% CI	*P* value[a]	Variable adjusted OR	95% CI	*P* value[a]	PS adjusted OR	95% CI	*P* value[a]
MDM2										
Wt	183/509	1.000			1.000			1.000		
Del	6/7	2.246	0.663-7.609	0.193	4.447	0.446-44.382	0.194	3.679	0.532-25.444	0.182
Amp	4/2	5.562	1.010-30.640	0.049	23.331	0.744-731.689	0.073	**15.578**	**1.520-159.672**	**0.021**
Del *v.s.* wt+amp		2.206	0.651-7.474	0.203	4.068	0.435-38.032	0.210	3.535	0.520-24.048	0.192
Amp *v.s.* del+wt		5.476	0.994-30.162	0.051	21.638	0.701-667.632	0.079	**14.999**	**1.477-152.326**	**0.022**
Del+amp *v.s.* wt		3.028	1.138-8.057	0.027	6.577	0.910-47.559	0.061	5.842	1.221-27.905	0.028
SKP2										
Wt	180/471	1.000			1.000			1.000		
Del	6/31	0.467	0.175-1.248	0.129	0.146	0.009-2.361	0.147	0.249	0.050-01.245	0.086
Amp	7/16	1.186	0.480-2.933	0.712	0.882	0.214-3.645	0.862	0.965	0.268-3.477	0.956
Del *v.s.* wt+amp		0.464	0.174-1.239	0.125	0.147	0.009-2.350	0.147	0.250	0.051-1.228	0.084
Amp *v.s.* del+wt		1.226	0.496-3.028	0.659	0.981	0.245-3.931	0.979	1.045	0.297-3.672	0.946
Del+amp *v.s.* wt		0.714	0.363-1.402	0.327	0.345	0.076-1.570	0.153	0.446	0.144-1.378	0.151
FBXW7										
Wt	156/421	1.000			1.000			1.000		
Del	18/49	1.008	0.537-1.890	0.980	0.866	0.308-2.431	0.782	0.947	0.382-2.348	0.906
Amp	19/48	1.034	0.576-1.857	0.910	1.131	0.494-2.590	0.770	1.021	0.444-2.349	0.960
Del *v.s.* wt+amp		1.004	0.535-1.885	0.989	0.854	0.300-2.430	0.764	0.945	0.376-2.376	0.903
Amp *v.s.* del+wt		1.034	0.575-1.858	0.911	1.147	0.496-2.650	0.747	1.027	0.441-2.392	0.950
Del+amp *v.s.* wt		1.021	0.659-1.580	0.927	1.005	0.539-1.876	0.987	0.982	0.544-1.772	0.952
β-TRCP										
Wt	175/485	1.000			1.000			1.000		
Del	6/10	1.468	0.473-4.554	0.505	0.590	0.046-7.512	0.663	0.758	0.093-6.151	0.784
Amp	12/23	1.446	0.703-2.974	0.317	1.980	0.600-6.537	0.260	1.654	0.495-5.532	0.408
Del *v.s.* wt+amp		1.439	0.465-4.455	0.526	0.569	0.045-7.118	0.640	0.740	0.093-5.915	0.764
Amp *v.s.* del+wt		1.431	0.696-2.941	0.329	2.006	0.621-6.485	0.244	1.665	0.503-5.508	0.398
Del+amp *v.s.* wt		1.456	0.780-2.721	0.238	1.395	0.417-4.672	0.578	1.276	0.404-4.031	0.666
NEDD4-1										
Wt	168/471	1.000			1.000			1.000		
Del	7/17	1.069	0.420-2.722	0.889	0.747	0.129-4.328	0.739	0.803	0.179-3.599	0.771
Amp	18/30	1.613	0.856-3.039	0.139	1.087	0.344-3.440	0.884	0.997	0.369-2.697	0.995
Del *v.s.* wt+amp		1.031	0.405-2.621	0.949	0.740	0.131-4.175	0.727	0.806	0.184-3.532	0.771
Amp *v.s.* del+wt		1.610	0.855-3.030	0.140	1.100	0.352-3.441	0.866	1.006	0.376-2.693	0.990
Del+amp *v.s.* wt		1.420	0.828-2.435	0.203	0.966	0.330-2.826	0.947	0.932	0.379-2.292	0.875

CI, confidence interval; OR, odds ratio; PS, propensity score. [a] *P* value calculated using unconditional Logistic regression analysis, *P* values < 0.025 were considered statistically significant.

Figure 3. Subgroup analysis by tumor location for the associations between gene CNVs and CRC risk in different adjusted models. **A.** in colon cancer and **B.** in rectal cancer. The forest plot showed the estimated ORs of the five genes associated with CRC risk and the bold squares indicated statistically significant.

regression combined with the effect of age, gender, family history and 10 SNPs with overall participants (42103 individuals) showed that the AUC range was 0.57-0.59 [38], while our CNV model showed that the AUC range was 0.814-0.818. As a regional variation of genes rather than single nucleotides variation, CNV probably has a stronger association with CRC risk and may contain more abundant information for CRC risk prediction. Despite being limited by our relative low frequency of CNVs in the five genes, enlarging the number of related gene CNV detections may be facilitative to improve the prediction efficiency.

A.

Duke's Stage A+B

Gene	Cases(315)/Controls(518)	Unadjusted OR	95% CI	*P* value[a]	Variable adjusted OR	95% CI	*P* value[a]	PS adjusted OR	95% CI	*P* value[a]
MDM2										
Wt	304/509	1.000			1.000			1.000		
Del	7/7	1.875	0.613-5.732	0.270	4.157	0.563-30.688	0.158	3.538	0.614-20.394	0.155
Amp	4/2	3.358	0.613-18.404	0.163	16.013	0.888-288.776	0.060	9.068	0.830-99.106	0.071
Del *v.s.* wt+amp		1.858	0.608-5.679	0.277	3.927	0.541-28.506	0.172	3.457	0.605-19.748	0.161
Amp *v.s.* del+wt		3.321	0.613-17.986	0.167	14.933	0.829-269.149	0.067	8.776	0.809-95.176	0.074
Del+amp *v.s.* wt		2.224	0.876-5.650	0.093	5.837	0.951-35.844	0.056	4.612	1.029-20.676	0.046
SKP2										
Wt	298/471	1.000			1.000			1.000		
Del	12/31	0.617	0.309-1.231	0.171	0.350	0.073-1.672	0.169	0.359	0.121-1.068	0.065
Amp	5/16	0.442	0.146-1.334	0.147	0.249	0.052-1.194	0.082	0.270	0.063-1.153	0.077
Del *v.s.* wt+amp		0.628	0.315-1.253	0.187	0.365	0.077-1.723	0.183	0.373	0.126-1.105	0.074
Amp *v.s.* del+wt		0.452	0.150-1.363	0.158	0.266	0.056-1.261	0.095	0.289	0.068-1.231	0.093
Del+amp *v.s.* wt		0.558	0.307-1.014	0.055	0.316	0.096-1.043	0.058	**0.330**	**0.137-0.794**	**0.014**
FBXW7										
Wt	266/421	1.000			1.000			1.000		
Del	27/49	0.872	0.455-1.672	0.667	1.029	0.410-2.580	0.950	0.940	0.384-2.298	0.889
Amp	22/48	0.704	0.405-1.223	0.213	0.488	0.216-1.102	0.084	0.527	0.243-1.145	0.105
Del *v.s.* wt+amp		0.899	0.473-1.708	0.735	1.089	0.430-2.757	0.853	0.997	0.402-2.472	0.995
Amp *v.s.* del+wt		0.714	0.415-1.228	0.223	0.487	0.214-1.111	0.087	0.530	0.242-1.165	0.113
Del+amp *v.s.* wt		0.788	0.492-1.261	0.309	0.700	0.396-1.237	0.219	0.697	0.401-1.211	0.200
β-TRCP										
Wt	289/485	1.000			1.000			1.000		
Del	8/10	1.275	0.482-3.372	0.624	0.729	0.110-4.837	0.736	0.682	0.139-3.350	0.634
Amp	18/23	1.274	0.670-2.422	0.461	1.161	0.411-3.282	0.778	1.053	0.381-2.908	0.920
Del *v.s.* wt+amp		1.260	0.477-3.326	0.641	0.724	0.110-4.759	0.730	0.681	0.139-3.328	0.631
Amp *v.s.* del+wt		1.267	0.666-2.407	0.471	1.172	0.418-3.288	0.762	1.064	0.387-2.923	0.904
Del+amp *v.s.* wt		1.272	0.736-2.199	0.388	1.001	0.375-2.674	0.998	0.907	0.366-2.248	0.832
NEDD4-1										
Wt	269/471	1.000			1.000			1.000		
Del	9/17	0.850	0.318-2.269	0.743	0.601	0.123-2.949	0.518	0.575	0.102-3.251	0.507
Amp	37/30	**2.153**	**1.275-3.638**	**0.004**	1.686	0.750-3.793	0.205	1.458	0.661-3.217	0.347
Del *v.s.* wt+amp		0.795	0.297-2.126	0.643	0.569	0.119-2.715	0.467	0.554	0.100-3.073	0.476
Amp *v.s.* del+wt		**2.163**	**1.281-3.652**	**0.004**	1.715	0.766-3.840	0.189	1.485	0.681-3.241	0.318
Del+amp *v.s.* wt		1.696	1.062-2.709	0.027	1.293	0.620-2.693	0.489	1.158	0.550-2.437	0.692

CI, confidence interval; OR, odds ratio; PS, propensity score. [a] *P* value calculated using unconditional Logistic regression analysis, *P* values < 0.025 were considered statistically significant.

B.

Duke's Stage C+D

Gene	Cases(203)/Controls(518)	Unadjusted OR	95% CI	*P* value[a]	Variable adjusted OR	95% CI	*P* value[a]	PS adjusted OR	95% CI	*P* value[a]
MDM2										
Wt	194/509	1.000			1.000			1.000		
Del	4/7	1.714	0.464-6.329	0.418	2.303	0.228-23.309	0.473	1.536	0.184-12.823	0.686
Amp	5/2	**6.566**	**1.263-34.131**	**0.025**	**26.230**	**1.722-399.581**	**0.019**	**12.843**	**1.339-123.143**	**0.027**
Del *v.s.* wt+amp		1.678	0.454-6.193	0.436	2.027	0.210-19.516	0.536	1.495	0.181-12.351	0.704
Amp *v.s.* del+wt		**6.507**	**1.253-33.782**	**0.026**	**24.943**	**1.666-373.407**	**0.020**	**12.750**	**1.331-122.15**	**0.027**
Del+amp *v.s.* wt		2.860	1.074-7.613	0.035	5.517	0.871-34.954	0.069	3.389	0.697-16.481	0.129
SKP2										
Wt	192/471	1.000			1.000			1.000		
Del	7/31	0.511	0.177-1.473	0.207	0.159	0.017-1.497	0.098	0.262	0.060-1.147	0.073
Amp	4/16	0.685	0.226-2.077	0.503	0.608	0.116-3.180	0.553	0.572	0.131-2.498	0.456
Del *v.s.* wt+amp		0.516	0.179-1.488	0.214	0.161	0.017-1.508	0.099	0.266	0.062-1.149	0.074
Amp *v.s.* del+wt		0.706	0.233-2.140	0.538	0.659	0.131-3.350	0.614	0.620	0.145-2.642	0.517
Del+amp *v.s.* wt		0.573	0.261-1.257	0.162	0.273	0.062-1.199	0.081	0.345	0.109-1.091	0.068
FBXW7										
Wt	165/421	1.000			1.000			1.000		
Del	23/49	1.172	0.645-2.131	0.598	0.989	0.332-2.950	0.984	1.035	0.371-2.882	0.945
Amp	16/48	0.845	0.454-1.574	0.595	1.077	0.445-2.604	0.869	0.958	0.408-2.251	0.922
Del *v.s.* wt+amp		1.191	0.654-2.168	0.563	0.983	0.323-2.988	0.975	1.039	0.362-2.980	0.940
Amp *v.s.* del+wt		0.830	0.445-1.547	0.557	1.079	0.438-2.659	0.868	0.955	0.395-2.311	0.917
Del+amp *v.s.* wt		1.010	0.653-1.562	0.963	1.033	0.548-1.949	0.919	0.997	0.552-1.801	0.992
β-TRCP										
Wt	189/485	1.000			1.000			1.000		
Del	3/10	0.618	0.061-6.302	0.661	0.295	0.018-4.714	0.363	0.313	0.021-4.769	0.373
Amp	12/23	1.281	0.613-2.677	0.510	1.657	0.513-5.352	0.397	1.615	0.544-4.792	0.385
Del *v.s.* wt+amp		0.611	0.060-6.198	0.652	0.284	0.018-4.489	0.347	0.306	0.020-4.684	0.364
Amp *v.s.* del+wt		1.290	0.618-2.692	0.498	1.713	0.534-5.501	0.364	1.648	0.558-4.872	0.364
Del+amp *v.s.* wt		1.094	0.535-2.238	0.804	1.051	0.376-2.940	0.924	1.040	0.388-2.784	0.937
NEDD4-1										
Wt	182/471	1.000			1.000			1.000		
Del	7/17	1.110	0.355-3.468	0.852	0.680	0.141-3.284	0.626	0.660	0.171-2.548	0.543
Amp	15/30	1.240	0.629-2.445	0.535	1.464	0.363-5.899	0.566	1.253	0.329-4.775	0.719
Del *v.s.* wt+amp		1.094	0.348-3.441	0.873	0.659	0.135-3.229	0.601	0.649	0.167-2.529	0.530
Amp *v.s.* del+wt		1.235	0.624-2.445	0.544	1.486	0.365-6.055	0.552	1.273	0.332-4.885	0.701
Del+amp *v.s.* wt		1.195	0.681-2.097	0.534	1.154	0.432-3.085	0.768	1.016	0.394-2.620	0.973

CI, confidence interval; OR, odds ratio; PS, propensity score. [a] *P* value calculated using unconditional Logistic regression analysis, *P* values < 0.025 were considered statistically significant.

Figure 4. Subgroup regression analysis by tumor Duke's Stages for the associations between gene CNVs and CRC risk in different adjusted models. **A.** in Duke's Stage A+B. **B.** in Duke's Stage C+D. The forest plot showed the estimated ORs of the five genes associated with CRC risk and the bold squares indicated statistically significant.

We calculated NRI and IDI, involving the classification of case and control in risk categories and determining how the new model should be reclassified when adding new biomarkers [62]. Additionally, NRI is sensitive to arbitrary cut-off values [51], so the cut-off points were set as 0.3 to 0.6 to explore the model calibration. The BI+Var-model resulted in the reclassification of 1.7% of the subjects into more accurate risk categories. If small increases in the AUC can bring significant improvement in reclassified NRI and steady growth in IDI, although improvements in AUC are very limited, it is worth incorporating such a factor into the prediction model [51].

In the stratified analysis, we observed the associations between *MDM2* amplification and increased risk in the rectal tumors, as well as between the *SKP2* (del+amp) genotype and reduced CRC risk in colon cancer. Studies have proposed that differences in gene expression levels exist between the colon and rectal cancer [63, 64], and overexpression of p53 is found more often in rectal cancer than colon cancer [64, 65]. *MDM2* has been well recognized as a key regulator of *p53* [54] and the close relationship may affect the abnormal expression of *MDM2* in rectal cancer. Due to many cell cycle regulatory proteins being degraded by *SKP2*, in addition to microarray data analysis having identified cell cycle genes being mainly expressed in the colon rather than the rectum [63], it is reasonable that the protective function of *SKP2* mainly occurs in colon cancer.

MDM2 amplification was associated with an increased CRC risk in advanced stages, and *SKP2* deletion had a correlation with decreased CRC risk in early CRC stages. A Japanese study showed that *MDM2* amplification in tissues was only 16 of 211 (7.5%), and the incidence of it in Duke's Stage C was significantly higher than that in early A and B [66]. The dysregulation of *SKP2* expression may occur in the precancerous stage, prior to obtaining an invasive phenotype during development [10]. Colorectal carcinoma forms from dysplasia of mucosal epithelial cells, *SKP2* disordered copy number may also function at an early stage of CRC, and its level fluctuates as worsening grades of the disease progression.

Our analysis still had several limitations. First, this is a retrospective study, the selection and observation bias may still have affected the results. Second, we did not add gene-dietary interactions to the predictive models because our analysis was based on the variables and outcomes collected from previous data, and some environmental factors were obtained by frequency rather than quantity, possibly weakening the efficiency of the analysis. Finally, the study was limited by the sample size and the percentage of the detectable gene CNVs, so the statistical performance needs to be improved in further studies.

Despite these limits, the strengths of this study are clear. First, considering many potential confounding factors by applying PS adjustment, we concluded that *MDM2* amplification and *SKP2* CNVs are associated with increased and decreased CRC risk, respectively. Second, we were also the first to consider the effectiveness of different CNV patterns and introduced them into a CRC risk predictive model. Our results indicated that an abnormal CNV-combined pattern may be more accurate for predicting CRC risk, and further research needs to be conducted to validate the efficiency of gene CNV models in CRC risk prediction.

Abbreviations

CRC: colorectal cancer; CNV: Copy number variation; E3s: ubiquitin ligases E3; SNPs: single nucleotide polymorphisms; AUC: areas under the curve; ROC: receiver operating characteristic; PS: propensity score; OR: odds ratio; CI: confidence interval; NRI: reclassification improvement; IDI: integrated discrimination improvement.

Supplementary Material

Supplementary figures and tables.
http://www.jcancer.org/v10p3291s1.pdf

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Human Research and Ethics Committee of Harbin Medical University.

Author Contributions

Y.S.Z. and F.L.H. designed the study, directed its implementation (including quality assurance and control), and reviewed the manuscript critically for important intellectual content. H.R.B. and L.Y.P did the data analysis and wrote the manuscript. H.R.B and T.T. did the main experiments, contributed to the experimental data acquisition and compiled the data. T.T.X, R.P, and Y.W.Z helped with questionnaire data

collection and conducting experiments, and also contributed to the data analysis and draft checking.

Competing Interests

The authors have declared that no competing interest exists.

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