Published in final edited form as:

Endocr Relat Cancer. 2020 November ; 27(11): 641–646. doi:10.1530/ERC-20-0310.

High Plasma Levels of Pro-NT Are Associated with Increased Colon Cancer Risk

Li Li, MD, PhD^{1,*†}, Heidi L. Weiss, PhD^{2,*}, Jing Li, MD², Zhengyi Chen, PhD³, Leslie Donato, PhD⁴, B. Mark Evers, MD^{2,†}

¹Department of Family Medicine, University of Virginia, Charlottesville, VA

²Markey Cancer Center, University of Kentucky, Lexington, KY

³Case Western Reserve University, 10900 Euclid Ave, Cleveland, OH

⁴Mayo Clinic, Department of Laboratory Medicine and Pathology, Rochester, MN

Abstract

Emerging data support a potential role of neurotensin (NT) in the development of obesity, obesity-associated comorbidities, and certain cancers. The association of NT with colon cancer risk has not been explicitly explored. We determined plasma levels of pro-NT, a stable NT precursor fragment, in 223 incident colon cancer patients and 223 age-, gender-, BMI-matched population controls participating in a population-based case-control study of colon cancer. On average, the cases have significantly higher levels of pro-NT than the controls (median = 205.6 pmol/L vs. 183.1 pmol/L, respectively; $p = 0.02$). Multivariate logistic regression models, adjusted for age, gender, BMI, family history of colorectal cancer, smoking, diabetes mellitus, alcohol, and NSAIDs use, show statistically significant risk associations: for continuous measure of pro-NT, the OR estimate was 1.30 (95% CI = 1.03 – 1.64; $p = 0.026$) for each increment of 175 pmol/L; for dichotomized measure of pro-NT, the OR estimate was 1.75 (95% CI = 1.12 – 2.74; $p = 0.025$) for those in the top quartile comparing to the other participants. Our results support circulating levels of pro-NT as a novel risk biomarker for colon cancer.

Keywords

pro-NT; biomarker; obesity; intestinal hormone; Kentucky Colon Cancer Genetic Epidemiology study

Introduction

Neurotensin (NT or NTS), a tridecapeptide hormone originally described by Carraway and Leeman in 1973 from bovine hypothalamic extracts (Carraway and Leeman 1973), is

[†]Correspondence: B. Mark Evers, 800 Rose Street CC140, University of Kentucky, Lexington, KY 40536 (phone: 859-323-4331) Mark.evers@uky.edu; Li Li, Department of Family Medicine, University of Virginia, School of Medicine, Charlottesville, VA 22908 (434-982-3975) LL8NV@Virginia.edu.

*Co-first authors

Conflict of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

predominantly localized to the central nervous system and enteroendocrine cells (N cells) in the small intestine (Polak, *et al.* 1977). NT is released in response to ingested fats (Ferris, *et al.* 1985) and has numerous physiological functions in the gastrointestinal tract including facilitation of fatty acid translocation from the intestine, inhibition of intestinal motility, stimulation of pancreaticobiliary secretions and proliferation of normal intestinal mucosa (Mustain, *et al.* 2011).

In addition to its proliferative effect on normal tissues, NT also stimulates growth and progression of a variety of human cancers that express the high-affinity NT receptor 1 (NTR1 or NTSR1) (Bossard, *et al.* 2007; Carraway and Plona 2006; Evers 2006; Wu, *et al.* 2012). In particular, the role of NT on colorectal cancer (CRC) growth and progression has been extensively studied. Notably, approximately 93% of CRCs express NTR1 and 57% express both NT and NTR1 (Dupouy, *et al.* 2011). NT stimulates the growth of certain human CRCs, both *in vitro* and *in vivo* (Maoret, *et al.* 1999; Qiu, *et al.* 2017; Yoshinaga, *et al.* 1992). Treatment with either SR48692, an NTR1 inhibitor, or siRNA directed to NTR1 decreased cell growth and migration in human CRC cells (Kim, *et al.* 2017). NT is also known to enhance colon cancer cell migration by increasing IL-8 expression and secretion, an action that is blocked by SR48692 or curcumin (Wang, *et al.* 2006). Additionally, NT appears to promote carcinogenesis in the colon of rats given weekly injections of azoxymethane, a potent carcinogen (Iishi, *et al.* 1989). Collectively, these functional analyses strongly implicate a clinical role for NT in the growth and progression of human CRCs.

Plasma levels of pro-NT, a stable NT precursor fragment produced in equimolar amounts relative to NT (Ernst, *et al.* 2006), are associated with risk of diabetes, cardiovascular disease and mortality, and development of obesity (Li, *et al.* 2016; Melander, *et al.* 2012), as well as breast cancer (Melander, *et al.* 2012; Melander, *et al.* 2014). Although it has been postulated, based on limited clinical data, that circulating levels of NT may serve as a biomarker for CRC, the risk association of pro-NT with CRC has not been explicitly and fully examined.

Methods

Study Population

To test the hypothesis that high plasma levels of pro-NT are associated with increased risk of colon cancer, we utilized data from the Kentucky Colon Cancer Genetic Epidemiology Study, a population case-control study based on the Kentucky Cancer Registry (KCR) (Li, *et al.* 2008). Recruitment of participants was conducted between April 2003 and December 2010. One thousand and forty incident colon cancer cases and 1,750 population-based controls completed the study with collection of comprehensive lifestyle and epidemiological data, pathology information, and fasting blood samples. Cases were defined as individuals diagnosed with histopathologically confirmed incident primary colon cancer (excluding patients with rectal or syndromic cancers) who were invited to participate in the study within three months of KCR registration. Cases were eligible to participate if they: 1) had a non-recurrent diagnosis; 2) had no known family history or diagnosis of familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNPCC), Peutz-Jeghers, or Cowden disease; 3) had no known diagnosis of inflammatory bowel disease such as Crohn's

disease or ulcerative colitis; 4) were at least 21 years of age at the time of diagnosis; 5) had contact information listed in the KCR database; 6) were willing to complete two questionnaires. Individuals diagnosed with rectal cancer were excluded. The majority of participants completed data collection within 12 months (median of 5 months) of their colon cancer diagnosis.

Random digit dialing and referrals were utilized to recruit controls representative of the general Kentucky population. Controls consisted of frequency-matched individuals who have never been diagnosed with any cancer except non-melanoma skin cancer and are over the age of 30, preferably 50 years old. For cases and controls, self-reported inflammatory bowel disease (e.g., Crohn's disease or ulcerative colitis), family history of FAP, and HNPCC were excluded in the recruitment. The response rate for cases and controls who answered the phone and allowed eligibility determination was 70.8% and 66.7%, respectively. The study was approved by the Institutional Review Boards of the University of Kentucky and University of Virginia. All participants provided written informed consent.

For the present analysis, 223 cases and 223 controls frequency-matched on age, gender and body mass index (BMI) were randomly selected from the above Kentucky Colon Cancer Genetic Epidemiology study using the propensity score matching technique (Ho, *et al.* 2011).

Data Collection and Risk Factors

Eligible cases and controls donated one blood sample and answered self-administered questionnaires. A two-step approach was used to collect blood samples and lifestyle risk factor data. First, a pre-packed phlebotomy kit with detailed written instructions for blood sample collection and written consent forms was sent to each case subject. Participants were instructed to go to their physician offices or adjacent medical facilities for blood draw after overnight fasting. The samples were collected in purple-top (K3EDTA) blood collection tubes and shipped overnight on frozen ice pack. Upon receipt, the blood tubes were spun for 15 min at 600g and aliquots of plasma and concentrated buffy coat were prepared and frozen at -80°C . Second, a self-administered lifestyle risk factor questionnaire developed by the National Cancer Institute Colon Cancer Familial Cancer Registry (http://epi.grants.cancer.gov/documents/CFR/center_questionnaires/Colon/LA/ColonRiskFactor_USC.pdf) was sent to each participant to collect detailed information on demographics and lifestyle risk factors such as age, gender, smoking status, alcohol use, body mass index (BMI), family history of colorectal cancer, diabetes mellitus, and nonsteroidal anti-inflammatory drug (NSAID) use.

For cases, age was defined as age at colon cancer diagnosis, and for controls as age at recruitment. Positive family history of colorectal cancer was defined as reporting colorectal cancer in one or more first-degree relatives. BMI was calculated based on self-reported weight (kg) two years prior to colon cancer diagnosis (cases)/participation (controls) divided by height in meters squared (kg/m^2). Smoking status was coded as “never regular smoker,” “former regular smoker” or “current regular smoker.” “Regular” smoker was defined as ever smoking at least one cigarette a day for 3 months or longer. “Current” smoker for controls was defined as regularly smoking at the time of study participation, and “current” smoker for

cases was defined as regularly smoking two years prior to diagnosis of colon cancer. Alcohol use status was coded as “never drinker” and “ever drinker”. “Ever” drinker was defined as consumption of any alcoholic beverages at least once a week for 6 months or longer. For NSAIDs use (“yes” or “no”), two questions were asked, including use for at least twice a week for more than a month and duration of use. For this study, NSAIDs use was defined as self-reported ever usage at least twice a week for 1 month or longer. Diabetes mellitus was based on self-reported of diagnosis (“yes” or “no”).

Pro-NT Hormone Assay

Pro-NT was measured in fasting plasma samples using the Sphingotest pro-NT immunoluminometric assay (Sphingotec GmbH, Hennigsdorf, Germany) (Melander, *et al.* 2012) to detect a pro-NT precursor fragment (pro-NT 1–117) (Ernst, *et al.* 2006). The assay has an analytical measuring range of 40–500 pmol/L with between-assay variance of approximately 6%. The laboratory personnel were blinded to disease status of the participants.

Statistical Analysis

Descriptive characteristics were summarized by case vs. control status and compared using the chi-square test or Wilcoxon rank sum test. First, the univariate association of continuous levels of pro-NT with colon cancer was assessed using logistic regression model along with estimates of odds ratio (OR) and 95% confidence interval (CI) for each 175 pmol/L increment of pro-NT (approximately, one standard deviation of pro-NT for the study sample). Furthermore, the distribution of pro-NT in the control samples was utilized to categorize pro-NT levels into quartiles as well as dichotomized groups (quartile 4 vs. quartiles 1–3); univariate logistic regression was likewise employed. Multivariate logistic regression was utilized to assess the association of pro-NT adjusted for known CRC risk factors including age, gender, BMI, family history of CRC, smoking status, alcohol use, diabetes mellitus, and NSAIDs use. Tests for multiplicative interactions between pro-NT and other variables were assessed by adding a cross-product term of the two variables in the model. Sub-group analysis was performed for each of distal and proximal anatomical locations and separate multivariate logistic regressions were employed adjusted for similar CRC risk factors. Statistical analyses were performed using the SAS system version 9.4 (Cary, NC).

Results

Table 1 shows the descriptive characteristics of study participants. Cases and controls were well matched for age, gender, and BMI. The proportions of smokers among cases and controls were 64% and 57%, respectively ($p = 0.18$); 87% of cases were alcohol users vs. 84% in controls ($p = 0.50$); 33% of cases had a family history of CRC compared to 26% in controls ($p = 0.10$); 23% of cases reported to have diabetes mellitus as compared to 17% in controls ($p = 0.12$); NSAIDs use was slightly higher in controls (74%) than that in cases (67%) ($p = 0.08$).

Pro-NT levels were significantly higher in cases vs. controls (median = 205.6 pmol/L and 183.1 pmol/L, respectively; $p=0.02$). The distribution of pro-NT is depicted in Figure 1 which shows that pro-NT quartile values are consistently higher in cases compared to controls.

In unadjusted analyses, each 175 pmol/L increment of pro-NT corresponds to a 37% increased risk of colon cancer ($p = 0.006$) (Table 2). Categorized analyses show that the fourth (vs. the first) quartile of pro-NT was associated with an 81% increased risk of colon cancer ($p = 0.009$), while a dichotomized level of pro-NT comparing the fourth vs. the first three quartiles was associated with a 1.72-fold increased risk of colon cancer ($p = 0.009$).

Multivariate logistic regression was employed to determine the effect of pro-NT on CRC risk controlling for other known CRC risk factors. For continuous measure of pro-NT, each 175 pmol/L increment of pro-NT was associated with a 30% increased risk of colon cancer ($p = 0.026$). In categorized analyses, the top quartile of pro-NT was associated with a 1.65-fold ($p = 0.087$) increased risk of colon cancer, while a dichotomized level of pro-NT comparing the fourth vs. the first three quartiles was associated with a 1.75-fold increased risk of colon cancer ($p = 0.015$).

Sub-group analyses of colon cancer risk by anatomical location show that the associations were comparable for distal (multivariate OR = 1.32, 95% CI: 1.02 – 1.71, $p = 0.032$; $n = 100$ distal, $n = 202$ controls) and proximal colon cancer (multivariate OR = 1.24, 95% CI: 0.96 – 1.61, $p = 0.099$; $n = 104$ proximal, $n = 202$ controls). Tests for multiplicative interactions of pro-NT with other colon cancer risk factors were not statistically significant (data not shown).

Discussion

In the present analysis nested within a population-based case-control study, we found that elevated levels of plasma pro-NT were associated with an increased risk of colon cancer, which was independent of other known CRC risk factors. Consistently, in a small clinical-scale study, plasma NT levels were noted to be 3.7-fold higher in 14 patients with various colon pathology as compared to 12 healthy controls (Kontovounisios, *et al.* 2017). The same group more recently reported that higher plasma NT levels were found in 46 individuals with colonic polyps and cancers (Qiu, *et al.* 2019) as compared to disease-free controls. Both of these previous studies measured plasma NT, which has a short half-life and is quickly degraded in the bloodstream. In contrast, we analyzed plasma pro-NT, a more stable precursor form of NT that has been identified as a potential predictive biomarker for obesity, cardiovascular disease, diabetes mellitus, and breast cancer (Melander, *et al.* 2012; Melander, *et al.* 2014). Our analysis, based on a much larger sample size randomly selected from a well-characterized, population-based, incident case-control study where all data and blood samples were uniformly collected, confirms a positive NT-colon cancer risk association. Moreover, the selected controls in our study were matched to the cases by age, gender, and BMI, thus minimizing the confounding effects of the known CRC risk factors on which data were available. Caution must be exercised in causal interpretation of our results. Given the retrospective nature of case-control studies, reversal causality cannot be

completely excluded. Large prospective studies are warranted to further confirm our findings.

Conclusion

Our study clearly shows that elevated levels of pro-NT are significantly associated with increased risk of colon cancer. Together with the expression of NT and NTR1 in a majority of human CRCs and the strong experimental data showing that NT stimulates growth of NTR1-positive CRCs, our data strongly support pro-NT as a novel risk biomarker of colon cancer.

Acknowledgement

The authors wish to thank Donna Gilbreath in the Markey Cancer Center Research Communications Office for assistance in manuscript preparation.

Funding

This study was supported by the National Institutes of Health (grant numbers R01 DK112034 to B.M.E.; U01 CA181770 and P20 CA233216 to L.L.). In addition, we acknowledge support from the Biostatistics and Bioinformatics Shared Resource Facility of the University of Kentucky Markey Cancer Center (funded by National Cancer Institute grant number P30 CA177558 to B.M.E.).

References

- Bossard C, Souaze F, Jarry A, Bezieau S, Mosnier JF, Forgez P & Labois CL 2007 Over-expression of neurotensin high-affinity receptor 1 (NTS1) in relation with its ligand neurotensin (NT) and nuclear beta-catenin in inflammatory bowel disease-related oncogenesis. *Peptides* 28 2030–2035. [PubMed: 17870207]
- Carraway R & Leeman SE 1973 The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalamus. *J Biol Chem* 248 6854–6861. [PubMed: 4745447]
- Carraway RE & Plona AM 2006 Involvement of neurotensin in cancer growth: evidence, mechanisms and development of diagnostic tools. *Peptides* 27 2445–2460. [PubMed: 16887236]
- Dupouy S, Mourra N, Doan VK, Gompel A, Alifano M & Forgez P 2011 The potential use of the neurotensin high affinity receptor 1 as a biomarker for cancer progression and as a component of personalized medicine in selective cancers. *Biochimie* 93 1369–1378. [PubMed: 21605619]
- Ernst A, Hellmich S & Bergmann A 2006 Proneurotensin 1–117, a stable neurotensin precursor fragment identified in human circulation. *Peptides* 27 1787–1793. [PubMed: 16519961]
- Evers BM 2006 Neurotensin and growth of normal and neoplastic tissues. *Peptides* 27 2424–2433. [PubMed: 16904238]
- Ferris CF, Carraway RE, Hammer RA & Leeman SE 1985 Release and degradation of neurotensin during perfusion of rat small intestine with lipid. *Regul Pept* 12 101–111. [PubMed: 3840906]
- Ho D, Imai K, King G & Stuart EA 2011 MatchIt: Nonparametric Preprocessing for Parametric Causal Inference. *Journal of Statistical Software* 42.
- Iishi H, Tatsuta M, Baba M & Taniguchi H 1989 Promotion by ethanol of gastric carcinogenesis induced by N-methyl-N'-nitro-N-nitrosoguanidine in Wistar rats. *Br J Cancer* 59 719–721. [PubMed: 2736206]
- Kim JT, Weiss HL & Evers BM 2017 Diverse expression patterns and tumorigenic role of neurotensin signaling components in colorectal cancer cells. *Int J Oncol* 50 2200–2206. [PubMed: 28498396]
- Kontovounisios C, Qiu S, Rasheed S, Darzi A & Tekkis P 2017 The role of neurotensin as a novel biomarker in the endoscopic screening of high-risk population for developing colorectal neoplasia. *Updates in Surgery* 69 397–402. [PubMed: 28560510]

- Li J, Song J, Zaytseva YY, Liu Y, Rychahou P, Jiang K, Starr ME, Kim JT, Harris JW, Yiannikouris FB, et al. 2016 An obligatory role for neurotensin in high-fat-diet-induced obesity. *Nature* 533 411–415. [PubMed: 27193687]
- Li L, Plummer SJ, Thompson CL, Tucker TC & Casey G 2008 Association between phosphatidylinositol 3-kinase regulatory subunit p85alpha Met326Ile genetic polymorphism and colon cancer risk. *Clinical Cancer Research* 14 633–637. [PubMed: 18245521]
- Maoret JJ, Anini Y, Rouyer-Fessard C, Gully D & Laburthe M 1999 Neurotensin and a non-peptide neurotensin receptor antagonist control human colon cancer cell growth in cell culture and in cells xenografted into nude mice. *Int J Cancer* 80 448–454. [PubMed: 9935189]
- Melander O, Maisel AS, Almgren P, Manjer J, Belting M, Hedblad B, Engstrom G, Kilger U, Nilsson P, Bergmann A, et al. 2012 Plasma proneurotensin and incidence of diabetes, cardiovascular disease, breast cancer, and mortality. *Journal of the American Medical Association* 308 1469–1475. [PubMed: 23047361]
- Melander O, Belting M, Manjer J, Maisel AS, Hedblad B, Engstrom G, Nilsson P, Struck J, Hartmann O, Bergmann A, et al. 2014 Validation of plasma proneurotensin as a novel biomarker for the prediction of incident breast cancer. *Cancer Epidemiology Biomarkers and Prevention* 23 1672–1676.
- Mustain WC, Rychahou PG & Evers BM 2011 The role of neurotensin in physiologic and pathologic processes. *Curr Opin Endocrinol Diabetes Obes* 18 75–82. [PubMed: 21124211]
- Polak JM, Sullivan SN, Bloom SR, Buchan AM, Facer P, Brown MR & Pearse AG 1977 Specific localisation of neurotensin to the N cell in human intestine by radioimmunoassay and immunocytochemistry. *Nature* 270 183–184. [PubMed: 337160]
- Qiu S, Pellino G, Fiorentino F, Rasheed S, Darzi A, Tekkis P & Kontovounisios C 2017 A Review of the Role of Neurotensin and Its Receptors in Colorectal Cancer. *Gastroenterol Res Pract* 2017 6456257. [PubMed: 28316623]
- Qiu S, Nikolaou S, Fiorentino F, Rasheed S, Darzi A, Cunningham D, Tekkis P & Kontovounisios C 2019 Exploratory Analysis of Plasma Neurotensin as a Novel Biomarker for Early Detection of Colorectal Polyp and Cancer. *Hormones and Cancer* 10 128–135. [PubMed: 31093954]
- Wang X, Wang Q, Ives KL & Evers BM 2006 Curcumin inhibits neurotensin-mediated interleukin-8 production and migration of HCT116 human colon cancer cells. *Clin Cancer Res* 12 5346–5355. [PubMed: 17000667]
- Wu Z, Martinez-Fong D, Tredaniel J & Forgez P 2012 Neurotensin and its high affinity receptor 1 as a potential pharmacological target in cancer therapy. *Front Endocrinol (Lausanne)* 3 184. [PubMed: 23335914]
- Yoshinaga K, Evers BM, Izukura M, Parekh D, Uchida T, Townsend CM Jr. & Thompson JC 1992 Neurotensin stimulates growth of colon cancer. *Surg Oncol* 1 127–134. [PubMed: 1341243]

Quartile 1:	38.40–125.40	14.40–136.60
Quartile 2:	125.40–183.10	136.60–205.60
Quartile 3:	183.10–248.70	205.60–303.10
Quartile 4:	248.70–848.0	303.10–1834.0

Figure 1.

Violin plots of pro-NT distribution by case and control status. $P_{\text{difference}} = 0.02$ based on the Wilcoxon rank sum test.

Table 1.

Descriptive Characteristics of Study Participants and Plasma Levels of pro-NT by Case and Control Status

Variables	Cases (n=223)	Controls (n=223)	Total (n=446)	p-value
Gender, n (%)				0.64[*]
Female	110 (49.3%)	105 (47.1%)	215 (48.2%)	
Male	113 (50.7%)	118 (52.9%)	231 (51.8%)	
Smoking Status, n (%)				0.18[*]
Ever	142 (63.7%)	128 (57.4%)	270 (60.5%)	
Never	81 (36.3%)	95 (42.6%)	176 (39.5%)	
Alcohol Use, n (%)				0.50[*]
Ever	193 (86.5%)	188 (84.3%)	381 (85.4%)	
Never	30 (13.5%)	35 (15.7%)	65 (14.6%)	
Family History of CRC¹, n (%)				0.10[*]
Yes	73 (32.7%)	57 (25.6%)	130 (29.1%)	
No	150 (67.3%)	166 (74.4%)	316 (70.9%)	
Diabetes Mellitus, n (%)				0.12[*]
Yes	50 (22.7%)	34 (16.7%)	84 (19.9%)	
No	170 (77.3%)	169 (83.3%)	339 (80.1%)	
Missing	3	20	23	
NSAID Use², n (%)				0.08[*]
Yes	148 (66.7%)	165 (74.3%)	313 (70.5%)	
No	74 (33.3%)	57 (25.7%)	131 (29.5%)	
Missing	1	1	2	
Age (years)³				0.70⁺
Mean (SD)	62.5 (9.6)	62.3 (8.7)	62.4 (9.1)	
Median	64.0	63.0	63.0	
Range	35.0, 80.0	38.0, 81.0	35.0, 81.0	
BMI (kg/m²)⁴				0.51⁺
Mean (SD)	29.2 (6.2)	28.9 (6.2)	29.1 (6.2)	
Median	27.5	27.8	27.5	
Range	19.2, 57.4	17.1, 56.9	17.1, 57.4	
Pro-NT Measure, (pmol/L)				0.02⁺
n	222	223	445	
Mean (SD)	256.9 (209.3)	209.2 (128.4)	233.0 (175.0)	
Median	205.6	183.1	191	
Range	14.4, 1834.0	38.4, 848.0	14.4, 1834.0	

¹Family history of colorectal cancer (CRC) in 1st relatives.

²Self-reported ever usage of non-steroidal anti-inflammatory drugs at least twice a week for 1 month or longer.

³Age at diagnosis for cases; at recruitment for controls.

⁴Body mass index at recruitment.

* Chi-square test

⁺ Wilcoxon rank sum test

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2.

Association of Plasma Levels of pro-NT with Colon Cancer Risk

Univariate Models ¹	Unadjusted Odds Ratio (95% CI)	p-value
Pro-NT (pmol/L) (Continuous)²	1.37 (1.10 – 1.71)	0.006
Pro-NT (Quartiles)		
Q1	1.00 (Reference)	
Q2	1.04 (0.6 – 1.81)	0.402
Q3	1.11 (0.64 – 1.92)	0.633
Q4	1.81 (1.08 – 3.05)	0.009
Pro-NT (Dichotomized)		
Q1–Q3	1.0 (Reference)	
Q4	1.72 (1.14 – 2.59)	0.009
Multivariate Models ³	Adjusted Odds Ratio (95% CI)	p-value
Pro-NT (pmol/L) (Continuous)²	1.30 (1.03 – 1.64)	0.026
Pro-NT (Quartiles)		
Q1	1.0 (Reference)	
Q2	0.93 (0.52 – 1.66)	0.801
Q3	0.92 (0.52 – 1.64)	0.780
Q4	1.65 (0.93 – 2.94)	0.087
Pro-NT (Dichotomized)		
Q1–Q3	1.0	
Q4	1.75 (1.12 – 2.74)	0.015

¹Univariate logistic regression models with pro-NT.²Odds ratio for each 1 SD (~175 pmol/L) change of pro-NT.³Multivariate logistic regression models with pro-NT adjusted for age, gender, BMI, diabetes mellitus, family history of CRC, NSAIDs use, smoking status, and alcohol use.