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Heterozygosity for the Cys282Tyr mutation in the HFE gene and the risk of colorectal cancer (Netherlands)

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Abstract

Background & Aims: Heterozygosity for the Cys282Tyr transition in the HFE-gene is associated with slightly increased iron levels and may therefore be a potential risk factor for colorectal cancer.
Methods: We studied the relationship between Cys282Tyr-heterozygosity and colorectal cancer using a case–control design. The 240 colorectal cancer cases and 635 controls in our study were derived from a prospective cohort study of 12,242 postmenopausal women, who were invited for an experimental breast cancer screening program in Utrecht, the Netherlands. The women were age 51–69 at time of inclusion and were followed for a period of 20 years. HFE genotyping was performed by PCR and allele-specific oligonucleotide (ASO) hybridization.
Results: The risk of colorectal cancer was higher for women who were heterozygous for the Cys282Tyr mutation, than for those who were Cys282Tyr-wildtypes, although this was not statistically significant (Age-adjusted OR = 1.2, 95% CI: 0.6–2.2). Cys282Tyr-heterozygotes who smoked seemed to be at higher risk of colorectal cancer, although the *p*-value for interaction was not significant (*p*-value 0.42).
Conclusions: The Cys282Tyr mutation is not associated with an increased risk for colorectal cancer in postmenopausal women, although in combination with smoking a slightly increased risk cannot be excluded.

Introduction

Epidemiological studies have shown a relation between iron exposure and extrahepatic cancer like lung cancer [1, 2], colon/rectal cancer [2–8], hematologic malignancies [3], gastric cancer [3], esophageal cancer [9] and melanoma of the skin [9]. A plausible biological mechanism for these findings could be the catalyst activity of iron in the formation of hydroxyl radicals [10], and iron's abilities to suppress host defense cells activity [11, 12] and to promote cancer cell multiplication [13].

Hereditary hemochromatosis (HH) is a genetic iron overload disorder with an autosomal recessive mode of inheritance [14]. It is characterized by excessive iron absorption from the gut and subsequent accumulation of iron in parenchymal cells of many organs leading to hepatic fibrosis and cirrhosis, diabetes mellitus, arthropathy, hypogonadism, skin pigmentation and cardiomyopathy. The estimated disease frequency in populations of northern European descent varies from one in 200 to one in 500 and depends to a large extent on the disease definition. Clinical expression of iron overload is less and occurs at a later age in women compared to men, due to menstruation, pregnancy and less dietary iron intake.

In 1996 the HFE-gene, encoding for a major histocompatibility class I-like molecule, was identified as a candidate gene for HH [15]. A single base pair mutation leads to a G → A transition at nucleotide 845 of the open reading frame and converts a cysteine to a tyrosine at amino acid 282 (Cys282Tyr). The majority of European patients with HH are homozygous for this mutation (52–96%) [16].

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Generally, iron in plasma is tightly bound to transferrin and therefore non-toxic, but when it is unbound, it may act as a catalytic agent in the formation of harmful hydroxyl radicals. This non-transferrin-bound iron (NTBI) is present at high levels in serum of HH-homozygotes and is, compared to normal controls, also significantly elevated in HH-heterozygotes [17]. Heterozygosity for the Cys282Tyr mutation may therefore be a common genetic marker of life-long moderate iron overload.

Smoking increases oxidative stress and may lead to an overexposure of oxygen radicals in women heterozygous for the Cys282Tyr mutation.

We examined the association between heterozygosity for the Cys282Tyr mutation and colorectal cancer risk in a population-based case–control study of postmenopausal women. We also investigated whether smoking enhanced risk in Cys282Tyr-heterozygous women.

Materials and methods

Population

Between December 1974 and October 1980, all 20,555 women, born between 1911 and 1925 living in the city of Utrecht, The Netherlands, were invited for an experimental program for breast cancer screening, the so called Diagnostisch Onderzoek Mammacarcinoom (DOM) project [18]. The women were invited for repeat examinations at one to six year intervals. At the second examination (n = 12,242) a self-administered questionnaire on reproductive factors and smoking was filled out and anthropometric measurements were taken. Women also donated an overnight urine sample, which was stored at −20 °C. All women gave oral consent to use their data and urine for future scientific research. The study was approved by the Institutional Review Board of the University Medical Center, Utrecht, The Netherlands.

The cases comprised 240 women who died of colorectal cancer or were diagnosed with colorectal cancer and the control group consisted of 635 women randomly chosen from the total group of 12,002 women who were free of colorectal cancer at end of follow-up at 01-01-1996. Urine samples of 49 colorectal cancer cases and 51 women of the control group were not available or suitable for DNA analysis. The final study comprised 191 colorectal cancer cases and 574 controls.

Data

Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Women were classified as ever smokers when they reported to be current smokers or having smoked in the past. Menopause was defined as cessation of menstrual bleeding for at least 12 months.

Endpoints

Municipal registries informed the Department of Epidemiology (currently the Julius Center for Health Sciences and Primary Care) about migration and mortality of DOM cohort participants. Cause of death was inquired from the women's general practitioners. At the end of follow-up (01-01-1996), 7928 (64.8%) women were still alive and 2052 women (16.8%) had moved outside the recruitment area. During follow up, 2262 (18.5%) women died: 770 from neoplasms (codes 140–239 of the International Classification of Diseases, Ninth Revision; ICD-9). From 1987 onwards data on newly diagnosed cases of colorectal cancer became available through linkage with the data base of the IKMN, a regional cancer registry which is part of the Netherlands Cancer Registry. Follow-up until 01-01-1996 revealed a total number of 240 colorectal cancer cases, consisting of 175 colon cancer cases (ICD 153) and 65 rectal cancer cases (ICD 154).

Assessment of Cys282Tyr genotype

Full details of the Cys282Tyr-genotyping were previously published [19] and are only briefly described here. Genomic DNA was isolated from urine samples by salt and ethanol precipitation [20]. A 268-bp fragment containing the Cys282Tyr mutation was amplified by PCR using the following pair of primers: 5′-CCTCCTTTGGTGAAGGTGACA-3′ (forward) and 5′-CACAATGAGGGGCTGATCCA-3′ (reverse). Distinction between the Cys282 and Tyr282 allele was made by hybridization with allele-specific oligonucleotides (ASO): HFECys282: 5′-GATATACGT**G**CCAGGTGGA-3′ and HFETyr282: 5′-GATATACGT**A**CCAGGTGGA-3′.

Data analysis

Means and proportions of baseline characteristics were computed for colorectal cancer cases and controls separately. Differences in means were analyzed using Student's t test. Differences in proportions were analyzed using Pearson's χ^2 test. Initial observer variability in genotype assessment was measured by Cohen's κ, yielding the chance-adjusted percentage of agreement.

The association between colorectal cancer and Cys 282Tyr-heterozygosity was evaluated using logistic regression analysis. All analyses were adjusted for age at

baseline. To test for significance of interaction, Wald's test was performed for models with and without terms for the cross product of smoking with Cys282Tyr-genotype. Age-adjusted odds ratios for colorectal cancer were calculated in four subgroups according to smoking status (never smoker or ever smoker) and Cys282Tyr-genotype (wildtype or heterozygote).

Analyses were performed using the statistical package SPSS (SPSS for Windows, Rel. 10.1.4. 2001. Chicago: SPSS Inc.).

Results

Baseline characteristics

The general characteristics of the study population at baseline are presented in Table 1. Colorectal cancer cases and controls did not differ in mean age, BMI, age at menopause, parity, and percentages of women who were ever smokers.

Table 1. Baseline characteristics of the study population

	Colorectal cancer cases (n = 240)	Controls (n = 635)
	Mean (sd)	Mean (sd)
Age at baseline (years)	59.4 (4.1)	58.2 (4.1)
Age at menopause (years)	48.7 (4.5)	48.8 (4.5)
	n (%)	n (%)
Genotype		
Cys282Tyr wildtype	175 (91.6)	532 (92.7)
Cys282Tyr heterozygote	16 (8.4)	39 (6.8)
Cys282Tyr homozygote	0	3 (0.5)
Body mass index (kg m^{-2})		
<25	98 (40.8)	241 (38.0)
25–26	27 (11.3)	78 (12.3)
>26	115 (47.9)	315 (49.7)
Age at menopause (years)		
≤46	60 (28.0)	138 (26.1)
47–49	40 (18.7)	100 (18.9)
50–51	48 (22.4)	141 (26.7)
≥52	66 (30.8)	150 (28.4)
Parity (no.)		
None	42 (17.5)	117 (18.4)
1	29 (12.1)	97 (15.3)
2	58 (24.2)	132 (20.8)
3	42 (17.5)	112 (17.6)
4	34 (14.2)	67 (10.6)
≥5	35 (14.6)	110 (17.3)
Smoking[a]		
Ever	69 (29.9)	185 (30.2)
Never	162 (70.1)	427 (69.8)

[a] Ever smoking was defined as reported to be a current smoker or having smoked in the past.

Allele frequencies

The frequency of the tyrosine allele in the control group of 574 women was 3.9% (95% CI: 2.9–5.2). The prevalence of Cys282Tyr-heterozygotes in the control group was 6.8% and the prevalence of Cys282Tyr-homozygotes was 0.5%. Observer variability in mutation analysis was measured. Before disagreements were resolved in conference, the kappa (κ) was 0.84, reflecting excellent agreement [21].

Colorectal cancer risk

Women who were heterozygous for the Cys282Tyr mutation had an age-adjusted OR for colorectal cancer of 1.2 (95% CI: 0.6–2.2) compared to women who were Cys282Tyr-wildtypes. (Table 2)

The age-adjusted odds ratios for colorectal cancer according to smoking status and Cys282Tyr-genotype are presented in Table 3. Women who were 'ever smokers' and were Cys282Tyr-heterozygotes had a 2.3-fold risk of colorectal cancer compared to women who were 'never smokers' and were Cys282Tyr-wildtypes, although this was not statistically significant (95% CI: 0.7–7.4). We tested whether smoking modified the association between Cys282Tyr-heterozygosity and colorectal cancer. The *p*-value of the cross product of Cys282Tyr-genotype and smoking was 0.42.

Discussion

The results of this case–control study showed no increased risk of colorectal cancer for women who were heterozygous for the Cys282Tyr mutation compared with women who were Cys282Tyr-wildtypes. No clear signs of effect modification by smoking on the relation between Cys282Tyr-heterozygosity and colorectal cancer were observed in our study.

Table 2. Odds ratio and 95% confidence interval (CI) for the risk of colorectal cancer associated with Cys282Tyr-genotype

	Cases (n)	Controls (n)	OR[a]	95% CI
Cys282Tyr-wildtypes (Cys282/Cys282)	175	532	1.0	
Cys282Tyr-heterozygotes (Cys282/Tyr282)	16	39	1.2	(0.6–2.2)

[a] Adjusted for age at baseline.

Table 3. Cys282Tyr-genotype and smoking: separate and combined effects on colorectal cancer

Cys282Tyr-genotype	Smoking status[a]	Cases (n)	Controls (n)	OR[b]	95% CI
Cys282Tyr-wildtype	Never smoker	116	370	1	–
Cys282Tyr-wildtype	Ever smoker	50	162	1.2	(0.8–1.8)
Cys282Tyr-heterozygote	Never smoker	11	31	1.1	(0.5–2.3)
Cys282Tyr-heterozygote	Ever smoker	5	8	2.3	(0.7–7.4)

[a] Ever smoking was defined as reported to be a current smoker or having smoked in the past.
[b] Adjusted for age at baseline.

Before we can interpret our results, some issues need to be addressed. A limitation of our study is that no blood samples were collected at baseline to verify whether iron parameters like serum iron, transferrin saturation and ferritin levels were indeed increased in Cys282Tyr-heterozygous subjects. One study suggests that these levels are higher in Cys282Tyr-heterozygotes, although still within the normal range, than in control subjects [22]. Furthermore, no information on dietary factors was available, and we were therefore not able to take the dietary intake of iron into account.

However, the design of our study has some advantages compared to other designs. Case and control status was determined by development of disease instead of a selection procedure followed by the investigator. The possibility of selection bias is hereby reduced to a minimum. In addition, the problem of information-bias is overcome, since information on determinants was collected before the cases arose. The fact that not every individual in the cohort needs to be genotyped saves time, money and valuable biologic material. Finally, linkage with the regional cancer registry made it possible to detect newly diagnosed cases of colorectal cancer and not only fatal cases therefore covering most part of the diagnoses within this cohort.

We tested by χ^2 statistics whether the distribution of alleles was in Hardy Weinberg equilibrium ($\chi^2 = 5.51$; 1df, $p = 0.02$) [23, 24]. The test showed a significant deviation from HWE, but when expected numbers are too small, and this is the case for the Cys282Tyr-homozygotes, the χ^2 test does not give reliable values. However, observed numbers of Cys282Tyr-heterozygotes (n = 39) and Cys282Tyr-homozygotes (n = 3) were in line with the expected numbers (n = 43 resp n = 1).

The association between smoking and colorectal cancer is weak and this may be attributed to the relatively low dose and short duration of smoking in these elderly women in 1974. Studies on smoking and colorectal cancer risk among women only found increased risks for long-term heavy smokers [25]. It is possible that the combination of carcinogens present in tobacco smoke and slightly higher iron levels in the blood causes an overexposure to oxygen radicals, leading to an increased risk of colorectal cancer in Cys282Tyr-heterozygous women who smoke. The results of our study point in this direction, but show no statistically significant interaction (p-value 0.42).

Several studies showed significantly increased risks of colorectal neoplasms by increased iron exposure, measured by dietary iron intake and various parameters reflecting body iron stores, like serum ferritin and transferrin saturation [26]. One prospective cohort study demonstrated that parents of hemochromatosis patients have an increased risk of colorectal cancer [3]. Later studies, in which heterozygosity was determined on the basis of the Cys282Tyr mutation, found no evidence for an association [27–29].

In conclusion, our study suggests that Cys282Tyr-heterozygosity does not appear to be an important risk factor for colorectal cancer in postmenopausal women, although combined with smoking a slightly increased risk cannot be excluded.

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