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Germline HOXB13 p.Gly84Glu Mutation and Risk of Colorectal Cancer

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Abstract

Introduction—The *HOXB13* p.Gly84Glu mutation has recently been associated with an increased risk of prostate cancer but the association of other cancer sites with this allele has not been assessed. Data has suggested that *HOXB13* expression levels are decreased in colorectal cancer (CRC) cell lines indicating **this** gene may be involved in colorectal tumourigenesis.

Methods—To evaluate a potential association of this mutation with CRC, we genotyped the mutation in 2,695 CRC cases and 4,593 controls from population-based registries in Canada and Australia.

Results—The *HOXB13* p.Gly84Glu mutation was more common in CRC cases than controls (0.48% vs. 0.17%, p=0.02) indicating a significant association between the *HOXB13* variant and CRC risk (OR = 2.8; 95%CI: 1.2-6.8). This association was attenuated but remained significant with the inclusion of previously published and publicly available genotype data. Pedigree analysis

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of cases and controls revealed that 7/21 *HOXB13* mutation carriers had a family history of prostate cancer.

Discussion—This report is the first to suggest a risk of CRC associated with mutations in the *HOXB13* gene. These findings require further validation but may be of importance in the screening and genetic counseling of families known to carry the *HOXB13* p.Gly84Glu mutation.

Keywords

Colorectal cancer; risk; HOXB13 gene; genetic polymorphism

Introduction

Up to one third of colorectal cancer (CRC) cases have a family history of the disease suggesting a heritable component in the cases. Mutations in *APC, MUTYH* and mismatch repair genes account for only a minority of familial cases, while results from recent genome-wide association studies have identified multiple low-penetrance variants that when combined explain a proportion of the heritability of CRC (1). Despite this relatively advanced understanding of CRC genetics, the etiology of the vast majority of familial cases remains unexplained. Recently, a novel germline mutation, p.Gly84Glu (rs138213197), in exon one of the *HOXB13* gene was shown to increase risk of prostate cancer by 5-10 fold (2, 3). The risk was higher in association with familial cases. *HOXB13* is a transcription factor gene that belongs to the *HOXB* gene cluster at chromosome 17 (4), is involved in embryonic development of different organs including the digestive tract (5) and regulates transcription of androgen receptor (AR) target genes (6). Previous studies suggest that *HOXB13* might be involved in colorectal tumorigenesis. *HOXB13* expression levels are decreased in colon tumour cells compared to normal cells (7), and hypermethylation of a CpG island upstream of *HOXB13* was reported as a potential mechanism for down regulation of *HOXB13* in CRC (8). Based on these data, we hypothesized that the recently described HOXB13 p.Gly84Glu mutation may be associated with CRC risk.

Materials and Methods

We genotyped the HOXB13 p.Gly84Glu mutation in germline DNA of 2,695 population-based CRC cases and in 4,593 controls. Subjects included 1,952 CRC cases and 1,197 controls from the Ontario Familial Colon Cancer Family Registry (OFCCR) and 743 CRC cases and 246 controls from Australasian Colorectal Cancer Family Registry (ACCFR). The OFCCR and ACCFR are two population-based sites of the National Cancer Institute supported Colorectal Cancer Family Registries consortium. The details of this consortium including recruitment strategies have been previously published (9). Briefly, the OFCCR recruited incident CRC cases (1997-2002) from the population-based Ontario Cancer Registry. Cases were stratified into high risk (Amsterdam criteria) (n=106), intermediate risk (n=920) and low risk (n=926) according to family histories, age of onset and pathologic characteristics; all high and intermediate risk cases and a 25% random sample of low-risk cases were subsequently recruited. For the Ontario cases, controls with no prior personal history of cancer were recruited through residential telephone lists as well as the Ontario Ministry of Finance property-assessment file for the year 2000 (10). The ACCFR recruited CRC cases aged 18-59 from the Victoria Cancer Registry and controls were identified through local electoral roles. Those cases (n=106) that met Amsterdam Criteria were screened for mutations on mismatch repair genes and 23 cases were identified to have a pathogenic mutation in one of the mismatch repair genes. In both registries, Cases of confirmed or suspected familial adenomatous polyposis were excluded and control subjects were age- and sex-frequency matched to CRC cases. The population frequency of the

HOXB13 p.Gly84Glu variant is reported to be very low (<1%) in previous studies and therefore we sought to enlarge our control group to generate a precise estimate. In addition, 925 female controls were obtained from the Health Watch (HW) program at Womens College hospital. These are healthy women with no prior history of cancer who had attended a multimodal screening clinic for well women at Women's College Hospital in Toronto (but not for colorectal screening); and 2,225 male controls from a case-control study in Toronto that have been previously genotyped (3). These men had an elevated PSA but had a negative prostate biopsy. The study was approved by the ethics review board at all participating institutions.

In a second phase, we also used the allele frequency of the mutation reported among 6,481 study subjects of the NHLBI (National Heat, lung and blood Institute) Exome Sequencing Project (ESP6500) (11) and 1,128 individuals from the 1000 Genomes Project (G1000, release7) (12).

Genotyping of the *HOXB13* p.Gly84Glu mutation among cases and controls was performed using a combination of TaqMan assay on ABI 7500 fast and ViiA7 real-time systems (Applied Biosystems Co., Foster City, CA, USA) and iPLEX chemistry on a MALDI-TOF MassARRAY system (Sequenom Inc., San Diego, CA, USA). All mutation carriers identified by genotyping were confirmed by direct sequencing using the BigDye Terminator Cycle Sequencing kit on an ABI 3500XL DNA Analyzer (Applied Biosystems Co., Foster City, CA, USA). We compared the frequency of the *HOXB13*p.Gly84Glu mutation between cases and controls using Fisher's exact test and calculated odds ratios (OR) and their 95% confidence intervals (CI) based on 2×2 table analysis of cases and controls.All statistical tests were two-sided and p values <0.05 were considered statistically significant. Because of the very small number of carriers of the variant alleles in the control population, we combined the control groups to provide a composite estimate of prevalence and it was not practical to perform subset analyses. Adjustment for ethnicity, age and gender using a multivariable logistic regression model was not possible given the low number of positive observations of the mutation.

Results

The characteristics of cases and controls are shown in Table 1. Thirteen heterozygote carriers of the *HOXB13* p.Gly84Glu mutation were identified among 2,695 cases (0.48%) and 8 carriers were seen among 4,593 controls (0.17%) (OR = 2.8, 95% CI = 1.2 - 6.7, P = 0.02). Several carriers among the cases and controls had a family history of CRC and/or prostate cancer (See Table 2). Among the 13 CRC mutation carriers, 5 had a family history of CRC and 4 had a family history of prostate cancer. Of the 8 control subjects with HOXB13 mutation for whom we had family history information (OFCCR, ACCFR, HW and Prostate study controls), 3 had a family history of CRC and 3 had a family history of prostate cancer. When the analysis was limited to 1,395 cases who had a family history of CRC or prostate cancer, the odds ratio was 3.3 (0.57% vs. 0.17%, 95%CI: 1.2-8.8, P = 0.01). Based on prior testing, none of the *HOXB13* mutation carriers harboured mutations in *MUTYH* and none of the Ontario cases tested had truncating mutations in *APC*, although cases of polyposis were excluded from both studies. Two *HOXB13* mutation carriers were found to have mutations in *MLH1* and *MSH6*, respectively, and their tumors demonstrated high microsatellite instability.

Given the small number of controls with the mutation (n = 8) we sought to incorporate mutation frequency data from publicly available sources. The minor allele frequency (MAF) of HOXB13 p.Gly84Glu among the 1,128 individuals with available exome sequencing data in release 7 of the G1000 project is 0.001, equal to 2 mutated alleles. Twenty mutated alleles

were also reported among the 6,481 individuals of ESP6500 project. Since all carriers in these two studies were heterozygous, there are 22 carriers in total among the 7,609 individuals in G1000 and ESP6500 projects combined (0.29%). Including these controls with our own set of controls and comparing the mutation frequency with that in CRC cases shows that HOXB13 p.Gly84Glu is associated with increased risk of colon cancer, but with a lower odds ratio (OR = 2.0, 95%CI = 1.0 to 3.8, $P = 0.04$). Combining the data presented by Alanee et al. (13) with the case-control data from our series yields an odds ratio of 2.6 (95%CI 1.12-6.01, p=0.03)

Discussion

We are the first to report a significant association between the HOXB13 p.Gly84Glu mutation and CRC risk. We identified a carrier frequency of 0.48% among CRC cases from two distinct population-based CRC registries. Many of the carriers identified had a family history of colon and/or prostate cancer further suggesting that this variant may prediscpose to both colorectal and prostate cancer. The carrier frequency among controls was 0.17% among subjects tested and 0.29% among subjects in publicly available databases. The carrier frequency in all controls combined in our series (0.17%) is similar to that of 0.1% and 0.2% seen in controls genotyped by Ewing et al. (2) and the 0.21% reported by Breyer et al (14**).** Alanee et al (13) reported a 0.1% carrier rate among 1,052 CRC cases and 1,650 controls; while this report did not suggest an increased risk of CRC; combining the data from Alanee et al (13) and this series still produces a significant association between HOXB13 and CRC risk. Given the rarity of this variant, our findings require further validation in other series of CRC cases and controls.

Two of the HOXB13 p.Gly84Glu carriers in our study also carried a mutation on one of the mismatch repair genes. This is not expected to confound the association of HOXB13 with colon cancer, since none of the mismatch repair genes are located in a same chromosome with HOXB13 and their inheritance is totally independent from HOXB13. However, observing the concurrent mutations on HOXB13 and mismatch repair genes among our study subjects raises a question about synergistic effect between carrying mutations on these two sets of genes together that could not be answered with small number of carriers we observed.

Since genes harbouring risk alleles can be expressed in multiple tissues, it is not surprising that some mutations may predispose to multiple different cancers. Mutations in DNA mismatch repair genes are associated with colorectal, endometrial and ureteric cancers; while *APC* mutations are associated with colorectal and multiple extracolonic neoplasms. Due to its low allele frequency (0.14%), this *HOXB13* mutation accounts for only a small fraction of familial CRC cases. However, the effect size (OR: 2.0-2.8) of the HOXB13 p.Gly84Glu mutation that we have shown to be associated with CRC risk is similar or greater compared to that of other known CRC risk alleles (e.g. APC I1307K) or those identified via genome-wide association studies (15). In addition, since the recruitment of cases to the OFCCR favoured subjects with a family history of CRC**,** it is possible that this may lead to an overestimation of the population-based risk for this variant. The increased recruitment of familial cases may be responsible for the increased mutation frequency in these cases and enhanced our ability to detect a risk associated with this mutation. None the less, our finding of an association between *HOXB13* mutations and CRC risk may lead to future investigations of the role of androgens and AR response genes in CRC and, if confirmed, will have implications for counseling families found to be carrying this mutation.

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Abbreviations

CRC	colorectal cancer
OFCCR	Ontario Familial Colon Cancer Family Registry
ACCFR	Australasian Colorectal Cancer Family Registry
HW	Health Watch Study
OR	odds ratio
CI	confidence interval

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Table 1

Characteristics of the case and control subjects

Variable		Cases		Controls				p-value
		OFFCR	ACCFR	OFFCR	ACCFR	Health Watch	Prostate Study	
Total Number, n		1,952	743	1,197	246	925	2,225	
Age[a], Mean (range)		56.8 (15-90)	47.0 (18-59	63.6 (29-79)	48.5 (20-61)	56.0 (27-95)	70.9 (44-97)	<0.001
Gender, n (%)	Male	884 (45.3)	389 (52.3)	622 (52.0)	130 (52.8)	0 (0.0)	2,225 (100)	<0.001
	Female	1,068 (54.7)	354 (47.7)	575 (48.0)	116 (47.2)	925 (100)	0 (0.0)	
Ethnicity, n (%)	White	1,815 (93.0)	704 (94.7)	1,126 (94.1)	236 (95.9)	884 (95.6)	1,787 (80.3)	<0.001
	Other[b]	137 (7.0)	39 (5.3)	71 (5.9)	10 (4.1)	41 (4.4)	438 (19.7)	
HOXB13 p.Gly84Glu carriers, n (%)		11 (0.6)	2 (0.2)	4 (0.3)	1 (0.4)	1 (0.1)	2 (0.1)	0.02

[a] Age for cases is their age of diagnosis and for controls is their age of consent

[b] Including south and east Asians, south Americans and Africans.

Table 2

Clinical characteristics of 21individuals carrying germline *HOXB13*G84E mutation

No.	Study[a]	Subjects	Gender	Age[b] at diagnosis, y	Family history[c]
1	OFCCR	Case	M	73	None
2	OFCCR	Case	M	63	None
3	OFCCR	Case	F	66	None
4	OFCCR	Case	M	51	Father, 5 Paternal Uncles (PrCa, 74, 60, 63, 72, 74, 74)), Maternal Grandfather (CRC, 81)
5	OFCCR	Case	F	59	Paternal Uncle, Maternal Grandfather (PrCa, 78, 80)
6	OFCCR	Case	M	45	2 Maternal Uncles (CRC, 69, 75), 2 Paternal Uncles (PrCa,UK, UK)
7	OFCCR	Case	F	59	None
8	OFCCR	Case	M	42	Paternal Uncle (CRC, 60)
9	OFCCR	Case	F	69	Maternal Uncle, Maternal Grandfather (CRC, UK, UK)
10	OFCCR	Case	M	39	None
11[d]	OFCCR	Case	M	40	Father, Mother, Sister, Maternal Uncle (CRC, 58, 54, 56, 54)
12[e]	ACCFR	Case	M	22	None
13	ACCFR	Case	F	42	Father, Brother (PrCa, 64, 49)
14	ACCFR	Control	M	52	Father, Paternal Uncle, Maternal Uncle (CRC, 44, UK, UK)
15	OFCCR	Control	M	59	Father (PrCa)
16	OFCCR	Control	F	73	None
17	OFCCR	Control	M	68	None
18	OFCCR	Control	F	54	Father, Mother, Maternal Grandfather (CRC), Maternal Uncle (PrCa)
19	HW	Control	F	57	Brother (CRC, 54)
20	PC	Control	M	59	Father, Uncle (PrCa, 79, 85)
21	PC	Control	M	66	None

[a] Ontario Colon Cancer Family Registry (OFCCR), Australasian Colorectal Cancer Family Registry (ACCFR), Health Watch Study (HW), Prostate Cancer Study (PC)

[b] Age for cases is their age of diagnosis and for controls is their age of consent

[c] Family history of colorectal cancer (CRC) and prostate cancer (PrCa) among the first and second degree relatives of the probands. The age at diagnosis was provided for whom it was available.UK= age of diagnosis is unkown

[d] This individual carried a *MLH1* mutation (c.1217_23dupGTCAGCC, p.Q409fsX85)

[e] This individual carried a *MSH6* mutation (c.3311_3312delTT, p.Phe1104TrpfsX3)