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Germline variation of the melanocortin-1 receptor does not explain shared risk for melanoma and thyroid cancer

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Abstract

Background—Recently, germline variants of the melanocortin-1 receptor (MC1R) have been shown to be associated with an increased risk for BRAF mutant but not BRAF wild-type cutaneous melanoma. Similar to melanoma, BRAF mutations are also commonly found in papillary thyroid carcinomas. Furthermore, patients with melanoma have an increased risk for thyroid carcinoma and vice versa.

Methods—To determine whether MC1R variation also represents a risk factor for BRAF mutant thyroid carcinomas, we sequenced BRAF and MC1R in two separate case-control cohorts.

Results—We demonstrate that MC1R is expressed in normal and neoplastic thyroid epithelial cells, albeit at lower levels than in melanocytes. In the first cohort of 66 follicular (FTC) and 62 papillary thyroid carcinomas (PTC), and 128 matched controls from the San Francisco Bay Area we found no association between the number of MC1R variant alleles and thyroid cancer. Patients with BRAF mutated tumors had a higher frequency of MC1R variant alleles than their matched controls (p=0.039). However contrary to the findings in melanoma, the odds ratio for having a BRAF mutant cancer decreased from 3.9 for carriers of one MC1R allele to 1.5 for carriers of two or more alleles. As the frequency of MC1R alleles varies highly among different ethnic populations, we analyzed a second, ethnically more homogeneous cohort from Spain and Portugal, and found no association with PTC nor with BRAF mutated PTC.

Conclusion—Our data indicates that the strong association between *BRAF* mutations and *MC1R* variants previously found in melanoma does not extend to thyroid cancer.

Keywords

MC1R; *BRAF*; thyroid carcinoma; melanoma; susceptibility gene

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Introduction

BRAF is a serine/tyrosine kinase in the MAP kinase signaling pathway that is frequently mutated in different types of cancer (1). In cutaneous melanoma its mutation frequency varies dramatically depending on anatomic site and sun exposure (2). We and collaborators recently showed that germline variants of the melanocortin-1 receptor (*MC1R*), a G-protein coupled receptor that responds to alpha-melanocyte stimulating hormone (α-MSH), confer a highly increased risk for the development of *BRAF* mutated melanomas but not *BRAF* wild-type melanomas in intermittently sun-exposed skin (3;4). *MC1R* is highly polymorphic throughout all ethnicities, with about 50% Caucasians carrying at least one variant allele, and a spectrum of less frequent and different variations in Asians and Africans (5–10). Variants have reduced signaling ability, contributing to distinct phenotypic traits such as fair skin, freckling, and red hair. *MC1R* variations have been shown to be melanoma risk factors (10), even beyond their effect on pigmentation (11;12).

The mechanism of the gene-gene interaction between germline *MC1R* variants and somatic *BRAF* mutations in melanoma rendering *MC1R* variants a critical predisposing factor for *BRAF*-driven melanomas is currently unclear. Several lines of evidence suggested that the gene-gene interaction between *MC1R* and *BRAF* in melanoma may extend to other cancers. Animal models show that reduced *MC1R* signaling results in an increased cancer incidence. For example, in mice, activating mutations of the agouti signaling protein gene (ASIP), an inhibitor of *MC1R*, result in yellow-orange coat color and a significantly increased incidence of mammary-gland tumors and liver carcinoma (13–16). In the human, *BRAF* mutations are also commonly found in papillary thyroid carcinomas (17), which occur more frequently in Caucasians. Most interestingly, patients with melanoma have a higher risk of thyroid cancer, and vice versa (18;19), indicating a possible common susceptibility mechanism. *MC1R* is expressed in the thyroid gland, and other tissues in levels comparable to the skin (20). We hypothesized that impaired *MC1R* signaling may have more far reaching cancer promoting consequences than its impact on skin pigmentation.

Material and Methods

Real-Time Quantitative PCR

To test whether *MC1R* is expressed in thyroid epithelial cells, we performed real-time quantitative PCR (qPCR) on mRNA from 11 normal thyroid tissue samples, 20 papillary thyroid carcinoma samples, both extracted from frozen thyroid cancer surgery specimen, and from two foreskin melanocyte cell cultures. Total RNA was prepared by TRIZOL extraction (Invitrogen, Carlsbad, CA). A total of 123 ng of total RNA were reverse-transcribed using the RT script cDNA synthesis kit (USB, Cleveland, OH). Real-time quantitative qPCR was used to measure mRNA expression levels normalized to *GUS* mRNA expression. The PCR primers and probes for the genes were purchased from Applied Biosystems (Assay-on-Demand kit; Foster City, CA). All quantitative qPCR reactions were performed in triplicate.

Patients and Control Cohorts

A first cohort of 128 thyroid carcinomas (66 follicular thyroid carcinomas (FTC), and 62 papillary thyroid carcinomas (PTC), median age 49, range 15 to 96) from the archives of the Department of Pathology at UCSF, and 128 gender and ethnicity matched controls from the DNA bank at UCSF (103 female, 25 male; 6 African American, 22 Asian, 87 Caucasian, 13 Latino) was assembled. The study was approved by the institutional review board of the University of California, San Francisco.

The second cohort of 74 PTC and 74 age- and gender-matched controls (57 female, 17 male, median age PTC 41 years, controls 44 years) was from Portugal and Spain and previously described (21). The study was approved by the institutional review board of the University of Porto, Portugal.

Sequence analysis

For cases, DNA was extracted from micro-dissected paraffin embedded tissue sections, and *BRAF* exon 15 and the entire *MC1R* gene (5 amplicons) were amplified from tumor DNA and neighboring normal tissue DNA, respectively, as published previously (3). Micro-dissection of the first cohort was performed in San Francisco, for the second cohort in Porto. For the controls, *MC1R* was amplified form peripheral blood lymphocyte DNA. PCR products were purified using ExoSAP-IT (USB Corporation, Cleveland, OH) and sequenced directly using the ABI BigDye v3.1 dye terminator sequencing chemistry and an ABI PRISM 3730xl capillary DNA analyzer (Applied Biosystems, Foster City, CA).

Statistical analysis

Standard univariate statistical tests were performed using STATA 9.2 (StataCorp, College Station, TX). All p-values were two-sided, and $p<0.05$ was considered statistically significant. As the functional impairment of individual *MC1R* variants is not entirely clear, we classified the *MC1R* genotype for statistical analysis as “0” for consensus sequence, “1” for one for any one variant allele, and “2” for two or more variants. In a second, alternative analysis we classified *MC1R* variants according to their associated phenotype as red-hair (R151C, R160W, and D294H), non-red-hair (all other variants), and consensus sequence.

Results

Expression of *MC1R* in normal thyroid and thyroid cancer

Overall, in benign and malignant thyroid tissue the expression levels of *MC1R* were lower than in cultures of normal human melanocytes, with mean *MC1R* expression levels of 2.1 in normal thyroid tissue, 1.8 in PTC, and 8.5 in foreskin melanocyte cultures (Figure 1). In spite of the fact that different sample preparation techniques might have added certain variability to our results (frozen tissue vs. cultured cells), we conclude that *MC1R* is expressed in benign and malignant thyroid tissue, however at lower levels than in melanocyte cultures. Hence we decided to proceed and investigate the possibility of *MC1R* variation as a risk factor for *BRAF* mutant thyroid cancers.

Sequence Analysis

First study cohort from the San Francisco Bay Area—As expected, the frequency of *BRAF* mutations was significantly higher in papillary than follicular thyroid carcinomas (69.4% vs. 3.0%, respectively). All mutations in exon 15 were V600E mutations. There was no association between *MC1R* genotype and thyroid cancer as a whole or within the PTC or FTC subgroups. Similarly, no association was found when the analysis was restricted to Caucasians or Asians (Table 1). Other ethnic groups were too small to be analyzed.

By contrast, we found a higher frequency of *MC1R* alleles in patients with *BRAF* mutated tumors compared to their matched controls (Pearson chi-square test, $p=0.039$). The corresponding odds ratios were 3.9 (95% confidence interval 1.3 to 12.3) for carriers of one *MC1R* variant allele compared to *MC1R* consensus sequence carriers, but only 1.5 (95% confidence interval 0.5 to 4.4) for two or more variants (Table 2) When this analysis was restricted to Caucasians a similar trend was seen ($p=0.062$). By contrast, when *MC1R* alleles were stratified by red-hair or non-red-hair alleles no significant association was found.

Second study cohort from Portugal and Spain—In the second, ethnically more homogeneous cohort 32 of 74 (43.2%) PTCs harbored a V600E *BRAF* mutation. The frequency of *MC1R* variants was almost identical between cases and controls (Table 3). Similarly, no significant differences were found when the *MC1R* genotype was stratified as red or non-red hair variants.

Discussion

Our analysis of the effect of germline variations of *MC1R* on somatic mutations of *BRAF* in thyroid tissue indicate that the strong association previously found in melanoma does not exist in thyroid cancer. Whereas patients from the San Francisco Bay Area with *BRAF*-mutant carcinomas tended to have a higher frequency of *MC1R* alleles than the corresponding controls, this association did not replicate in the ethnically more homogenous cohort of PTCs from Portugal and Spain. Further, the pattern of association found in the first cohort differs significantly from our prior findings in melanoma, where the odds ratios increased with the number of alleles as well as from weak (non-red-hair) to strong (red-hair) alleles. It is therefore likely that the well documented strong variation of *MC1R* alleles among ethnic populations has resulted in a spurious association between *MC1R* and *BRAF* in the first cohort. The sample sizes of the two cohorts analyzed in the present study were larger than the two cohorts that were used to show a statistically significant correlation between *BRAF* mutation and *MC1R* variants in melanoma (3). Based on the hypothesis of an equally strong association in thyroid carcinoma, the present study should have been able to show a correlation, or at least a clear trend.

In summary, we conclude that although *MC1R* is expressed in the thyroid it is unlikely to affect the mutation frequency of *BRAF* present in thyroid carcinoma.

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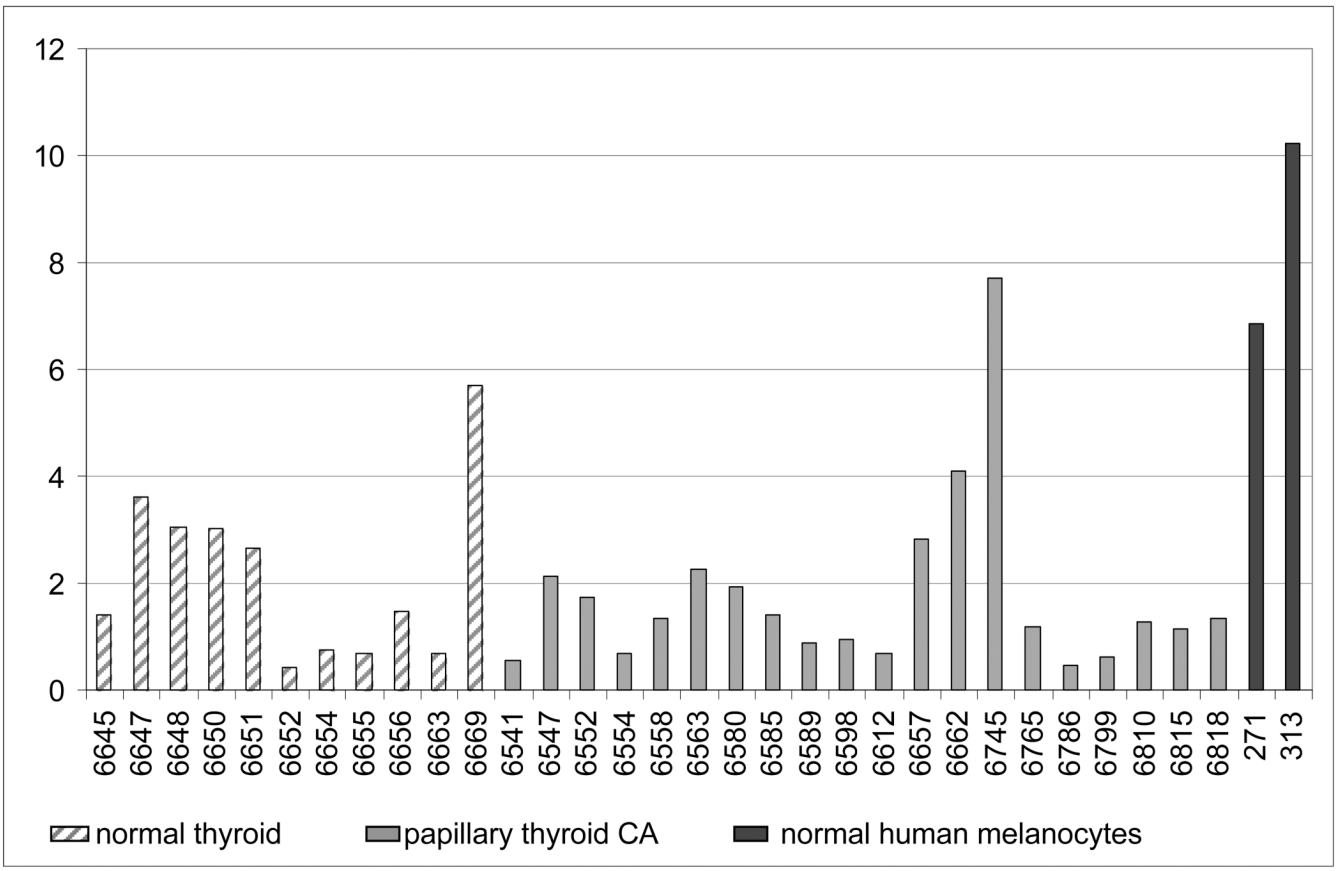

Figure 1.
qPCR analysis of *MC1R* mRNA expression relative to *GUS* mRNA in 11 normal thyroid tissue samples, 20 papillary thyroid carcinoma, and normal human melanocytes.

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Table 1

Analysis of the San Francisco Bay Area cohort independently of ethnicity and BRAF mutation status.

Tumors	Papillary thyroid carcinoma			Follicular thyroid carcinoma		
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
MC1R consensus	12	20	reference	13	10	reference
1 *MC1R* variant	26	16	2.708 (1.049 to 6.994)	29	26	0.858 (0.322 to 2.286)
≥ 2 *MC1R* variants	24	26	1.539 (0.622 to 3.805)	24	30	0.615 (0.230 to 1.646)
total	62	62	p = 0.108*	66	66	p = 0.543*

Given are odds ratios for papillary (PTC) and follicular (FTC) thyroid carcinoma depending on the number of *MC1R* variants compared to the matched control cohort.

*Pearson chi-square test.

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Table 2

Analysis of the San Francisco Bay Area cohort independently of ethnicity and tumor type (PTC and FTC included).

Tumors	*BRAF* mutant			*BRAF* wild-type		
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
MC1R consensus	8	15	reference	17	15	reference
1 *MC1R* variant	21	10	3.938 (1.2572 to 12.3322)	34	32	0.938 (0.4024 to 2.1839)
≥ 2 *MC1R* variants	16	20	1.5 (0.5089 to 4.4213)	32	36	0.784 (0.3379 to 1.8202)
total	45	45	p = 0.039*	83	83	p = 0.810*

Given are odds ratios for *BRAF* mutant and *BRAF* wild-type thyroid carcinoma depending on the number of *MC1R* variants compared to the matched control cohort.

*Pearson chi-square test.

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Table 3

Analysis of the Portuguese and Spanish cohort consisting of PTC cases only.

Tumors	***BRAF* mutant or wild-type**			***BRAF* mutant**		
	Cases	**Controls**	**OR (95% CI)**	**Cases**	**Controls**	**OR (95% CI)**
MC1R consensus	41	40	reference	21	22	reference
1 *MC1R* variant	26	26	0.976 (0.486 to 1.958)	8	8	1.048 (0.3324 to 3.302)
≥ 2 *MC1R* variants	7	8	0.854 (0.283 to 2.575)	3	2	1.571 (0.2382 to 10.3653)
total	74	74	p = 0.961*	32	32	p = 0.894*

Given are odds ratios for any *BRAF* mutation status and for *BRAF* mutant cases only, depending on the number of *MC1R* variants compared to the matched control cohort.

*Pearson chi-square test.

Table 4

Frequency of MC1R variant alleles in the two cohorts from the San Francisco Bay Area and Portugal and Spain.

	San Francisco Cases					San Francisco Controls					Portugal & Spain	
	African American	Asian	Caucasian	Latino	All ethnicities	African American	Asian	Caucasian	Latino	All ethnicities	Cases	Controls
n (individuals)	**6**	**22**	**87**	**13**	**128**	**6**	**22**	**87**	**13**	**128**	**74**	**74**
Variants												
V60L	-	-	22	3	25	-	-	26	3	29	15	22
R67Q	-	-	-	-	-	-	1	-	-	1	-	-
D84E	-	-	3	-	3	-	-	1	-	1	-	1
V92M	-	16	19	1	36	-	9	15	5	29	13	5
I120T	-	4	-	-	4	-	3	-	-	3	1	-
V122M	-	-	1	-	1	-	-	-	-	-	-	1
S131N	-	-	-	-	-	-	-	1	-	1	-	-
R142H	-	-	-	-	-	-	-	2	-	2	1	1
R151C	-	-	10	-	10	-	-	16	1	17	4	3
I155T	-	-	-	-	-	-	-	1	-	1	-	-
V156L	-	-	-	-	-	1	-	-	-	1	-	-
R160W	-	-	13	-	13	-	-	14	-	14	1	-
R163Q	1	22	14	9	46	-	32	9	9	50	3	6
A166G	-	-	-	-	-	-	1	-	-	1	-	-
S172I	-	-	-	-	-	-	-	1	-	1	-	-
Y183D	-	-	-	-	-	-	-	1	-	1	-	-
A198P	-	-	-	-	-	-	-	-	1	1	-	-
R213W	-	-	-	-	-	-	-	-	-	-	1	-
G248D	-	-	-	-	-	-	-	-	-	-	-	2
P256S	-	-	-	1	1	-	-	-	-	-	-	-
D294H	1	-	3	1	5	-	-	2	-	2	3	1
T308M	-	1	-	-	1	-	-	-	-	-	-	-
C315S	-	-	1	-	1	-	-	-	-	-	-	-
insA726	-	-	-	-	-	-	-	1	-	1	-	-

	San Francisco Cases					San Francisco Controls					Portugal & Spain	
	African American	Asian	Caucasian	Latino	All ethnicities	African American	Asian	Caucasian	Latino	All ethnicities	Cases	Controls
n (individuals)	**6**	**22**	**87**	**13**	**128**	**6**	**22**	**87**	**13**	**128**	**74**	**74**
all variants	2	43	86	15	146	1	46	90	19	156	42	42

Highlighted in grey are the “red-hair variants”.