Endocrine CONNECTIONS

The *HABP2* G534E polymorphism does not increase nonmedullary thyroid cancer risk in Hispanics

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Abstract

Familial nonmedullary thyroid cancer (NMTC) has not been clearly linked to causal germline variants, despite the large role that genetic factors play in risk. Recently, *HABP2* G534E (rs7080536A) has been implicated as a causal variant in NMTC. We have previously shown that the *HABP2* G534E variant is not associated with TC risk in patients from the British Isles. Hispanics are the largest and the youngest minority in the United States and NMTC is now the second most common malignancy in women from this population. In order to determine if the *HABP2* G534E variant played a role in NMTC risk among Hispanic populations, we analyzed 281 cases and 1105 population-matched controls from a multicenter study in Colombia, evaluating the association through logistic regression. We found that the *HABP2* G534E variant was not significantly associated with NMTC risk ($P=0.843$) in this Hispanic group. We also stratified available clinical data by multiple available clinicopathological variables and further analyzed the effect of *HABP2* on NMTC presentation. However, we failed to detect associations between *HABP2* G534E and NMTC risk, regardless of disease presentation ($P\geq0.273$ for all cases). Therefore, without any significant associations between the *HABP2* G534E variant and NMTC risk, we conclude that the variant is not causal of NMTC in this Hispanic population.

Key Words
- *HABP2*
- G534E
- thyroid cancer
- Hispanics

Endocrine Connections (2016) **5**, 123–127

Introduction

Thyroid cancer (TC) is becoming epidemic, growing in incidence both nationally and internationally (1). Nonmedullary thyroid cancer (NMTC) makes up over 95% of all cases, making it the most common endocrine malignancy (2). Among Hispanics in the United States, the country's largest minority, NMTC is now the second most common cancer among women, accounting for 9% of all incident female cancer cases in this minority (3).

http://www.endocrineconnections.org
DOI: 10.1530/EC-16-0017

Even though NMTC is a highly familial malignancy (4), there has not been conclusive evidence for the discovery of highly penetrant NMTC genes.

Recently, two studies implicated a germline missense variant (G534E) in the *HABP2* gene as the cause of familial NMTC (5, 6). Gara and coworkers (5) first reported the cosegregation of *HABP2* G534E variant with either papillary TC (PTC) or follicular adenomas among seven members of the same family. The authors also demonstrated that *HABP2* G534E had a dominant-negative function and based on cosegregation and functional data concluded that *HABP2* G534E was causal of NMTC in the family. More recently, Zhang and Xing (6) also concluded that *HABP2* G534E was causal of NMTC. This conclusion was based on the presence of the variant in three individuals from the same family and in four additional individuals belonging to four different kindred out of total 29 kindred studied (6). Since then, the role of *HABP2* G534E in NMTC has been highly contested given its relatively high frequency in the general population (7, 8, 9, 10).

In order to assess the NMTC risk conferred by *HABP2* G534E, we previously carried out an association study using over 2000 NMTC cases and over 5000 population controls from the British Isles (11). We found that *HABP2* G534E was present at a relatively high frequency (4.6%) in the population and it was not associated with NMTC risk, even when the data were stratified by age of onset and NMTC subtypes (11). Other groups have also reported similar negative results in sporadic and familial PTC cases of European (12) and Chinese (13) ancestries. Intrigued by these conflicting reports on the role of *HABP2* G543E in NMTC, we decided to analyze this variant in South American Hispanics, a population of admixed American Indian, European and African ancestries (14).

Materials and methods

Study subjects

All cases and controls were recruited in five Colombian cities (Bogotá, Ibagué, Neiva, Medellín and Pasto) between 2010 and 2014 as part of a population-based multicenter study of thyroid cancer in the country. Ethics committees from the University of Tolima, Hospital Federico Lleras Acosta, Hospital Fernando Moncaleano, and Hospital Pablo Tobón Uribe approved the research protocol used in the study. These recruitment hospitals are tertiary healthcare centers in major Colombian cities, and in most instances, they represent the only regional centers offering radiation and surgical therapy for thyroid cancer. Given that medicine is socialized in Colombia, these hospitals attend patients from all socioeconomic classes in the country. Thus, these cases recruited in the study are representative of the general Colombian population. In total, 281 histologically verified NMTC cases (48 males and 233 females) and 1105 population-matched controls were included in the study. All controls were recruited at the same hospitals, were cancer-free and older than 50 years at the time of recruitment. The mean age of cases was 46.89 years (s.d.=1.66) and mean age of controls was 64.15 years (s.d.=0.59). Clinical data such as age of onset, NMTC subtype, family history of cancer, presence or absence of local or distal metastasis, and histological characteristics such as vascular and capsule invasion, focality, and bilaterality were available for most of the cases ($n=241$).

Genotyping

Genomic DNA was isolated from whole blood samples using Promega Maxwell 16 system (Promega). *HABP2* G534E variant was genotyped using competitive allele-specific KASP genotyping chemistry (LGC Genomics, Teddington, UK) as described previously (11). The genotyping call rates were >98%, with controls derived from replicates with verified genotypes used in all assays. Four additional markers, two upstream of G534E (rs10787491, rs932650) and two downstream of G534E (rs10885478, rs1885434), were genotyped in five G534E heterozygous individuals for haplotype analysis.

Statistical analysis

All genotype frequencies and association testing using logistic regression models were performed using PLINK (15). Genotypes were in Hardy–Weinberg equilibrium.

Results

Association between *HABP2* G534E and TC risk in Hispanic population

In this study, we genotyped the *HABP2* G534E variant in 281 NMTC cases and 1105 population- and sex-matched controls from Colombia. We found that *HABP2* G534E was polymorphic in both cases (frequency=1.4%) and controls (frequency=1.3%). All heterozygous carrier controls were >50 years. The mean age of heterozygous

http://www.endocrineconnections.org
DOI: 10.1530/EC-16-0017

carrier cases was 43.57 years (s.d. = 16.066) and the age of heterozygous carrier controls was 65.44 years (s.d. = 4.33). Consistent with the similar frequency of these variants in cases and controls, we failed to detect an association between *HABP2* G534E and NMTC risk in our Hispanic case–control study (odds ratio, OR = 0.92, 95% confidence interval, CI: 0.4–2.11, $P = 0.843$).

Association between *HABP2* G534E and TC risk in cases stratified by clinical variables

We further looked into any possible association between *HABP2* G534E and NMTC risk by stratifying the cases by clinical variables. As shown in Table 1, the variant was not significantly associated with early (<45 years) or late (≥45 years) disease onset, presence or absence of family history of cancer, or with any other tumor histological feature (size, focality, capsular invasion, vascular invasion, lymph node metastasis or extrathyroid extension).

The frequency of the *HABP2* G534E allele varies across population of different ancestries (11). This variant is absent from African and East Asian populations but is present at ~5% frequency in populations of European ancestry (11). Colombians are a population of mixed European, Amerindian and African ancestries, where genetic ancestry correlates with geographical origin and socioeconomic status (SES) (14, 16, 17, 18). To account for a potentially confounding role of geographical origin and SES, we carried out analyses that incorporated recruitment center and SES. We failed to detect an association between *HABP2* G534E and TC risk even when these covariates were incorporated into the analyses ($P = 0.60$, data not shown).

Haplotype analysis

We also investigated the haplotype surrounding *HABP2* G534E to test the possibility of different origins of G534E allele, using previously described methods (11) in five cases who were *HABP2* G534E heterozygous. These five individuals shared the same haplotype that we previously identified in the British Isles (11), suggesting a European ancestry of this variant in Hispanics (data not shown).

Table 1 Association between *HABP2* G534E and different clinical variables.

Variable	***HABP2* genotype**		**G534E frequency**	**OR** (95% CI)*
	Heterozygous	Wild type		
Age at diagnosis				
≥45	4 (2.4)	165 (97.6)	169 (0.012)	0.87 (0.3–2.49)
<45	3 (2.7)	108 (97.3)	111 (0.014)	1 (0.3–3.29)
Family history of cancer*				
Present	3 (3.4)	84 (96.6)	87 (0.017)	1.28 (0.39–4.22)
Absent	4 (2.1)	189 (97.9)	193 (0.01)	0.76 (0.27–2.17)
Histologic type				
FTC	4 (3.7)	105 (96.3)	109 (0.018)	1.36 (0.47–3.89)
PTC	3 (1.8)	168 (98.2)	171 (0.009)	0.64 (0.2–2.12)
Tumor size				
Small <2 cm	5 (3.7)	131 (96.3)	136 (0.018)	1.36 (0.52–3.54)
Large ≥2 cm	2 (1.9)	102 (98.1)	104 (0.01)	0.71 (0.17–2.97)
Focality				
Multifocal	4 (4.5)	85 (95.5)	89 (0.022)	1.67 (0.58–4.8)
Unifocal	3 (2)	148 (98)	151 (0.01)	0.73 (0.22–2.42)
Capsular invasion				
Absent	3 (2.2)	133 (97.8)	136 (0.011)	0.81 (0.25–2.67)
Present	4 (3.8)	100 (96.2)	104 (0.019)	1.43 (0.5–4.08)
Vascular invasion				
Absent	6 (3.2)	182 (96.8)	188 (0.016)	1.18 (0.49–2.85)
Present	1 (1.9)	51 (98.1)	52 (0.01)	0.71 (0.1–5.22)
Lymph node metastasis				
Absent	5 (3)	161 (97)	166 (0.015)	1.11 (0.43–2.88)
Present	2 (2.7)	72 (97.3)	74 (0.014)	1 (0.24–4.21)
Extrathyroid extension				
Absent	6 (3)	194 (97)	200 (0.015)	1.11 (0.46–2.68)
Present	1 (2.5)	39 (97.5)	40 (0.013)	0.92 (0.12–6.83)

*None of the variables were found to be statistically significant (all two-sided *P* values ≥0.273).

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DOI: 10.1530/EC-16-0017

Discussion

In this study, we tested the association between *HABP2* G534E and NMTC in a Hispanic population-based study using SNP genotyping and logistic regression methods. We also investigated the associations between this variant and disease manifestation. We did so as recent reports have suggested conflicting results about the role of *HABP2* G534E and NMTC risk. Here, we failed to detect an association between *HABP2* G534E and NMTC risk in our Hispanic population-based study.

NMTC is the second most common malignancy in the Hispanic population and there is an urgent need for effective clinical management of this common endocrine malignancy. Considering that around 45% of male and 60% of female NMTC cases can be attributed to over-diagnosis (19), it is becoming extremely important to develop better risk stratification tools that can guide aggressive or conservative treatment of this cancer. On this background, finding novel genetic variants that truly increase the risk of NMTC will be very beneficial. *HABP2* G534E is a low- to moderate-frequency variant that occurs in 2–6% of the population. Here, consistent with previous studies in European and Chinese populations, we failed to replicate association between *HABP2* G534E and TC risk in Hispanic population as well. We, however, acknowledge that our study may have limited power to detect the low-penetrance effect of a variant that is relatively rare in the population and that future studies, using larger sample sizes, will be required. However, our data indicate that *HABP2* G534E is unlikely to be causal (i.e. a moderate- to high-penetrance effect) to NMTC in populations of diverse ethnicity, and caution should be taken in interpreting TC causality associated with this common variant.

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

Funding
The University of California, Davis and Universidad del Tolima provided principal funding for this study (Grants to L G C C and M E). A P E receives funding from Programa de Becas Doctorales from the Colombian National Science Foundation (Colciencias). L G C C receives funding from the V Foundation for Cancer Research and the National Cancer Institute (Paul Calabresi Career Development Award for Clinical Oncology K12 at UC Davis, award number K12CA138464) of the National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. M B, R P and M E receive funding from COLCIENCIAS and the Research Office of Universidad del Tolima (Projects 400111 and 360113).

Author contribution statement
M E Bohórquez, A P Estrada, J Stultz and R Sahasrabudhe contributed equally to this work.

Acknowledgements
We would like to thank all the individuals who participated in the study.

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Received in final form 4 April 2016
Accepted 19 April 2016

http://www.endocrineconnections.org
DOI: 10.1530/EC-16-0017