Evidence for the founder effect of RET533 as the common Greek and Brazilian ancestor spreading multiple endocrine neoplasia 2A

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Abstract

Objectives: About one-quarter of patients with medullary thyroid cancer (MTC) have inherited disease due to mutations in the *RET* gene. A rare mutation in exon 8 (G533C) of *RET*, previously described in a large Brazilian family with MEN2A, also appeared to be clustering in Greece, whereas it was rarely reported in other ethnic groups. The aim of this study was to identify a possible common ancestry between these carriers.

Patients and methods: Twelve *RET* G533C mutation carriers, four randomly selected from the Brazilian cohort and eight from apparently unrelated Greek families, were studied for a possible common ancestral origin. *RET* flanking microsatellite markers at chromosome 10q (D10S197, D10S196, D10S1652 and D10S537) were used.

Results: Genomic DNA analysis using these markers showed that many of these apparently unrelated individuals shared a common haplotype indicating a common ancestral origin.

Conclusion: Our data suggest that Brazilian and Greek patients with MTC carrying the G533C mutation in exon 8 of *RET* gene originate from a common ancestor. Due to historical reasons, we speculate that the more plausible explanation for the origin of this mutation is in Greece.

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Introduction

More than 25% of medullary carcinoma patients (MTC) have familial disease, which is due to germ line mutations of the *RET* gene. The clinical presentation varies according to the type of *RET* mutation. After the routine application of genetic analysis in all MTC cases (1), it became apparent that some apparently sporadic cases were in fact familial as they carried a *RET* mutation. Furthermore, a wider distribution of mutations across the *RET* gene was identified, which were different from those originally described. In this context, we recently described a large family with medullary thyroid carcinoma (MTC) carrying a missense mutation in exon 8 of *RET*, which corresponds

to a glycine-to-cysteine substitution at codon 533 (2); this was later confirmed to be MEN2A. This family comprises 728 members of Caucasian origin with ancestors from Catalonia, Spain, who immigrated to Brazil at the end of the 19th century. Most of the family members live in Southeastern Brazil and have been followed at an outpatient Endocrine Unit either in Vitoria or São Paulo by the same team of physicians for over fifteen years. During this long-term follow-up, molecular and clinical characteristics of these patients have been described in detail (3, 4). However, the ancestry related to the origin of G533C *RET* mutation remains to be elucidated.

Figure 1

(A) Schematic distribution of *RET* microsatellite markers in representative family members of apparently unrelated kindreds from Brazil and Greece. Microsatellite markers D10S197, D10S196, D10S1652 and D10S537, and their distance in cm, distributed throughout *RET* genomic region as haplotype analysis. (B) Phylogenetic tree of Brazilian and Greek families and controls.

In 2007, Bethanis *et al.* reported a Greek patient with MEN 2A harboring the same *RET* G533C mutation (5). A few years later, Sarika *et al.* identified this mutation in 10 out of 129 patients with apparently sporadic medullary thyroid carcinoma in Greece (6). Gathering these results, we hypothesized whether Brazilian and Greek patients carrying the same *RET* mutation could share a common ancestor.

We proceeded with the investigation of putative founder effect as a point of coalescence between Brazilian and Greek affected families.

Patients and methods

Twelve patients with the G533C *RET* mutation were enrolled in the study: 4 randomly selected from the Brazilian cohort and 8 randomly selected from apparently unrelated Greek families and followed at the Endocrine Unit of Athens University Medical School (Athens, Greece). A control group comprising 29 unrelated thyroid-healthy Brazilian individuals was also studied. Blood samples were obtained from all participants, and all laboratory procedures were performed at the Laboratory of Molecular and Translational Endocrinology at the Federal University of Sao Paulo – UNIFESP, Brazil. The study protocol was approved by the internal review board of UNIFESP (São Paulo, Brazil) and University of Athens (Athens, Greece). All patients received genetic counseling before and after *RET* testing.

Genomic DNA was extracted from peripheral-blood leucocytes as previously reported (7). Haplotype analysis

using four *RET* flanking microsatellite markers (D10S197, D10S196, D10S1652 and D10S537) was performed, as described by Qi *et al.* (8), to track a founder effect. The first marker was placed at position 50.04cm and the other three at positions 70.07, 80.61 and 89.16cm (Fig. 1A). The chromosome map distances were derived from the deCODE map (URL:<http://www.ncbi.nlm.nih.gov/probe>).

Haplotypes of patients and controls were reconstructed using the statistical software package PHASE, version 2.1 (<http://stephenslab.uchicago.edu/software.html>) (9). We constructed the phylogenetic tree of the studied families with POPTREE2 software by estimating the genetic distance between them (10). Chi-square test was used to yield differences in allele distribution among Greek patients, Brazilian patients and Brazilian controls.

Table 1 Clinical characteristics of the cohort of G533C patients.

Patient number	Origin	Age at diagnosis (years)	Gender	pTNM staging	Status at the last follow-up visit
1	Brazil	57	F	T1N0Mx	NED
2	Brazil	44	F	T2N1aMx	NED
3	Brazil	33	M	No MTC	NED
4	Brazil	53	M	T3N1aM0	NED
5	Greece	36	F	T1N0M0	NED
6	Greece	34	F	T1bN0M0	NED
7	Greece	55	M	T1N0M0	NED
8	Greece	37	M	T1N0M0	NED
9	Greece	35	F	T1N0M0	NED
10	Greece	35	F	T1N0M0	NED
11	Greece	24	F	T3N1aM0	NED
12	Greece	50	F	T1N0M0	NED

NED, no evidence of disease.

Table 2 Polymorphic microsatellite markers and allele frequency in Brazilian and Greek patients with medullary thyroid carcinoma bearing the *RET* G533C mutation.

Marker	Allele	<i>RET</i> G533C Brazilian patients		<i>RET</i> G533C Greek patients		P value
		n	%	n	%	
D10S197	162	0	0	3	37.5	0.223
	166	0	0	1	12.5	
	168	4	100	4	50	
D10S196	96	3	75	4	50	0.634
	98	0	0	1	12.5	
	100	1	25	3	37.5	
D10S1652	157	1	25	0	0	0.312
	166	0	0	1	12.5	
	170	0	0	2	25	
	173	3	75	5	62.5	
D10S537	139	2	50	1	12.5	0.262
	144	1	25	0	0	
	148	1	25	5	62.5	
	152	0	0	1	12.5	
	154	0	0	1	12.5	

GenePop was used to perform Hardy–Weinberg exact test (11), and all loci were in Hardy–Weinberg equilibrium.

Results

This study included 8 Greek patients (2 men and 6 women) and 4 Brazilian patients (2 men and 2 women). Clinical and biochemical data of these patients are summarized in Table 1. As can be seen from the table, all the examined cases had excellent long-term follow-up compatible with the relatively mild phenotype that has already been reported for carriers of this mutation (12). Furthermore, the age at presentation was in all cases relatively late.

Four MTC patients (3 from Greece and one from Brazil) shared a common haplotype (D10S197-RET-D10S196-D10S1652: 168-RETG533C-100-173-148, Fig. 1A and Table 2), which was not seen in chromosomes bearing wild-type alleles ($P < 0.005$). In fact, Brazilian patients

presented similar distribution of *RET* polymorphic microsatellite markers compared to Greek patients, suggesting that patients from both nationalities may share a unique genetic signature (Table 2). When comparing Brazilian and Greek affected patients to the control group, we observed a different distribution of D10S197 ($P = 0.016$), D10S196 ($P = 0.042$), D10S1652 ($P < 0.001$) and D10S537 alleles ($P = 0.003$; Table 3). These results reveal a genetic progenitor similarity between Brazilian and Greek *RET* G533C patients regarding *RET* flanking regions that was not observed in unrelated thyroid-healthy controls.

According to the analysis of the phylogenetic tree of the MTC patients and controls, Brazilian and Greek patients pertained to a common root, corresponding to a putative most recent common ancestor (Fig. 1B). As a matter of fact, the control group shared reduced genetic similarity with the MTC patients.

Discussion

After the original identification that the exon 8 G533C *RET* mutation is associated with inherited MTC in our large Brazilian pedigree, there followed very few relative reports from the Mediterranean region and especially from Greece. Interestingly, the one family harboring this mutation in the USA proved to be of Greek ethnic origin (13). Our data suggest that Brazilian patients with MTC caused by the G533C *RET* mutation have an ancestor common to the Greek patients that share the same *RET* genotype. Indeed, by tracking the founder effect, we brought scientific shreds of evidence to this sense. Probably, the G533C mutation observed in Brazilian patients, originally from Catalonia, Spain, was settled by a migratory movement in Europe, either from Greece or from those same who established the Greek population.

Since ancient times, more than thirty Greek city-states had several colonies over the Mediterranean Sea,

Table 3 Comparison of *RET* haplotype frequency among Brazilian and Greek RET533 patients with thyroid-healthy controls.

Marker	Allele	<i>RET</i> G533C Brazilian patients	<i>RET</i> G533C Greek patients	Thyroid-healthy controls	P value ^a	P value ^b	P value ^c
D10S197	168	4	4	18	0.013	0.424	0.016
	Other alleles	0	8	40			
D10S196	96	3	4	14	0.059	0.199	0.042
	Other alleles	1	4	44			
D10S1652	173	3	5	1	<0.001	<0.001	<0.001
	Other alleles	1	3	57			
D10S537	148	1	5	8	0.472	0.004	0.003
	Other alleles	3	3	50			

^aBrazilian patients vs controls; ^bGreek patients vs controls; ^cBrazilian patients vs Greek patients vs controls.

including in the Iberian Peninsula, where Catalonia is located (14). In our first description of the G533C *RET* mutation, we speculated that 'this large family has a Caucasian background from Catalonia, and it is conceivable that relatives living in Europe might also bear the same mutation; hence, it is likely to address a founder effect from Spain, and it is feasible that other relatives also migrated to other countries by the time the ancestor moved to Brazil at the end of the 19th century' (2).

The fact that Brazilian (from Catalonia, Spain) and Greek patients have a common ancestor is fascinating and suggests that, historically, the more plausible explanation for the origin of this mutation is in Greece as Greek migratory currents have occurred in the Mediterranean Sea for many centuries; furthermore, the substantial proportion of the G533C *RET* mutation among the hereditary MTC patients in Greece reinforces this hypothesis (6).

It is worth noting that the world is a melting pot, in which our genetically heterogeneous population can be described as a complex fusion of ethnicities, nationalities and cultures. In this scenario, cultural assimilation in the setting of immigration is important not only for the maintenance of national unity but also for the understanding of the genetic distribution of deleterious alleles. This ethnographic consideration highlights the impact of the present study on public health.

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.

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Author contribution statement

Lucas L Cunha: Collection and assembly of data, data analysis and interpretation and manuscript writing; Susan C Lindsey: Clinical data analysis, interpretation and manuscript writing; Maria Inez C França: Provision of study patients; Leda Sarika: Provision of study patients, clinical data analysis and interpretation; Alexandra Papathoma: Technical support; Ilda S Kunii: Technical support; Janete M Cerutti: Data analysis and interpretation and manuscript writing; Magnus R Dias-da-Silva: Design, data analysis and interpretation and manuscript writing; Maria

Alevizaki: Conception and design and manuscript writing; Rui M B Maciel: Conception and design and manuscript writing.

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