RESEARCH LETTER

Geographic epidemiology of medullary thyroid cancer families: unearthing European ancestral heritage

Dear Editor,

Some 50 years ago, astute clinicians identified a familial medullary thyroid cancer (MTC) syndrome associated with pheochromocytoma and/or pseudonodular parathyroid hyperplasia. These hereditary conditions now are subsumed under the umbrella term of multiple endocrine neoplasia type 2A or MEN 2A (Wells *et al.* 2015). The genetic cause of MEN 2A was identified in the 1990s: missense mutations in the rearranged during transfection (*RET*) proto-oncogene on chromosome 10q11.2, encoding the RET transmembrane tyrosine kinase receptor. This seminal discovery offered unprecedented insights, beyond genotype-phenotype correlations (Eng *et al.* 1996, Machens *et al.* 2013a), into the molecular epidemiology of MEN 2A (Machens *et al.* 2009, 2013b, Romei *et al.* 2010).

Recent ethnographic fieldwork and haplotype analysis, published in 2016 and 2017, traced founder mutations RET p.M918V and p.G533C from Brazil back to the Iberian Peninsula, identifying:

- A p.M918V ancestor from Portugal (city of Braga) who migrated to Northeastern Brazil (state of Ceará) in the 1600s (Martins-Costa *et al.* 2016);
- A p.G533C ancestor from Spain (Catalunia region) who migrated to Southeastern Brazil in the late 1800s (Cunha *et al.* 2017).

Two Greek p.G533C families also were identified in Southern Central Greece and the Peloponnese. Remarkably, p.G533C carriers from Greece were distantly related to the Spanish p.G533C ancestor who migrated to Southeastern Brazil in the late 1800s (Cunha *et al.* 2017).

In Europe, most RET variants seemingly are distributed more or less evenly across countries (Machens *et al.* 2009). Although national screening programs and case ascertainment vary from country to country, distinct RET

variants, typically associated with a sporadic appearing, less-aggressive MTC in the fifth and sixth decade of life, are more frequent in some European countries than others based on comparative literature review (Machens *et al.* 2009):

- p.L790F: 12% in Germany; 1–4% in Italy and France; 0% in Czech Republic and Poland;
- p.Y791F: 0% in France and Italy; 7% in Germany; 9% in Czech Republic; 19% in Poland;
- p.V804: 4% in Poland; 6% in Germany; 15% in France and 20% in Italy in which haplotype analysis excluded a founder effect (Romei *et al.* 2010).

Based on historic settlement areas, these comparative genomic data may hint at a putative ‘Germanic’ ancestry (RET p.L790F), a putative ‘Slavic’ ancestry (RET p.Y791F) and a putative ‘Roman’ ancestry (RET p.V804), subject to further validation. Arguably, such ethnic constructs may simply reflect longstanding inheritance of RET variants created centuries ago in a geographic region.

Twenty-five years into the genomic era, we explored the geographic epidemiology of these three RET variants in present-day Germany in relation to historic Roman and Slavic settlement areas. For the purpose of this study, ‘variant’ is used as a neutral term regardless of whether current evidence indicates ligand-independent activation of the RET receptor protein, qualifying these variants as mutations (e.g., p.L790F or p.V804M) or no longer (e.g., Y791F). For retrospective analysis of existing data sets from routine patient care, no institutional review board approval is required under German law and applicable institutional regulations.

Kindreds from 209 unrelated families carrying RET variants commonly associated with MEN 2A (Wells *et al.* 2015) underwent standard MEN 2A-related interventions

Figure 1

Distribution by historic settlement areas. (A) RET p.L790F (c.2410G>A) and 'Roman' area (shaded). *recent de novo mutation ~1968. (B) RET p.Y791F and 'Slavic' area (shaded). (C) RET p.V804M and 'Roman' area (shaded).

This geographic epidemiological study lends further credence to comparative evidence from the literature pointing toward a 'Roman' ancestry of RET p.V804M and a 'Slavic' ancestry of RET p.Y791F. For each RET variant, our findings were consistent across all three control groups (Table 1), attesting to the robustness of the association. Owing to limitations in study design, specifically the Germanic ancestral heritage of present-day Germany, our data were inconclusive regarding a putative 'Germanic' ancestry of RET p.L790F/c.2370G>T.

RET screening in Germany has been fully covered by both private and statutory health insurance from inception (Machens et al. 2013b). Even under quasi-ideal conditions, subjects carrying RET mutations with low transforming activity may not be captured because they do not have pertinent family histories and have not developed MTC. Unless the whole population of a country is screened, which is logistically unfeasible and not cost-effective, under-ascertainment of RET families is inevitable.

Population-based screening, even if instituted now, would not aid much in tracing RET mutations far back in time because at least one parent, grandparent or great-grandparent may have deceased or be unavailable for genomic testing. Because RET p.L790F/c.2370G>T, p.Y791F and p.V804M phenotypes are less penetrant, parents and grandparents who reportedly were free of a disease that was neither suspected nor screened for cannot be assumed not to harbor the trait. Considering our

limited data, only two RET mutations in this study came into being more recently: p.L790F/c.2370G>T and C634R. These exceptional instances of *de novo* mutations were detected on routine screening in two MTC patients born in 1968 and 1983 whose parents each had negative RET gene test results, ruling out longstanding transmission of the trait.

Haplotype analysis may have helped establish relatedness of German families carrying the same RET variant. In Germany, genomic screening raises difficult issues due to German data protection and privacy regulations. Barring unusually fortunate circumstances, it proved challenging to trace mutations back in time over more than four generations, an insurmountable boundary for most ordinary ancestral research.

Misclassification of distantly related RET families as 'unrelated' cannot be ruled out completely but is set to have been non-differential, affecting families carrying the same RET variant and controls equally. Another method to control for variation was calibration of families with the same RET variant against other RET families, using controls modeled closer to the respective mutational profile.

When exploring rare, less penetrant genomic variants such as RET p.L790F/c.2370G>T, p.Y791F and p.V804M, inadequate statistical power always is an issue. First, evidence of absence (negative test result) cannot be taken as absence of evidence (no association). Second, many genetic association studies,

including this one, are somewhat constrained by small sample size and thus have limited statistical power. A statistically significant finding in an underpowered study is more likely to be false positive due to chance than is such a finding in an adequately powered study. In this situation, some researchers may consider correction for multiple testing, although it helps guard against false-positive findings, as too conservative. This is why independent validation of our geographical epidemiological associations remains important. Intriguingly, the association between RET p.V804M and the Roman historic settlement area would have survived correction for multiple testing.

There is a need for additional geographical epidemiological studies, in particular from countries that include historic Germanic settlement areas and have equally comprehensive national RET screening programs. Because the RET p.L790F variant was found in at least 19 families from France (Bihan *et al.* 2013) and 8 families from Italy (Romei *et al.* 2010), these countries may be ideally suited to elucidate the European ancestry of the RET L790F variant.

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Declaration of interest

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