The penetrance of MEN2 pheochromocytoma is not only determined by RET mutations

Dear Editor,

Multiple endocrine neoplasia type 2 (MEN2) is a rare syndrome subdivided into 2 main entities: MEN2A and MEN2B (Donis-Keller et al. 1993, Mulligan et al. 1993, Eng et al. 1996). Genetic results can predict the natural history of medullary thyroid carcinoma (MTC) depending on the mutation of RET. This is the basis for ATA guidelines giving different ages to perform early thyroidectomy in such patients (Wells et al. 2015). MEN2A and MEN2B are also characterized by the occurrence of pheochromocytoma (PHEO), though less frequent than MTC. PHEO is a chromaffin tumor arising from the medullar zone of the adrenals and responsible for mortality if left undiagnosed (Lenders et al. 2005). Precise comparative large-scale epidemiological data on PHEO penetrance in different geographical zones are however missing in the literature as the majority of published studies were coming from a single Center or a single country.

To further describe the natural history of MEN2A PHEO, investigators from South America (Argentina, Chile, Brazil), Southern Europe (Italy, Spain, Portugal, Greece), Western Europe (France, Germany, The Netherlands) and Central Europe (Ukraine, Poland, Slovenia, Czech Republic) were contacted and asked to retrospectively collect demographic, clinical and genetic data about PHEO in MEN2 patients carrying mutations in exons 10 and 11 of RET. The aim was to better characterize the main epidemiological characteristics of MEN2 PHEO in these 4 different geographical areas. Follow-up policy was the same for all Centres: plasma and/or urinary metanephrine measurements were performed in symptomatic patients or in asymptomatic patients every 6–12 months depending on each investigator; imaging was done systematically when metanephrine concentrations increased, and every 3–5 years in patients with normal metanephrine values based upon investigators’ decision.

In accordance with the institutional review board, all patients provided signed written informed consent for genetics and analyses of the results. Exceptions for this second point were the centers in Groningen and Utrecht in the Netherlands, and Lisbon in Portugal, where the participants’ identities were anonymized and protected by unique codes that were known only by two dedicated data managers; therefore, no further institutional review board approval was needed at these centers. The database was established from November 1, 2012 to October 1, 2013. Full characterization of the database was previously reported (Castinetti et al. 2014). Patients included in the database were enrolled at the participating centers from 1968 to 2013. Patients included in the database had to be diagnosed as carriers of germline pathogenic mutations of RET. Additionally, first-degree relatives with histologically proven MTC and PHEO (defined by the association of increased urinary or plasma metanephrines and adrenal tumor) were included.

Continuous variables are presented as median (IQR). Age-dependent penetrance estimates of PHEO were performed with the Kaplan–Meier method: we compared disease-free survival with the log-rank analysis. Statistical comparisons of quantitative data were performed with Student’s t test or ANOVA. For dichotomous data, χ² test was used. All statistical tests were two sided, and P values of less than 0.05 were considered significant. Analyses were done with PRISM 6 software (GraphPad Software, 2014).

The cohort included 812 patients (446 females, 366 males) with MEN2A due to RET mutations in exons 10 and 11. Genetic analysis of the whole cohort showed that 239 patients (29%) carried exon 10 mutations and 573 patients (71%) exon 11 mutations (RET 634 codon). At last follow-up, 462 patients (56.9%) were presenting with at least 1 PHEO, while 350 (43.1%) were safe of...
PHEO (with negative biochemistry) at a median age of 35 years (min, 6; max, 87). Among the 462 patients with at least 1 PHEO, 412 carried mutations in exon 11 (71.9% of the 573 patients with RET 634) while 50 carried mutations in exon 10 (20.9% of 239 patients) ($P < 0.001$ for exon 10 vs exons 11). Median age at first diagnosis was 42 (IQR, 17) and 34 (IQR, 18) years for exons 10 and 11 respectively ($P < 0.001$ for exon 10 vs exons 11, data not shown). At least 1 pheochromocytoma was observed in 50% of patients with exon 11 by age 38 years, while the same penetrance was observed in 50% of patients with exon 10 by age 65 ($P < 0.0001$, data not shown).

At last follow-up, 290 patients (62.8%) had bilateral pheochromocytoma: 210 (72.5%) presented synchronous bilateral pheochromocytoma, while 80 (27.5%) presented metachronous bilateral pheochromocytoma after a median time of 72 months (IQR, 84). Among these 290 patients, 270 carried exon 11 (93.1%) while 20 carried exon 10 (6.9%) ($P < 0.001$). Interestingly, among patients with exon 10 and 11 mutations, there was no significant difference in the median age at first diagnosis of pheochromocytoma whatever the codon or the amino-acid substitution ($P = 0.207$, data not shown).

We subdivided the whole cohort of patients into 4 different geographical areas: South America ($n = 268$), Southern Europe ($n = 178$), Western Europe ($n = 192$) and Central Europe ($n = 174$) to determine the phenotypic characteristics of MEN2 pheochromocytoma in each area. There was a significant difference in the number of patients with pheochromocytoma and exons 10 (codon 620) and 11 (codon 634) mutations as shown in Table 1. At last follow-up (not significantly different between all areas), for exon 11, only 53% of patients had presented at least 1 pheochromocytoma in South America, while they were 75–81% in the other 3 areas ($P = 0.038$). There was a significant difference for unilateral and bilateral pheochromocytoma penetrance between South America and the 3 European areas: 50% of patients presented unilateral pheochromocytoma at 33, 36, 37 and 43 and bilateral pheochromocytoma at 44, 44, 45 and 61 years of age, respectively, in Southern Europe, Central Europe, Western Europe and South America ($P < 0.0001$, $P = 0.0017$ for South America vs other areas) (Fig. 1).

Our data are of importance mainly because PHEO has progressively become the main MEN2A disease: Indeed, early thyroidectomy has modified the outcome of MEN2

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**Table 1** Characteristics of pheochromocytoma based on geographic area.

<table>
<thead>
<tr>
<th></th>
<th>Southern Europe</th>
<th>Central Europe</th>
<th>Western Europe</th>
<th>South America</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>185</td>
<td>174</td>
<td>190</td>
<td>263</td>
<td></td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>93 (52%)</td>
<td>118 (68%)</td>
<td>141 (74%)</td>
<td>110 (41%)</td>
<td>0.038</td>
</tr>
<tr>
<td>Unilateral</td>
<td>35 (38%)</td>
<td>44 (37%)</td>
<td>45 (32%)</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>Bilateral</td>
<td>58 (62%)</td>
<td>74 (63%)</td>
<td>96 (68%)</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>Synchronous</td>
<td>51 (88%)</td>
<td>48 (65%)</td>
<td>70 (73%)</td>
<td>42 (68%)</td>
<td>0.012</td>
</tr>
<tr>
<td>Metachronous</td>
<td>7 (12%)</td>
<td>26 (35%)</td>
<td>26 (27%)</td>
<td>20 (32%)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

| Patients with mutations in | | | |
| Exon 10               | 17/90 (19%)     | 9/35 (25%)     | 15/36 (42%)    | 9/78 (12%)  | 0.021|
| Exon 11               | 76/95 (81%)     | 109/139 (75%)  | 126/154 (81%)  | 101/185 (53%) | 0.033|

| Patients with mutations in codon | | | |
| 609                               | 3/25 (12%)      | 3/10 (30%)     | 3/4 (75%)      | 0/0     | ns   |
| 611                               | 2/2 (100%)      | 1/6 (17%)      | 3/3 (100%)     | 0/6     | ns   |
| 618                               | 3/18 (17%)      | 3/11 (27%)     | 8/27 (30%)     | 4/17 (25%) | ns   |
| 620                               | 9/45 (20%)      | 2/8 (25%)      | 1/2 (50%)      | 5/55 (9%)  | 0.049|
| 634                               | 76/95 (80%)     | 109/139 (78%)  | 126/154 (81%)  | 101/185 (55%) | 0.038|

| Mean age at last follow-up (years) (min–max) | | | |
| 45 (12–90)                           | 42 (7–79)       | 51 (6–95)      | 43 (7–96)     | ns     |

Percent patients with bilateral pheochromocytoma

Figure 1

Bilateral pheochromocytoma penetrance in patients with RET 634 mutation based on geographic area. The table shows the number of subjects at risk initially, and then at 20 and 40 years of age. There was a significant difference in terms of penetrance depending on the geographical area ($P < 0.0001$ for unilateral PHEO, $P = 0.0017$ for bilateral PHEO).
patients in the way that endocrinologists taking care of familial cases identified by early genetic screening will mainly have to deal with PHEO follow-up and management (Waguespack et al. 2011). MEN2 PHEO can be diagnosed in a same way as sporadic PHEO with the main advantage of being usually discovered at an early stage due to the regular follow-up of MEN2 patients. We recently reported that patients with MEN2 PHEO could take benefit of adrenal sparing surgery, a technique aimed at sparing a piece of cortical tissue while taking off the PHEO, allowing for a normal post-surgical cortical function in more than 50% of patients (Castinetti et al. 2014). A proper follow-up of MEN2 patients is thus mandatory to optimize their treatment: as such, endocrinologists need to know perfectly the natural history of MEN2 PHEO, but specific series on pheochromocytoma are lacking (Imai et al. 2013, Thosani et al. 2013).

Our study emphasizes the fact that RET mutation is not the only determinant of the natural history of PHEO in MEN2. Indeed, we report for the first time a different natural history of MEN2 PHEO between Europe and South America. As all the centers had the same policy in terms of PHEO follow-up, this difference cannot be explained by different diagnostic approaches. The difference is highly significant: 50% of Central Europe patients will have a PHEO by age 33 years, while it is only by the age of 43 years that the same percent will be obtained in South America. More importantly from a management viewpoint, a delay of more than 15 years was observed between the age at which patients would present bilateral PHEO (and thus a definite adrenal insufficiency when treated by bilateral adrenalectomy) in Europe vs South America. This could be due to modifier variants in RET or in another gene: Siqueira and coworkers had suggested that RET polymorphisms (L769L, S836S, and G691S/S904S) might modify the natural history of MEN2 PHEO: patients with 2 of these polymorphisms indeed presented a younger age at diagnosis (Siqueira et al. 2014). Protective alleles could also explain this different natural history: protective alleles are rare and potentially ancestry specific, and in our cohort, they could be identified in patients from South America. To further ascertain this hypothesis, large multiethnic studies should be performed. Finally, one further explanation could also be the influence of the environment, but this remains hypothetical for MEN2.

To conclude, the differences reported in this study thus suggest that the natural history of MEN2 PHEO could be influenced by modifying factors (genetic or environmental). Future studies should be aimed at better defining these factors, now that the main epidemiological characteristics of MEN2 PHEO have been well defined.

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