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► **To cite this version:**

Frederic Castinetti, Jeffrey Moley, Lois Mulligan, Steven G Waguespack. A COMPREHENSIVE REVIEW ON MEN2B. *Endocrine-Related Cancer*, 2017. hal-01724177

**HAL Id: hal-01724177**

**<https://amu.hal.science/hal-01724177>**

Submitted on 6 Mar 2018

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1 **A COMPREHENSIVE REVIEW ON MEN2B**

2

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15

16 **Short title:** Multiple endocrine neoplasia type 2B

17 **Keywords:** medullary thyroid cancer, pheochromocytoma, ganglioneuromas, RET, Marfanoid

18 **Word count:** 4651 words

19

20

21 **ABSTRACT**

22

23 MEN2B is a very rare autosomal dominant hereditary tumor syndrome associated with  
24 medullary thyroid carcinoma (MTC) in 100% cases, pheochromocytoma in 50% cases, and  
25 multiple extra-endocrine features, many of which can be quite disabling. Only few data are  
26 available in the literature. The aim of this review is to try to give further insights into the  
27 natural history of the disease and to point out the missing evidence that would help  
28 clinicians optimize the management of such patients. MEN2B is mainly characterized by the  
29 early occurrence of MTC, which led the American Thyroid Association to recommend  
30 preventive thyroidectomy before the age of 1. However, as the majority of mutations are *de*  
31 *novo*, improved knowledge of the non-endocrine signs would help to lower the age of  
32 diagnosis and improve long-term outcomes. Future large scale studies will be aimed at  
33 characterizing more in detail the main characteristics and outcomes of MEN2B.

34

35 **1. INTRODUCTION**

36

37 While the first clinical association between pheochromocytoma and medullary thyroid  
38 carcinoma (MTC) was described in 1961 by Sipple *et al.* (Sipple 1961), it was only 5 years  
39 later that the first description of oral mucosal neuromas in 2 patients with MTC and  
40 pheochromocytoma was reported in the English literature (Williams and Pollock 1966). The  
41 term multiple endocrine neoplasia type 2 was proposed by Steiner *et al.* in 1968 (Steiner, et  
42 al. 1968) and the rare association of hereditary MTC with a mucosal neuroma phenotype  
43 was eventually named multiple endocrine neoplasia type 2B (MEN2B) by Chong *et al.* in  
44 1975 (Chong, et al. 1975). The phenotypic specificities of MEN2B compared with MEN2A  
45 include prominent extra-endocrine features, a more aggressive presentation of MTC, and  
46 the lack of primary hyperparathyroidism. Because of its very low point prevalence (0.9-1.65  
47 per million) and incidence (1.4-2.6 per million live births per year) (Machens, et al. 2013;  
48 Mathiesen, et al. 2017b; Znaczo, et al. 2014), MEN2B (OMIM #162300) remains poorly  
49 described in the literature. As a consequence, the information provided for MEN2B is  
50 derived from large studies that primarily address MEN2A or from large, poorly described  
51 genetic studies with little clinical detail. Three large genetic registries of patients with *RET*  
52 mutations have identified MEN2B in 21/141 (15%, Germany), 20/246 (8.1%, Italy), and 4/145  
53 (2.8%, EUROMEN) patients (Frank-Raue, et al. 1996; Machens, et al. 2003; Romei, et al.  
54 2010), emphasizing the difficulty in collecting robust data about the outcome of patients  
55 with MEN2B. Up to now, the largest published descriptive study of MEN2B was based on  
56 only 44 patients (Brauckhoff, et al. 2014). The aim of this review is to give detailed insights

57 on the genetics, natural history and management of MEN2B, in addition to specific points  
58 that should be detailed in future large-scale studies.

59

## 60 **2. GENETICS OF MEN2B**

61

62 MEN2B, similar to MEN2A, is due to autosomal dominant, activating germline mutations of  
63 the *RET* proto-oncogene.(Carlson, et al. 1994b; Hofstra, et al. 1994). The *RET* proto-  
64 oncogene has 21 exons and encodes a tyrosine-kinase receptor expressed in thyroid  
65 parafollicular C cells. Hyperactivation of the receptor leads to the induction of downstream  
66 signals responsible for oncogenesis (Mise, et al. 2006). While it has long been held that the  
67 parafollicular C cells are of neural crest origin, a recent lineage tracing study suggested that  
68 anterior endoderm, and not the neural crest, is the only source of differentiated C cells in  
69 mice (Johansson, et al. 2015).

70 MEN2B is primarily due to a methionine to threonine substitution at codon 918 in the  
71 tyrosine-kinase domain of RET (Carlson et al. 1994b; Hofstra et al. 1994). Over 90% of  
72 patients present with *de novo* mutations (Brauckhoff et al. 2014), which might be due to a  
73 reduced fertility of the patients carrying the MEN2B phenotype. *De novo* mutations have  
74 also been found to be of paternal origin and associated with advanced paternal age,  
75 suggesting a differential susceptibility of *RET* to mutation in paternally and maternally  
76 derived DNA (Carlson, et al. 1994a; Choi, et al. 2012). Other mutations involving codon 883  
77 (*A883F*) or rare double heterozygotes involving mutations of codon 804 in combination with  
78 other *RET* mutations have also been reported in patients with a MEN2B phenotype. The  
79 increased aggressiveness of *M918T* compared with other *RET* mutations may in part be due  
80 to the location of amino-acid 918 within the catalytic core of the RET kinase, where it leads

81 to increases in ATP binding and the enzymatic activity of the kinase and the ability to be  
82 activated without receptor dimerization (Gujral, et al. 2006; Plaza-Menacho, et al. 2014). *In*  
83 *vitro* studies indeed suggest that the M918T-mutated receptor can be activated and  
84 autophosphorylated in the absence of any ligand. Finally, the M918T-mutated receptor can  
85 be further activated by its endogenous ligand, even if it is already hyperactivated  
86 spontaneously, and this might explain the more aggressive clinical presentation, or at least  
87 the very early age of MTC diagnosis, in patients with the *M918T* mutation (Gujral et al.  
88 2006).

89 Notably, the precise mechanisms leading to MTC remain imperfectly understood. While it is  
90 obvious that activating *RET* mutations lead to C-cell hyperplasia, the precise mechanisms  
91 leading to MTC tumorigenesis remain to be determined. As *RET* is a proto-oncogene, a single  
92 germline mutation should be enough to lead to MTC. However, the phenotypic variability of  
93 MEN2B patients could be explained by the occurrence of associated somatic mutations that  
94 might accelerate the oncogenic process and modify the aggressiveness of MTC (Eng 1996;  
95 Mulligan 2014) or differentially regulate targeted genes (Maliszewska, et al. 2013). Recently,  
96 a whole gene-expression profile of MEN2A and MEN2B MTC samples identified 3 genes  
97 differently deregulated, which might explain the different phenotypes: *NNAT* (tumor  
98 suppressor gene), *CD14B* (cell cycle control) and *NTRK3* (tyrosine receptor kinase) (Oczko-  
99 Wojciechowska, et al. 2017).

100 Finally, the underlying *RET* mutation also determines the extra-thyroidal features of MEN2,  
101 likely imparting a different susceptibility of adrenal or parathyroid cells to a given mutant  
102 receptor; this might explain the lack of primary hyperparathyroidism in MEN2B or, for  
103 example, the difference in the prevalence of pheochromocytoma in *M918T* patients  
104 compared with patients who harbor exon 10 germline *RET* mutations (Frank-Raue, et al.

105 2011; Gujral and Mulligan 2006). Of note, the *M918T RET* mutation has also been identified  
106 somatically in a high percentage of sporadic MTC (Eng and Mulligan 1997; Marsh, et al.  
107 1996). The fact that MEN2A mutations are uncommon in sporadic MTC might suggest that  
108 they have their effects primarily in specific developmental windows, while the *M918T*  
109 mutation would be a stronger oncogenic driver with similar effects whether occurring in the  
110 germline or somatically.

111

### 112 **3. PHENOTYPE OF PATIENTS WITH A GERMLINE *M918T RET* MUTATION**

113

114 The *M918T RET* mutation is the most frequent etiology (>95%) of MEN2B (Hansford and  
115 Mulligan 2000). Patients with MEN2B usually present with very early-onset MTC, a 50%  
116 lifetime risk of pheochromocytoma and universal extra-endocrine features, mainly bowel  
117 problems due to diffuse intestinal ganglioneuromatosis (constipation, feeding difficulties in  
118 infancy, megacolon) and alacrima, both of which can be the earliest presenting features, in  
119 addition to mucosal neuromas and a marfanoid body habitus (note that both may not be  
120 become clinically apparent until several years of age) (Brauckhoff, et al. 2008; Cohen, et al.  
121 2002; Eng, et al. 1996; O'Riordain, et al. 1994) (Table 1).

122

#### 123 **a. Medullary Thyroid Carcinoma in *M918T*-mutated MEN2B**

##### 124 Epidemiology, diagnosis and prognosis

125 MTC is a malignant neuroendocrine tumor arising from the parafollicular C-cells of the  
126 thyroid gland. It develops within the first years of life in 100% of MEN2B patients and  
127 remains the leading cause of death in MEN2B. It is usually the first disease to be diagnosed,  
128 and the average age of diagnosis of MTC is in the second decade of life, about 10 years

129 earlier than that seen in individuals with MEN2A (Brandi, et al. 2001; O'Riordain et al. 1994).  
130 The size of the tumor at diagnosis is not significantly different between MEN2A and MEN2B  
131 (Thosani, et al. 2013). Compared with sporadic MTC, MEN2B-associated MTC is usually  
132 multifocal, bilateral, and accompanied by C-cell hyperplasia, the initial step of tumorigenesis  
133 in a well-defined oncologic cascade similar to MEN2A (Figure 1) (Metz and Asa 2013;  
134 Waguespack, et al. 2011). MTC has been reported as early as the first few months of life and  
135 lymph node metastases have been identified within the first year (Zenaty, et al. 2009).  
136 Among MEN2 patients, those with MEN2B have a worse prognosis, with a 10-year survival of  
137 75.5% compared with 97.4% in MEN2A (Modigliani, et al. 1998). Some analyses have  
138 suggested that the more aggressive MEN2B phenotype is due to the stage at diagnosis (more  
139 advanced disease due to its earlier onset), rather than an intrinsically more aggressive tumor  
140 due to the *M918T* mutation. This hypothesis may also hold true for MEN2A, given a recent  
141 study showing that high-risk *RET* exon 11 and moderate-risk exon 10 mutations had similar  
142 overall survival and time to development of distant metastatic disease after the diagnosis of  
143 MTC (Voss, et al. 2017). In the series by Leboulleux *et al.*, based on 18 patients with MEN2B,  
144 the overall outcome of MTC did not appear more aggressive than for sporadic or other  
145 heritable MTC (Leboulleux, et al. 2002). The majority of patients presented with stage III  
146 MTC at diagnosis, and only three had an undetectable plasma calcitonin level after surgery,  
147 including one who had a further increase of calcitonin 8 years after surgery. The probability  
148 of surgical cure is undoubtedly lower in MEN2B than in MEN2A, but it is likely due to an  
149 earlier age of MTC appearance and a delayed clinical diagnosis (Wray, et al. 2008). Of note,  
150 5- and 10-year overall survival rates were 85 and 75%, respectively (Leboulleux et al. 2002).  
151 The largest series ever reported on MEN2B was based on 44 patients (including three with  
152 inherited mutations, and 41 with *de novo* mutations). The three patients with an inherited



153 *M918T* mutation were operated on before 1 year of age and were biochemically cured. In  
154 the remaining 41 cases, patients were cured only when the diagnosis was made before 4  
155 years of age. To avoid the risk of incurable MTC, the current American Thyroid Association  
156 (ATA) guidelines recommend early thyroidectomy before the age of 1 in children carrying  
157 the *M918T* mutation (Wells, et al. 2015). However, except for familial cases, this is difficult  
158 to achieve in current practice, as the vast majority of patients with MEN2B carry *de novo*  
159 mutations, leading to delayed diagnosis and making early intervention difficult. Improving  
160 the outcome of MEN2B is thus mainly based on appropriate education of pediatricians and  
161 other health care professionals to recognize the early, nonendocrine manifestations of  
162 disease (Brauckhoff et al. 2008; O'Riordain, et al. 1995).

### 163 Management

164 Surgery is the first line treatment of MTC in the majority of patients with MEN2, as it can  
165 modify the clinical course and long-term prognosis of the tumor (Brandi et al. 2001). The  
166 surgical treatment of clinical disease in hereditary MTC is the same as for sporadic MTC  
167 (Wells et al. 2015). However, one of the main issues with MEN2B-related MTC is the very  
168 early age of appearance of the MTC precursor lesion, C Cell hyperplasia (Sanso, et al. 2002).  
169 Indeed, Zenaty *et al.* reported that bilateral millimetric MTC associated with unilateral lymph  
170 node micrometastases were already present in some of the patients with MEN2B operated  
171 before the age of 1 (Zenaty et al. 2009).

172 Prophylactic thyroidectomy (i.e. thyroidectomy in presymptomatic carriers) before the  
173 appearance of MTC was first advised in patients with MEN2 more than 20 years ago (Wells,  
174 et al. 1994) and then confirmed to be a viable approach 10 years later (Skinner, et al. 2005).

175 Less data is available regarding MEN2B patients in this setting, although it appears that long-  
176 term cure can also be achieved with prophylactic thyroidectomy in this group (Waguespack,

177 et al. 2011). In any case, the oncologic goal of early surgery is not so much to prevent  
178 malignancy from occurring in the first place but to remove the thyroid before MTC  
179 metastasis occurs, and recent data would suggest that this goal may be achieved in a  
180 majority of MEN2B patients treated before age 4 years (Brauckhoff et al. 2014). The reality  
181 remains, however, that very few children with MEN2B will actually have a true prophylactic  
182 thyroidectomy.

183 As previously mentioned, in MEN2B, the ATA recommends thyroidectomy before 1 year of  
184 age (Wells et al. 2015). At this age, surgical complications can be high (Kluijfhout, et al.  
185 2015), underscoring the need for these surgeries to be performed only by experienced  
186 thyroid surgeons in tertiary care centers where multidisciplinary expertise exists. The extent  
187 of surgery should be based on the clinical data available, including the presence of thyroid  
188 nodules/abnormal lymph nodes on ultrasonographic examination and the basal calcitonin  
189 level, recognizing that calcitonin levels are higher in children less than age three (Basuyau, et  
190 al. 2004). In the prophylactic setting and in the absence of suspicious lymph nodes, the  
191 performance of a central neck dissection should be determined by the treating surgeon  
192 (Wells et al. 2015). This decision depends on whether the parathyroid glands can be  
193 identified and left *in situ* or autotransplanted, recognizing that the parathyroid glands are  
194 very difficult to localize in infants (Moley, et al. 2015). A central neck dissection is  
195 recommended for overt clinical disease, and lateral cervical lymph node dissection can also  
196 be considered in children with MEN2B and clinically apparent disease or significantly  
197 elevated calcitonin levels (Jin and Moley 2016; Waguespack et al. 2011; Wells et al. 2015).  
198 Specific complications of thyroidectomy in very young MEN2 patients remain difficult to  
199 determine as few studies have evaluated this specific issue, and previous studies generally  
200 included older patients (primarily MEN2A patients in whom thyroidectomy should be

201 performed around 5 years of age). We can thus only extrapolate the potential consequences  
202 of surgery for MEN2B at a very young age. In 50 patients with MEN2A aged less than 19 at  
203 the time of surgery, 3 patients presented with permanent hypocalcemia after surgery  
204 (Skinner et al. 2005). In another series of 44 children aged 17 years or younger, including  
205 three with MEN2B, 4 of 11 patients younger than 3 (44%) suffered from transient  
206 hypocalcemia, while 2 had permanent hypocalcemia. This rate was significantly higher than  
207 that observed for older patients (Kluijfhout et al. 2015). Finally, in a recent series dealing  
208 with a large number of preventive thyroidectomies, permanent hypoparathyroidism  
209 occurred in only 1 of 102 children (<1%) operated on by an experienced surgeon with the  
210 intent to preserve the parathyroid glands *in situ* with an intact vascular pedicle (Moley et al.  
211 2015).

212 Post-surgical follow-up should incorporate calcitonin and carcinoembryonic antigen (CEA)  
213 levels, cervical ultrasound (if calcitonin positive), and further imaging dictated by the level  
214 and trend of calcitonin and CEA (Taieb, et al. 2014; Wells et al. 2015). In the majority of  
215 cases, residual disease will primarily be in cervical lymph nodes, but other sites of metastatic  
216 disease include intrathoracic lymph nodes, lungs, liver, bones, and rarely the brain. While  
217 the specific treatment of advanced, metastatic MTC in the context of MEN2B is beyond the  
218 scope of this review, it is however necessary to discuss the two commercially available,  
219 molecular targeted small molecule kinase inhibitors approved for the treatment of MTC:  
220 vandetanib and cabozantinib. In adults, Wells *et al.* reported in 2012 (Wells, et al. 2012) the  
221 results of a randomized, double blind placebo-controlled phase III study on Vandetanib in  
222 331 patients with metastatic MTC. The median progression free survival was significantly  
223 different in the vandetanib vs placebo groups (30.5 [predicted] vs 19.3 months, respectively;  
224  $p < 0.001$ ). Specifically, in the subgroup of patients with nonhereditary MTC and a somatic

225 *M918T* mutation (n=101), an objective response rate of 54.5% was observed. Fox and  
226 coworkers also reported in 2013 the first phase I/II clinical trial of vandetanib in 16 children  
227 and adolescents with metastatic MTC, 15 of whom had MEN2B and the *M918T* mutation. All  
228 15 patients had a decrease in tumor size, and 7 had a confirmed partial response, including  
229 two who had ultimate progression of disease after 44 and 48 cycles of vandetanib. Clinical  
230 response was also documented by a mean decline of 59% (35-84) in calcitonin levels (Fox, et  
231 al. 2013). Cabozantinib was studied in a randomized, double blind placebo-controlled phase  
232 III study (Elisei, et al. 2013) of 330 patients, which showed a significant improvement of  
233 median progression-free survival (11.2 vs 4.0 months in the cabozantinib and placebo  
234 groups, respectively). Sherman *et al.* subsequently reported, in a subgroup analysis of the  
235 cabozantinib phase III trial, that patients with a germline or somatic *M918T* mutation had  
236 the greatest progression free survival benefit vs placebo, in comparison with patients with  
237 no identified *RET* mutation (Sherman, et al. 2016).

238

239 In summary, MTC is the major component of MEN2B. Presumably more aggressive, it  
240 appears much earlier than in any other form of hereditary MTC. ATA guidelines suggest  
241 thyroidectomy before age 1, a surgical procedure that should be performed only by highly-  
242 experienced thyroid surgeons. As the majority of the patients present with *de novo*  
243 mutations and hence have a delayed clinical diagnosis, thyroid surgery is rarely curative. For  
244 patients presenting with symptomatic or progressive MTC metastases, targeted therapy  
245 using the commercially available tyrosine kinase inhibitors has provided hope for better  
246 long-term outcomes.

247

248 **b. Pheochromocytoma in *M918T*-mutated MEN2B**

249 Limited data are available on the outcomes of pheochromocytoma in MEN2B. This explains  
250 why some physicians may fear the possibility of a more aggressive pheochromocytoma  
251 clinical outcome in MEN2B compared with MEN2A, as if the outcome was comparable to  
252 that of MTC. In MEN2B, the youngest age reported for pheochromocytoma was 12 years old  
253 (Nguyen, et al. 2001), and the current ATA guidelines suggest starting routine  
254 pheochromocytoma screening (plasma or 24-hour urine fractionated metanephrines) at age  
255 11 years (Wells et al. 2015). There have also been rare cases of adrenal ganglioneuroma  
256 identified in MEN2B, a tumor that can be mistaken for pheochromocytoma (Lora, et al.  
257 2005). The largest dedicated study on MEN2B pheochromocytoma was based on 15  
258 patients. The median age at pheochromocytoma diagnosis was 25 years (18-40) compared  
259 to 34 years (17-60) in the 70 patients with MEN2A ( $p<0.05$ ) (Thosani et al. 2013). At  
260 diagnosis, the median size of pheochromocytoma in the MEN2B patients was smaller than  
261 for patients with MEN2A (25 vs 38 mm) ( $p<0.01$ ), but this could be due to stricter  
262 surveillance in patients with MEN2B, thus leading to an earlier diagnosis in asymptomatic  
263 patients. After a mean follow-up of 57 months, none of the patients presented with delayed  
264 metastasis.

265

### 266 **c. Extra-endocrine features of M918T-mutated MEN2B**

267 The penetrance of the extra-endocrine features in MEN2B may be incomplete for a given  
268 patient, but all MEN2B patients will have one or more of these nonendocrine manifestations  
269 (Brauckhoff et al. 2014; O'Riordain et al. 1995). The association of several of these clinical  
270 signs in a patient should prompt the astute clinician to measure a calcitonin level and pursue  
271 genetic testing for MEN2B. Early recognition of these signs should decrease the age at which  
272 thyroidectomy will be performed and theoretically lead to better long-term outcomes.

### 273 Marfanoid habitus and other skeletal features

274 The skeletal phenotype (Figure 2) usually includes a variable expression of a marfanoid body  
275 habitus characterized by taller stature, long limbs, a thin elongated face and arachnodactyly  
276 of the fingers and toes. Other skeletal abnormalities such as lordosis, kyphosis, scoliosis,  
277 joint hypermobility, pes cavus, pectus excavatum (linked to overgrowth of the ribs), high-  
278 arched palate, and slipped capital femoral epiphysis (SCFE) can also be associated.

### 279 Mucosal neuromas

280 Mucosal neuromas (Figure 2) may be evident in many MEN2B cases at birth. Although their  
281 appearance may be delayed until an older age (Brauckhoff et al. 2008), they occur in the  
282 majority of cases within the first decade (Gorlin, et al. 1968). Mucosal neuromas are  
283 described as multiple, small soft papules in or around the oral cavity, including the tip of the  
284 tongue and lips, the nasal and laryngeal mucosae, and the conjunctivae. The lesions appear  
285 as multiple 2–7 mm yellow to white sessile painless nodules. When in enough numbers,  
286 labial lesions give a “blubbery” appearance.

### 287 Ophthalmological signs

288 In the series reported by Brauckhoff et al. (Brauckhoff et al. 2014), ocular manifestations  
289 (Figure 2) were present in all patients with detailed clinical information, the most frequent  
290 sign being alacrima (“tearless crying”). Mild ptosis, eversion of upper eyelids, conjunctival  
291 neuromas and prominent corneal nerves are also a component of the MEN2B phenotype  
292 (Parker, et al. 2004).

### 293 Gastrointestinal signs

294 Over 40 years ago, Carney *et al.* first focused on the gastrointestinal signs associated with  
295 MEN2B (Carney, et al. 1976). Out of 16 patients with likely MEN2B, symptomatic  
296 gastrointestinal signs were present in 10 cases at birth or shortly thereafter, while a total of

297 12 patients complained of constipation and/or diarrhea during follow-up. Gastrointestinal  
298 signs, especially constipation, usually represent the first nonspecific manifestation of  
299 MEN2B; feeding intolerance can be observed in infancy. The gastrointestinal issues are due  
300 to diffuse intestinal ganglioneuromatosis and impaired colonic motility that leads to an  
301 intestinal pseudo-obstruction and the ultimate development of megacolon. Indeed, in  
302 contrast with Hirschsprung disease, in which a part of the bowel (typically the rectosigmoid  
303 colon) is aganglionic, megacolon is due to enteric and extrinsic nerve hyperplasia and  
304 ganglioneuromas of the submucosal and myenteric plexuses that lead to distension of the  
305 colon by loss of normal bowel tone (Gibbons, et al. 2016). Rectal biopsy can lead to the  
306 diagnosis of intestinal ganglioneuromatosis (Gfroerer, et al. 2016). Of note, increased  
307 secretion of catecholamines (such as would be seen in MEN2B-associated  
308 pheochromocytoma) can worsen constipation and rarely lead to toxic megacolon (Thosani,  
309 et al. 2015). MEN2B patients can also present with upper GI symptomatology and  
310 esophageal manifestations (Cohen et al. 2002; Gibbons et al. 2016). In a previously reported  
311 study of 28 MEN2B patients, 39% had difficulty swallowing and 14% had vomiting,  
312 suggesting a diagnosis of esophageal abnormalities. Medical management (dietary  
313 adjustments, laxatives, fiber supplements) of MEN2B patients can be difficult and some  
314 patients (about a third) will ultimately require hospital admission and/or surgery (Cohen et  
315 al. 2002; Gibbons et al. 2016). Finally, intestinal manifestations may also be predictive of  
316 MTC aggressiveness, as suggested by some authors showing that a worse MTC prognosis  
317 was associated with more severe gastrointestinal signs (Brauckhoff, et al. 2004).

318 Other signs: Other associations have also been reported in MEN2B, including coarse facies,  
319 tooth malposition, abnormal feet with a long first toe, and an increased space between the  
320 1<sup>st</sup> and the 2<sup>nd</sup> toe (Martucciello, et al. 2012). Underweight, chronic pain and asthenia, and

321 delayed puberty can also be seen.

322

#### 323 **4. THE SPECIFIC FEATURES OF PATIENTS CARRYING THE *M918V RET* MUTATION**

324 Recently, the phenotype of 50 Brazilian patients from eight kindreds carrying the rare  
325 *M918V RET* mutation was reported (Martins-Costa, et al. 2016). None of the patients  
326 presented with extra-endocrine features characteristics of MEN2B. Age at diagnosis of MTC  
327 varied from 24 to 59 years, with incomplete penetrance identified. While only two patients  
328 presented with distant metastases at last follow-up, the majority of operated patients  
329 (12/20) had lymph node metastases at the time of surgery. None of the patients presented  
330 with pheochromocytoma or hyperparathyroidism. Together, these data suggest that this  
331 variant is not responsible for a true MEN2B phenotype. Thus, the authors recommended  
332 classifying this variant as a moderate risk in the current ATA classification.

333

#### 334 **5. THE SPECIFIC FEATURES OF PATIENTS CARRYING THE *A883F RET* MUTATION**

335 The codon 883 alanine to phenylalanine (*A883F RET*) mutation is responsible for less than  
336 5% cases of MEN2B. International guidelines on MTC management have recently reclassified  
337 the *A883F RET* mutation as a high risk variant, recommending early thyroidectomy by 5  
338 years of age. Until recently, however, only isolated case reports had been published with  
339 patients carrying this rare mutation. Mathiesen and coworkers recently reported the  
340 outcome of 13 unique *A883F* carriers from 8 different families (Mathiesen, et al. 2017a).  
341 Three patients with C-cell hyperplasia and one with normal thyroid pathology who were  
342 operated on at a median age of 7.5 years were considered cured at last follow-up. Only 4/11  
343 evaluable patients (two patients without original pathology available) had lymph node  
344 metastases at the time of initial surgery (Median age, 20.5 years; range 10-39 years). The



345 earliest age at distant metastasis was 20 years. Ten-year survival of the nine patients with  
346 MTC (median follow-up, 12 years) was 88%. At last follow-up, 38% of the patients had  
347 presented with pheochromocytoma (median age at diagnosis, 34 years). Other extra-  
348 endocrine features of MEN2B were inconsistently observed. Mucosal neuromas were  
349 present in 11 patients and marfanoid body habitus in 6. These data thus suggest that the  
350 clinical phenotype and MTC aggressiveness of the *RET A883F* mutation is not as severe as  
351 the classical *M918T* mutation. Rather, the onset and disease course of MTC can be highly  
352 variable, and very early thyroidectomy (< 1 year old) is not necessary.

353

#### 354 **6. THE SPECIFIC FEATURES OF PATIENTS CARRYING TANDEM *RET* MUTATIONS**

355 Four reports have described MEN2B phenotypes in patients without a mutation in *RET*  
356 codon 883 or 918. Instead, the patients presented with compound *RET* mutations including a  
357 common mutation in codon 804 in combination with a second substitution mutation in  
358 codon 781, 806, 904 or 905 (Cranston, et al. 2006; Menko, et al. 2002; Miyauchi, et al. 1999;  
359 Nakao, et al. 2013). In all these cases, the patient presented with MTC, extra-endocrine  
360 features suggesting MEN2B, but no pheochromocytoma. These cases are rare and clearly  
361 much less severe than classical MEN2B, but the possibility of a double *RET* mutation should  
362 be kept in mind in patients with a MEN2B phenotype but without a classical *RET* 883 or 918  
363 mutation.

364

#### 365 **7. PERSPECTIVES AND CONCLUSIONS**

366 In contrast with MEN2A, the overall outcome of MEN2B remains relatively poor because  
367 MTC, the disease that primarily determines overall prognosis, is not readily modified by early  
368 thyroidectomy, given the typical delay in clinical diagnosis (due to the high rate of *de novo*

369 disease) and the early development of metastatic disease in MEN2B-associated MTC.  
370 Nevertheless, total thyroidectomy and clinically-appropriate compartment-oriented neck  
371 dissection should still be considered for most patients with MEN2B, whatever the age at  
372 diagnosis. The one exception might be the patient with an extensive distant metastatic  
373 burden (in whom there is no immediate concern about symptomatic progression in the  
374 neck) who might benefit from upfront systemic therapy. The difficulty in properly evaluating  
375 the clinical features and outcomes of MEN2B relates to the low number of patients  
376 reported. This explains why, despite its first description over 50 years ago, the natural  
377 history of MEN2B remains imprecise. In particular, the outcome of patients undergoing  
378 surgery at different ages seems highly variable.

379 Our understanding of the clinical spectrum, the underlying genetic causes and the optimal  
380 management of patients MEN2B has progressed, but there is still much that needs to be  
381 clarified. Future retrospective studies will require a large-scale, international network of  
382 specialized centers, given the rarity of MEN2B, and should be aimed at:

- 383 • Educating pediatricians and other primary care providers: improving their knowledge  
384 of the extra-endocrine features of MEN2B to allow earlier patient identification and  
385 management. As such, better description of the non-endocrine features, specifically  
386 their prevalence and clinical presentation, will be of major help. Though MEN2B is a  
387 rare disease, each provider should be aware of the red flags (marfanoid habitus and  
388 other skeletal features, mucosal neuromas, constipation, alacrima) that will lead to  
389 the clinical and genetic diagnosis of MEN2B.
- 390 • Improving understanding of the outcomes of MTC in MEN2B, defining the predictors  
391 of a more aggressive clinical course, and determining how patient outcomes differ  
392 depending on the age at initial surgery. While MEN2B has always been considered a

393 fatal disease, better knowledge of the natural history of MTC may inform the  
394 development of new treatments and provide better understanding as to the timing  
395 of therapy. Interestingly, some patients with MEN2B, despite diagnosis at a later age,  
396 can present with stable disease for many years whereas others who were identified  
397 and operated on at a younger age will develop progressive metastatic disease on  
398 long-term follow-up. The major advances introduced by tyrosine kinase inhibitors will  
399 be more fully exploited when the outcome of operated MEN2B MTC is better known.

400 • Improving understanding of the outcome of MEN2B pheochromocytoma. The  
401 hypothesis that the aggressiveness of MTC could be extrapolated to the  
402 aggressiveness of pheochromocytoma has been raised. The natural history of  
403 pheochromocytoma in MEN2B and the optimal surgical approach should be major  
404 goals of future studies.

405

406 Finally, future prospective studies should be aimed at understanding the reasons for the  
407 wide phenotypic variability observed in patients with MEN2, especially MEN2B.  
408 Transcriptomic studies have begun to suggest reasons why the phenotype might be different  
409 depending on the mutation. MEN2 is also characterized by an intra-familial variability that  
410 may be explained by modifying genes or polymorphisms. A complete dataset of these  
411 modifiers will help tailor the treatment and the follow-up of such patients. In conclusion,  
412 despite the rather old age of MEN2B, there are still lots of things to explore before we  
413 achieve a more complete understanding of the pathophysiology of this rare disease.

414

415 Disclosure: The authors have nothing to disclose.

416 Funding: This research did not receive any specific grant from any funding agency in the  
417 public, commercial or not-for-profit sector.

418

419

420

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**TABLE 1:** Multiple Endocrine Neoplasia Type 2A and type 2B phenotypic characteristics and lifetime risk of development.

	<b>Risk of Medullary thyroid carcinoma</b>	<b>Pheochromocytoma</b>	<b>Primary Hyperparathyroidism</b>	<b>Other extra-endocrine signs</b>
<b>MEN2A</b>	90-100%	0-50% (risk depends on genotype)	0-20% (risk depends on genotype)	<5% (Hirschprung disease, Cutaneous lichen amyloidosis)
<b>MEN2B (M918T, A883F, Tandem mutations)</b>	100%	50% (risk depends on genotype)	0%	100% Gastrointestinal Ophthalmological Skeletal Manifestations Mucosal neuromas

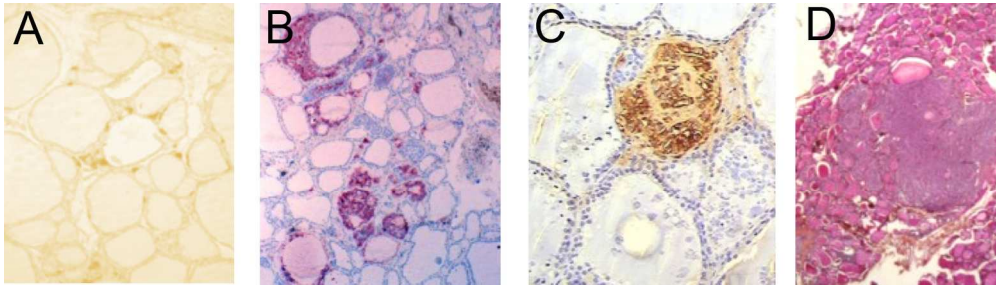
For further details on the clinical manifestations of MEN2B, please refer to the text.

## LEGEND TO FIGURES

**Figure 1:** The development of Medullary thyroid carcinoma (MTC). From normal C-cells (A), the tumor follows an asymptomatic hyperplastic state (B) before the development of microMTC (C, size  $\leq 1$  cm) and then macroMTC (D, size  $> 1$  cm).

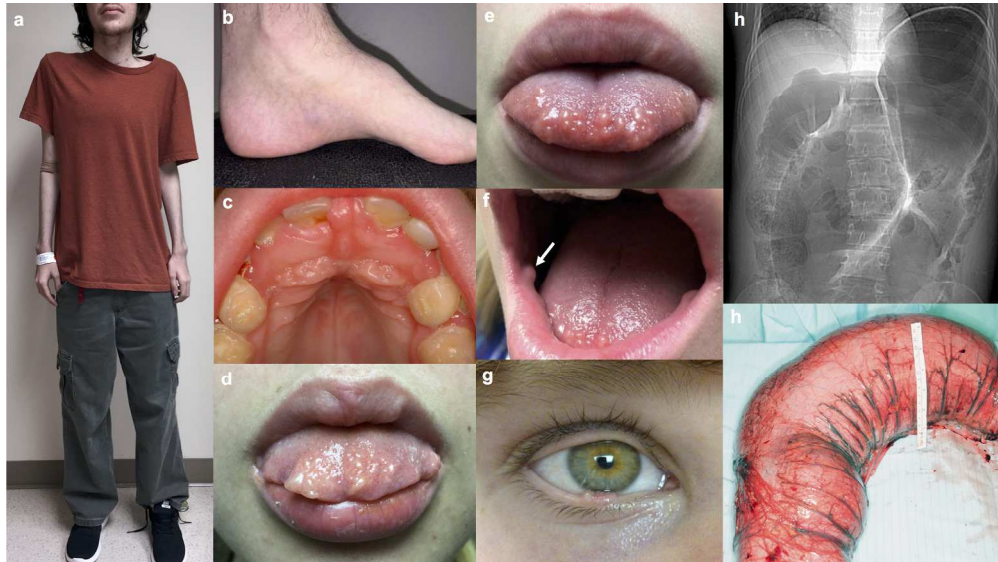
**Figure 2:** The pathognomonic clinical phenotype of MEN2B: 1) a marfanoid body habitus (a) and other skeletal features including scoliosis (a), pes cavus (b), and high-arched palate (c); 2) thickened lips and neuromas affecting the tongue (d and e), the oral mucosa, (f) the conjunctiva (g), and other mucosal surfaces; 3) ophthalmological signs including ptosis and everted upper eyelids (g); and 4) gastrointestinal problems primarily related to impaired colonic motility due to diffuse intestinal ganglioneuromatosis that can lead to megacolon (h). For additional clinical manifestations of MEN2B, please refer to the text.





The development of Medullary thyroid carcinoma (MTC). From normal C-cells (A), the tumor follows an asymptomatic hyperplastic state (B) before the development of microMTC (C, size  $\leq 1$  cm) and then macroMTC (D, size  $> 1$  cm).

187x52mm (300 x 300 DPI)



The pathognomonic clinical phenotype of MEN2B: 1) a marfanoid body habitus (a) and other skeletal features including scoliosis (a), pes cavus (b), and high-arched palate (c); 2) thickened lips and neuromas affecting the tongue (d and e), the oral mucosa, (f) the conjunctiva (g), and other mucosal surfaces; 3) ophthalmological signs including ptosis and everted upper eyelids (g); and 4) gastrointestinal problems primarily related to impaired colonic motility due to diffuse intestinal ganglioneuromatosis that can lead to megacolon (h). For additional clinical manifestations of MEN2B, please refer to the text.

279x156mm (300 x 300 DPI)