Risk Profile of the *RET* A883F Germline Mutation: An International Collaborative Study

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Context: The A883F germline mutation of the rearranged during transfection (*RET*) proto-oncogene causes multiple endocrine neoplasia 2B. In the revised American Thyroid Association (ATA) guidelines for the management of medullary thyroid carcinoma (MTC), the A883F mutation has been reclassified from the highest to the high-risk level, although no well-defined risk profile for this mutation exists.

Objective: To create a risk profile for the A883F mutation for appropriate classification among the ATA risk levels.

Design: Retrospective analysis.

Setting: International collaboration.

Patients: Included were 13 A883F carriers.

Intervention: The intervention was thyroidectomy.

Main Outcome Measures: Earliest age of MTC, regional lymph node metastases, distant metastases, age-related penetrance of MTC and pheochromocytoma (PHEO), overall and disease-specific survival, and biochemical cure rate.

Results: One and three carriers were diagnosed at age 7 to 9 years (median, 7.5 years) with a normal thyroid and C-cell hyperplasia, respectively. Nine carriers were diagnosed with MTC at age 10 to 39

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Abbreviations: ATA, American Thyroid Association; MEN2, multiple endocrine neoplasia 2; MTC, medullary thyroid carcinoma; PHEO, pheochromocytoma; TNM, tumor-node-metastasis; WorldMEN, International Workshop on Multiple Endocrine Neoplasia.

years (median, 19 years). The earliest age of MTC, regional lymph node metastasis, and distant metastasis was 10, 20, and 20 years, respectively. Fifty percent penetrance of MTC and PHEO was achieved by age 19 and 34 years, respectively. Five- and 10-year survival rates (both overall and disease specific) were 88% and 88%, respectively. Biochemical cure for MTC at latest follow-up was achieved in 63% (five of eight carriers) with pertinent data.

Conclusions: MTC of A883F carriers seems to have a more indolent natural course compared with that of M918T carriers. Our results support the classification of the A883F mutation in the ATA high-risk level. **(*J Clin Endocrinol Metab* 102: 2069–2074, 2017)**

Multiple endocrine neoplasia 2 (MEN2) is an autosomal-dominant, inherited cancer syndrome that is subdivided into MEN2A and MEN2B. MEN2A associates medullary thyroid carcinoma (MTC), pheochromocytoma (PHEO), hyperparathyroidism, cutaneous lichen amyloidosis, and Hirschsprung disease. MEN2B associates MTC, PHEO, ganglioneuromatosis of the aerodigestive tract, and facial, ophthalmologic, and skeletal abnormalities (1).

In 1993 and 1994, activating missense germline mutations of the rearranged during transfection (*RET*) proto-oncogene were discovered to cause MEN2A and MEN2B (2–6). MEN2A is most commonly caused by mutations of codon 634 (7), whereas MEN2B is caused by the M918T and A883F mutation in approximately 95% and <5% of cases, respectively (1).

Shortly after these pivotal discoveries, strong genotype-phenotype correlations were recognized (8, 9). This provided the basis for individual risk profiles and establishment of the American Thyroid Association (ATA) MTC risk levels, according to the specific mutations (1, 10).

In MEN2A, several risk profiles for mutations of codon 10, 11, and 15 have been well defined (11–13). In MEN2B, almost all published reports concern the M918T mutation, and little is known about the aggressiveness of the A883F mutation. Despite the lack of a well-defined risk profile, the A883F mutation has been reclassified from the highest to the high-risk level in the revised ATA guidelines for the management of MTC (1).

We conducted an international cohort study of A883F carriers for the purpose of creating a risk profile for appropriate classification in the ATA risk levels.

Patients and Methods

Patients

This international retrospective cohort study included 13 unique A883F carriers from eight unrelated families.

At the International Workshop on Multiple Endocrine Neoplasia (WorldMEN) 2014 in Vienna, Austria, the WorldMEN 2016 workshop in Utrecht, the Netherlands, and during various other scientific meetings, all known MEN2 centers were asked to contribute relevant data to this study. Eleven A883F carriers were identified.

Additionally, a systematic literature search was performed. Of 199 unique citations, 12 reported A883F germline carriers (14–25). The reference list of each citation was scrutinized to uncover carriers published more than once. When this was insufficient, an author of the concerned publication was contacted for clarification. Six of the 12 citations described an A883F carrier who had been reported previously (14–16, 19, 22, 23). The remaining six citations were found to be the original reports of 12 A883F carriers (17, 18, 20, 21, 24, 25), two of whom had not been identified by the inquiries at the WorldMEN workshops. Thus, a total of 13 unique carriers were identified and included.

Data collection was performed using a uniform data sheet for each registrant. One carrier had been lost to follow-up several years before, and only data already published in relation to this carrier could be retrieved (15, 18, 19).

This study was approved by the respective institutional review boards for human subjects' protection in accordance with the ethical standards of each country and center.

Methods

The follow-up period was calculated from the date of MTC diagnosis to the date of death or latest follow-up. The date of MTC diagnosis was recorded as the date of initial thyroid surgery.

TNM (tumor-node-metastasis) staging was performed according to the seventh edition of the American Joint Committee on Cancer Staging Manual (26).

Biochemical cure was defined as basal serum calcitonin below the upper reference limit of the respective calcitonin assays at latest follow-up.

Statistical analysis

Continuous variables were calculated as the median and range. The Kaplan-Meier method was used for estimating age-related penetrance of MTC and PHEO, overall survival, and disease-specific survival. All analyses were performed using Stata 14.1 (StataCorp, College Station, TX).

Results

A total of 13 carriers from eight unrelated families were included. Demographic, clinical, surgical, and follow-up data are shown in Table 1. *De novo* mutations were recorded in five (45%) of the 11 carriers with pertinent data.

Carriers and families originated from the United States (four carriers from one family), United Kingdom (four carriers from three families), France (two carriers from one family), Germany (one carrier from one family),

Table 1. Demographic, Clinical, Surgical, and Follow-Up Data for 13 A883F Carriers

Carrier No.	Family No.	Sex	Index Carrier	Mucosal Neuroma	Marfanoid Habitus	At Initial Adrenal Surgery		At Initial Thyroid Surgery				At Latest Follow-Up		Reference
						Age, y	Pathology	Age, y	Procedure	Pathology	TNM[a]	Age, y	BC[b]	
1	1	F	No	No	No			7	TTX	CCH	T0N0M0	8	Yes	24
2	1	F	No	No	No			7	TTX	CCH	T0N0M0	8	Yes	24
3	2	M	No	Yes	No			8	TTX + LND	CCH	T0N0M0	8	Yes	17
4	1	M	No	Yes	No			9	TTX	Normal	T0N0M0	10	Yes	24
5	7	F	Yes	Yes	Yes			10	TTX	MTC	TxNxMx	37	Yes	21
6	6	F	Yes	Yes	No		NA	10	TTX	MTC	TxN0M0[c]	22	NA	15, 18, 19
7	4	M	Yes	Yes	Yes	11	PHEO	10	TTX + LND	MTC	T1aN1bM0	19	No	25
8	5	F	Yes	Yes	Yes	34	PHEO	13	TTX	MTC	T1N1M0	34	Yes	18
9	8	F	Yes	Yes	Yes	23	PHEO	19	TTX	MTC	TxNxMx[d]	47	No	21
10	3	F	Yes	Yes	Yes	44	PHEO	25	TTX	MTC	T2N0M0	60	Yes	20
11	2	F	Yes	Yes	No			28	TTX + LND	MTC	T4aN1bM1	32	No[e]	17
12	3	M	No	Yes	Yes			32	TTX + LND	MTC	T2N0M0	37	Yes	
13	1	F	Yes	Yes	No	39	PHEO	39	TTX + LND	MTC	T3N1bM0	44	Yes	24

Abbreviations: BC, biochemical cure; CCH, C-cell hyperplasia; F, female; LND, lymph node dissection; M, male; NA, not available; TTX, total thyroidectomy.

[a]According to the 7th edition of the American Joint Committee on Cancer Staging Manual.

[b]Defined as basal serum calcitonin lower than the upper reference limit.

[c]N1b and M1 (liver) at 12 and 20 years of age, respectively.

[d]M1 (mediastinum) at 29 years of age.

[e]Died as a result of MTC.

Denmark (one carrier from one family), and Australia (one carrier from one family).

Normal/C-cell hyperplasia

Upon prophylactic thyroidectomy, carrier no. 4 was diagnosed with a normal thyroid, and carriers no. 1 to 3 were diagnosed with C-cell hyperplasia (Table 1). Median age at diagnosis was 7.5 years (range, 7 to 9 years).

MTC

Nine carriers were diagnosed with MTC. Median age at diagnosis was 19 years (range, 10 to 39 years).

Full TNM status at initial thyroid surgery was available in six carriers. MTC without metastasis (T1-4N0M0) was diagnosed in two carriers with a median age of 28.5 years (range, 25 to 32 years). MTC with regional lymph node metastasis but without distant metastasis (T1-4N1M0) was diagnosed in three carriers with a median age of 13 years (range, 10 to 39 years). Distant metastasis (T1-4N0M1 or T1-4N1M1) was diagnosed in one carrier at age 28 years. However, the earliest observed age of distant metastasis was 20 years, 10 years after initial thyroid surgery (carrier no. 6).

Twenty-five percent, 50%, and 75% penetrance for MTC was achieved at 10, 19, and 28 years, respectively (Fig. 1).

Median follow-up of the nine carriers with MTC was 12 years (range, 4 to 35 years). One carrier died (as a result of MTC) at 32 years of age after four years of follow-up. Eight carriers were still alive at latest follow-up. Five- and 10-year survival rates (both overall and disease specific) were 88% and 88%, respectively.

Basal serum calcitonin at latest follow-up was available for 12 of 13 A883F carriers. Nine of the 12 (75%) had achieved biochemical cure. Among the seven index carriers with pertinent data, four (57%) had achieved biochemical cure. Among the A883F carriers with MTC and pertinent data, biochemical cure was seen in five of eight (63%) (Table 1).

PHEO

Five of 13 (38%) carriers were diagnosed with PHEO. The median age at diagnosis was 34 years (range, 11 to 44 years). Twenty-five percent, 50%, and 75% penetrance was achieved at 23, 34, and 39 years, respectively (Fig. 1). All PHEOs were bilateral and adrenal in location. None were malignant. All carriers had been diagnosed with MTC prior to PHEO. The median interval between the

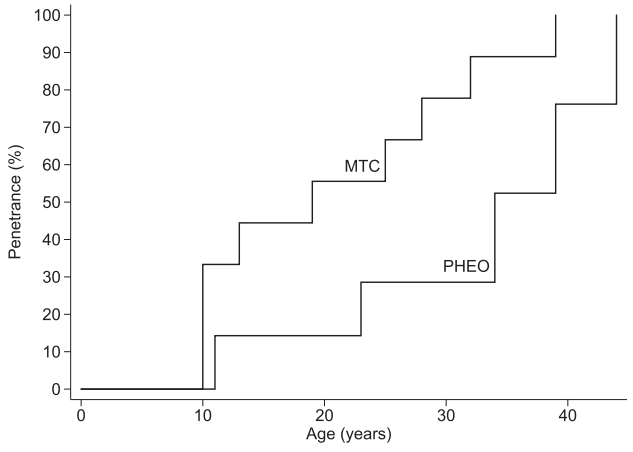

Figure 1. Age-related penetrance in 13 A883F carriers.

diagnosis of MTC and the diagnosis of PHEO was five years (range, 0 to 22 years).

Nonendocrine manifestations

Mucosal neuromas were shown in all but two carriers. Only the two youngest females, age 7 years, had no visible mucosal neuromas. Marfanoid habitus, thick eyelids, and narrow faces were described in six of the 13 patients. Pes cavus was diagnosed in three carriers.

Discussion

On the basis of this international retrospective cohort study of 13 A883F carriers from eight unrelated families, we created a risk profile for the A883F *RET* mutation. Our data show that the age-related penetrance of disease manifestations such as MTC and PHEO occur later in life than formerly thought.

Concerning age-related penetrance for MTC in our series, 50% penetrance of MTC in the A883F carriers was achieved by 19 years of age (Fig. 1). This is clearly later in life than in M918T carriers, who are grouped as having the highest risk mutation in the recently revised ATA guidelines; in this highest risk level, 50% penetrance of MTC was described at age 12 years (27). More similar to our results, the 50% penetrance for carriers of the C634R, C634W, and C634Y mutations (classified at the ATA high-risk level) has been reported at approximately 19, 30, and 33 years of age (12, 28). Considerably more favorable was the age-related penetrance of MTC in exon 10 codons 609, 611, 618, and 620 (ATA moderate-risk level), ranging from age 34 to 44 years (Table 2) (11). Thus, in regard to age-related penetrance for MTC, the A883F mutation resembles the other mutations in the ATA high-risk level.

In regard to the earliest onset of MTC, a normal thyroid and C-cell hyperplasia without MTC were diagnosed in four carriers of age 7 to 9 years. The earliest age of diagnosis of MTC, regional lymph node, and distant metastasis was 10, 10, and 20 years, respectively. This is distinctively later than in the ATA highest-risk level. The earliest ages of MTC, regional, and distant metastasis in M918T carriers (ATA highest-risk level) have been reported at 0.17, 0.25, and 5 years of age, respectively (29–31). For mutation carriers of codon 634 (ATA high-risk level), the corresponding ages have been reported at 0.8, 5, and 20 years (30, 32, 33). The earliest ages of MTC, regional metastasis, and distant metastasis reported in carriers of mutations classified at the ATA moderate-risk level have been reported at age 1, 6, and 6 years, respectively (34, 35). However, considerable variability in the age of earliest onset in regard to mutated codons and mutated amino acids has been reported in carriers belonging to the ATA moderate-risk level category (36). If one follows the rule of earliest onset, the A883F mutation appears to be most appropriately classified in the ATA high-risk or even ATA moderate-risk level.

Table 2. Age of 50% Penetrance for MTC According to *RET* Mutation and ATA Risk Level

ATA Risk Level	Mutation	Age at 50% Penetrance for MTC, y	Reference
MOD	C609F/G/R/S/Y[a]	~40	11
	C611F/Y/W[a]	~44	11
	C618F/G/R/S/Y[a]	~35	11
	C620F/G/R/S/Y[a]	~34	11
H	C634R	~19	28
	C634W	~30[b]	12
	C634Y	~33	28
	A883F	~19	Present study
HST	M918T[c]	~12[d]	27

Abbreviations: H, high; HST, highest; MOD, moderate.

[a] Mutations of the given codon are pooled.

[b] Age-related penetrance at 52%.

[c] Eighteen patients with MEN2B in the study. The 16 *RET*-tested patients were all M918T carriers.

[d] Calculated using the Kaplan-Meier method from the data available in the reference.

Biochemical cure at the latest follow-up was achieved in 75% and 57% of our total cohort and our index carriers with pertinent data, respectively. In a study of M918T carriers, biochemical cure was found less frequently, in 23% (10 of 44) and 17% (seven of 41) of the total cohort and the index carriers, respectively (37). Thus, the proportion of A883F carriers achieving biochemical cure appears large compared with that of the M918T carriers. In a recent study, biochemical cure in C634R and C634Y carriers with MTC was seen in 58% (32 of 55) and 63% (22 of 35), respectively (38). Correspondingly, biochemical cure was achieved in 63% of the A883F carriers with MTC. When using biochemical cure for risk classification, the A883F cohort resembles the high-risk level.

Five- and 10-year survival rates (both overall and disease specific) were 88% and 88%, respectively. In a study of 18 patients with MEN2B and MTC, all of the 16 *RET*-tested patients were M918T carriers. Five- and 10-year overall survival rates were 85% and 75%, respectively (27). Accordingly, the overall survival of A883F carriers seems favorable compared with that of M918T carriers, which also justifies classification of this mutation in the high-risk level.

PHEO was diagnosed at a median age of 34 years in the A883F carriers. This was 12 years later than seen in M918T carriers and eight years earlier than in exon 10 mutation carriers (11, 27). Therefore, with respect to

PHEO, stratifying the A883F mutation in the ATA high-risk level seems justified.

This study shares limitations that are inherent to retrospective multinational studies of rare diseases. Small sample sizes limiting generalization are often seen when studying rare diseases, as in this study. To increase sample size, carriers of different amino acid substitutions within the same codon can be pooled (11). To the best of our knowledge, there has only been one report of a mutation in codon 883 other than the A883F mutation (39). However, inclusion of patients carrying the A883T mutation from this report was not appropriate, given that no MEN2B phenotype was described. The A883T mutation showed a very low transforming activity, as demonstrated by the absence of the MTC phenotype in heterozygous carriers. MTC was only present in two homozygous carriers (39). Retrospective studies are frequently associated with missing data. However, no data were missing with regard to key variables in this study, such as ages, procedure, and pathology at initial thyroid surgery. Data on status and biochemical cure at latest follow-up were present in 89% (eight of nine) of carriers with MTC. Full TNM status at initial thyroid surgery was available in 67% (six of nine) of carriers with MTC. MEN2B nonendocrine manifestations were present to some extent in all A883F carriers, but with this small data set, we were not able to describe if manifestations develop later or in a more discrete variant in A883F compared with M918T carriers.

Conclusion

MTC of A883F *RET* mutation carriers seems to have a more indolent natural course compared with that of M918T carriers. PHEO manifests later in A883F carriers than in M918T carriers. Our results support the classification of the A883F mutation in the ATA high-risk level.

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