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Primary hyperparathyroidism as first manifestation in multiple endocrine neoplasia type 2A: an international multicenter study

Louise Velund Larsen1, Delphine Mirebeau-Prunier2, Tsuneo Imai3, Cristina Alvarez-Escola4, Kornelia Hasse-Lazar5, Simona Censi6, Luciana A Castroneves7, Akihiro Sakurai8, Minoru Kihara3, Kiyomi Horiiuch10, Véronique Dorine Barbu11,12, François Borson-Chazot12,13, Anne-Paule Gimenez-Roqueplo12,14,15, Pascal Pigny12,16, Stephane Pinson12,17, Nelson Wohlk18, Charis Eng19, Berna Imge Aydogan20, Dhananjaya Saranath21, Sarka Dvorakova22, Frederic Castinetti23,24, Attila Patocs25, Damjan Bergant26, Thera P Links27, Mariola Peczewska28, Ana O Hoff2, Caterina Mian2, Trisha Dwight29, Barbara Jarzab30, Hartmut P H Neumann31, Mercedes Robledo32,33, Shinya Uchino34, Anne Barlier12,35, Christian Godballe1 and Jes Sloth Mathiesen3,36

1Department of ORL Head & Neck Surgery and Audiology, Odense University Hospital, Odense, Denmark
2Laboratoire de Biochimie et Biologie Moléculaire, CHU Angers, Université d’Angers, UMR CNRS 6015, INSERM U1083, MITOVASC, Angers, France
3Department of Breast & Endocrine Surgery, National Hospital Organization, Higashinagoya National Hospital, Nagoya, Japan
4Endocrinology and Nutrition Department, University Hospital ‘La Paz’, Madrid, Spain
5Department of Nuclear Medicine and Endocrine Oncology, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Gliwice, Poland
6Endocrinology Unit, Department of Medicine (DIMED), University of Padua, Padua, Italy
7Department of Endocrinology, Endocrine Oncology Unit, Instituto do Cancer do Estado de Sao Paulo, Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, Brazil
8Department of Medical Genetics and Genomics, Sapporo Medical University School of Medicine, Sapporo, Japan
9Department of Surgery, Kuma Hospital, Kobe, Hyogo, Japan
10Department of Breast and Endocrine Surgery, Tokyos Women’s Medical University, Tokyo, Japan
11AP-HP, Sorbonne Université, Laboratoire Commun de Biologie et Genétique Moléculaires, Hôpital St Antoine & INSERM CRSA, Paris, France
12Réseau TenGen, Marseille, France
13Fédération d’Endocrinologie, Hospices Civils de Lyon, Université Lyon 1, France
14Service de Génétique, AP-HP, Hôpital européen Georges Pompidou, Paris, France
15Université de Paris, PARCC, INSERM, Paris, France
16Laboratoire de Biochimie et Oncologie Moléculaire, CHU Lille, Lille, France
17Laboratoire de Génétique Moléculaire, CHU Lyon, Lyon, France
18Endocrine Section, Hospital del Salvador, Santiago de Chile, Department of Medicine, University of Chile, Santiago, Chile
19Genomic Medicine Institute, Lerner Research Institute and Taussig Cancer Institute, Cleveland Clinic, Cleveland, Ohio, USA
20Department of Endocrinology And Metabolic Diseases, Ankara University School of Medicine, Ankara, Turkey
21Department of Research Studies & Additional Projects, Cancer Patients Aid Association, Dr. Vithaldas Parmar Research & Medical Centre, Worli, Mumbai, India
22Department of Molecular Endocrinology, Institute of Endocrinology, Prague, Czech Republic
23Aix-Marseille Université, Institut National de la Santé et de la Recherche Médicale (INSERM), U1251, Marseille Medical Genetics (MMG), Marseille, France
24Department of Endocrinology, Assistance Publique-Hôpitaux de Marseille (AP-HM), Hôpital de la Conception, Centre de Référence des Maladies Rares de l’hypophyse HYPO, Marseille, France
25HAS-SE Momentum Hereditary Endocrine Tumors Research Group, Semmelweis University, Budapest, Hungary
26Department of Surgical Oncology, Institute of Oncology, Ljubljana, Slovenia
27Department of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands
28Department of Hypertension, Institute of Cardiology, Warsaw, Poland
29Cancer Genetics, Kolling Institute, Royal North Shore Hospital and University of Sydney, Sydney, New South Wales, Australia
30Department of Nuclear Medicine and Endocrine Oncology, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Gliwice, Poland
31Section for Preventive Medicine, Medical Center-University of Freiburg, Faculty of Medicine, Albert Ludwigs-University of Freiburg, Freiburg, Germany
32Hereditary Endocrine Cancer Group, Spanish National Cancer Research Center (CNIO), Madrid, Spain
33Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain
34Department of Endocrine Surgery, Noguchi Thyroid Clinic and Hospital Foundation, Beppu, Oita, Japan
35Aix Marseille Univ, APHM, INSERM, MMG, Laboratory of Molecular Biology, Hospital La Conception, Marseille, France
36Department of Clinical Research, University of Southern Denmark, Odense, Denmark

Correspondence should be addressed to J S Mathiesen: jes.mathiesen@yahoo.dk

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Abstract

Objective: Multiple endocrine neoplasia type 2A (MEN 2A) is a rare syndrome caused by RET germline mutations and has been associated with primary hyperparathyroidism (PHPT) in up to 30% of cases. Recommendations on RET screening in patients with apparently sporadic PHPT are unclear. We aimed to estimate the prevalence of cases presenting with PHPT as first manifestation among MEN 2A index cases and to characterize the former cases.

Design and methods: An international retrospective multicenter study of 1085 MEN 2A index cases. Experts from MEN 2 centers all over the world were invited to participate. A total of 19 centers in 17 different countries provided registry data of index cases followed from 1974 to 2017.

Results: Ten cases presented with PHPT as their first manifestation of MEN 2A, yielding a prevalence of 0.9% (95% CI: 0.4–1.6). 9/10 cases were diagnosed with medullary thyroid carcinoma (MTC) in relation to parathyroid surgery and 1/10 was diagnosed 15 years after parathyroid surgery. 7/9 cases with full TNM data were node-positive at MTC diagnosis.

Conclusions: Our data suggest that the prevalence of MEN 2A index cases that present with PHPT as their first manifestation is very low. The majority of index cases presenting with PHPT as first manifestation have synchronous MTC and are often node-positive. Thus, our observations suggest that not performing RET mutation analysis in patients with apparently sporadic PHPT would result in an extremely low false-negative rate, if no other MEN 2A component, specifically MTC, are found during work-up or resection of PHPT.

Introduction

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant inherited cancer syndrome caused by germline mutations of the rearranged during transfection (RET) proto-oncogene (1, 2, 3, 4, 5, 6). The syndrome is divided into MEN 2A and MEN 2B with a point prevalence of 13–24 per million and 1–2 per million, respectively (7, 8, 9, 10). Virtually all patients with MEN 2A develop medullary thyroid carcinoma (MTC), while lower numbers develop pheochromocytoma, primary hyperparathyroidism (PHPT), cutaneous lichen amyloidosis (CLA) and Hirschsprung disease (HSCR) (11).

For identification of new MEN 2A index cases and families, RET screening has been recommended for years in all patients with apparently sporadic MTC, pheochromocytoma, CLA and infants with HSCR (11, 12, 13, 14). However, for patients with apparently sporadic PHPT, recommendations on RET screening are less clear. Thus, in 2001 the consensus guidelines from the seventh international workshop on MEN recommended against RET screening in these patients (13), while the issue lacks mentioning in the 2009 and 2015 guidelines by the American Thyroid Association (11, 12).

To ascertain if all patients with apparently sporadic PHPT should be RET screened, a valuable estimate would be the prevalence of MEN 2A index cases presenting with PHPT as first manifestation in an unselected population-based cohort of apparently sporadic PHPT cases, who have all been RET screened. To our knowledge, however, no such cohorts exist. Instead, a surrogate cohort study is to examine the prevalence of MEN 2A index cases presenting with PHPT as the first manifestation in an unselected cohort of MEN 2A index cases. Based on the experience from previous MEN 2A PHPT series (15, 16), we hypothesized that this prevalence would be low.

Consequently, we aimed to estimate the prevalence of MEN 2A index cases presenting with PHPT as first manifestation in an unselected cohort of MEN 2A index cases. Additionally, we aimed to characterize the cases presenting with PHPT as their first manifestation.

Methods

Study design and participants

This investigation is an international retrospective multicenter study of 1085 MEN 2A index cases. We invited experts from 40 MEN 2 centers all over the world to participate. This yielded a total of 19 centers in...
17 different countries, including Denmark, providing data of index cases followed from 1974 to 2017 (Supplementary Table 1, see section on supplementary materials given at the end of this article). Data were retrieved from June 2017 to September 2019.

**Data sources**

Data were drawn from the registry of each center. Some of the patients have been reported on previous occasions and updated data were obtained (17, 18, 19, 20, 21, 22, 23, 24, 25, 26).

**Variables**

Patients were defined as having MEN 2 if they had tested positive for a RET germline sequence change classified as pathogenic (mutation) in the ARUP MEN 2 database on February 1, 2020 (27). For inclusion of only the MEN 2A patients, we excluded those with mutations pathognomonic of MEN 2B (RET M918T and A883F) (28, 29). An index case was defined as a clinically affected individual through whom attention is first drawn to MEN 2A in a family (https://www.cancer.gov/publications/dictionaries/genetics-dictionary/def/index-case). The first manifestation in MEN 2A was defined by the symptoms or biochemistry leading to initial endocrine work-up and was judged by the MEN 2 experts participating in the study. PHPT had to be both biochemically (hypercalcemia and an elevated or inappropriately normal parathyroid hormone level (30)) and histologically proven, while MTC, pheochromocytoma, CLA and HSCR were considered by histology only. TNM staging was performed according to the seventh edition of the American Joint Committee on Cancer Staging Manual (31). Biochemical cure was regarded as undetectable basal calcitonin at last biochemical follow-up.

**Statistical analysis**

Continuous data were presented as median and range. All analyses were done using Stata® 15.1 (StataCorp LP).

**Ethics**

Informed consent was given by all patients participating in the study for RET screening. Ethical approval was obtained from the institutional review boards of all participating centers when required: French National Commission for Computerized Data and Individual Freedom, Institutional Ethical Review Board of Shinshu University School of Medicine (Matsumoto, Japan), Comité de bioética y bienestar animal of the Instituto de Salud Carlos III, Northern Sydney Local Health District Human Research Ethics Committee, ICESP/HCFMUSP, Ethics Committee of the Institute of Cardiology (Warsaw, Poland), Regional Committee on Health Research Ethics for Southern Denmark, Scientific and Research Committee of the Medical Research Council of Hungary, Ethics Committee of Aix Marseille University, Ethics Committee of the Institute of Endocrinology (Prague, Czech Republic), Ethics Committee of Reliance Life Sciences (Navi Mumbai, India), Local Ethics Committee of Ankara University Faculty of Medicine, Cleveland Clinic Institutional Review Board for Human Subjects Protection and Ethical Committee (Santiago, Chile). This was in accordance with the ethical standards of each country and center.

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

**Results**

A total of 1085 MEN 2A index cases were included in the study. The distribution of RET germline mutations in these cases is shown in Table 1. The most frequent site of mutations was exon 11 (53%), followed by exon 10 (25%), exon 14 (12%), exon 13 (7%), exon 15 (3%), exon 8 (1%) and exon 16 (0%). Of the 1085 cases, 10 had presented with PHPT as first manifestation of the syndrome, yielding a prevalence of 0.9% (95% CI: 0.4–1.6).

Characteristics of the ten cases are depicted in Table 2. In these cases, the female-to-male ratio was 4.0 (95% CI: –2.2–10.2), while the median age at diagnosis of PHPT was 34.5 years (range, 14–68). All cases were diagnosed with PHPT between 1993 and 2012. Of these, seven were diagnosed in the new millennium.

All cases with pertinent data (n = 9) were symptomatic at diagnosis of PHPT with symptoms being nephrolithiasis (n = 8) and polyuria (n = 1). MTC was diagnosed in 10/10 cases. 9/10 were diagnosed in relation to parathyroid surgery as a synchronous MTC and 1/10 was diagnosed 15 years after parathyroid surgery, as a metachronous MTC. In three cases, MTC was not suspected during preoperative PHPT work-up, but diagnosed during parathyroid surgery. 7/9 cases with full TNM data available had regional lymph node metastases at time of
Table 1  Distributions of RET mutations among 1085 MEN 2A index cases.

| RET mutation | n  | (%) |
|--------------|----|-----|
| Exon 8       |    |     |
| C531R        | 3  | (0) |
| G533C        | 5  | (0) |
| G548S        | 2  | (0) |
| Exon 10      |    |     |
| C609F/G/R/S/Y| 19 | (2) |
| C611F/G/W/Y  | 48 | (4) |
| C618F/G/R/S/W/Y| 113| (10)|
| C620F/G/R/S/W/Y| 87 | (8) |
| Exon 11      |    |     |
| C630R/Y      | 4  | (0) |
| D631Y        | 3  | (0) |
| C634F/G/L/S/R/W/Y | 562 | (52) |
| K666E/N/T    | 6  | (1) |
| Exon 13      |    |     |
| E768D        | 18 | (2) |
| Q781R        | 1  | (0) |
| L790F        | 52 | (5) |
| Exon 14      |    |     |
| V804L/M      | 132| (12)|
| Exon 15      |    |     |
| S891A        | 28 | (3) |
| Exon 16      |    |     |
| R912P        | 1  | (0) |
| M918V        | 1  | (0) |
| Total        | 1085 | (100)|

Due to rounding up, not all sums of the numbers fit.

MTC diagnosis. Biochemical cure was achieved only in the node-negative cases (n=2).

Discussion

This large international retrospective multicenter study found that 0.9% of cases had PHPT as their first manifestation of MEN 2A. In the cases presenting with PHPT as first manifestation, MTC was coexistent and had metastasized to regional lymph nodes in 7/9 cases.

Prevalence

In this study, we found 0.9% of our MEN 2A index cases presented with PHPT as the first manifestation of the syndrome. To our knowledge, no similar studies on MEN 2A index cases have been reported, rendering comparisons difficult. However, there exist several studies, in which the study cohorts comprise only MEN 2A cases with PHPT. In these cohorts the prevalence of MEN 2A cases presenting with PHPT as a first manifestation ranges 0–11% (15, 16, 32, 33, 34, 35). Considering the selection of these cohorts and the fact that they included index and non-index cases, presumably a majority of the latter, our prevalence of 0.9% appears as a solid estimate. This is in line with the experience of other smaller series, that PHPT rarely was the first diagnosed manifestation (16, 36). In fact, there seems to be a decrease in the overall prevalence of PHPT in MEN 2A cohorts reported over time, possibly explained by inclusion of more patients with the full-blown syndrome (MTC, pheochromocytoma and PHPT) in the earliest series (6, 33, 37).

In our overall cohort, the most frequently mutated codon was 634, followed by codons 804, 618, 620, 790, 611, 891, 609, 768 and other rarely mutated codons. With only minor differences, likely accounted for by founder effects, the distribution of mutations in our cohort is, by and large, comparable to that of series in the literature (7, 17, 19, 20, 21, 38, 39, 40, 41, 42, 43, 44, 45).

Characteristics of cases

Our study depicts the characteristics of MEN 2A index cases presenting with PHPT as first manifestation. Age at diagnosis is by and large similar to that of other MEN 2A PHPT cohorts (15, 16, 32, 33, 35, 46). Our female-to-male ratio of 4.0 is higher than that (1.3–1.9) reported by others (15, 16, 32, 34). This may be a question of sample size, but may also indicate that female MEN 2A cases in comparison to males are more prone to present with PHPT as first manifestation.

In our cohort all cases with pertinent data were symptomatic at diagnosis of PHPT. This is in contrast with other MEN 2A PHPT cohorts, in which most cases (58–84%) are asymptomatic (15, 16, 32, 33, 34). A likely explanation is the difference in cohorts, where our cohort solely comprises index cases presenting with PHPT as first manifestation, while the other cohorts presumably comprise mainly non-index cases diagnosed with PHPT by screening before they become symptomatic.

Nine of our ten cases were diagnosed with MTC, either due to a suspected or unsuspected finding in relation to parathyroid surgery. As a consequence, RET screening would be prompted by the MTC, if not instigated by the PHPT diagnosis. To our knowledge, the MTC TNM stage of the cases has not previously been reported in MEN 2A PHPT cohorts. In our cohort, 7/9 cases with available data were MTC node positive. This may reflect an over-representation of codon 634 mutation carriers (6/10), who generally have earlier age at MTC onset compared with other MEN 2A patients (47, 48). The over-representation...
To assess if all cases with apparently sporadic PHPT should be RET screened, one could have estimated the prevalence of MEN 2A index cases presenting with PHPT as first manifestation. Thus, we cannot rule out that our study cohort consists of already recognized MEN 2A index cases. The issue that underlies the fact that our study cohort consists of already recognized MEN 2A index cases is still unsolved. MEN 2A index cases could be RET screened, rendering such a study unfeasible. Instead, we sought to estimate the prevalence of MEN 2A index cases presenting with PHPT as their first manifestation in the largest series of MEN 2A index cases seen to date. As the first RET-gene mutation screening in MEN 2A families arising from de novo mutations likely occurs (3), one may argue that the pool of unrecognized MEN 2A families arising from de novo mutations is very small, thus minimizing the selection bias in the current study. In several other multicenter studies on MEN 2, including all MEN 2 centers in the world is an immensely difficult and time-consuming task. However, formation of a consortium including all MEN 2 centers worldwide may be helpful for future studies.

**Table 2  Characteristics of MEN 2A index cases presenting with PHPT as first manifestation.**

| Patient no. | Sex | RET mutation | Age (yrs) | PHPT* | MTC* | PHEO* | HSCR* | CLA* | Follow-up |
|-------------|-----|--------------|----------|-------|------|-------|-------|------|-----------|
| 1           | F   | C634Y        | 14       | Hyperplasia | 14  | T2N1M0 | None | N    | 19        | Alive    |
| 2           | F   | C634R        | 18       | Adenoma | 18  | T2N1M0 | Bilateral | N  | 30        | Dead     |
| 3           | M   | C634Y        | 19       | Adenoma | 19  | T2N0M0 | Unilateral | N  | 30        | Alive    |
| 4           | F   | C634R        | 28       | Hyperplasia | 28  | T1N1M0 | Unilateral | N  | 38        | Alive    |
| 5           | F   | C634R        | 31       | Adenoma | 46  | T1N0N0 | Unilateral | N  | 57        | Alive    |
| 6           | F   | C634R        | 38       | Adenoma | 38  | T2N1M0 | Bilateral | N  | 47        | Alive    |
| 7           | M   | C611Y        | 40       | Adenoma | 40  | T1N1M0 | Unilateral | N  | 47        | Alive    |
| 8           | F   | C620R        | 61       | Adenoma | 61  | T3N1M1 | None | N    | 75        | Dead     |
| 9           | F   | E768D        | 61       | Adenoma | 61  | T1N1M0 | None | N    | 66        | Alive    |
| 10          | F   | C618F        | 68       | Adenoma | 68  | T2NxMx | Unilateral | N  | 90        | Alive    |

*Defined by biochemistry (30) and histology. Defined by histology. 
†Staging was based on the American Joint Committee on Cancer seventh edition (31). 
‡Malignant.

CLA, cutaneous lichen amyloidosis; HSCR, Hirschsprung disease; MEN 2A, multiple endocrine neoplasia type 2A; MTC, medullary thyroid carcinoma; N, no; NA, not available; PHEO, pheochromocytoma; PHPT, primary hyperparathyroidism; RET, rearranged during transfection; Y, yes.
A limitation of the study is the lack of preoperative data, especially regarding ultrasonography and serum calcitonin. This hinders the elaborations on reasons for the preoperative suspicion of MTC during PHPT work-up and makes it difficult to assess potential diagnostic bias. High-resolution ultrasonography is routinely used in the preoperative setup for PHPT patients, while measurements of serum calcitonin are not (64). In some patients the preoperative serum calcitonin will likely be measured as a consequence of thyroid nodules found by ultrasonography (65, 66, 67, 68, 69). Some authors have suggested systematically preoperative calcitonin measurements in patients with apparently sporadic PHPT to exclude potential MEN 2 cases (70). Such a strategy in all PHPT patients or in PHPT patients with synchronous thyroid tumors found by ultrasonography would likely prove more cost effective than systematically carrying out RET mutation analysis. However, to our knowledge no evidence for or against this strategy exists.

Conclusion

Our data suggest that the prevalence of MEN 2A index cases that present with PHPT as their first manifestation is very low. The majority of index cases presenting with PHPT as first manifestation, have synchronous MTC, often node-positive. Thus, our observations suggest that not performing RET mutation analysis in patients with apparently sporadic PHPT would result in an extremely low false negative rate, if no other MEN 2A component, specifically MTC, are found during work-up or resection of PHPT.

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References

1 Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JE, Goodfellow P & Wells Jr SA. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Human Molecular Genetics 1993 2 851–856. (https://doi.org/10.1093/hmg/2.7.851)
2 Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK & Papi L. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature 1993 363 458–460. (https://doi.org/10.1038/363458a0)
3 Carlson KM, Dou S, Chi D, Scavarda N, Toshima K, Jackson CE, Wells Jr SA, Goodfellow PJ & Donis-Keller H. Single missense mutation in the tyrosine kinase catalytic domain of the RET protooncogene is associated with multiple endocrine neoplasia type 2B. PNAS 1994 91 1579–1583. (https://doi.org/10.1073/pnas.91.4.1579)
4 Hofstra RM, Landsvater RM, Coccinelli I, Stulp RP, Stelwagen T, Luo Y, Pasini B, Hoppenr JW, van Amstel HK & Romeo G. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 1994 367 375–376. (https://doi.org/10.1038/367375a0)
5 Eng C, Smith DF, Mulligan LM, Nagai MA, Healey CS, Ponder MA, Gardner E, Scheunemann GF, Jackson CE & Tunnacliffe A. Point mutation within the tyrosine kinase catalytic domain of the RET protooncogene in multiple endocrine neoplasia type 2B and related sporadic tumours. Human Molecular Genetics 1994 3 237–241. (https://doi.org/10.1093/hmg/3.2.237)
6 Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RE, van Amstel HK, Lips CJ, Nishisho I, Takai SI, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA 1996 276 1575–1579.
7 Opsahl EM, Brauckhoff M, Schlichting E, Helset K, Svarberg J, Brauckhoff K, Maehle L, Engelbreken LE, Sigstad E, Groholtt KK, et al. A Nationwide study of multiple endocrine neoplasia type 2A in Norway: predictive and prognostic factors for the clinical course of medullary thyroid carcinoma. Thyroid 2016 26 1225–1238. (https://doi.org/10.1089/thy.2015.0673)
8 Mathiesen JS, Kroustrup JP, Vestergaard P, Kochtahl K, Poulsen PL, Rasmussen ÅK, Feldt-Rasmussen U, Schytte S, Mathiesen PB, et al. Incidence and prevalence of multiple endocrine neoplasia 2A in Denmark 1901–2014: a nationwide study. Clinical Epidemiology 2018 10 1479–1487. (https://doi.org/10.2147/CEPES.174606)
9 Mathiesen JS, Kroustrup JP, Vestergaard P, Madsen M, Stochholm K, Poulsen PL, Krogh Rasmussen Å, Feldt-Rasmussen U, Schytte S, Poulsen HB, et al. Incidence and prevalence of multiple endocrine neoplasia 2B in Denmark: a nationwide study. Endocrine-Related Cancer 2017 24 L39–L42. (https://doi.org/10.1530/ERC-17-0122)
10 Znaczko A, Donnelly DE & Morrison PJ. Epidemiology, clinical features, and genetics of multiple endocrine neoplasia type 2B in a complete population. Oncologist 2014 19 1284–1286. (https://doi.org/10.1634/theoncologist.2014-0277)
L V Larsen et al. PHPT as first manifestation of MEN 2A

11 Wells Jr SA, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, Machens A, Moley JE, Pacini F, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid* 2015 **25** 567–610. (https://doi.org/10.1089/thy.2014.0335)

12 Kroo RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JE, Pacini F, Ringel MD, et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid* 2009 **19** 565–612. (https://doi.org/10.1089/thy.2008.0403)

13 Brandt ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri RG, Libhosa A, et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 5658–5671. (https://doi.org/10.1210/jcem.86.12.8070)

14 Traugott AL & Moley JF. Multiple endocrine neoplasia type 2: clinical manifestations and management. *Cancer Treatment and Research 2010** 153 321–337. (https://doi.org/10.1007/978-1-4419-0857-5_18)

15 Raue F, Kraljms JL, Dralle H, Cougaard P, Proye C, Frilling A, Limbert E, Londero I & Niederle B. Primary hyperparathyroidism in multiple endocrine neoplasia type 2A. *Journal of Internal Medicine* 1995 **238** 369–373. (https://doi.org/10.1111/j.1365-2796.1995.tb01212.x)

16 Kraljms JL, Denizot A, Caraille B, Henry JE, Proye C, Bacourt F, Sarafit E, Dupond JL, Maes B, Travagl J, et al. Primary hyperparathyroidism in multiple endocrine neoplasia type Ila: retrospective French multicentric study. Groupe d'Etude des Tumeurs a Calcitonine. *World Journal of Surgery* 1996 **20** 808–812; discussion 812. (https://doi.org/10.1007/s002689900125)

17 Lebeault M, Pinson S, Guillaume-Bataille M, Gimenez-Roqueplo AP, Carrie A, Barba V, Piginy P, Bezeau S, Rey JM, Delvincourt C, et al. Nationwide French study of RET variants detected from 2003 to 2013 suggests a possible influence of polymorphisms as modifiers. *Thyroid* 2017 **27** 1511–1522. (https://doi.org/10.1089/thy.2016.0399)

18 Imai T, Uchino S, Okamoto T, Suzuki S, Kosugi S, Kikumori T, Sakurai A & MEN Consortium of Japan. High penetrance of RET A883F germline mutation: an international collaborative study. *Journal of Clinical Endocrinology and Metabolism* 2017 **102** 2069–2074. (https://doi.org/10.1210/jc.2016-3640)

19 Castinetti F, Waguespack SG, Machens A, Uchino S, Hasse-Lazar K, Sanco G, Else T, Dvorakova S, QI XP, Elisei R, et al. Natural history, treatment, and long-term follow up of patients with multiple endocrine neoplasia type 2B: an international, multicentre, retrospective study. *Lancet: Diabetes and Endocrinology* 2019 **7** 213–220. (https://doi.org/10.1016/S2213-8587(18)30336-X)

20 Khan AA, Hanley DA, Rizzoli R, Bollerslev J, Youen J, Rejnmark L, Thakker R, D’Amour P, Paul T, Vow U, et al. Primary hyperparathyroidism: review and recommendations on evaluation, diagnosis, and management. A Canadian and international consensus. *Osteoporosis International* 2017 **28** 1–19. (https://doi.org/10.1007/s00198-016-3716-2)

21 Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL & Trotti A (eds). *AJCC Cancer Staging Manual*, 7th ed. New York, NY, USA: Springer, 2010.

22 Twigt BA, Chaolten A, Valk GD, Rinckes IH & Vriens MR. Differences between sporadic and MEN related primary hyperparathyroidism; clinical expression, preoperative workup, operative strategy and follow-up. *Orphanet Journal of Rare Diseases* 2013 **8** 50. (https://doi.org/10.1186/1750-1172-8-50)

23 Schuffenecker I, Virally-Monod M, Brohet R, Goldgar D, Conte-Devolx B, Leclerc L, Chabre O, Boneu A, Caron J, Houdent C, et al. Risk and penetrance of primary hyperparathyroidism in multiple endocrine neoplasia type 2f families with mutations at codon 634 of the RET protooncogene. Groupe d'Etude des Tumeurs a Calcitonine. *Journal of Clinical Endocrinology and Metabolism* 1999 **83** 487–491. (https://doi.org/10.1210/jcem.83.2.4529)

24 Herfarth KK, Bartsch D, Doherty GM, Wells Jr SA & Laimore TC. Surgical management of hyperparathyroidism in patients with multiple endocrine neoplasia type 2A. *Surgery* 1996 **120** 966–973; discussion 973–964. (https://doi.org/10.1016/s0039-6060(96)y00420-0)

25 Howe JR, Norton JA & Wells Jr SA. Prevalence of pheochromocytoma and hyperparathyroidism in multiple endocrine neoplasia type 2A: results of long-term follow-up. *Surgery 1993** 114** 1070–1077.

26 Frank-Rauhe K, Leidig-Bruckner G, Lorenz A, Rondot S, Haag C, Schulze E, Buchler M & Raue F. Hereditary variants of primary hyperparathyroidism – MEN1, MEN2, HPT-JT, FHH, FHPPT. *Deutsche Medizinische Wochenschrift* 2011 **136** 1889–1894. (https://doi.org/10.1055/s-0031-1286358)

27 Machens A & Dralle H. Advances in risk-oriented surgery for multiple endocrine neoplasia type 2. *Endocrine-Related Cancer* 2018 **25** 741–752. (https://doi.org/10.1530/ERC-17-0202)

28 Sanika HL, Papathoma A, Garofalaki M, Saltikov K, Pappa T, Pazzitou-Panayiotou K, Anastasiou E & Alevizaki M. Genetic screening of...
patients with medullary thyroid cancer in a referral center in Greece during the past two decades. European Journal of Endocrinology 2015 172 501–509. (https://doi.org/10.1530/EJE-14-0817)

39 Machens A, Lorenz K, Sekulla C, Hoppner W, Frank-Raue K, Raue F & Drahle H. Molecular epidemiology of multiple endocrine neoplasia 2: implications for RET screening in the new millennium. European Journal of Endocrinology 2013 168 307–314. (https://doi.org/10.1530/EJE-12-0919)

40 Giacche M, Panarotto A, Tacchetti MC, Tosini R, Campana F, Mori L, Cappelli C, Ptrola I, Lombardi D, Pezzola DC, et al. p.Ser891A RET gene mutations in medullary thyroid cancer: phenotypical and genealogical characterization of 28 apparently unrelated kindreds and founder effect uncovering in Northern Italy. Human Mutation 2019 40 926–937. (https://doi.org/10.1002/humu.23754)

41 Elisei R, Tacito A, Ramone T, Ciampi R, Bottici V, Cappagli V, Viola D, Matrone A, Lorusso L, Valerio L, et al. Twenty-five years experience on RET genetic screening on hereditary MTC: an update on the prevalence of germline RET mutations. Genes 2019 10 698. (https://doi.org/10.3390/genes10090698)

42 Mathiesen JS, Kroustrup JP, Stockholm K, Poulsen ML, Rasmussen ÅK, Feldt-Rasmussen U, Gaustadnes M, Omortf TF, Rossing M, et al. Founder effect of the RET(C611Y) mutation in multiple endocrine neoplasia 2A in Denmark: a nationwide study. Thyroid 2017 27 1505–1510. (https://doi.org/10.1089/thy.2017.0404)

43 Cunha LL, Lindsay SC, Franca MC, Sarika L, Paphathoma A, Kunist IS, Cerutti JM, Dias-da-Silva MR, Alevizaki M & Maciel RMB. Evidence for the founder effect of RET533 as the common Greek and Brazilian ancestor spreading multiple endocrine neoplasia 2A. European Journal of Endocrinology 2017 176 515–519. (https://doi.org/10.1530/EJC-16-1021)

44 Martins-Costa MC, Cunha LL, Lindsay SC, Camacho CP, Dotto RP, Furuzawa GK, Sousa MS, Kasamatsu TS, Kunist IS, Martins MM, et al. M918V RET mutation causes familial medullary thyroid carcinoma: study of 8 affected kindreds. Endocrine-Related Cancer 2016 23 909–920. (https://doi.org/10.1530/EJC-16-0141)

45 Machens A, Lorenz K, Weber F & Drahle H. Geographic epidemiology of MTC families: unearthing European ancestral heritage. Endocrine-Related Cancer 2018 25 L27–L30. (https://doi.org/10.1530/ERC-17-0514)

46 Machens A, Lorenz K & Drahle H. Peak incidence of pheochromocytoma and primary hyperparathyroidism in multiple endocrine neoplasia 2: need for age-adjusted biochemical screening. Journal of Clinical Endocrinology and Metabolism 2013 98 E336–E345. (https://doi.org/10.1210/jc.2012-3192)

47 Raue F, Bruckner T & Frank-Raue K. Long-term outcomes and aggressiveness of hereditary medullary thyroid carcinoma: 40 years of experience at one center. Journal of Clinical Endocrinology and Metabolism 2019 104 4264–4272. (https://doi.org/10.1210/jc.2019-00516)

48 Machens A, Lorenz K, Weber F & Drahle H. Genotype-specific progression of hereditary medullary thyroid cancer. Human Mutation 2018 39 860–869. (https://doi.org/10.1002/humu.23430)

49 Mathiesen JS, Kroustrup JP, Vestengaard P, Stockholm K, Poulsen PL, Rasmussen ÅK, Feldt-Rasmussen U, Schytte S, Londero SC, et al. Survival and long-term biochemical cure in medullary thyroid carcinoma in Denmark 1997–2014: a nationwide study. European Thyroid Journal 2019 8 159–166. (https://doi.org/10.1159/000499018)

50 Opsahl EM, Akslen LA, Schlichting E, Aas T, Brauckhoff K, Hagen AL, Rosenlund AF, Sigstad E, Groholt KK, Jørgensen LH, et al. The role of calcitonin in predicting the extent of surgery in medullary thyroid carcinoma: a nationwide population-based study in Norway. European Thyroid Journal 2019 8 159–166. (https://doi.org/10.1159/000499018)

51 Scuffenecker I, Ginot N, Goldgar D, Eng C, Chambe B, Boneu A, Houdenc T, Pidlo D, Schlumberger M, Thivolet C, et al. Prevalence and parental origin of de novo RET mutations in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma. The Groupe d’Etude des Tumeurs a Calcitonine. American Journal of Human Genetics 1997 60 233–237

52 Castinetti F, Maia AL, Peczkowska M, Barontini M, Hasse-Lazar K, Links TP, Toledo RA, Dvorakova S, Mian C, Bugallo MJ, et al. The penetrance of MEN2p pheochromocytoma is not only determined by RET mutations. Endocrine-Related Cancer 2017 24 L63–L67. (https://doi.org/10.1530/ERC-17-0189)

53 Frank-Raue K, Rybicki LA, Eric L, Schweizer H, Winder A, Milos I, Toledo SP, Toledo RA, Tavares MR, Alevizaki M, et al. Risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germline RET mutations located in exon 10. Human Mutation 2011 32 51–58. (https://doi.org/10.1002/humu.21385)

54 Milos IN, Frank-Raue K, Wohllk N, Maia AL, Pusiol E, Patocs A, Robledo M, Biemes J, Barontini M, Links TP, et al. Age-related neoplastic risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germ line RET Cys634Trp mutation. Endocrine-Related Cancer 2008 15 1035–1041. (https://doi.org/10.1677/ERC-08-0105)

55 Machens A, Nicolli-Sire P, Hoegel J, Frank-Raue K, van Vroonhoven TJ, Roher HD, Wahl RA, Lamesch P, Raue F, Conte-Devols B, et al. Early malignant progression of hereditary medullary thyroid cancer. New England Journal of Medicine 2003 349 1517–1525. (https://doi.org/10.1056/NEJMoa021915)

56 Eng C, Mulligan LM, Smith DP, Healey CS, Frilling A, Raue F, Neumann HP, Ponder MA & Ponder BA. Low frequency of germline mutations in the RET proto-oncogene in patients with apparently sporadic medullary thyroid carcinoma. Clinical Endocrinology 1995 43 123–127. (https://doi.org/10.1111/j.1365-2265.1995.tb01903.x)

57 Modigliani E, Vesen HM, Raue K, Drahle H, Frilling A, Gheri RG, Brandi ML, Libert M, Niederle B & Forgas L. Pheochromocytoma in multiple endocrine neoplasia type 2: European study. The Euromen Study Group. Journal of Internal Medicine 1995 238 363–367. (https://doi.org/10.1111/j.1365-2969.1995.tb01211.x)

58 Mulligan LM, Marsh DJ, Robinson BG, Scuffenecker I, Zedenius J, Lips CJ, Gagel RF, Takai SI, Noll WW & Fink M. Genotype-phenotype correlation in multiple endocrine neoplasia type 2: report of the International RET Mutation Consortium. Journal of Internal Medicine 1995 238 343–346. (https://doi.org/10.1111/j.1365-2769.1995.tb01208.x)

59 Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JB, Gardner E, Ponder MA, Frilling A, Jackson CE & Lehner H. Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. Nature Genetics 1994 4 60–70. (https://doi.org/10.1038/ng0194-70)

60 Mulligan LM, Eng C, Attie T, Lyonne N, Marsh DJ, Hyland VJ, Robinson BG, Frilling A, Verellen-Dumoulin C & Safar A. Diverse phenotypes associated with exon 10 mutations of the RET proto-oncogene. Human Molecular Genetics 1994 3 2163–2167. (https://doi.org/10.1093/hmg/3.12.2163)

61 Castinetti F, Qi XP, Walz MK, Maia AL, Sanzo G, Peczowski M, Hasse-Lazar K, Links TP, Dvorakova S, Toledo RA, et al. Outcomes of adrenal-sparing surgery or total adrenalectomy in pheochromocytoma associated with multiple endocrine neoplasia type 2: an international retrospective population-based study. Lancet: Oncology 2014 15 648–655. (https://doi.org/10.1016/S1470-2045(14)70154-8)

62 Bilezikian JP, Bandeira L, Khan A & Casano NE. Hyperparathyroidism. Lancet 2018 391 168–178. (https://doi.org/10.1016/S0140-6736(17)31430-7)
65 Verbeek HH, de Groot JW, Sluiter WJ, Muller Kobold AC, van den Heuvel ER, Plukker JT & Links TP. Calcitonin testing for detection of medullary thyroid cancer in people with thyroid nodules. Cochrane Database of Systematic Reviews 2020 3 CD010159. (https://doi.org/10.1002/14651858.CD010159.pub2)

66 Opsahl EM, Akslen LA, Schlichting E, Aas T, Brauckhoff K, Hagen AI, Rosenlund AF, Sigstad E, Groholt KK, Maehle L, et al. Trends in diagnostics, surgical treatment, and prognostic factors for outcomes in medullary thyroid carcinoma in Norway: a nationwide population-based study. European Thyroid Journal 2019 8 31–40. (https://doi.org/10.1159/000493977)

67 Machens A & Dralle H. Surgical cure rates of sporadic medullary thyroid cancer in the era of calcitonin screening. European Journal of Endocrinology 2016 175 219–228. (https://doi.org/10.1530/EJE-16-0325)

68 Saltiki K, Rentziou G, Stamatelopoulos K, Georgiopoulos G, Stavrianos C, Lambrinoudaki E & Alevizaki M. Small medullary thyroid carcinoma: post-operative calcitonin rather than tumour size predicts disease persistence and progression. European Journal of Endocrinology 2014 171 117–126. (https://doi.org/10.1530/EJE-14-0076)

69 Elisei R & Romei C. Calcitonin estimation in patients with nodular goiter and its significance for early detection of MTC: European comments to the guidelines of the American Thyroid Association. Thyroid Research 2013 6 (Supplement 1) S2. (https://doi.org/10.1186/1756-6614-6-S1-S2)

70 Skandarajah A, Barlier A, Morlet-Barlat N, Sebag F, Enjalbert A, Conte-Devolx B & Henry JF. Should routine analysis of the MEN1 gene be performed in all patients with primary hyperparathyroidism under 40 years of age? World Journal of Surgery 2010 34 1294–1298. (https://doi.org/10.1007/s00268-009-0388-5)

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