

Title Page

**PRIMARY HYPERPARATHYROIDISM AS FIRST MANIFESTATION IN MEN 2A: AN INTERNATIONAL
MULTICENTER STUDY**

¹Louise Vølund Larsen, ²Delphine Mirebeau-Prunier, ³Tsuneo Imai, ⁴Cristina Alvarez-Escola, ⁵Kornelia Hasse-Lazar, ⁶Simona Censi, ⁷Luciana A. Castroneves, ⁸Akihiro Sakurai, ⁹Minoru Kihara, ¹⁰Kiyomi Horiuchi, ¹¹Véronique Dorine Barbu, ¹²Francoise Borson-Chazot, ¹³Anne-Paule Gimenez-Roqueplo, ¹⁴Pascal Pigny, ¹⁵Stephane Pinson, ¹⁶Nelson Wohllk, ¹⁷Charis Eng, ¹⁸Berna Imge Aydogan, ¹⁹Dhananjaya Saranath, ²⁰Sarka Dvorakova, ²¹Frederic Castinetti, ²²Attila Patocs, ²³Damijan Bergant, ²⁴Thera P. Links, ²⁵Mariola Peczkowska, ²⁶Ana O. Hoff, ²⁷Caterina Mian, ²⁸Trisha Dwight, ²⁹Barbara Jarzab, ³⁰Hartmut P. H. Neumann, ³¹Mercedes Robledo, ³²Shinya Uchino, ³³Anne Barlier, ³⁴Christian Godballe, ³⁵Jes Sloth Mathiesen

¹Louise Vølund Larsen, MD

Department of ORL Head & Neck Surgery and Audiology, Odense University Hospital, Odense, Denmark.

louise.voelund91@gmail.com

²Delphine Mirebeau-Prunier, MD

Laboratoire de Biochimie et Biologie Moléculaire, CHU Angers, Université d'Angers, UMR CNRS 6015, INSERM U1083, MITOVASC, Angers, France.

Réseau TenGen, France.

deprunier@chu-angers.fr

³Tsuneo Imai, MD, PhD

Department of Breast & Endocrine Surgery, National Hospital Organization, Higashinagoya National Hospital, 5-101 Umemorizaka, Meito-ku, Nagoya, 465-8620, Japan.

timai@aichi-med-u.ac.jp

⁴Cristina Alvarez-Escola, MD, PhD

Endocrinology and Nutrition Department, University Hospital "La Paz", Madrid, Spain.

escola.cristina@gmail.com

⁵Kornelia Hasse-Lazar, MD

Department of Nuclear Medicine and Endocrine Oncology, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Gliwice, Poland.

kornelia.hasse-lazar@io.gliwice.pl

⁶Simona Censi, MD

Endocrinology Unit, Department of Medicine (DIMED), University of Padua, Padua, Italy.

ssimonacensi@gmail.com

⁷Luciana A. Castroneves, MD

Department of Endocrinology, Endocrine Oncology Unit, Instituto do Cancer do Estado de São Paulo, Faculdade de Medicina da Universidade de São Paulo.

lu_audi@hotmail.com

⁸Akihiro Sakurai, MD, PhD

Department of Medical Genetics and Genomics, Sapporo Medical University School of Medicine,
South 1 West 17, Chuo-ku, Sapporo, 060-8556, Japan

sakurai.akihiro@sapmed.ac.jp

⁹Minoru Kihara, MD, PhD

Department of Surgery, Kuma Hospital, 8-2-35, Shimoyamate-dori, Chuo-ku, Kobe, Hyogo 650-0011,
Japan

kihara@kuma-h.or.jp

¹⁰Kiyomi Horiuchi, MD, PhD

Department of Breast and Endocrine Surgery, Tokyo Women's Medical University, 8-1 Kawada-cho
Shinjuku-ku, Tokyo 162-8666, Japan

horouchi.kiyomi@twmu.ac.jp

¹¹Véronique Dorine Barbu, MD, PhD

AP-HP. Sorbonne Université, Laboratoire Commun de Biologie et Génétique Moléculaires, Hôpital St Antoine
& INSERM CRSA, Paris, France.

Réseau TenGen, France.

veronique.barbu@inserm.fr

¹²Francoise Borson-Chazot, MD, PhD

Fédération d'Endocrinologie, Hospices Civils de Lyon, HESPER EA 7425 et Université Lyon 1, France.

Réseau TenGen, France.

francoise.borson-chazot@chu-lyon.fr

¹³Anne-Paule Gimenez-Roqueplo, MD, PhD

Service de Génétique, AP-HP, Hôpital européen Georges Pompidou, F-75015 Paris, France

Université de Paris, PARCC, INSERM, F-75015 Paris, France

Réseau TenGen, France.

anne-paule.gimenez-roqueplo@aphp.fr

¹⁴Pascal Pigny, PhD

Laboratoire de Biochimie et Oncologie Moléculaire, CHU Lille, France.

Réseau TenGen, France.

pascal.pigny@chru-lille.fr

¹⁵Stephane Pinson, MD, PhD

Laboratoire de Génétique Moléculaire, CHU Lyon, France.

Réseau TenGen, France.

stephane.pinson@chu-lyon.fr

¹⁶Nelson Wohllk, MD

Endocrine Section, Hospital del Salvador, Santiago de Chile, Department of Medicine

University of Chile, Chile.

nwohllk@gmail.com

¹⁷Charis Eng, MD, PhD

Genomic Medicine Institute, Lerner Research Institute and Taussig Cancer Institute, Cleveland Clinic,

Cleveland, Ohio, USA.

engc@ccf.org

¹⁸Berna Imge Aydogan, MD

Ankara University School of Medicine, Department of Endocrinology And Metabolic Diseases, Ankara
06410, Turkey.

imgehalici@gmail.com

¹⁹Dhananjaya Saranath, MD, PhD

Department of Molecular Medicine, Reliance Life Sciences Pvt. Ltd., Dhirubhai Ambani Life Sciences
Centre, Navi Mumbai, India.

saranathd@aol.com

²⁰Sarka Dvorakova, MD, PhD

Department of Molecular Endocrinology, Institute of Endocrinology, Prague, Czech Republic.

sarka@obloha.cz

²¹Frederic Castinetti, MD, PhD

Aix-Marseille Université, Institut National de la Santé et de la Recherche Médicale (INSERM), U1251,
Marseille Medical Genetics (MMG), Marseille, France.

Assistance Publique-Hôpitaux de Marseille (AP-HM), Department of Endocrinology, Hôpital de la
Conception, Centre de Référence des Maladies Rares de l'hypophyse HYPO, 13005, Marseille,
France.

frederic.castinetti@ap-hm.fr

²²Attila Patocs, MD, PhD

HAS-SE Momentum Hereditary Endocrine Tumors Research Group, Semmelweis University, Budapest, Hungary.

patocs.attila@med.semmelweis-univ.hu

²³Damijan Bergant, MD

Department of Surgical Oncology, Institute of Oncology, Zaloska 2, 1000 Ljubljana, Slovenia.

damijan.bergant@mf.uni-lj.si

²⁴Thera P. Links, MD, PhD

Department of Endocrinology, University of Groningen, University Medical Center Groningen, Netherlands.

t.p.links@umcg.nl

²⁵Mariola Peczkowska, MD

Department of Hypertension, Institute of Cardiology, Warsaw, Poland.

mpeczkowska@ikard.pl

²⁶Ana O. Hoff, MD, PhD

Department of Endocrinology, Endocrine Oncology Unit, Instituto do Cancer do Estado de São Paulo, Faculdade de Medicina da Universidade de São Paulo.

ana.hoff@hc.fm.usp.br

²⁷Caterina Mian, MD, PhD

Endocrinology Unit, Department of Medicine (DIMED), University of Padua, Padua, Italy.

caterina.mian@unipd.it

²⁸Trisha Dwight, PhD

Cancer Genetics, Kolling Institute, Royal North Shore Hospital and University of Sydney, Sydney, New South Wales, Australia.

trisha.dwight@sydney.edu.au

²⁹Barbara Jarzab, MD, PhD

Department of Nuclear Medicine and Endocrine Oncology, Maria Sklodowska-Curie National Research Institute of Oncology, Gliwice Branch, Gliwice, Poland.

barbara.jarzab@io.gliwice.pl

³⁰Hartmut P. H. Neumann, MD

Section for Preventive Medicine, Medical Center-University of Freiburg, Faculty of Medicine, Albert Ludwigs-University of Freiburg, Freiburg, Germany.

hartmut.neumann@uniklinik-freiburg.de

³¹Mercedes Robledo, PhD

Hereditary Endocrine Cancer Group, Spanish National Cancer Research Center (CNIO), Madrid, Spain.
Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Madrid, Spain.

mrobledo@cnio.es

³²Shinya Uchino, MD, PhD

Department of Endocrine Surgery, Noguchi Thyroid Clinic and Hospital Foundation, 7-52 Aoyama, Beppu, Oita 874-0902, Japan.

uchino@noguchi-med.or.jp

³³Anne Barlier, MD, PhD

Aix Marseille Univ, APHM, INSERM, MMG, Laboratory of Molecular Biology, Hospital La Conception, APHM, Marseille, France.

Réseau TenGen, France.

anne.barlier@ap-hm.fr

³⁴Christian Godballe, MD, PhD

Department of ORL Head & Neck Surgery and Audiology, Odense University Hospital, Odense, Denmark.

christian.godballe@rsyd.dk

³⁵Jes Sloth Mathiesen, MD, PhD (corresponding author)

Department of ORL Head & Neck Surgery and Audiology, Odense University Hospital, Odense, Denmark.

Department of Clinical Research, University of Southern Denmark, Odense, Denmark.

jes_mathiesen@yahoo.dk

Short title: PHPT as first manifestation of MEN 2A

Keywords: primary hyperparathyroidism, multiple endocrine neoplasia type 2A, *RET*, medullary thyroid carcinoma, pheochromocytoma

Word count: 2204

Abstract

OBJECTIVE: Multiple endocrine neoplasia type 2A (MEN 2A) is a rare syndrome caused by *RET* germline mutations, and have 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 centres 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, 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.

Main body

INTRODUCTION

Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant inherited cancer syndrome caused by germline mutations of the *RE*arranged during *Transfection* (*RET*) proto-oncogene¹⁻⁶. The syndrome is divided into MEN 2A and MEN 2B with a point prevalence of 13-24 per million and 1-2 per million, respectively⁷⁻¹⁰. 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 HSCR¹¹⁻¹⁴. 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 patients¹³, 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 series including only those with PHPT^{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 centres 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. 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 ¹⁷⁻²⁶.

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, 2019 ²⁷. For inclusion of MEN 2A patients only, 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 biochemical (hypercalcemia and an elevated or inappropriately normal parathyroid hormone level

³⁰) and histological 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, College Station, TX, USA).

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 centres 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, 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 10 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 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 that 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, 32-35}. 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, 19-21, 38-45}.

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, 32-34}. 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 in this cohort of MEN2A index cases presenting with PHPT as first manifestation is expected, as carriers of codon 634 mutations are regarded as having the highest penetrance of PHPT^{6, 46}. Given the fact, that long-term biochemical cure only rarely occurs in node-positive MTC⁴⁹, the likelihood of cure as indicated by our cohort is supposedly very low for MEN 2A index cases that present with PHPT as their first manifestation. Due to the high prevalence of regional lymph node metastases in these cases, neck dissection is often warranted already at primary surgery for better local control. Although controversial, the preoperative serum calcitonin level may also guide this decision, despite the fact that high levels not always guarantee metastases⁵⁰⁻⁵². On a general comment, the cohort of cases presenting with PHPT as first manifestation is small making generalizations difficult.

Limitations

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 in an

unselected population-based cohort of cases with apparently sporadic PHPT, in which all had been *RET* screened. To our knowledge, no such cohorts exist, 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.

An issue that may underestimate the prevalence is the fact that our study cohort consists of already recognized MEN 2A index cases. Thus, we cannot rule out that some MEN 2A index cases presenting with PHPT as first manifestation, are still unrecognized as MEN 2A cases, if they have not been *RET* screened and instead are still regarded as sporadic PHPT cases. To comply with this, a study cohort of apparently sporadic PHPT cases is needed as previously described. However, as the first *RET* germline mutations causing MEN 2A were discovered >25 years ago^{1, 2} combined with the fact, that de novo mutations rarely occur⁵³, one may argue that the pool of unrecognized MEN 2A families arising from de novo mutations likely is very small, thus minimizing the issue.

As in several other multicenter studies on MEN 2, selection bias in the current study cannot be ruled out^{6, 15, 28, 29, 54-63}. 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.

A limitation of the study is the lack of preoperative data, especially regarding ultrasonography and serum calcitonin. This hinders 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 not⁶⁴. In some patients the preoperative serum calcitonin will likely be measured as a consequence of thyroid nodules found by ultrasonography⁶⁵⁻⁶⁹. Some authors have suggested systematically preoperative calcitonin measurements in patients with apparently sporadic PHPT to exclude potential MEN 2 cases⁷⁰. 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.

DECLARATION OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

FUNDING

SD received a national grant (AZV 16-32665A).

AUTHOR CONTRIBUTION STATEMENT

JS. Mathiesen conceived the study, drafted, revised and approved the manuscript. LV. Larsen collected the data, revised and approved the manuscript. The remaining authors contributed data, critically revised and gave final approval of the manuscript.

ACKNOWLEDGEMENTS

AP would like to thank Dr. Judit Toke, Pr. Dr. Miklós Tóth, Pr. Dr. Péter Igaz and Prof. Dr. Károly Rác (2nd Department of Medicine, Semmelweis University, Budapest, Hungary) for their help in collecting clinical data. TPL thanks Dr. Maran Olderode-Berends (Department of Medical Genetics, University Medical Center Groningen) for supporting the genetic data. BIA and VB would like to thank Prof. Murat Faik Erdoğan (Department of Endocrinology and Metabolism, Ankara University School of Medicine, Ankara, Turkey) and Prof. Jacques Azorin (Service de Chirurgie Thoracique et Vasculaire, Hôpital Avicenne, Bobigny, France), respectively. LVL, CG and JSM thank the Danish Thyroid Cancer Study Group (DATHYRCA) and the Danish MEN 2 group.

REFERENCES

1. Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P & Wells SA, Jr. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. *Humun Molecular Genetics* 1993 **2** 851-856.
2. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L *et al.* Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 1993 **363** 458-460.
3. Carlson KM, Dou S, Chi D, Scavarda N, Toshima K, Jackson CE, Wells SA, Jr., 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. *Proceedings of the National Academy of Sciences* 1994 **91** 1579-1583.
4. Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, Pasini B, Hoppener JW, van Amstel HK, Romeo G *et al.* A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. *Nature* 1994 **367** 375-376.
5. Eng C, Smith DP, Mulligan LM, Nagai MA, Healey CS, Ponder MA, Gardner E, Scheumann GF, Jackson CE, Tunnacliffe A *et al.* Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours. *Humun Molecular Genetics* 1994 **3** 237-241.
6. E Eng C, Clayton D, Schuffenecker I, Lenoir G, Cote G, Gagel RF, 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, Svartberg J, Brauckhoff K, Maehle L, Engebretsen LF, Sigstad E, Groholt KK *et al.* 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.
8. Mathiesen JS, Kroustrup JP, Vestergaard P, Stochholm K, Poulsen PL, Rasmussen AK, Feldt-Rasmussen U, Schytte S, Pedersen HB, Hahn CH et al. Incidence and prevalence of multiple endocrine neoplasia 2A in Denmark 1901-2014: a nationwide study. *Clinical Epidemiology* 2018 **10** 1479-1487.
 9. Mathiesen JS, Kroustrup JP, Vestergaard P, Madsen M, Stochholm K, Poulsen PL, Krogh Rasmussen A, Feldt-Rasmussen U, Schytte S, Pedersen HB et al. Incidence and prevalence of multiple endocrine neoplasia 2B in Denmark: a nationwide study. *Endocrine-Related Cancer* 2017 **24** L39-l42.
 10. Znaczk 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.
 11. Wells SA, Jr., Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, Lee N, Machens A, Moley JF, Pacini F et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid* 2015 **25** 567-610.
 12. Kloos RT, Eng C, Evans DB, Francis GL, Gagel RF, Gharib H, Moley JF, Pacini F, Ringel MD, Schlumberger M et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid* 2009 **19** 565-612.
 13. Brandi ML, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri RG, Libroia 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.
 14. Traugott AL & Moley JF. Multiple endocrine neoplasia type 2: Clinical manifestations and management. *Cancer Treatment and Research* 2010 **153** 321-337.

15. Raue F, Kraimps JL, Dralle H, Cougard P, Proye C, Frilling A, Limbert E, Llenas LF & Niederle B. Primary hyperparathyroidism in multiple endocrine neoplasia type 2A. *Journal of Internal Medicine* 1995 **238** 369-373.
16. Kraimps JL, Denizot A, Carnaille B, Henry JF, Proye C, Bacourt F, Sarfati E, Dupond JL, Maes B, Travagli JP *et al.* Primary hyperparathyroidism in multiple endocrine neoplasia type IIa: retrospective French multicentric study. Groupe d'Etude des Tumeurs a Calcitonine (GETC, French Calcitonin Tumors Study Group), French Association of Endocrine Surgeons. *World Journal Surgery* 1996 **20** 808-812.
17. Lebeault M, Pinson S, Guillaud-Bataille M, Gimenez-Roqueplo AP, Carrie A, Barbu V, Pigny P, Bezieau 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.
18. Imai T, Uchino S, Okamoto T, Suzuki S, Kosugi S, Kikumori T & Sakurai A. High penetrance of pheochromocytoma in multiple endocrine neoplasia 2 caused by germ line RET codon 634 mutation in Japanese patients. *European Journal of Endocrinology* 2013 **168** 683-687.
19. Romei C, Mariotti S, Fugazzola L, Taccaliti A, Pacini F, Opocher G, Mian C, Castellano M, degli Uberti E *et al.* Multiple endocrine neoplasia type 2 syndromes (MEN 2): results from the ItaMEN network analysis on the prevalence of different genotypes and phenotypes. *European Journal of Endocrinology* 2010 **163** 301-308.
20. Maciel RMB, Camacho CP, Assumpcao LVM, Bufalo NE, Carvalho AL, de Carvalho GA, Castroneves LA, de Castro FM, Jr., Ceolin L, Cerutti JM *et al.* Genotype and phenotype landscape of MEN2 in 554 medullary thyroid cancer patients: the BrasMEN study. *Endocrine Connections* 2019 **8** 289-298.
21. Mathiesen JS, Kroustrup JP, Vestergaard P, Stochholm K, Poulsen PL, Rasmussen AK, Feldt-Rasmussen U, Gaustadnes M, Orntoft TF, van Overeem Hansen T *et al.* Distribution of RET

- Mutations in Multiple Endocrine Neoplasia 2 in Denmark 1994-2014: A Nationwide Study. *Thyroid* 2017 **27** 215-223.
22. Bergant D, Hocevar M, Besic N, Glavac D, Korosec B & Caserman S. Hereditary medullary thyroid cancer in Slovenia--genotype-phenotype correlations. *Wiener Klinische Wochenschrift* 2006 **118** 411-416.
23. Patocs A, Klein I, Szilvasi A, Gergics P, Toth M, Valkusz Z, Forizs E, Igaz P, Al-Farhat Y, Tordai A et al. Genotype-phenotype correlations in Hungarian patients with hereditary medullary thyroid cancer. *Wiener Klinische Wochenschrift* 2006 **118** 417-421.
24. Sharma BP & Saranath D. RET gene mutations and polymorphisms in medullary thyroid carcinomas in Indian patients. *Journal of Biosciences* 2011 **36** 603-611.
25. Aydogan BI, Yuksel B, Tuna MM, Navdar Basaran M, Akkurt Kocaeli A, Ertorer ME, Aydin K, Guldiken S, Simsek Y, Cihan Karaca Z et al. Distribution of RET Mutations and Evaluation of Treatment Approaches in Hereditary Medullary Thyroid Carcinoma in Turkey. *Journal of Clinical Research in Pediatric Endocrinology* 2016 **8** 13-20.
26. Diaz RE & Wohlk N. Multiple endocrine neoplasia: the Chilean experience. *Clinics (Sao Paulo)* 2012 **67 Suppl 1** 7-11.
27. Margraf RL, Crockett DK, Krautscheid PM, Seamons R, Calderon FR, Wittwer CT & Mao R. Multiple endocrine neoplasia type 2 RET proto-oncogene database: repository of MEN2-associated RET sequence variation and reference for genotype/phenotype correlations. *Human Mutation* 2009 **30** 548-556.
28. Mathiesen JS, Habra MA, Bassett JHD, Choudhury SM, Balasubramanian SP, Howlett TA, Robinson BG, Gimenez-Roqueplo AP, Castinetti F, Vestergaard P et al.. Risk Profile of the RET A883F Germline Mutation: An International Collaborative Study. *Journal of Clinical Endocrinology and Metabolism* 2017 **102** 2069-2074.

29. Castinetti F, Waguespack SG, Machens A, Uchino S, Hasse-Lazar K, Sanso 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.
30. Khan AA, Hanley DA, Rizzoli R, Bollerslev J, Young JE, Rejnmark L, Thakker R, D'Amour P, Paul T, Van Uum S *et al.* Primary hyperparathyroidism: review and recommendations on evaluation, diagnosis, and management. A Canadian and international consensus. *Osteoporosis International* 2017 **28** 1-19.
31. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A & (Eds.). *AJCC cancer staging manual 7th ed.* New York, NY: Springer, 2010.
32. Twigt BA, Scholten A, Valk GD, Rinkes 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.
33. 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 2A families with mutations at codon 634 of the RET proto-oncogene. Groupe D'etude des Tumeurs a Calcitonine. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 487-491.
34. Herfarth KK, Bartsch D, Doherty GM, Wells SA, Jr. & Lairmore TC. Surgical management of hyperparathyroidism in patients with multiple endocrine neoplasia type 2A. *Surgery* 1996 **120** 966-973; discussion 973-964.
35. Howe JR, Norton JA & Wells SA, Jr. Prevalence of pheochromocytoma and hyperparathyroidism in multiple endocrine neoplasia type 2A: results of long-term follow-up. *Surgery* 1993 **114** 1070-1077.

36. Frank-Raue 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, FIHPT]. *Deutsche Medizinische Wochenschrift* 2011 **136** 1889-1894.
37. Machens A & Dralle H. Advances in risk-oriented surgery for multiple endocrine neoplasia type 2. *Endocrine-Related Cancer* 2018 **25** T41-t52.
38. Sarika HL, Papathoma A, Garofalaki M, Saltiki K, Pappa T, Pazaitou-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.
39. Machens A, Lorenz K, Sekulla C, Hoppner W, Frank-Raue K, Raue F & Dralle H. Molecular epidemiology of multiple endocrine neoplasia 2: implications for RET screening in the new millenium. *European Journal of Endocrinology* 2013 **168** 307-314.
40. Giacche M, Panarotto A, Tacchetti MC, Tosini R, Campana F, Mori L, Cappelli C, Pirola I, Lombardi D, Pezzola DC *et al.* p.Ser891Ala 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.
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 (Basel)* 2019 **10**.
42. Mathiesen JS, Kroustrup JP, Vestergaard P, Stochholm K, Poulsen PL, Rasmussen AK, Feldt-Rasmussen U, Gaustadnes M, Orntoft 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.
43. Cunha LL, Lindsey SC, Franca MI, Sarika L, Papathoma A, Kunii IS, Cerutti JM, Dias-da-Silva MR, Alevizaki M & Maciel RM. 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.
44. Martins-Costa MC, Cunha LL, Lindsey SC, Camacho CP, Dotto RP, Furuzawa GK, Sousa MS, Kasamatsu TS, Kunii 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.
 45. Machens A, Lorenz K, Weber F & Dralle H. Geographic epidemiology of MTC families: unearthing European ancestral heritage. *Endocr Relat Cancer* 2018.
 46. Machens A, Lorenz K & Dralle 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** E336-345.
 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.
 48. Machens A, Lorenz K, Weber F & Dralle H. Genotype-specific progression of hereditary medullary thyroid cancer. *Human Mutation* 2018 **39** 860-869.
 49. Mathiesen JS, Kroustrup JP, Vestergaard P, Stochholm K, Poulsen PL, Rasmussen AK, Feldt-Rasmussen U, Schytte S, Londero SC, Pedersen HB *et al.* Survival and Long-Term Biochemical Cure in Medullary Thyroid Carcinoma in Denmark 1997-2014: A Nationwide Study. *Thyroid* 2019 **29** 368-377.
 50. Machens A & Dralle H. Surgical Treatment of Medullary Thyroid Cancer. *Recent Results in Cancer Research* 2015 **204** 187-205.
 51. Censi S, Cavedon E, Watutantrige-Fernando S, Barollo S, Bertazza L, Manso J, Iacobone M, Nacamulli D, Galuppini F, Pennelli G *et al.* Unique Case of a Large Indolent Medullary Thyroid Carcinoma: Time to Reconsider the Medullary Thyroid Adenoma Entity? *European Thyroid Journal* 2019 **8** 108-112.

52. Opsahl EM, Akslen LA, Schlichting E, Aas T, Brauckhoff K, Hagen AI, Rosenlund AF, Sigstad E, Groholt KK, Jorgensen 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.
53. Schuffenecker I, Ginet N, Goldgar D, Eng C, Chambe B, Boneu A, Houdent C, Pallo 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. Le Groupe d'Etude des Tumeurs a Calcitonine. *American Journal Human Genetics* 1997 **60** 233-237.
54. Castinetti F, Maia AL, Peczkowska M, Barontini M, Hasse-Lazar K, Links TP, Toledo RA, Dvorakova S, Mian C, Bugalho MJ *et al.* The penetrance of MEN2 pheochromocytoma is not only determined by RET mutations. *Endocrine-Related Cancer* 2017 **24** L63-L67.
55. Frank-Raue K, Rybicki LA, Erlic Z, Schweizer H, Winter 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.
56. Milos IN, Frank-Raue K, Wohllk N, Maia AL, Pusiol E, Patocs A, Robledo M, Biarnes 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 (TGC>TGG) mutation. *Endocrine-Related Cancer* 2008 **15** 1035-1041.
57. Machens A, Niccoli-Sire P, Hoegel J, Frank-Raue K, van Vroonhoven TJ, Roehler HD, Wahl RA, Lamesch P, Raue F, Conte-Devolx B *et al.* Early malignant progression of hereditary medullary thyroid cancer. *New England Journal of Medicine* 2003 **349** 1517-1525.
58. 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.

59. Modigliani E, Vasen HM, Raue K, Dralle H, Frilling A, Gheri RG, Brandi ML, Limbert E, 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.
60. Mulligan LM, Marsh DJ, Robinson BG, Schuffenecker I, Zedenius J, Lips CJ, Gagel RF, Takai SI, Noll WW, Fink M et al. Genotype-phenotype correlation in multiple endocrine neoplasia type 2: report of the International RET Mutation Consortium. *Journal of Internal Medicine* 1995 **238** 343-346.
61. Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JB, Gardner E, Ponder MA, Frilling A, Jackson CE, Lehnert H et al. Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. *Nature Genetics* 1994 **6** 70-74.
62. Mulligan LM, Eng C, Attie T, Lyonnet S, Marsh DJ, Hyland VJ, Robinson BG, Frilling A, Verellen-Dumoulin C, Safar A et al. Diverse phenotypes associated with exon 10 mutations of the RET proto-oncogene. *Human Molecular Genetics* 1994 **3** 2163-2167.
63. Castinetti F, Qi XP, Walz MK, Maia AL, Sanso G, Peczkowska 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.
64. Bilezikian JP, Bandeira L, Khan A & Cusano NE. Hyperparathyroidism. *Lancet* 2018 **391** 168-178.
65. Verbeek HH, de Groot JWB, 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.
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.
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.
68. Saltiki K, Rentziou G, Stamatelopoulos K, Georgiopoulos G, Stavrianos C, Lambrinouadaki 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.
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 Suppl 1** 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.

Table 1. Distributions of *RET* mutations among 1085 MEN 2A index cases.

<i>RET</i> 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.

Abbreviations: *RET*, REarranged during Transfection; MEN 2A, multiple endocrine neoplasia type 2A.

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

Patient no.	Sex	RET mutation	PHPT ^a			MTC ^b		PHEO ^b		HSCR ^b	CLA ^b	Follow-up	
			Age (yrs)	Histology	Symptoms	Age (yrs)	TNM ^c	Age (yrs)	Side			Age (yrs)	Status
1	F	C634Y	14	Hyperplasia	Y	14	T2N1M0		None	N	N	19	Alive
2	F	C634R	18	Adenoma	Y	18	T2N1M0	18	Bilateral ^d	N	N	30	Dead
3	M	C634Y	19	Adenoma	Y	19	T2N0M0	27	Unilateral	N	N	30	Alive
4	F	C634R	28	Hyperplasia	Y	28	T1N1M0	28	Unilateral	N	N	38	Alive
5	F	C634R	31	Adenoma	Y	46	T1N0N0	42	Bilateral	N	N	57	Alive
6	F	C634R	38	Hyperplasia	Y	38	T2N1M0	38	Bilateral	N	N	47	Alive
7	F	C611Y	40	Adenoma	Y	40	T1N1M0	40	Unilateral	N	N	47	Alive
8	M	C620R	61	Adenoma	Y	61	T3N1M1		None	N	N	75	Dead
9	F	E768D	61	Adenoma	Y	61	T1N1M0		None	N	N	66	Alive
10	F	C618F	68	Adenoma	NA	68	T2NxMx	80	Unilateral	N	N	90	Alive

Abbreviations: MEN 2A, multiple endocrine neoplasia type 2A; PHPT, primary hyperparathyroidism; *RET*, REarranged during Transfection; MTC, medullary thyroid carcinoma; PHEO, pheochromocytoma; HSCR, Hirschsprung disease; CLA, cutaneous lichen amyloidosis; Y, yes; N, no; NA, not available.

^aDefined by biochemistry³⁰ and histology.

^bDefined by histology.

^cStaging was based on the American Joint Committee on Cancer seventh edition³¹.

^dMalignant.

Appendix 1. MEN 2A index cases.

Center	Country	Principal investigator	Patients
TenGen	France	Delphine Prunier-Mirebeau	356
Matsumoto	Japan	Akihiro Sakurai	229
Madrid	Spain	Mercedes Robledo	80
Freiburg	Germany	Hartmut P. H. Neumann	75
Gliwice	Poland	Barbara Jarzab	57
Sydney	Australia	Trisha Dwight	40
Padua	Italy	Caterina Mian	36
Sao Paulo	Brazil	Ana O. Hoff	32
Warsaw	Poland	Mariola Peczkowska	28
Denmark	Denmark	Jes Sloth Mathiesen	28
Gronningen	Netherlands	Thera P. Links	23
Ljubljana	Slovenia	Damijan Bergant	22
Budapest	Hungary	Attila Patocs	16
Marseille	France	Frederic Castinetti	16
Prague	Czech Republic	Sarka Dvorakova	16
Mumbai	India	Dhananjaya Saranath	13
Ankara	Turkey	Berna Imge Aydogan	10
Cleveland	US	Charis Eng	5
Santiago de Chile	Chile	Nelson Wohlk	3

Abbreviations: MEN 2A, multiple endocrine neoplasia 2A.