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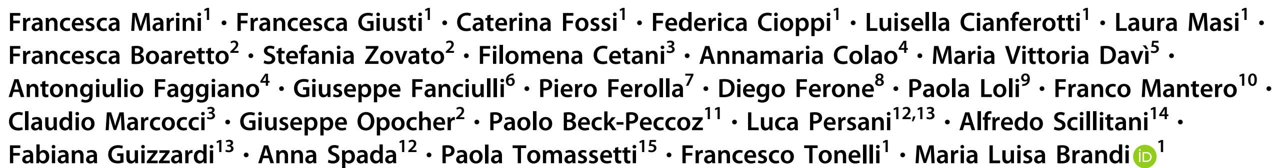
ORIGINAL ARTICLE

Multiple endocrine neoplasia type 1: analysis of germline *MEN1* mutations in the Italian multicenter MEN1 patient database



Francesca Marini[1] · Francesca Giusti[1] · Caterina Fossi[1] · Federica Cioppi[1] · Luisella Cianferotti[1] · Laura Masi[1] · Francesca Boaretto[2] · Stefania Zovato[2] · Filomena Cetani[3] · Annamaria Colao[4] · Maria Vittoria Davì[5] · Antongiulio Faggiano[4] · Giuseppe Fanciulli[6] · Piero Ferolla[7] · Diego Ferone[8] · Paola Loli[9] · Franco Mantero[10] · Claudio Marcocci[3] · Giuseppe Opocher[2] · Paolo Beck-Peccoz[11] · Luca Persani[12,13] · Alfredo Scillitani[14] · Fabiana Guizzardi[13] · Anna Spada[12] · Paola Tomassetti[15] · Francesco Tonelli[1] · Maria Luisa Brandi[1]

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Abstract

Purpose Multiple endocrine neoplasia type 1 (MEN1) is caused by germline inactivating mutations of the *MEN1* gene. Currently, no direct genotype–phenotype correlation is identified. We aim to analyze *MEN1* mutation site and features, and possible correlations between the mutation type and/or the affected menin functional domain and clinical presentation in patients from the Italian multicenter MEN1 database, one of the largest worldwide *MEN1* mutation series published to date.

Methods The study included the analysis of *MEN1* mutation profile in 410 MEN1 patients [370 familial cases from 123 different pedigrees (48 still asymptomatic at the time of this study) and 40 single cases].

Results We identified 99 different mutations: 41 frameshift [small intra-exon deletions (28) or insertions (13)], 13 nonsense, 26 missense and 11 splicing site mutations, 4 in-frame small deletions, and 4 intragenic large deletions spanning more than one exon. One family had two different inactivating *MEN1* mutations on the same allele. Gastro-entero-pancreatic tumors

The original version of this article was revised: The missing entries in Table 2 of the PDF version of the article has been added.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12020-018-1566-8) contains supplementary material, which is available to authorised users.

✉ Maria Luisa Brandi
marialuisa.brandi@unifi.it

[1] Department of Surgery and Translational Medicine, University of Florence, Florence, Italy

[2] Familial Cancer Clinic, Veneto Institute of Oncology IRCCS, Padua, Italy

[3] Department of Clinical and Experimental Medicine, Section of Endocrinology, University of Pisa, Pisa, Italy

[4] Endocrinology Unit, Department of Clinical Medicine and Surgery, University of Naples Federico II, Naples, Italy

[5] Internal Medicine, Section of Endocrinology, Department of Medicine, University of Verona, Verona, Italy

[6] NET Unit, Department of Clinical and Experimental Medicine, University of Sassari, Sassari, Italy

[7] Multidisciplinar NET Center, Umbria Regional Cancer Network, Azienda Ospedaliera di Perugia and University of Perugia, Perugia, Italy

[8] Endocrinology Unit, Department of Internal Medicine and Medical Specialties (DiMI), Policlinico San Martino, University of Genoa, Genoa, Italy

[9] Department of Endocrinology, Hospital Niguarda Ca' Granda, Milan, Italy

[10] Division of Endocrinology, Department of Medicine (DIMED), University of Padua, Padua, Italy

[11] University of Milan, Milan, Italy

[12] Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy

[13] Division of Endocrine and Metabolic Diseases, IRCCS Istituto Auxologico Italiano, Milan, Italy

[14] Unit of Endocrinology 'Casa Sollievo della Sofferenza' Hospital, IRCCS, San Giovanni Rotondo, Foggia, Italy

[15] Department of Medical and Surgical Sciences, S. Orsola-Malpighi University Hospital, Bologna, Italy

resulted more frequent in patients with a nonsense mutation, and thoracic neuroendocrine tumors in individuals bearing a splicing-site mutation.

Conclusions Our data regarding mutation type frequency and distribution are in accordance with previously published data: *MEN1* mutations are scattered through the entire coding region, and truncating mutations are the most common in MEN1 syndrome. A specific direct correlation between *MEN1* genotype and clinical phenotype was not found in all our families, and wide intra-familial clinical variability and variable disease penetrance were both confirmed, suggesting a role for modifying, still undetermined, factors, explaining the variable MEN1 tumorigenesis.

Keywords Multiple endocrine neoplasia type 1 · Genetic test · *MEN1* inactivating mutations · Genotype–phenotype correlation

Introduction

Multiple endocrine neoplasia type 1 (MEN1; MIM#131100) is an inherited rare endocrine tumor syndrome, affecting primarily the parathyroids, the anterior pituitary, and the neuroendocrine tissues of the gastro-entero-pancreatic (GEP) and thoracic tracts. MEN1 patients can develop varying combinations of more than 20 endocrine and non-endocrine tumors and lesions, presenting highly variable spectra of clinical manifestations even among members of the same family and identical twins. The responsible gene, the tumor suppressor *MEN1*, was identified in 1997 at 11q13.1 locus (OMIM gene/locus number 613,733), and it consists of 10 exons encoding a 610 amino acid nuclear protein, named menin. Germline inactivating mutations of the *MEN1* gene are responsible for the development of the syndrome. MEN1 is an autosomal dominant syndrome that can be inherited from the affected parent (familial form; over 90% of all reported cases) or, more rarely, occurs as a consequence of an embryonic de novo heterozygote *MEN1* mutation (non-familial form; accounting for less than 10% of cases) [1]. MEN1 mutations are identified in 80–90% of probands with familial disease, and in a smaller percentage of simplex cases. To date, over 1500 germinal and somatic mutations of the *MEN1* gene have been identified in familial and single cases [2, 3].

Mutation analysis of the *MEN1* gene can confirm the clinical diagnosis of the index case, and allow the early identification of asymptomatic mutation carriers, years before a MEN1-associated hormonal abnormality and/or tumor mass can be detected [2, 4]. During the last two decades, an increasingly frequent application of the genetic test has forwarded the diagnosis of the disease, allowing an early tumor-surveillance screening program of mutation carriers, and granting the reduction of syndrome-derived morbidity and mortality. Unfortunately, as reported by most worldwide epidemiology studies, a clear, direct genotype–phenotype correlation has not been identified, strongly reducing the possibility to foresee the exact future clinical manifestations associated with a specific gene mutation or protein mutated region [2, 5–7]. Recently, two studies by the "Groupe d'étude des tumors endocrines (GTE)" reported a trend for intra-familial correlation of the disease's clinical presentation and severity, and heritability of some MEN1 tumors, but without any direct genotype–phenotype correlation [8, 9]. The first study reported a two-fold increased risk of death in patients bearing a *MEN1* mutation affecting menin domain interacting with the transcription factor JunD [8]. The second evidenced a positive intra-familial heritability, but only for three specific MEN1-associated tumor types, namely pituitary adenomas, adrenal tumors, and thymic tumors [9]. However, this appears to be only as a minor intra-familial correlation that progressively decreases with the degree of the genetic relationship, suggesting, together with the absence of a direct genotype–phenotype correlation, the existence of other possible modifying genetic and epigenetic factors influencing MEN1 clinical phenotypes.

In this study, we described the *MEN1* mutation profile in 410 MEN1 patients [a total of 370 familial cases from 123 different pedigrees (of which 48 were still non-presenting any biochemical or imaging sign of tumors or any symptoms of MEN1 at the time of this study) and 40 single cases], collected through the Italian multicenter MEN1 patient database [10], one of the largest worldwide *MEN1* mutation series published to date. We described and analyzed the main characteristics of identified *MEN1* mutations and investigated possible direct correlations between the mutation type and/or the affected menin functional domain and the disease clinical presentation.

Materials and methods

Patients

Patients were, retrospectively and prospectively, collected in the "Italian MEN1 Database" [10] from 2011 to May 2017 (retrospective data included in the database were derived from the Italian Registry of Multiple Endocrine Neoplasia (RINEM), originally created in 1991), along with their birth date, gender, personal and familial clinical

history, first MEN1-manifestation (type and age of onset), age at MEN1 genetic and/or clinic diagnosis, all MEN1-associated endocrine and non-endocrine lesions, all MEN1-related surgical and medical treatments and the result of the *MEN1* genetic test. The Italian MEN1 database includes data from 14 different endocrine main referral centers located in 12 cities from 9 different Italian regions, covering the entire national territory [10]. This study selected a total of 410 MEN1 patients with an identified *MEN1* gene mutation (176 men and 234 women; aged 1–76 years at the time of MEN1 diagnosis). MEN1 patients without an identified *MEN1* mutation were excluded from the present study.

Selected patients consisted of 370 familial cases, from 123 different pedigrees, and 40 single cases. Forty-eight subjects, one single case and 47 familial cases from 28 pedigrees, were still without any biochemical or imaging sign of tumors or any symptoms of MEN1 at the time of this study (mean age 25.9 ± 16.0 years; range 5–75 years). They were diagnosed as MEN1 only by the genetic test (mean age 19.3 ± 15.8 years; range 1–75 years). They are included, in this study, as asymptomatic, and considered only for the analysis of the distribution and features of *MEN1* mutations, but not for the study about genotype–phenotype associations.

This study was initially approved by the Internal Review Board of the University of Florence (coordinating center) and then by the local ethics committee of each of the participating centers. All patients gave informed consent for genetic analyses. Data collected were made appropriately anonymous and each patient was identified, during this study, by a unique alphanumeric identification code; data were also analyzed as aggregates.

MEN1 gene mutation analysis

Mutational analysis of the *MEN1* gene had been previously performed in all MEN1 patients included in the database. Mutation screening included the PCR-based Sanger's sequencing analysis of the coding region (exons 2–10) and the exon-intron junctions (splicing sites) of the *MEN1* gene (this test usually fails in detecting a MEN1 in about 10–15% of affected individuals, not covering promoter and untranslated regions and not being able to identify large intragenic deletions/insertions) [11]. Obtained sequences were compared to the wild type reference sequence of the *MEN1* gene (OMIM 613733); mutations were classified using the standard nomenclature for the description of human DNA sequence variants. Benign *MEN1* polymorphisms [2] were distinguished from mutations, during sequencing analysis, for genetic diagnosis. Non-synonymous missense mutations have been considered as pathogenic if: (1) they were previously reported as associated with the development of the syndrome in MEN1 pedigrees, in published literature; (2) they were reported as pathogenic variations in mutation databases (i.e., Human Mutation Database, OMIM, etc); (3) they were novel but showed to segregate with the development of MEN1 within our pedigree/s. Exon–intron junction mutations were considered as pathological if they have been previously described in literature or reported in mutation databases as associated with the development of the syndrome. If novel, they were analyzed by specific in silico prediction tools of splicing site analysis (i.e., Human Splicing Finder Version 2.4.1 at http://www.umd.be/HSF/). One non-familial case was found with the IVS5 + 27 C > T variation in exon 5; in silico analysis failed to find any alteration of the splicing sites and no relatives were available for the analysis of disease co-segregation with genotype. She was considered as non-carrier of a pathogenic *MEN1* mutation and excluded from this study.

When a *MEN1* mutation was detected, the mutation screening was extended to first-degree relatives, independently of the presence of specific MEN1-related signs and symptoms. Four MEN1 pedigrees were analyzed by multiplex ligation-dependent probe amplification (MPLA), a probe-based method for the detection of gene copy number change and gross intra-genic deletions, insertions or rearrangements, after they resulted negative for *MEN1* mutation by the sequencing analysis.

Fifteen clinically affected MEN1 patients [4.0% of all the, genetically screened, affected patients (377); 6 familial cases from 3 pedigrees and 9 non-familial cases] resulted to be negative to the sequencing analysis of the *MEN1* gene. MPLA has not yet been performed, at the time of this study, in all these patients. Four *MEN1*-negative non-familial cases were analyzed for mutation of the *CDKN1B* gene and they all resulted to be negative.

No additional multigene panel screening was performed in all our patients, to date.

Data analysis

Mutation distribution and classification were analyzed by descriptive statistics; data are presented as nominal categories and percentages.

Correlation between age of onset of the first clinical manifestation and mutation type and/or mutated gene region was analyzed by Student's *t*-test, assuming a positive significance with $p < 0.05$ (only groups of mutations including more than 20 patients were included in the analyses).

Correlations between clinical data and mutation type and/or mutated gene region were analyzed by chi-squared test, assuming a positive significance with $p < 0.05$ (Yates' correction was applied for clinical categories with less than five cases).

Table 1 Localization of *MEN1* mutations identified in our series of patients

Exon/intron	Different mutations (*n*)	Type of mutation	Truncating mutations[a]	Non-truncating mutations[b]	Total MEN1 patients bearing mutations
Exon 2	21	12 Frameshift (6 deletions; 6 insertions)	15	6	72
		1 In-frame deletion			
		2 Nonsense			
		5 Missense			
		1 Splicing site			
Intron 2	1	1 Splicing site	1	0	1
Exon 3	11	4 Frameshift (4 deletions)	4	7	37
		1 In-frame deletion			
		6 Missense			
Intron 3	2	2 Splicing site	2	0	5
Exon 4	4	1 Frameshift (1 deletion)	1	3	11[c]
		3 Missense			
Intron 4	3	3 Splicing site	3	0	28
Exon 5	3	1 In-frame deletion	1	2	10
		1 Nonsense			
		1 Missense			
Intron 5	2	2 Splicing site	2	0	3
Exon 6	3	2 Frameshift (2 deletions)	2	1	7
		1 Missense			
Intron 6	0	NA	NA	NA	0
Exon 7	6	4 Frameshift (3 deletions; 1 insertion)	5	1	19
		1 Nonsense			
		1 Missense			
Intron 7	2	2 Splicing site	2	0	4
Exon 8	5	3 Frameshift (3 deletions)	3	2	20[c]
		2 Missense			
Intron 8	0	NA	NA	NA	0
Exon 9	14	3 Frameshift (3 deletions)	6	8	100
		1 In-frame deletion			
		3 Nonsense			
		7 Missense			
Intron 9	0	NA	NA	NA	0
Exon 10	18	12 Frameshift (6 deletions; 6 insertions)	18	0	89
		6 Nonsense			
Total	95		65	30	402 Mutated patients[c]

NA non-applicable

[a]Truncating mutations include frameshift, nonsense, splicing and large intra-genic deletions

[b]Non-truncating mutations include missense and in-frame deletions

[c]Four patients bear one mutation in exon 4 and one mutation in exon 8

Results

Sequencing analysis of the *MEN1* gene identified 95 different inactivating mutations located in the coding region and splicing sites of the gene; MPLA analysis identified four large intra-genic deletions within the *MEN1* gene, all of them including more than one exon.

Table 2 Main characteristics of *MEN1* mutations in our MEN1 patients

Mutation	Reference	Type	Exon	Intron	Affected codon	Amino acid substitution	Premature stop codon	Main effect on menin protein	Number of MEN1 families bearing the mutation (total members)	Number of non-familial MEN1 cases bearing the mutation	Total number of MEN1 patients bearing the mutation
g.197delA	Novel[b]	Frameshift (deletion)	2	NA	29	NA	118 (TGA)	Shortened menin of only 117 amino acids, lacking all three NLSs	1 (2)	0	2
g.301_305dup5	Bassett [17]	Frameshift (insertion)	2	NA	67	NA	120 (TGA)	Shortened menin of only 119 amino acids, lacking all three NLSs	0	1	1
g.302 insA	Concolino [3]	Frameshift (insertion)	2	NA	64	NA	116 (TGA)	Shortened menin of only 115 amino acids, lacking all three NLSs	1 (4)	0	4
g.308_312ins5	Giraud [18]	Frameshift (insertion)	2	NA	66	NA	114 (TGA)	Shortened menin of only 113 amino acids, lacking all three NLSs	2 (5)	0	5
g.317_320ins4	Vannucci [19]	Frameshift (insertion)	2	NA	69	NA	117 (TGA)	Shortened menin of only 116 amino acids, lacking all three NLSs	1 (6)	0	6
g.317_321ins5	Cebrian [20]	Frameshift (insertion)	2	NA	69	NA	120 (TGA)	Shortened menin of only 119 amino acids, lacking all three NLSs	3 (7)	1	8
g.317delC	Agarwal [21]	Frameshift (deletion)	2	NA	69	NA	118 (TGA)	Shortened menin of only 117 amino acids, lacking all three NLSs	2 (8)	0	8
g.335delA	Novel[b]	Frameshift (deletion)	2	NA	76	NA	118 (TGA)	Shortened menin of only 117 amino acids, lacking all three NLSs	0	1	1
g.357_360del4	Agarwal [21]	Frameshift (deletion)	2	NA	83	NA	118 (TGA)	Shortened menin of only 116 amino acids, lacking all three NLSs	0	1	1
g.359_362del4	Sakurai [22]	Frameshift (deletion)	2	NA	83	NA	117 (TGA)	Shortened menin of only 116 amino acids, lacking all three NLSs	4 (10)	0	10
g.445insC	Novel[b]	Frameshift (insertion)	2	NA	111	NA	116 (TGA)	Shortened menin of only 115 amino acids, lacking all three NLSs	0	1	1
g.531delC	Novel[b]	Frameshift (deletion)	2	NA	140	NA	184 (TAG)	Shortened menin of only 183 amino acids, lacking all three NLSs	1 (1)	0	1
g.579delG	Nuzzo [23]	Frameshift (deletion)	3	NA	157	NA	184 (TAG)	Shortened menin of only 183 amino acids, lacking all three NLSs	1 (4)	0	4
g.613delT	Novel[b]	Frameshift (deletion)	3	NA	167	NA	184 (TAG)	Shortened menin of only 183 amino acids, lacking all three NLSs	1 (3)	0	3
g.734_737del4	Novel[b]	Frameshift (deletion)	3	NA	208	NA	222 (TGA)	Shortened menin of only 221 amino acids, lacking all three NLSs	1 (5)	0	5
g.738_741del4	Chandrasekharappa [24]	Frameshift (deletion)	3	NA	210	NA	222 (TGA)	Shortened menin of only 221 amino acids, lacking all three NLSs	2 (4)	0	4
g.868delC	Novel[b]	Frameshift (deletion)	4	NA	253	NA	279 (TAG)	Shortened menin of only 278 amino acids, lacking all three NLSs	1 (1)	1	2
g.953_954delGA	Novel[b]	Frameshift (deletion)	6	NA	281	NA	315 (TGA)	Shortened menin of only 314 amino acids, lacking all three NLSs	1 (3)	0	3
g.1005delC	Ellard [25]	Frameshift (deletion)	6	NA	299	NA	367 (TAG)	Shortened menin of only 366 amino acids, lacking all the three NLSs	0	1	1
g.1059delC	Morelli [26]	Frameshift (deletion)	7	NA	317	NA	367 (TAG)	Shortened menin of only 366 amino acids, lacking all three NLSs	0	1	1
g.1060insC	Novel[b]	Frameshift (insertion)	7	NA	317	NA	368 (TAG)	Shortened menin of only 367 amino acids, lacking all three NLSs	0	1	1
g.1061delC	Novel[b]	Frameshift (deletion)	7	NA	317	NA	367 (TAG)	Shortened menin of only 366 amino acids, lacking all three NLSs	1 (3)	0	3
g.1071delT	Morelli [26]	Frameshift (deletion)	7	NA	321	NA	367 (TAG)	Shortened menin of only 366 amino acids, lacking all three NLSs	1 (2)	0	2

Table 2 (continued)

Mutation	Reference	Type	Exon	Intron	Affected codon	Amino acid substitution	Premature stop codon	Main effect on menin protein	Number of MEN1 families bearing the mutation (total members)	Number of non-familial MEN1 cases bearing the mutation	Total number of MEN1 patients bearing the mutation
g.1181delC[a]	Vannucci [19]	Frameshift (deletion)	8	NA	357	NA	367 (TAG)	Shortened menin of only 366 amino acids, lacking all three NLSs	1 (5)	0	5
g.1264delC	Morelli [26]	Frameshift (deletion)	8	NA	385	NA	444 (TAG)	Shortened menin of only 443 amino acids, lacking all three NLSs	1 (7)	0	7
g.1284delG	Agarwal [21]	Frameshift (deletion)	8	NA	392	NA	444 (TAG)	Shortened menin of only 443 amino acids, lacking all three NLSs	1 (1)	1	2
g.1364delC	Hai [27]	Frameshift (deletion)	9	NA	418	NA	444 (TAG)	Shortened menin of only 443 amino acids, lacking all three NLSs	1 (5)	0	5
g.1434delC	Novel[b]	Frameshift (deletion)	9	NA	441	NA	444 (TAG)	Shortened menin of only 443 amino acids, lacking all three NLSs	0	1	1
g.1449_1459del11	Giraud [18]	Frameshift (deletion)	9	NA	447	NA	526 (TGA)	Shortened menin of only 525 amino acids, lacking NLSa (AA 546–572) and NLS2 (AA 588–608) and with NLS1 (AA 479–497) altered because of aminoacidic changes from codon 447	1 (24)	0	24
g.1528_1534del7	Novel[b]	Frameshift (deletion)	10	NA	473	NA	530 (TGA)	Shortened menin of only 529 amino acids, lacking NLSa (AA 546–572) and NLS2 (AA 588–608) and with NLS1 (AA 479–497) altered because of aminoacidic changes from codon 473	1 (2)	0	2
g.1555insG	Morelli [26]	Frameshift (insertion)	10	NA	481	NA	530 (TGA)	Shortened menin of only 529 amino acids, lacking NLSa (AA 546–572) and NLS2 (AA 588–608) and with NLS1 (AA 479–497) altered because of aminoacidic changes from codon 481	3 (9)	0	9
g.1571delC	Novel[b]	Frameshift (deletion)	10	NA	487	NA	529 (TGA)	Shortened menin of only 528 amino acids, lacking NLSa (AA 546–572) and NLS2 (AA 588–608) and with NLS1 (AA 479–497) altered because of aminoacidic changes from codon 487	1 (2)	0	2
g.1631delG	Novel[b]	Frameshift (deletion)	10	NA	507	NA	558 (TGA)	Shortened menin of only 557 amino acids, lacking NLSa (AA 546–572) and NLS2 (AA 588–608). NLS1 (AA 479–497) remains intact	1 (2)	0	2
g.1656insC	Agarwal [21]	Frameshift (insertion)	10	NA	516	NA	530 (TGA)	Shortened menin of only 529 amino acids, lacking NLSa (AA 546–572) and NLS2 (AA 588–608). NLS1 (AA 479–497) remains intact	9 (25)	2	27
g.1659insG	Bartsch [28]	Frameshift (insertion)	10	NA	517	NA	530 (TGA)	Shortened menin of only 529 amino acids, lacking NLSa (AA 546–572) and NLS2 (AA 588–608). NLS1 (AA 479–497) remains intact	1 (2)	0	2
g.1671_1680del11	Concolino [3]	Frameshift (deletion)	10	NA	521	NA	526 (TGA)	Shortened menin of only 525 amino acids, lacking NLSa (AA 546–572) and NLS2 (AA 588–608). NLS1 (AA 479–497) remains intact	3 (7)	0	7
g.1690delG	Novel[b]	Frameshift (deletion)	10	NA	527	NA	561 (TGA)	Shortened menin of only 560 amino acids lacking part of NLSa (AA 546–572) and all NLS2 (AA 588–608). NLS1 (AA 479–497) remains intact	1 (1)	0	1
g.1705_1706insGG	Novel[b]	Frameshift (insertion)	10	NA	532	NA	561 (TGA)	Shortened menin of only 560 amino acids, lacking part of NLSa (AA 546–572) and all NLS2 (AA 588–608). NLS1 (AA 479–497) remains intact	1 (3)	0	3
g.1757insA	Novel[b]	Frameshift (insertion)	10	NA	549	NA	556 (TGA)	Shortened menin of only 555 amino acids, lacking part of NLSa (AA 546–572) and all NLS2 (AA 588–608). NLS1 (AA 479–497) remains intact	1 (1)	0	1
g.1786delA	Asteria [29]	Frameshift (deletion)	10	NA	559	NA	560 (TGA)		1 (5)	0	5

Table 2 (continued)

Mutation	Reference	Type	Exon	Intron	Affected codon	Amino acid substitution	Premature stop codon	Main effect on menin protein	Number of MEN1 families bearing the mutation (total members)	Number of non-familial MEN1 cases bearing the mutation	Total number of MEN1 patients bearing the mutation
								Shortened menin of only 559 amino acids, lacking part of NLSa (AA 546–572) and all NLS2 (AA 588–608). NLS1 (AA 479–497) remains intact.			
g.1937insC	Novel[b]	Frameshift (insertion)	10	NA	609	Stop611Leu	Loss of stop codon (TGA) at position 611	Insertion of a cytosine after codon 609 that leads to the loss of the stop codon at position 611. A novel codon stop (TGA) is generated at codon 632, presumably leading to a menin protein longer than normal (631 amino acids)	1 (3)	0	3
g.468_470del3	Agarwal [21]	In-frame deletion	2	NA	120	Lys120del	NA	Loss of Lysine at position 120, affecting binding site of SMAD3 (aa 40–278) and NM23H1 (aa 1–486). Menin protein of 609 amino acids.	0	1	1
g.674_691del18	Giacché [30]	In-frame deletion	3	NA	189–194	Loss of amino acids 189–194	NA	Loss of amino acids at position 189–194, affecting binding sites of JUND (aa 39–242), NMHCII-A (aa 154–306), SMAD3 (aa 40–278), HDAC1 (aa 145–450) and NM23H1 (aa 1–486). Menin protein of 604 amino acids	0	1	1
g.908_910del3	Papi [31]	In-frame deletion	5	NA	266–267	Leu267loss	NA	Loss of Leucine at position 267, affecting binding sites of NMHCII-A (aa 154–306), FANCD2 (aa 219–395), SMAD3 (aa 40–278), HDAC1 (aa 145–450) and NM23H1 (aa 1–486). Menin protein of 609 amino acids	1 (3)	0	3
g.1433_1438del6	Novel[b]	In-frame deletion	9	NA	441–443	Loss of Gln442 and Ser443	NA	Loss of Glutamine at position 442 and Serine at position 443, affecting binding sites of NM23H1 (aa 1–486), RPA2 (aa 286–448) and CHES1 (aa 428–610). Menin protein of 608 amino acids	1(2)	0	2
Gln64Stop	Langer [32]	Nonsense	2	NA	64 (CAG > TAG)	NA	64 (TAG)	Shortened menin of only 63 amino acids, lacking all three NLSs	1 (3)	0	3
Trp126Stop	Bassett [17]	Nonsense	2	NA	126 (TGG > TAG)	NA	126 (TAG)	Shortened menin of only 125 amino acids, lacking all three NLSs	2 (4)	0	4
Glu274Stop	Novel[b]	Nonsense	5	NA	274 (GAA > TAA)	NA	274 (TAA)	Shortened menin of only 273 amino acids, lacking all three NLSs	1 (1)	0	1
Thr341Stop	Cebrian [33]	Nonsense	7	NA	341 (TGG > TGA)	NA	341 (TGA)	Shortened menin of only 340 amino acids, lacking all three NLSs	1 (3)	0	3
Arg415Stop	Lemmens [34]	Nonsense	9	NA	415 (CGA > TGA)	NA	415 (TGA)	Shortened menin of only 414 amino acids, lacking all three NLSs	7 (33)	2	35
Gln442Stop	Shimizu [35]	Nonsense	9	NA	442 (CAG > TAG)	NA	442 (TAG)	Shortened menin of only 441 amino acids, lacking all three NLSs	2 (3)	0	3
Gln450Stop	Hai [36]	Nonsense	9	NA	450 (CAG > TAG)	NA	450 (TAG)	Shortened menin of only 449 amino acids, lacking all three NLSs	2 (4)	2	6
Arg460Stop	Agarwal [21]	Nonsense	10	NA	460 (CGA > TGA)	NA	460 (TGA)	Shortened menin of only 459 amino acids, lacking all three NLSs	1 (13)	2	15
Glu474Stop	Corbetta [37]	Nonsense	10	NA	474 (GAA > TAA)	NA	474 (TAA)	Shortened menin of only 473 amino acids, lacking all three NLSs	0	1	1

Table 2 (continued)

Mutation	Reference	Type	Exon	Intron	Affected codon	Amino acid substitution	Premature stop codon	Main effect on menin protein	Number of MEN1 families bearing the mutation (total members)	Number of non-familial MEN1 cases bearing the mutation	Total number of MEN1 patients bearing the mutation
Gly508Stop	Morelli [26]	Nonsense	10	NA	508 (CAG > TAG)	NA	508 (TAG)	Shortened menin of only 507 amino acids, lacking NLSa (aa 546–572) and NLS2 (aa 588–608). NLS1 remains intact	1 (2)	0	2
Arg527Stop	Chandrasekharappa [24]	Nonsense	10	NA	527 (CGA > TGA)	NA	527 (TGA)	Shortened menin of only 526 amino acids, lacking NLSa (aa 546–572) and NLS2 (aa 588–608). NLS1 remains intact	1 (2)	0	2
Glu556Stop	Jap [38]	Nonsense	10	NA	556 (GAG > TAG)	NA	556 (TAG)	Shortened menin of only 555 amino acids, lacking part of NLSa (aa 546–572) and the entire NLS2 (aa 588–608). NLS1 remains intact	0	1	1
Lys557Stop	Novel[b]	Nonsense	10	NA	557 (AAG > TAG)	NA	557 (TAG)	Shortened menin of only 556 amino acids, lacking part of NLSa (aa 546–572) and the entire NLS2 (aa 588–608). NLS1 remains intact	2 (4)	0	4
Met1Val	Villablanca [39]	Missense	2	NA	1 (ATG > GTG)	Met > Val	NA	Initial Metionin at codon 1 is substituted by a Valine. Usually initial Metionine is enzimatically removed at post-traduction level. The presence of a Valine at position 1 affects binding sites of JUND (aa 1–40), NM23H1 (aa 1–486) and RPA2 (aa 1–40). Menin protein of 611 amino acids	1 (1)	0	1
Glu45Gln	Griniatsos [40]	Missense	2	NA	45 (GAG > CAG)	Glu > Gln	NA	Single amino acid substitution at position 45, affecting binding sites of NM23H1 (aa 1–486) and SMAD3 (aa 40–278)	2 (7)	0	7
Glu45Lys	Morelli [26]	Missense	2	NA	45 (GAG > AAG)	Glu > Lys	NA	Single amino acid substitution at position 45, affecting binding site of NM23H1 (aa 1–486) and SMAD3 (aa 40–278)	0	1	1
Arg137Trp	Novel[b]	Missense	2	NA	137 (CGG > TGG)	Arg > Trp	NA	Single amino acid substitution at position 137, affecting binding sites of NM23H1 (aa 1–486) and SMAD3 (aa 40–278)	1 (2)	0	2
Phe146Ser	Vannucci [19]	Missense	2	NA	146 (TTC > TCC)	Phe > Ser	NA	Single amino acid substitution at position 146, affecting binding sites of JUND (aa 139–242), NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)	1 (2)	0	2
Asp153Glu	Filopanti [41]	Missense	3	NA	153 (GAC > GAG)	Asp > Glu	NA	Single amino acid substitution at position 153, affecting binding sites of JUND (aa 139–242), NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)	4 (8)	0	8
Gly163Arg	Novel[b]	Missense	3	NA	163 (GGG > AGG)	Gly > Arg	NA	Single amino acid substitution at position 163, affecting binding sites of JUND (aa 139–242), NMHCII-A (aa 154–306) NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)	1 (1)	0	1
His181Asp	Novel[b]	Missense	3	NA	181 (CAT > GAT)	His > Asp	NA	Single amino acid substitution at position 181, affecting binding sites of JUND (aa 139–242), NMHCII-A (aa 154–306) NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)	0	1	1
Val196Gly	Vannucci [19]	Missense	3	NA	196 (GTC > GGC)	Val > Gly	NA	Single amino acid substitution at position 196, affecting binding sites of JUND (aa 139–242), NMHCII-A (aa 154–306) NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)	1 (7)	0	7
His199Asp	Novel[b]	Missense	3	NA	199 (CAC > GAC)	His > Asp	NA	Single amino acid substitution at position 199, affecting binding sites of JUND (aa 139–242), NMHCII-A (aa 154–306) NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)	0	1	1
Val215Met	Morelli [26]	Missense	3	NA		Val > Met	NA	Single amino acid substitution at position 215, affecting binding sites of JUND (aa 139–242), NMHCII-A (aa 154–306)	1 (2)	0	2

Table 2 (continued)

Mutation	Reference	Type	Exon	Intron	Affected codon	Amino acid substitution	Premature stop codon	Main effect on menin protein	Number of MEN1 families bearing the mutation (total members)	Number of non-familial MEN1 cases bearing the mutation	Total number of MEN1 patients bearing the mutation
					215 (GTG > ATG)			NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)			
Trp220Arg	Novel[b]	Missense	4	NA	220 (TGG > CGG)	Trp > Arg	NA	Single amino acid substitution at position 220, affecting binding sites of JUND (aa 139–242), FANCD2 (aa 219–395), NMHCII-A (aa 154–306) NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)	1 (2)	1	3
Leu249Pro[a]	Vannucci [19]	Missense	4	NA	249 (CTG > CCG)	Leu > Pro	NA	Single amino acid substitution at position 249, affecting binding sites of NM23H1 (aa 1–486), FANCD2 (aa 219–395), NMHCII-A (aa 154–306), SMAD3 (aa 40–278) e HDAC1 (aa 145–450)	1 (5)	0	5
Leu256Phe	Tham [42]	Missense	4	NA	256 (CTT > TTT)	Leu > Phe	NA	Single amino acid substitution at position 256, affecting binding sites of FANCD2 (aa 219–395), NMHCII-A (aa 154–306) NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)	0	1	1
Leu273Pro	Novel[b]	Missense	5	NA	273 (CTG > CCG)	Leu > Pro	NA	Single amino acid substitution at position 273, affecting binding sites of FANCD2 (aa 219–395), NMHCII-A (aa 154–306) NM23H1 (aa 1–486), SMAD3 (aa 40–278) and HDAC1 (aa 145–450)	1 (6)	0	6
Ala284Val	Novel[b]	Missense	6	NA	284 (GCA > GTA)	Ala > Val	NA	Single amino acid substitution at position 284, affecting binding sites of FANCD2 (aa 219–395), NMHCII-A (aa 154–306) NM23H1 (aa 1–486) and HDAC1 (aa 145–450)	1 (3)	0	3
Thr344Arg	Agarwal [21]	Missense	7	NA	344 (ACG > AGG)	Thr > Arg	NA	Single amino acid substitution at position 344, affecting binding sites of JUND (aa 323–428) and NF-kB (aa 305–381), FANCD2 (aa 219–395), RPA2 (aa 286–448), NM23H1 (aa 1–486) and HDAC1 (aa 145–450)	3 (8)	1	9
Cys354Phe	Vannucci [19]	Missense	8	NA	354 (TGC > TTC)	Cys > Phe	NA	Single amino acid substitution at position 354, affecting binding sites of JUND (aa 323–428) and NF-kB (aa 305–381), FANCD2 (aa 219–395), RPA2 (aa 286–448), NM23H1 (aa 1–486) and HDAC1 (aa 145–450)	1 (4)	0	4
Pro390Arg	Novel[b]	Missense	8	NA	390 (CCG > CGG)	Pro > Arg	NA	Single amino acid substitution at position 354, affecting binding sites of JUND (aa 323–428), mSin3A (aa 371–387), FANCD2 (aa 219–395), RPA2 (aa 286–448), NM23H1 (aa 1–486) and HDAC1 (aa 145–450)	1 (2)	0	2
Leu413Pro	Asteria [29]	Missense	9	NA	413 (CTG > CCG)	Leu > Pro	NA	Single amino acid substitution at position 413, affecting binding sites of JUND (323–428), NM23H1 (1–486), RPA2 (286–448) and HDAC1 (aa 145–450)	0	1	1
Leu413Arg	Toledo [43]	Missense	9	NA	413 (CTG > CGG)	Leu > Arg	NA	Single amino acid substitution at position 413, affecting binding sites of JUND (323–428), NM23H1 (1–486), RPA2 (286–448) and HDAC1 (aa 145–450)	0	2	2
Asp418Asn	Bassett [17]	Missense	9	NA	418 (GAC > AAC)	Asp > Asn	NA	Single amino acid substitution at position 418, affecting binding sites of JUND (323–428), NM23H1 (1–486), RPA2 (286–448) and HDAC1 (aa 145–450)	2 (3)	1	4
Gly419Val	Novel[b]	Missense	9	NA	419 (GGC > GTC)	Gly > Val	NA	Single amino acid substitution at position 419, affecting binding sites of JUND (aa 323–428), NM23H1 (aa 1–486), RPA2 (286–448) and HDAC1 (aa 145–450)	0	1	1
Trp423Arg	Cebrian [20]	Missense	9	NA	423 (TGG > CGG)	Trp > Arg	NA	Single amino acid substitution at position 423, affecting binding sites of JUND (aa 323–428), NM23H1 (aa 1–486), RPA2 (aa 286–448) and HDAC1 (aa 145–450)	1 (3)	0	3
Leu444Pro	Cetani [44]	Missense	9	NA		Leu > Pro	NA		4 (11)	1	12

Table 2 (continued)

Mutation	Reference	Type	Exon	Intron	Affected codon	Amino acid substitution	Premature stop codon	Main effect on menin protein	Number of MEN1 families bearing the mutation (total members)	Number of non-familial MEN1 cases bearing the mutation	Total number of MEN1 patients bearing the mutation
Phe447Ser	Agarwal [21]	Missense	9	NA	444 (CTA > CCA) 447 (TTT > TCT)	Phe > Ser	NA	Single amino acid substitution at position 444, affecting binding sites of NM23H1 (aa 1–486), RPA2 (aa 286–448), HDAC1 (aa 145–450) and CHES1 (aa 428–610) Single amino acid substitution at position 447, affecting binding sites of NM23H1 (aa 1–486), RPA2 (aa 286–448), HDAC1 (aa 145–450) and CHES1 (aa 428–610)	1 (1)	0	1
g.104 G > A	Novel[b]	Splicing-site	2	NA	NA	NA	NA	Altering the splicing site 6 bases before the initial ATG codon within the exon 1, maybe altering binding sites of JUND (aa 1–40), NM23H1 (aa 1–486) and RPA2 (aa 1–40)	1 (3)	0	3
g.556-3 C > G	Burgess [45]	Splicing-site	NA	2	NA	NA	NA	Affecting the splicing site between intron 2 and exon 3	0	1	1
g.765-1 G > C	Balogh [46]	Splicing-site	NA	3	NA	NA	NA	Affecting the splicing site between intron 3 and exon 4	1 (4)	0	4
g.765-1 G > A	Novel[b]	Splicing-site	NA	3	NA	NA	NA	Affecting the splicing site between intron 3 and exon 4	0	1	1
g.893 + 1 G > A	Morelli [26]	Splicing-site	NA	4	NA	NA	NA	Affecting the splicing site between exon 4 and intron 4	1 (7)	0	7
g.893 + 1 G > C	Poncin [47]	Splicing-site	NA	4	NA	NA	NA	Affecting the splicing site between exon 4 and intron 4	1 (3)	0	3
g.894-9 G > A	Görtz [48]	Splicing-site	NA	4	NA	NA	NA	Affecting the splicing site between intron 4 and exon 5	5 (17)	1	18
g.935-2 A > G	Toliat [49]	Splicing-site	NA	5	NA	NA	NA	Affecting the splicing site between intron 5 and exon 6	1 (2)	0	2
g.935-18 delGA	Novel[b]	Splicing-site	NA	5	NA	NA	NA	Affecting the splicing site between intron 5 and exon 6	0	1	1
g.1159 + 1 G > A	Bassett [17]	Splicing-site	NA	7	NA	NA	NA	Affecting the splicing site between exon 7 and intron 7	1 (2)	1	3
g.1159 + 2 T > C	Han [50]	Splicing-site	NA	7	NA	NA	NA	Affecting the splicing site between exon 7 and intron 7	1 (1)	0	1
NA	NA	Large intra-genic deletion	Entire exon 1 and part of exon 2	1	NA	NA	NA	Loss of the entire exon 1 and part of exon 2. Loss of binding sites with JUND (aa 1–40), RPA2 (aa 1–40), NM23H1 (aa 1–486), SMAD3 (aa 40–278).	1 (2)	0	2
NA	NA	Large intra-genic deletion	1, 2 and 3	1 and 2	NA	NA	NA	Loss of exons 1, 2 and 3. Loss of binding sites with JUND (aa 1–40), NM23H1 (aa 1–486), NMHCII-A (aa 154–306), SMAD3 (aa 40–278) and HDAC1 (aa 145–450	1 (3)	0	3
NA	NA	Large multiple intra-genic deletions	Large intragenic deletions along the entire gene	NA	NA	NA	NA	Large intragenic deletions along the entire gene	2 (4)	0	4

NA non-applicable, *g.* genomic (nucleotide counting has been considered from the first nucleotide of the exon 1, excluding introns)

[a]These mutations were found in one family (bearing these two mutations on the same *MEN1* allele, inherited by the MEN1 index case from her paternal line)

[b]Mutations are indicated as "novel" if they are not reported in the Human Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) or they have never been published before this study

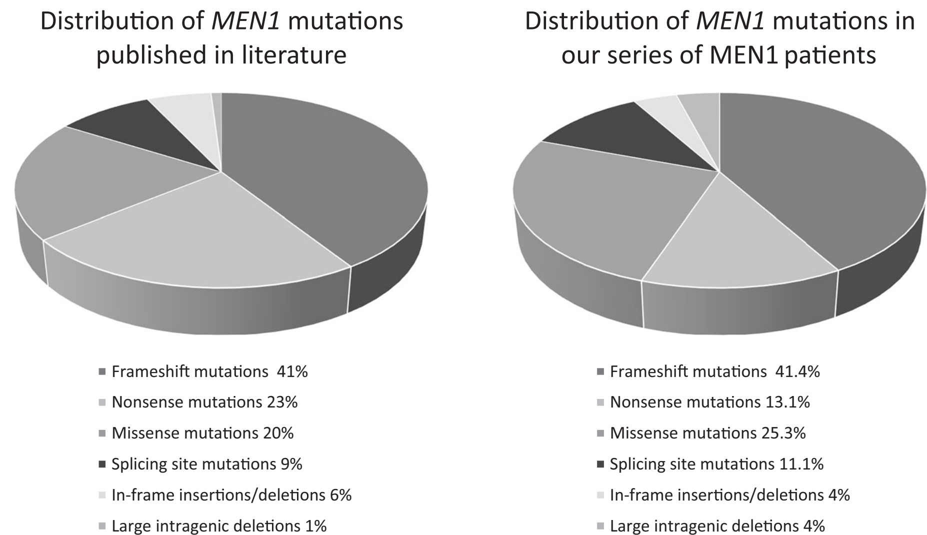

Fig. 1 Comparison of distribution of MEN1 mutation types in our series of patients with respect to published data [2, 3]

Identified mutations cover all the coding exons (2–10), and affect splicing sites at introns 2, 3, 4, 5, and 7. No specific mutational hot spots have been identified. Exons 2, 9, and 10 resulted to be the three most mutated exons, respectively with 21 [72 mutated patients: 65 familial cases (from 24 pedigrees) and 7 single cases], 14 [100 mutated patients: 89 familial cases (from 22 pedigrees) and 11 single cases], and 18 [89 mutated patients: 83 familial cases (from 29 pedigrees) and 6 single cases] different mutations (Table 1).

Our mutations included 41 (41.4%) frameshift mutations [of which 28 (28.3%) small intra-exon deletions and 13 (13.1%) insertions], 13 nonsense mutations (13.1%), 26 missense mutations (25.3%), 4 in-frame small deletions (4%), 11 splicing site mutations (11.1%) and 4 large intragenic deletions (4%) (Table 2; Fig. 1).

A common founder effect has not been identified (by reconstructing family history) for any of the families and/or non-familial cases bearing the same mutation.

We identified a MEN1 index case and four of her first-degree relatives (father, sister, and two of the sister's sons) as carriers of two different inactivating *MEN1* mutations, one in exon 4 (Leu249Pro missense mutation) and one in exon 8 (g.1181delC frameshift mutation). The genetic analysis of index case's parents identified the father as carrier of both the *MEN1* mutations, while the mother resulted to be wild type for the *MEN1* gene; both the mutations are located on the same *MEN1* allele, and inherited from the father. The double mutated index case and three mutated first-degree relatives (father, sister, and one of the sister's sons) are clinically affected, with first clinical manifestation appearing at 17, 60, 14, and 15 years, respectively, and all presenting a combination of primary hyperparathyroidism (PHPT) and active prolactinoma (PRLoma). The sister's mutated younger son (aged 13) was still asymptomatic at the time of the study.

Classification and main features of all identified *MEN1* mutations, as well as their distribution in our familial and non-familial patients, are reported, in detail, in Table 2. The distribution of MEN1-associated tumors, in our series of patients, is depicted in Tables 3 and 4, based on the *MEN1* mutation type or the *MEN1* mutated region, respectively.

No statistical correlation was found between disease age of onset and *MEN1* mutation type or localization (Table 5). No differences were found in the distribution of PHPT and pituitary tumors between different *MEN1* mutation types and localization. Statistical analyses evidenced a significantly higher percentage of GEP neuroendocrine tumors (GEP-NETs) in MEN1 clinically affected patients bearing a nonsense mutation (72.46%) with respect to frameshift mutations (51.85%; $\chi^2 = 8.44$, $p = 0.004$) and missense mutations (54.32%; $\chi^2 = 5.24$, $p = 0.022$). Also a significantly higher percentage of thoracic neuroendocrine tumors (NETs), previously referred as thoracic carcinoids, was reported in MEN1 clinically affected patients bearing a splicing-site mutation (18.42%) with respect to frameshift mutations (5.6%; $\chi^2 = 6.92$, $p = 0.009$). Conversely, no statistical association was found in the occurrence of the MEN1 main endocrine tumors, and in the number of different endocrine tumors per patient, between patients bearing a truncating mutation and patient with a non-truncating mutation (Table 6).

Nineteen patients (4.63%) died because of MEN1-related causes and malignant progression of MEN1 tumors. Thirteen died because of malignant gastrinomas [7 from the complications of uncontrolled acid peptic disease (4 intestinal perforations and 3 gastric hemorrhages) and 6 from multiple liver metastases and subsequent liver failure],

Table 3 MEN1-associated tumor distribution, in our series of patients, based on the *MEN1* mutation type

Type of mutation (*n*)	Mutated symptomatic patients	PHPT	Pituitary tumors	GEP-NETs	Thoracic NETs	Lipomas	Adrenocortical tumors/lesions[a]	Skin lesions	Other tumors/ lesions
Frameshift (41)	162[b]	155	48 PRLomas, 1 ACTH-secreting adenoma, 1 GH-PRL secreting adenoma, 14 NF adenomas, (total 64 pituitary tumors)	29 Gastrinomas, 10 insulinomas, 1 glucagonoma, 1 VIPoma, 42 NF pNETs, 1 NF gastric NET, (total 84 GEP-NETs)	7 Bronchopulmonary, 2 thymus, (total 9 thoracic NETs)	12 Multiple lipomatosis, 7 single lipomas, (total 19 cases of lipomas)	1 PHEO, 8 hyperplasias of adrenal glands, 9 adrenal gland adenomas, (total 18 cases)	3 Skin fibromas, 2 skin angiomas, 2 multiple skin angiofibromas, (total 7 skin lesions)	1 Thyroid adenoma, 3 meningiomas, 2 uterine myofibromas
In-frame deletion (4)	7	7	4 PRLomas, 1 GH-secreting adenoma, 1 NF adenoma, (total 6 pituitary tumors)	1 Gastrinoma, 2 insulinomas, 1 NF pNET, (total 4 GEP-NETs)	2 Bronchopulmonary	1 Multiple lipomatosis	1 Hyperplasia of adrenal glands, 1 adrenal gland adenoma, (total 2 cases)	0	0
Nonsense (13)	69	62	16 PRLomas, 2 ACTH-secreting adenomas, 5 NF adenomas, (total 23 pituitary tumors)	22 Gastrinomas, 6 insulinomas, 20 NF pNETs, 2 NF gastric NETs, (total 50 GEP-NETs)	4 Bronchopulmonary	2 Multiple lipomatosis, 1 single lipoma, (total 3 cases of lipomas)	2 Hyperplasias of adrenal glands, 3 adrenal gland adenomas, (total 5 cases)	2 Facial skin angiofibromas	2 Uterine myofibromas
Missense (26)	81[b]	79	23 PRLomas, 1 ACTH-secreting adenoma, 6 NF adenomas, (total 30 pituitary tumors)	18 Gastrinomas, 4 insulinomas, 2 glucagonoma, 1 VIPoma, 1 PPoma, 18 NF pNETs, (total 44 GEP-NETs)	6 Bronchopulmonary, 1 thymus, (total 7 thoracic NETs)	5 Multiple lipomatosis, 6 single lipomas, (total 11 cases of lipomas)	6 Hyperplasias of adrenal glands, 7 adrenal gland adenomas, (total 13 cases)	3 Skin angiomas, 1 skin fibroma, 1 facial skin angiofibromas, (total 5 skin lesions)	2 Uterine myofibroma, 1 uterine leiomyoma
Splicing-site mutation (11)	38	37	12 PRLomas, 1 GH-secreting adenoma, 3 NF adenomas, (total 16 pituitary tumors)	8 Gastrinomas, 3 insulinomas, 11 NF pNETs, 1 NF gastric NET, (total 23 GEP-NETs)	7 Bronchopulmonary	5 Multiple lipomatosis, 1 single lipoma, (total 6 cases of lipomas)	1 PHEO, 1 hyperplasia of adrenal glands, 3 adrenal gland adenomas, (total 5 cases)	1 Skin angiofibroma	0
Large intragenic deletion (4)	9	9	4 PRLomas, 1 ACTH-secreting adenoma, (total 5 pituitary tumors)	6 Gastrinomas, 3 NF pNETs, (total 9 GEP-NETs)	0	2 Multiple lipomatosis, 1 single lipoma, (total 3 cases of lipomas)	0	0	0

(*n*) number, *NA* non-applicable, *PHPT* primary hyperparathyroidism, *GEP-NETs* gastro-entero-pancreatic neuroendocrine tumors, *NETs* neuroendocrine tumors, *PRL* prolactin, *GH* growth hormone, *ACTH* adreno cortico tropic hormone, *VIP* vasoactive intestinal peptide, *NF* non-functioning, *pNETs* pancreatic neuroendocrine tumors, *NETs* neuroendocrine tumors, *PP* pancreatic polypeptide, *PHEO* pheochromocytoma

[a]All cases of adrenal hyperplasia reported in this study are primary diseases. No cases of bilateral hyperplasia secondary to an excess of ACTH secretion have been described in our patients affected by ACTH-secreting pituitary adenoma (we described 5 cases of ACTH-secreting adenomas whose reported comorbidities are: 5 PHPT, 1 post-surgical hypoparathyroidism, 1 gastrinoma, 1 insulinoma, 1 post-surgical diabetes mellitus, 1 NF-pNET, 1 PRLoma, 2 thoracic NETs (1 bronchopulmonary and 1 thymic), 1 lipoma, 1 hypothyroidism, 1 multinodular goiter and 2 cases of osteoporosis

[b]These numbers included also the same 4 affected patients bearing two different mutations (a frameshit and a missense)

Table 4 MEN1-associated tumor distribution, in our series of patients, based on the *MEN1* mutated region

Mutated exon/intron (*n*)	Mutated symptomatic patients	PHPT	Pituitary tumors	GEP-NETs	Thoracic NETs	Lipomas	Adrenocortical tumors/lesions[a]	Skin lesions	Other tumors/ lesions
Exon 2 (21)	63	60	16 PRLomas, 10 NF adenomas, (total 26 pituitary tumors)	9 Gastrinomas, 5 insulinomas, 2 VIPomas, 1 glucagonoma, 17 NF pNETs, (total 34 GEP-NETs)	4 Bronchopulmonary, 1 Thymus, (total 5 thoracic NETs)	5 Multiple lipomatosis, 3 Single lipomas, (total 8 cases of lipomas)	1 PHEO, 3 hyperplasias of adrenal glands, 2 adrenal gland adenomas, (total 6 cases)	1 Multiple skin angiofibroma	1 Thyroid adenoma, 1 uterine myofibroma
Intron 2 (1)	1	1	0	1 NF pNET	1 Bronchopulmonary	0	0	0	0
Exon 3 (11)	34	34	6 PRLomas, 1 NF adenoma, (total 7 pituitary tumors)	6 Gastrinomas, 6 insulinomas, 1 PPoma, 5 NF pNETs, (total 18 GEP-NETs)	3 Bronchopulmonary, 1 Thymus, (total 4 thoracic NETs)	2 Multiple lipomatosis, 1 Single lipoma, (total 3 cases of lipomas)	2 Hyperplasias of adrenal glands, 5 Adrenal gland adenomas, (total 7 cases)	2 Multiple skin angiomas, 1 multiple skin fibroma, (total 3 skin lesions)	1 Uterine myofibroma
Intron 3 (2)	5	5	4 PRLomas	1 Insulinoma	0	0	0	0	0
Exon 4 (4)	10[b]	10	9 PRLomas	1 Multiple NF pNET	1 Bronchopulmonary	2 Single lipomas	0	0	0
Intron 4 (3)	25	24	4 PRLomas, 1 GH-secreting adenoma, 1 NF adenoma, (total 6 pituitary tumors)	7 Gastrinomas, 1 insulinoma, 9 NF pNETs, 1 NF gastric NET, (total 17 GEP-NETs)	4 Bronchopulmonary	4 Multiple lipomatosis, 1 Single lipoma, (total 5 cases of lipomas)	1 PHEO, 1 hyperplasia of adrenal glands, 1 adrenal gland adenoma, (total 3 cases)	0	0
Exon 5 (3)	10	10	2 PRLomas, 1 GH-secreting adenoma, (total 3 pituitary tumors)	1 Gastrinoma, 1 Insulinoma, 1 Glucagonoma, (total 3 GEP-NETs)	1 Bronchopulmonary	0	0	0	0
Intron 5 (2)	3	3	2 PRLomas	0	1 Bronchopulmonary	1 Multiple lipomatosis	2 Adrenal gland adenomas	1 Skin angiofibroma	0
Exon 6 (3)	7	7	0	2 Gastrinomas, 1 Glucagonoma, (total 3 GEP-NETs)	0	0	0	0	0
Intron 6 (0)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Exon 7 (6)	15	15	2 PRLomas, 2 ACTH-secreting adenomas, 2 NF adenomas, (total 6 pituitary tumors)	6 Gastrinomas, 1 Insulinoma, 4 NF pNETs, (total 11 GEP-NETs)	2 Bronchopulmonary, 1 Thymus, (total 3 thoracic NETs)	2 Multiple lipomatosis, 2 Single lipomas, (total 4 cases of lipomas)	2 Hyperplasias of adrenal glands, 1 Adrenal gland adenoma, (total 3 cases)	1 Multiple skin angioma, 1 Skin multiple fibroma, (total 2 skin lesions)	1 Uterine myofibroma
Intron 7 (2)	3	3	1 PRLoma, 1 NF adenoma, (total 2 pituitary tumors)	1 Gastrinoma, 1 Insulinoma, (total 2 GEP-NETs)	0	0	0	0	0
Exon 8 (5)	16[b]	14			0	1 Multiple lipomatosis, 3	1 Hyperplasia of adrenal glands, 2	1 Skin angioma	1 Uterine myofibroma

Table 4 (continued)

Mutated exon/intron (n)	Mutated symptomatic patients	PHPT	Pituitary tumors	GEP-NETs	Thoracic NETs	Lipomas	Adrenocortical tumors/lesions[a]	Skin lesions	Other tumors/ lesions
			8 PRLomas, 1 NF adenoma, (total 9 pituitary tumors)	2 Gastrinomas, 5 NF pNETs, (total 7 GEP-NETs)		Single lipomas, (total 4 cases of lipomas)	adrenal gland adenomas, (total 3 cases)		
Intron 8 (0)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Exon 9 (14)	91	85	24 PRLomas, 7 NF adenomas, (total 31 pituitary tumors)	27 Gastrinomas, 8 Insulinomas, 27 NF pNETs, 1 NF gastric NET, 1 glucagonoma, (total 64 GEP-NETs)	1 Bronchopulmonary	4 Multiple lipomatosis, 1 Single lipoma, (total 5 cases of lipomas)	6 Hyperplasias of adrenal glands, 3 Adrenal gland adenomas, (total 9 cases)	1 Skin angioma, 3 facial skin angiofibromas, (total 4 skin lesions)	2 Meningiomas, 1 uterine leiomyoma
Intron 9 (0)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Exon 10 (18)	74	69	19 PRLomas, 2 ACTH-secreting adenomas, 1 GH-PRL secreting adenoma, 6 NF adenomas, (total 28 pituitary tumors)	15 Gastrinomas, 1 Insulinoma, 21 NF pNETs, 2 NF gastric NETs, 1 VIPoma, (total 39 GEP-NETs)	7 bronchopulmonary	5 Multiple lipomatosis, 2 Single lipomas, (total 7 cases of lipomas)	2 Hyperplasias of adrenal glands, 5 Adrenal gland adenomas, (total 7 cases)	1 Multiple skin angioma, 2 Multiple skin fibromas, (total 3 skin lesions)	2 Uterine leiomyomas, 1 meningioma
Multiple exon deletions (4)	9	9	4 PRLomas, 1 ACTH-secreting adenoma, (total 5 pituitary tumors)	6 Gastrinomas, 3 NF pNETs, (total 9 GEP-NETs)	0	2 Multiple lipomatosis, 1 Single lipoma, (total 3 cases of lipomas)	0	0	0

(n) number, *NA* non-applicable, *PHPT* primary hyperparathyroidism, *GEP-NETs* gastro-entero-pancreatic neuroendocrine tumors, *NETs* neuroendocrine tumors, *PRL* prolactin, *GH* growth hormone, *ACTH* adreno cortico tropic hormone, *VIP* vasoactive intestinal peptide, *NF* non-functioning, *pNETs* pancreatic neuroendocrine tumors, *NETs* neuroendocrine tumors, *PP* pancreatic polypeptide, *PHEO* pheochromocytoma

[a]All cases of adrenal hyperplasia reported in this study are primary diseases. No cases of bilateral hyperplasia secondary to an excess of ACTH secretion have been described in our patients affected by ACTH-secreting pituitary adenoma

[b]These numbers included also the same 4 affected patients bearing two different mutations (a frameshit and a missense)

Table 5 Mean age of onset of the first MEN1 clinical manifestation based on different type or localization of *MEN1* mutation

Mutation type or localization (number of affected patients)	Mean age of first clinical manifestation onset ± SD (years)
Frameshift (158)	38.1 ± 15.7
In-frame deletion (7)	40.1 ± 11.2
Nonsense (69)	36.0 ± 13.8
Missense (77)	37.3 ± 14.4
Splicing site mutations (38)	34.8 ± 11.3
Large intra-genic deletion (9)	40.6 ± 14.5
Double mutation (4)	26.5 ± 19.4
Truncating mutations (271)	37.2 ± 14.8
Non-truncating mutations (84)	37.5 ± 14.2
Exon 2 (63)	37.3 ± 14.9
Intron 2 (1)	NA
Exon 3 (34)	39.1 ± 16.1
Intron 3 (5)	30.2 ± 8.5
Exon 4 (6)	34.0 ± 11.7
Intron 4 (25)	36.8 ± 12.9
Exon 5 (10)	38.7 ± 13.9
Intron 5 (3)	32.7 ± 2.1
Exon 6 (7)	29.1 ± 9.7
Exon 7 (15)	39.5 ± 16.0
Intron 7 (3)	32.3 ± 6.0
Exon 8 (11)	35.3 ± 13.7
Exon 9 (91)	38.4 ± 14.2
Exon 10 (74)	36.5 ± 15.5

Truncating mutations include frameshift, nonsense, splicing and large intra-genic deletions; non-truncating mutations include missense and in-frame deletions. Results of Student's *t*-test: frameshift vs. nonsense $p = 0.36$; frameshift vs. missense $p = 0.71$; frameshift vs. splicing site mutations $p = 0.25$; splicing site mutations vs. nonsense $p = 0.66$; splicing site mutations vs. missense $p = 0.38$; missense vs. nonsense $p = 0.60$; truncating mutations vs. non-truncating mutations $p = 0.87$; Exon 2 vs. Exon 3 $p = 0.60$; Exon 2 vs. Exon 9 $p = 0.64$; Exon 2 vs. Exon 10 $p = 0.76$; Exon 3 vs. Exon 9 $p = 0.83$; Exon 3 vs. Exon 10 $p = 0.44$; Exon 9 vs. Exon 10 $p = 0.41$

SD standard deviation, *NA* non-applicable

2 because of kidney failure (due to an undiagnosed hypercalcemic hyperparathyroidism), 2 because of post-surgical complications of a macro PRLoma resection (one from severe pituitary insufficiency and one from post-surgical hyponatremia), and 2 because of cardiac complications due to electrolyte imbalance (cardiac arrest from ventricular fibrillation with hypokalemia). The average age at death was 59.1 years ± 14.5 (range 36–84 years). The average age at the first clinical manifestation was 43.1 ± 17.4 years (range 17–75 years), and the average age at diagnosis of MEN1 was 55.8 ± 15.2 years (range 33–80 years). There was no association between MEN1-related premature death, cause of death, and death age with the *MEN1* mutation type or localization.

Discussion

Our study analyzed a large series of 410 *MEN1*-mutated patients, bearing 95 different inactivating mutations and 4 gross intra-genic deletions. Analysis of mutation type and distribution were similar to data previously published for MEN1 syndrome [2, 3], identifying mutations (i.e., frameshift and nonsense mutations) that generate a truncated menin protein, unable to translate to the nucleus, as the most frequent mutations (54.5%) (Fig. 1). Frameshift mutations were confirmed to be the most common *MEN1* mutations, accounting alone for over 41% of all identified variations. Nonsense mutations were prevalently located in exon 10 (6 of 13), while exon 9 resulted to be the principal exon affected by missense mutations (7 of 26) (Table 1). No specific mutational hot spots were identified, even though we confirmed that exons 2, 9, and 10 were the three most mutated exons [2, 3]; in particular, a high concentration of insertion and deletion points was detected in exons 2 and 10, and they were presumably associated with short nucleotide repeats within these two exons.

No correlation was found between disease age of onset, distribution of PHPT and pituitary tumors and the *MEN1* mutation type or localization. We found that patients affected by GEP-NETs had a significantly higher frequency of nonsense than frameshift or missense mutations and that the presence of a thoracic NETs was significantly more common in patients bearing a splicing-site mutation with respect to those presenting a frameshift mutation. These findings could open interesting perspectives for prediction and prognosis, but further studies, including broad and different MEN1 populations, are needed to confirm a real increased risk/predisposition to develop GEP-NETs or thoracic NETs to carriers of nonsense or splicing-site mutations, respectively, and to exclude that they can be only accidental statistical associations. No selection biases can be suspected in the collection of our MEN1 patients, since they were obtained from 14 major referral centers for endocrine inherited tumors and MEN syndromes, which constantly visit and follow up MEN1 patients from all the 20 different Italian Regions. This multi-centric patients' collection grants to cover all the three main geographical areas of Italy (North, Center, South) and, thus, to obtain a large group of MEN1 patients who are representative of all affected individuals in Italy.

Despite these positive statistical associations, the detailed analysis of intra-familial clinical phenotypes, in all our 23 pedigrees, showed a high variability in disease age of onset and severity of the clinical manifestations, even in the presence of the same mutation, confirming the absence of a direct genotype–phenotype correlation. Recently, also another study failed to find a significant direct correlation between the type and location of *MEN1* mutations and

Table 6 Occurrence of the MEN1 main endocrine tumors/lesions in MEN1 truncating and non-truncating mutations

Type of mutation (*n* of affected patients)	PHPT	Pituitary tumors	GEP-NETs	Thoracic NETs	Adrenocortical tumors/lesions	Number of different main MEN1 endocrine manifestations/patient (number of cases)[a]
Truncating mutations (278)	263	108	166	20	28	5 (3)
						4 (18)
						3 (67)
						2 (95)
						1 (95)
Non-truncating mutations (88)	86	36	48	9	15	5 (0)
						4 (9)
						3 (21)
						2 (32)
						1 (26)

PHPT primary hyperparathyroidism, *GEP-NETs* gastro-entero-pancreatic neuroendocrine tumors, *NETs* neuroendocrine tumors

[a]This number is calculated considering the occurrence, with different combinations, of the five main endocrine tumors/lesions in MEN1 (PHPT, pituitary tumors, GEP-NETs, thoracic NETs, and adrenocortical tumors/lesions)

Truncating mutations include frameshift, nonsense, splicing and large intra-genic deletions; non-truncating mutations include missense and in-frame deletions

Results of chi-squared test of tumor occurrence in patients with a truncating mutation vs patients with a non-truncating mutation: PHPT $p = 0.36$; pituitary tumors $p = 0.73$; GEP-NETs $p = 0.39$; thoracic NETs = 0.36; adrenocortical tumors/lesions $p = 0.08$; five manifestations $p = 0.76$; four manifestations $p = 0.24$; three manifestations $p = 0.96$; two manifestations $p = 0.71$; one manifestation $p = 0.42$

clinical phenotypes in 54 MEN1 patients with Italian ancestry [12].

Our series of patients also included a pair of monozygous twins; at the time of the study (age under 35 years), they manifested PHPT only, being inconclusive for the study of genotype-phenotype correlation. A future clinical follow-up of these twin sisters would be very useful to evaluate the role of epigenetic or extrinsic factors in MEN1 tumorigenesis.

We reported a large MEN1 family (24 mutated members, of which 4 still asymptomatic at the time of this study), bearing the frameshift g.1449_1459 del11 in exon 9 (Table 2), presenting highly variable intra-familial disease phenotypes and varying onset of MEN1 manifestations. This family presented a relatively high mean age of MEN1 onset (mean age of first clinical manifestation 47.8 ± 15.2 years; median age of first clinical manifestation 51.5 years) with 50% of members developing the disease by the age of 52. Curiously, three members of this family did not develop PHPT at the age of 55, 57 and 64 years, respectively; but had a common involvement of the neuroendocrine tissues of the GEP tract (two with duodenal gastrinomas associated with Zollinger Ellison syndrome and one with non-functioning pancreatic NET).

We also identified a family with five members bearing one allele with two different mutations (Leu249Pro in exon 4 and g.1181delC in exon 8). The presence of a double mutation does not influence the severity of the disease in this pedigree, with respect to the other MEN1 families bearing a single mutation. Interestingly, this pedigree presented three members (the MEN1 index case, her sister and her sister's son) with a very early age of onset of the first MEN1 clinical manifestation (17, 15, and 14 years, respectively), with PRLoma being the first clinical sign in all of them. Conversely, the father of the index case presented PHPT as the first clinical sign of MEN1, at a later age of onset (60 years). This onset anticipation and pituitary first involvement, in the second and third generations, led us to suspect the presence of other influencing genetic factors (presumably inherited from maternal line by the index case and her sister). Another family, bearing a single, different, *MEN1* mutation (g.1364delC, in exon 9) showed a similar disease presentation to the family described above, with the pedigree MEN1 index case (father) presenting the first clinical manifestation, PHPT, at the age of 47 years, while his two daughters and one nephew manifested secreting PRLoma as the first MEN1 feature at the ages of 19, 15, and 18 years, respectively. A young nephew, genetically diagnosed at the age of 7 years, was still asymptomatic at the time of this study (24 years). The similar inter-familial MEN1 clinical presentation between these two families (even in presence of different mutations and affected gene region) would confirm the anticipation phenomenon of clinical expressivity evidenced in some MEN1 families, and previously described also for familial medullary thyroid carcinoma [13].

Forty-eight mutated individuals showed no sign and symptoms of MEN1 at the time of the study. The great

majority of them (40) were aged less than 40 years, and 4 were aged 42–46 years; presumably MEN1 has not manifested yet in all these subjects because of their age less than 50. Conversely, four of them were over 60 years of age (60, 68, 68, and 75, respectively); the absence of the disease in these individuals, despite the manifestation of the MEN1 phenotype in other members of the same pedigrees, not only confirmed the high intra-familial variability and penetrance of MEN1 tumorigenesis, but also suggested the possible existence of unknown protective factors, which presumably prevent the somatic loss of the wild type copy of *MEN1*, and, thus, the development of tumors.

In summary, our study tends to confirm the absence of a direct correlation between a specific *MEN1* mutation, mutation types and mutated regions of the gene, and the specific clinical presentation and penetrance of MEN1 syndrome, not allowing us to foresee the exact future tumor manifestation on the basis of genetic test results. However, more disrupting mutations (nonsense and splicing-site) appear to be significantly associated with more aggressive NETs.

In addition, our study strengthened the importance of the genetic test for an earlier diagnosis and, possibly, a reduction of morbidity and mortality of the syndrome due to the late recognition of MEN1-tumors and malignant progression. Indeed, all the 19 deceased patients had a late MEN1 diagnosis (a great majority of them were clinically diagnosed with MEN1 before 1998, before the availability of the genetic test), presenting at least two manifestations of the syndrome at the time of diagnosis, and indicating the late diagnosis (and the subsequent late therapeutic interventions) as one of the principal causes of mortality. The introduction of the genetic test was surely a landmark in the diagnosis of MEN1, allowing a significant decrease in lag time of diagnosis between a MEN1 index cases and his/her relatives [13]. During the last twenty years, the genetic test has progressively favored, in association with the constant progression in clinical diagnostic tools, surgery techniques and pharmaceutical therapies, a better management of patients and a subsequent reduced rate of morbidity and mortality [14, 15]. Today, MEN1 tumors in relatives of affected index cases are commonly diagnosed earlier, as a result of a more and more capillary application of the genetic test, an increased disease awareness (i.e., institution of MEN1 referral centers, creation of patients' associations) and a constant progression in diagnostic screening tools. In our series, the genetic testing allowed to identify 48 still asymptomatic carriers, who are, currently, under constant diagnostic screening, according to both the recommended and the suggested clinical, biochemical and imaging screenings of the international clinical practice guidelines for MEN1 [16].

Finally, data from the analysis of intra-familial MEN1 clinical phenotypes, age of onset, and disease penetrance in our pedigrees further enforced the hypothesis of the role of other genetic and, perhaps, epigenetic, still unknown, modifying factors, in the determination of individual MEN1 tumorigenesis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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