Results of a national survey on multiple endocrine neoplasia syndrome type 1 in Italy: a macroaggregate analysis

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Summary

In more than a decade of activity the Italian Register of Multiple Endocrine Neoplasias (RINEM = Registro Italiano delle Neoplasie Endocrine Multiple) made possible to collect both clinical and genetic data on Italian kindreds affected by Multiple Endocrine Neoplasia type 1 (MEN1) syndrome. Until the end of the 80thies no data were available for this syndrome in Italy. The number of cases referred to RINEM has been constantly increasing during the years. Data on 193 subjects, 41 sporadic and 152 familial cases from 44 MEN1 kindreds have been collected in RINEM. In 12 Italian informative MEN1 kindreds linkage analysis was initially performed before the cloning of *MEN1* gene. Thirty-two asymptomatic *MEN1* gene carriers have been originally identified and subsequently in 12 of these clinical confirmation within 1 to 3 years from the genetic test have been reported. The RINEM will focus in the future on the preparation of a detailed questionnaire on clinical, genetic and therapeutic approaches to MEN1 in our Country.

KEY WORDS: multiple endocrine neoplasia, clinical management of endocrine tumors, hereditary tumors, genetic diagnosis, antioncogene, MEN1.

Introduction

Rare or orphan diseases constitute a heterogeneous group of extremely infrequent human disorders that have been reported to approximately affect fewer than 200,000 people in United States (http://www.raredisease.org/).
Multiple Endocrine Neoplasia type 1 syndrome (MEN1; OMIM 131100) is a complex tumor-predisposing disorder inherited in an autosomal dominant manner with a high degree of penetrance, nearly 100% within 50 years of age. The syndrome exhibits a high grade of clinical variability, also in members from the same affected family. More than 20 combinations of both endocrine and nonendocrine tumors have been reported in MEN1 patients with three endocrine localizations constituting the "typical" clinical features of this syndrome: multiple tumors of parathyroid glands (generally all the parathyroid glands), pituitary adenomas and tumors of the neuroendocrine cells in the gastroenteropancreatic (GEP) tract. Due to the complexity of clinical expression a simple definition including all the tumoral combinations is lacking. Hence, a MEN1 case may be defined as a case with at least two of the principal MEN-related endocrine tumors, while familial form is defined by the presence of a MEN1 case, as described above, with a first degree relative showing one of the three characterizing tumors (1). MEN1 syndrome is most commonly diagnosed in the proband during the fourth or fifth decade of life with a considerable delay from the age of biochemically detectable onset, because symptoms are typically delayed for another 5-8 years (1-3). Early recognition of affected and at risk individuals within kindred is today facilitated by DNA-testing (1). However, the age of onset of MEN1 syndrome is extremely variable, ranging from 5 to 65 years, but the onset of the MEN1-associated primary hyperparathyroidism and the onset of MEN1-associated gastrinoma and insulinoma generally anticipate of 3 and 1 decades, respectively, the onset of the corresponding sporadic counterparts. Gastrinomas and carcinoids represent the more frequent causes of mortality in MEN1 patients. In Table I MEN1-associated tumors and their prevalence are described.
Advances in molecular biology and genetics have led to the identification of specific genetic defect that improves the under-

Table I - MEN1-related endocrine tumours and their prevalence.

Parathyroid adenomas (90%)	
GEP	Gastrinoma (40%) Insulinoma (10%) Others (VIPoma, PPoma, SSoma, glucagonoma) (2%) Non-functioning (20%)
Anterior pituitary	Functioning PRLoma (20%), GH-, GH/PRL-, TSH-, ACTH-secreting, or non-functioning (17%)
Foregut carcinoids	Thymic (2%) Bronchial (2%) Gastric (ECLoma) (10%)
Adrenal gland	Non functioning (20%)
Cutaneous tumors	Lipomas (they could be also visceral) (30%) Facial angiofibromas (85%) Collagenomas (70%)
Central Nervous System	Meningiomas (5%) Ependymomas (1%)
Others	Leyomiomas (10%)

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standing and ability to diagnose this syndrome. Particularly, after the cloning of *MEN1* gene the early detection of asymptomatic carriers dramatically decreases the morbidity and mortality of MEN1, providing the opportunity to initiate appropriate treatment at early stages. Paradoxically, the consequently longer life span may result in a rising cumulative morbidity and mortality from MEN1-associated malignancies. Unfortunately, the lack of genotype/phenotype correlation makes difficult the use of genetic information to predict clinical behavior, localization, early detection and prognosis of related tumors.

MEN1 gene

The responsible gene, MEN1, mapped to chromosome 11q12-13 region by combining linkage and tumoral microdeletion analyses and proposed as a tumor suppressor gene (4), has been cloned in 1997 (5). Its mutations, such as in-frame deletions, frame-shifts, missense and nonsense mutations, have been described at germline level in many of MEN1 affected kindreds (5), supporting its proposed oncosoppressor nature. At the present, more than 300 somatic MEN1 mutations have been reported in literature. *MEN1* is a tumor suppressor gene encoding a 610 amino acid protein named menin that does not reveal homology to any of the already known human proteins (5, 6). Recent advances on pathophysiological roles of menin disclose the existence of an intricate network composed by several molecular partners interacting with menin: JUND (7), Smad1, Smad3, Smad5, Runx2 (8), Sin3a, HDAC (9), Pem (10), COMPASS-like complex, RPA2 (11), FANCD2 (12)., Hsp70, CHIP (13), Hox (14), TGFβ (15), GFAP, vimentin (16), NF-kB (17), NM23H1 (18), ERK, JUNK, Elk-1 and c-Fos (19). However, it is still completely unknown how mutations in menin cause tumorigenesis, nor is the function of menin. Menin, mainly located in the nucleus (20), is widely expressed and may play different roles in different tissues and probably involved in the regulation of several cell functions, including DNA replication and repair and transcriptional machinery and so forth.

Italian background

In Italy neither clinical nor numerical data were available for MEN1 until the end of the 80thies. For this purpose, in 1991 the RINEM was established within the activities of the Study Group on Multiple Endocrine Neoplasia Syndromes of the Italian Society of Endocrinology. RINEM collects clinical records on Multiple Endocrine Neoplasia syndromes obtained from several Italian Clinical Centers[1] by compilation of a simple questionnaire. We noted a constantly increasing number of cases referred to RINEM during the years. The primary goal of this initiative was represented by the collection of both elementary clinical data and number of affected patients and the eventual geographic distribution of both MEN syndromes in our Country. However, as these affected subjects are not representative of whole Italian population both incidence and prevalence of these syndromes cannot be obtained by such a survey.
In particular, 44 MEN1 kindreds, 41 sporadic MEN1 cases have been reported to RINEM. A total of 193 subjects (152 familial and 41 sporadic cases) have been referred. Linkage analysis (before *MEN1* gene cloning) and subsequent mutational analysis of MEN1 gene have been performed in our Laboratory for several of the referred *MEN1* cases, after obtaining a signed informed consent by each subject.

Materials and methods

Questionnaires

A very simple questionnaire (Figure 1), specific for the syndrome, has been used in order to collect an as large as pos-

Family ID (Name/ Number)	Patient code	Degree of relationship	Date of birth	Eventual death (when, why)	GEP	Pituitary	Parathyroids	Other tissues and/or diseases	Genetic test (Yes/No) Mutation

Figure 1 - The simple questionnaire used to collect MEN1 cases at RINEM.

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sible number of MEN1 cases without need of a time-consuming compilation by collaborating physicians. MEN1 patients were registered in RINEM, as individual cases with well-documented clinical features or as hereditary cases (kindreds with at least two affected members), according to the above-described criteria. However, even if each referring physician strictly respected the clinical diagnostic criteria, we cannot exclude the possibility of having MEN1 phenocopies recorded as MEN1 cases. A MEN1 phenocopy resembles MEN1 but is not caused by *MEN1* mutation (1). Thus some families might have mutation in other genes, including *CaSR* for Familial Hypercalcemia Hypocalciuria (FHH) disorder (21) and the gene for the Hyperparathyroidism-Jaw Tumor (HPT-JT) syndrome (22).

MEN1 Linkage analysis

We performed linkage test in *MEN1* kindreds until 1997 when MEN1 gene was identified (5) and then mutational test has been introduced. For MEN1 linkage analysis DNA was prepared from at least two affected members, not affected first-degree relatives and corresponding partners of affected subjects for each kindred in order to identify asymptomatic carriers (23). Highly polymorphic DNA markers from 11q12-13 region, harboring *MEN1* gene, have been used to perform this genetic analysis (24-26), spanning an interval of 14 cM in this region. For the analysis, two polymorphic markers exhibited recombination 0 with the *MEN1* locus and flanking both sides of the *MEN1* gene have been chosen. The diagnostic accuracy to unravel an asymptomatic carrier can reach 99.5% when at least 3 DNA markers, two flanking on each side and one with recombination 0, result to be informative.
The identification of the MEN1 gene (5) allowed to replace linkage analysis with mutational analysis in affected families. Linkage analysis can be still carried out in those familial cases in which mutational analysis failed to detect mutations within the coding region of the gene. Currently, in informative kindreds we use six polymorphic microsatellite markers flanking the MEN1 locus at 11q13: cen-PYGM, D11S4946, D11S4940, D11S4938 and D11S4937, D11S449. The localization and order of all markers is based on the information presented in the Genome Database (www.gdb.org).

MEN1 Mutational analysis

The positional cloning of the *MEN1* gene in 1997 (5) made possible to perform mutational analysis of coding region and exon-intron junctions of the gene in both single subjects and familial cases. Consequently, the identification of an apparently sporadic form as the first case of a new MEN1 kindred has become easier.
All steps of such analysis, from gene amplification protocols to DNA sequencing, have been already accurately reported in literature (27). This method allows the identification of *MEN1* mutation carriers. Nevertheless, from 5% to 10% of MEN1 patients may not harbor mutations in the coding region of the *MEN1* gene (5, 28-32) but these individuals may have mutations in the promoter or untranslated regions (UTRs), which still remain to be investigated.
Genomic DNA from peripheral blood leukocytes is used to investigate coding region (exon 2-10) and exon-intron junctions of the *MEN1* gene. Each exon is amplified by Polymerase Chain Reaction (PCR) and an aliquot of each PCR product is sequenced, both in forward and reverse directions. Sequences are analyzed on the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and then compared to wild type reference sequence of the *MEN1* gene (U93237).

Results

MEN1: Clinical and numerical data

Data on a total of 193 subjects (152 familial and 41 sporadic cases) are available. The age range is 8-71 years and the female to male ratio is 1.2. Total affected subjects were 173, 132 of which were familial cases. Initially, the asymptomatic carriers at the time of the report to RINEM were 32. They are now 20 (12 have been lately report to clinically express the syndrome).
The distribution of typical anatomic sites is reported in Figure 2. The distribution of non-classical endocrinopathies, other endocrine tumors and of other clinical entities has been included in the analysis (Figures 3 and 4, and Table II). The referred causes of death of MEN1 patients are reported on Table III and they are aligned with already reported in literature.

Forty-four MEN1 kindreds were registered in RINEM. No differ-

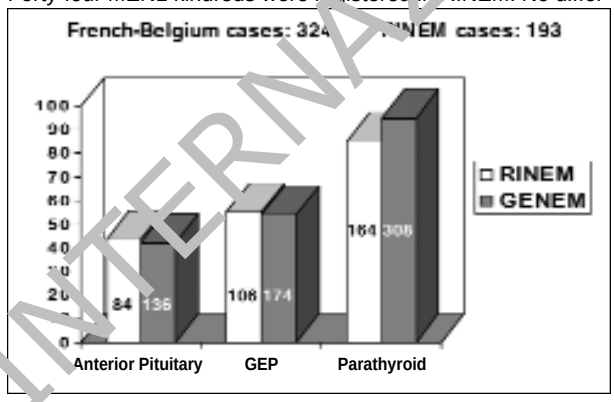

Figure 2 - Distribution of "typical" anatomic sites involved in MEN1. A comparison between the French-Belgium Multicentric study (ref. 33) and RINEM.

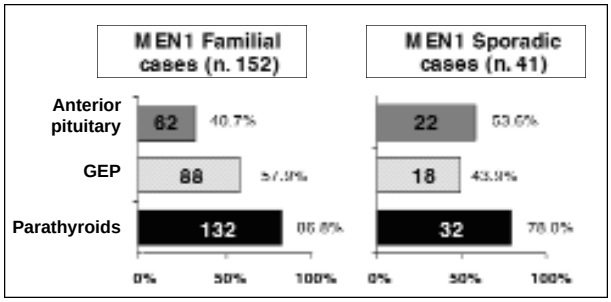

Figure 3 - Distribution of "typical" endocrinopathies in familial and sporadic MEN1 cases referred to RINEM.

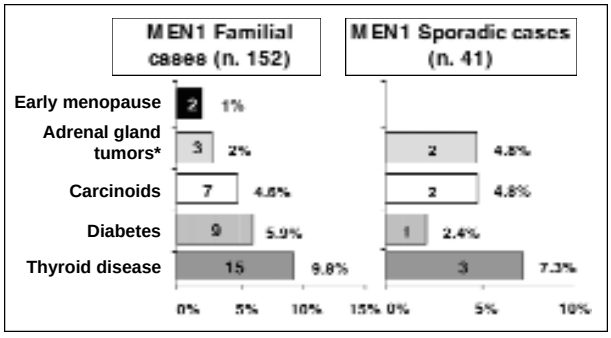

Figure 4 - Distribution of other endocrinopathies in familial and sporadic MEN1 cases referred to RINEM.

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Table II - Distribution of nonendocrine tumors and other clinical entities in familial and sporadic cases of MEN1.

	Familial % (n. 152 cases)	Sporadic % (n. 41 cases)
Lipomas	3.3	7.3
Meningiomas	1.3	–
Basalioma	0.6	2.4
Cutaneous angiofibromas	1.3	4.8
Prostatic tumor	0.6	2.4
Exocrine pancreatic tumor	1.3	2.4
Liver lesions	3.8	2.4
Acoustic neurinoma	–	2.4
Retroperitoneal neurofibromatosis	0.6	2.4
Lymphoma(s)	1.3	2.4
Mammary cancer	–	2.4
Ovaric cysts	–	2.4
Uterine fibromatosis	–	7.3
Blood hypertension	3.3	7.3
TBC	1	-

ences among sexes have been described, according to an autosomal pattern of inheritance. The involvement of typical anatomic sites (Figure 2) is similar to information from other Countries (33, 34). Even the distribution of MEN1-associated tumors by age groups, both in familial and sporadic cases, is similar to other international surveys. Thus, it has been possible to collect 25[illegible] typical MEN1-associated tumors from 173 patients. On the whole patients over 40 years of age exhibit primary hyperparathyroidism (PHPT) in 92% of cases, neuroendocrine neoplasms of gastroenteric tract in 65.5% and anterior pituitary tumors in 35%. As in sporadic cases even in familial forms non-classical endocrinopathies, non-endocrine tumors and other clinical entities were analyzed (Figure 3 and Table II). Of these 152 familial cases, 125 patients are still alive, while 27 are dead because of different causes (Table III), mainly represented by gastrinomas and carcinoids and in sporadic MEN1 cases 38 patients are alive and 3 died as reported in Table II.

Genetic analysis of MEN1

Linkage analysis

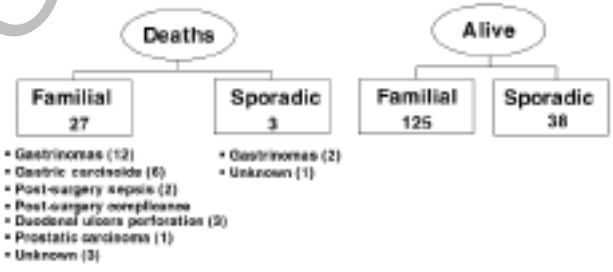
Before *MEN1* gene cloning, in 12 Italian informative kindreds out of 14 families collected through the RINEM linkage analysis of 11q13 region was performed (23). One hundred and sixty-two subjects were genotyped and of these ones 45 individuals were affected. Two families resulted to be not informative. Thirteen asymptomatic MEN1 carriers were identified and in 10 of these we had clinical confirmation within 1 to 3 years from the genetic test. The clinical history of these subjects showed PHPT to be the first manifestation (90%), as expected, in all but one who exhibited a PRL-secreting tumor. Interestingly, by RINEM we had the opportunity to genetically test a unique MEN1 kindred whose linkage analysis demonstrated that two unrelated affected parents generated three affected sons (two females and one male), with two of them being described as homozygotes for MEN1 mutation (35, 36).

MEN1 Gene mutational analysis

Mutational analysis of MEN1 gene was performed in 44 Italian unrelated MEN1 families (including the 14 kindreds previously described above) and in 41 isolated MEN1 patients, collected through RINEM. A total of 45 different heterozygous germline mutations were identified in 40 of 44 (91%) MEN1 families and in 20 of 41 (49%) cases referred as sporadic MEN1. These different mutations spread across most of the 9 translated exons and consisted of 9 nonsense, 1 missense, 4 splicing, 6 insertional and 15 deletional frameshift mutations. As reported in literature (1), 71% of MEN1 gene mutations at RINEM consist of nonsense and frameshift mutations, Specifically, 21.5% at exon 2, 7% at exon 3, 9% at exon 4/exon 4.intron 4, 1.7% at intron 4-exon 5, 3.5% at exon 6, 9% at exon 7, 5.3% at exon 8, 12.5% at exon 9 and 27% at exon 10. Thus, 61% of MEN1 mutations are prevalently located in the three larger exons (2, 9 and 10) of the gene, similarly to what observed by Kouvaraki et al. in a clinical series of MEN1 patients recorded at the Anderson Cancer Center of the University of Texas (Figure 5) (37).
The failure to detect mutations in all these cases may reflect large to complete deletion of the gene, or mutation in the untested parts such as regulatory or untranscribed regions, which are undetectable by current methods of screening. In fact, 3 of the 30 kindreds, not previously analyzed by linkage approach, resulted to be in linkage for polymorphic 11q13 loci when performed after the lack of mutation detection.
Moreover, some of the cases recorded at RINEM as MEN1 cases, especially sporadic cases, may be MEN1 phenocopies (21, 31, 38, 39).
The analysis of the clinical features in these families didn't suggest any correlation between genotype and phenotype. In general our data confirm those already published about the apparent lack of genotype-phenotype correlation (27).
Finally, mutational analysis of the above mentioned MEN1 kindred with the two linkage-based defined homozygotes revealed the presence of a MEN1 mutation only on the maternal side of pedigree. Thus, these homozygotes have to be considered as compound heterozygotes (27).

Discussion

Table III - Causes of death of MEN1 patients referred to RINEM.



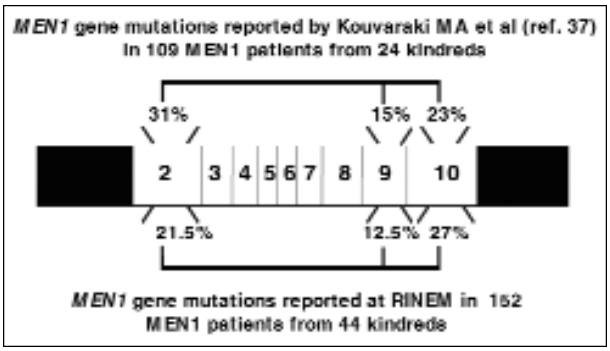


Figure 5 - Most of the Italian *MEN1* gene mutations reported at RINEM are located at exons 2, 9 and 10.

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Originally, geneticists predicted the equivalence: known mutation and known protein alteration = genotype-phenotype correlation. As for many other monogenic disorders even for MEN1 syndrome this correlation is incomplete. In fact, phenotypic variability within families has been described in several mendelian diseases (40), where the identical mutation is associated with a phenotype that may vary in features such as the age at onset of symptoms and the severity of symptoms. Moreover, intrafamilial phenotypic variability, all of whose members had the same mutation, suggest that additional genes, independently inherited, and/or environmental factors may influence the clinical expression. This is particularly true for familial MEN1 syndrome.

Modifying-phenotype factors have suggested in some cases (41) and may consist of polymorphism in other genes that may modulate expression and/or function of the candidate protein.

As described above for menin proteins, the primary mutant gene product is embedded within a highly complex system in which a multiplex of genetic polymorphisms, additional nonallelic mutations of genes, encoding for both directly or indirectly correlated molecular partners, and environmental influences, singularly or combined, might cause the differences among individuals.

Surprisingly, it is impressive the observation that occasional mutation does show a reliable correlation with phenotype, probably because the function of mutant gene product exceeds a threshold, above which systemic influences cannot compromise the collective operational integration, or, alternatively, below another threshold, beneath which the function of the mutant protein cannot be raised by other variables within the system. Between the two thresholds there is an indeterminate range in which mutant products have a level of residual function that may be influenced by additional systemic perturbations (41).

Thus, metabolic-control analysis demonstrated that simple Mendelian traits may substantially behave as complex traits (42, 43).

Metabolic pathways have a control shared among several steps, without a single rate-limiting step control, but with more than one step having significant influence on pathway flux (43). The activity of the particular steps in the pathway may be influenced by nonallelic polymorphisms and/or additional independent mutations or epigenetic influences so that individuals within the population will differ in flux through various steps in the pathway, determining an incredibly intricate "super-level" of genetic complexity, even if individuals are genetically identical. Each individual represents a highly complex collection of systems because of unique genetic and environmental contributions that may explain the frequent result in phenotypic differences among patients even within the same family.

Conclusions

The use of a simple questionnaire made possible to collect information on a large number of MEN1 cases in Italy, although MEN1 cases could be overestimated due to unrecognized phenocopies (21, 31, 38, 39). We confirm the existence of both a clear susceptibility to develop tumors in different types of endocrine and nonendocrine tissues and an intrafamilial phenotype variability of tumoral combinations in subjects affected by MEN1 syndrome. However, these results represent the background for RINEM future efforts, which will focus on the preparation of a detailed questionnaire on clinical, genetic and therapeutic approaches to MEN1 syndrome in our Country. Specific forms to help physicians to look for others MEN1-associated lesions, such as angiomas, angiofibromas, collagenomas and meningiomas (44), currently underestimated at RINEM, and to provide a more detailed clinical anamnesis will be prepared. The availability of a detailed clinical file on Italian MEN1 sporadic and familial cases will offer the unique possibility to perform homogenous prospective studies on both diagnostic and therapeutic strategies in a large number of patients. In particular, both basal and follow up clinical data on pituitary tumors are lacking and no information on pharmacological response of such neoplasms are available. It should be extremely interesting to compare Italian data to the ones from the French-Belgium Multicentric study that reported a pituitary involvement as the initial manifestation of *MEN1* in 17% of individuals and that pituitary adenomas were significantly more frequent in women than men (33).

Hopefully, new acquisitions on the genetic bases underlying MEN1 syndrome will provide clearer explanations on their pathogenesis extended to the pathogenesis of sporadic tumor counterparts. In fact, *MEN1* gene has been described to be involved also in the tumorigenesis of sporadic forms of the following endocrine neoplasms such as bronchial carcinoids, gastrinomas, parathyroid adenomas and insulinomas (44).

In order to implement the activity of RINEM concrete future developments will include the setting of a prospective database to be focused on: a) the bone mineral density variation/year and the response to pharmacological therapy in not surgically-treated MEN1-PHPT patients; b) the age-related variation of parathyroid glands size; c) the prognosis of MEN1-associated GEP tumors versus sporadic, non MEN1, GEP tumors; d) variation of gastrin circulating levels under pharmacological treatment; e) variation of circulating levels of prolactin under pharmacological therapy; f) the age-related variation of pituitary gland size.

Moreover, activities driving to the establishment of a specific Internet web site, preparation of guidelines for the clinical management and genetic counseling of MEN1 patients, and development of educational programs for general practitioners and specialists in Italian Regions that essentially need them will be foreseen.

Acknowledgements

This work was supported by grants from the Associazione Italiana per la Ricerca sul Cancro (AIRC to M.L.B.), from the National Council of Research (PF ACRO 95.00316.PF39 and 96.00520.PF.39) and from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica (MURST 40% and Fondo 60%). Authors are indebted to all the physicians and nurses who so carefully followed the patients and their families around the Country.

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