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Advances in Brief

Localization of the Multiple Endocrine Neoplasia Type I (*MEN1*) Gene Based on Tumor Loss of Heterozygosity Analysis

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Abstract

Multiple endocrine neoplasia type I (MEN1) is an inherited syndrome that results in parathyroid, anterior pituitary, and pancreatic and duodenal endocrine tumors as well as foregut carcinoids in affected patients. The gene responsible for the disease has been linked to chromosome 11q13. We analyzed loss of heterozygosity (LOH) in 188 tumors from 81 patients in an attempt to further define the location of the *MEN1* gene. Both tumors from MEN1 patients and corresponding sporadic tumors were analyzed. Tumor types included parathyroid, gastrinoma, pancreatic endocrine, pituitary, and lung carcinoid. Six tumors (three MEN1 and three sporadic tumors) were identified that provided important LOH boundaries. Four tumors (two parathyroid tumors, one gastrinoma, and one lung carcinoid tumor) showed allelic loss that placed the *MEN1* gene distal to marker *PYGM*. Two tumors (one gastrinoma and one parathyroid tumor) showed an LOH boundary that placed the gene proximal to *D11S449*, one of which further moved the telomeric boundary to *D11S4936*. Taken together, the present data suggest that the *MEN1* gene lies between *PYGM* and *D11S4936*, a region of approximately 300 kb on chromosome 11q13.

Introduction

Identification of the gene responsible for MEN1[2] is an important goal of the neuroendocrine tumor research community. Characterization of the biological function of the gene will likely yield new insights into the etiology and pathogenesis of these tumors. Additionally, the gene may serve as a future diagnostic or therapeutic target in the management of patients with inherited or sporadic neuroendocrine neoplasms.

The *MEN1* gene has been linked to chromosome 11q13 (1–4). High rates of allelic loss in this region have been observed in both tumors from MEN1 patients and from their sporadic counterparts (5–12). In an attempt to narrow the *MEN1* gene interval, we analyzed 188 tumors for LOH on 11q13. Both MEN1-associated tumors and sporadically arising tumors were included in the study. Analysis of tumor LOH was performed to define a minimal interval for the location of the *MEN1* gene.

Materials and Methods

Archival paraffin-embedded and freshly frozen tumor samples were collected from the files of the Laboratory of Pathology, National Cancer Institute. Tumor material was derived from patients seen at the NIH. Tumor samples included 149 MEN1 tumors from 42 patients and 39 sporadic tumors from 39 patients. Eighty-four parathyroid tumors (80 MEN1 and 4 sporadic tumors), 55 gastrinomas (36 MEN1 and 19 sporadic gastrinomas), 43 islet cell tumors (27 MEN1 and 16 sporadic tumors), and 3 MEN1 pituitary and 3 MEN1 lung carcinoids were studied.

Tissue microdissection was performed as described previously (12, 13) and was recently performed by laser capture microdissection (14). Tumor samples and normal cells were dissected from tissue sections, amplified by PCR, and analyzed by autoradiography. LOH was determined visually, based on complete or near complete loss of an allele. All samples were run in duplicate, and the six critical tumors that provided LOH boundaries were analyzed a minimum of two times.

Microsatellite markers included *D11S956*, *D11S480*, *D11S1883*, *D11S599*, *D11S457*, *PYGM*, *D11S1783*, *D11S449*, *D11S4907*, *D11S4908*, *D11S913*, *D11S2072*, and *INT-2* (15, 16). Previously unpublished markers included *D11S4938*, *D11S4939*, *D11S4936*, *D11S4941*, *D11S4933*, and *D11S4944*.[3]

Results and Discussion

Larsson and coworkers were instrumental in initially identifying chromosome 11q13 as the location of the gene responsible for MEN1, and two groups recently reported recombination analyses that substantially narrowed the candidate region (17, 18). The study by the European Consortium on MEN1 represents the smallest meiotic *MEN1* interval to date and localizes the gene to an approximately 2-Mb region (17).

The reliability of LOH analysis in further localizing the *MEN1* gene remains an open question. Previously published reports of neuroendocrine tumor LOH are conflicting, making it difficult to confidently assign a location of the *MEN1* gene based upon allelic loss (9, 10, 19–21). The recent report by the European Consortium on MEN1 questioned the reliability of tumor LOH in localizing the gene, primarily due to the discontinuous patterns of allelic loss that have been observed (17). Clearly, a tumor LOH boundary that provides an inaccurate interval will significantly damage efforts to locate the *MEN1* gene, thus tumor studies must be performed and interpreted with caution (12).

Certainly, LOH analysis of MEN1-related tumors is a difficult task. The combination of normal cell contamination (stromal, endothelial, and normal epithelial), multiple independently arising tumor clones within a single parathyroid gland, DNA recovered from archival formalin-fixed paraffin-embedded tissue, and PCR analysis must be carefully considered. These factors are compounded when newly developed PCR-based microsatellite markers are used that have not been tested extensively on patient tissue samples. Additionally, even with careful analysis of tumors, one must always be cautious of the background LOH that can occur in

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[2] The abbreviations used are: MEN1, multiple endocrine neoplasia type I; LOH, loss of heterozygosity.

[3] P. Manickam, S. C. Guru, L. V. Debelenko, S. K. Agarwal, S-E. Olufemi, J. Weissman, M. Boguski, J. Crabtree, B. Roe, I. A. Lubensky, M. B. Kester, A. L. Burns, A. M. Spiegel, S. J. Marx, L. A. Liotta, M. R. Emmert-Buck, F. S. Collins, and S. C. Chandrasekharappa. Eighteen new polymorphic markers in the MEN1 region, submitted for publication.

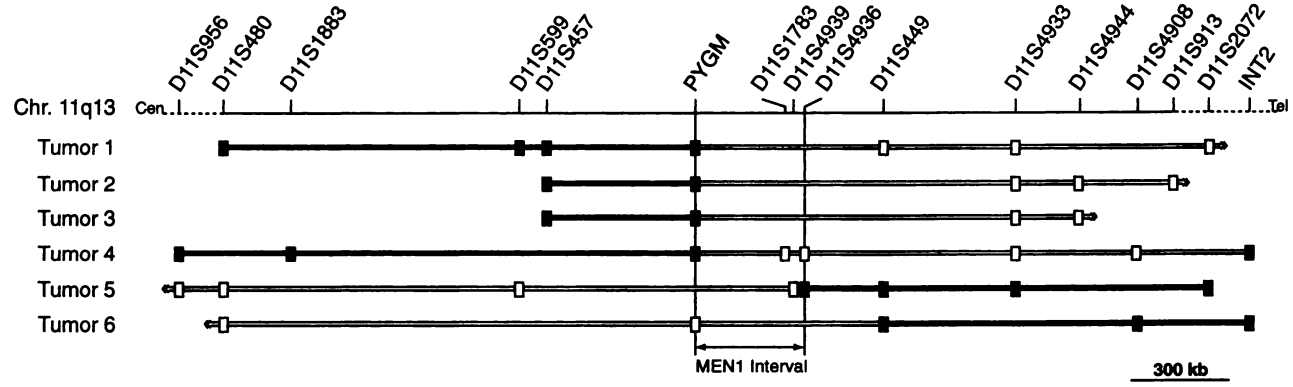

Fig. 1. Schematic diagram of chromosome 11q13 illustrating six tumors that provide LOH boundaries for localization of the *MEN1* gene. ■, allelic retention; □, LOH at informative polymorphic markers. *Tumors 1–4*, the *MEN1* gene is located distal to marker *PYGM*; *Tumor 5*, the gene is centromeric to *D11S4936*; *Tumor 6*, the gene is centromeric to marker *D11S449*. The region between *PYGM* and *D11S4936* is approximately 300 kb.

tumors, presumably due to genomic instability. Finally, the significance of LOH in sporadic neuroendocrine tumors is not entirely clear and could represent loss of a gene on 11q13 other than the one responsible for MEN1. Nonetheless, tumor LOH has been shown to correspond reliably to inactivating second mutations in other familial and sporadic tumors, including renal cell cancer and colon cancer (22, 23), and thus seems a reasonable approach in defining the location of the *MEN1* gene.

In the present study, we analyzed 188 MEN1 and sporadic neuroendocrine neoplasms and detected 6 tumors that provided LOH boundaries. Initially, all tumors were screened with eight polymorphic markers spanning chromosome 11q13. The majority of the MEN1 parathyroid, pituitary, carcinoid, and islet cell tumors showed LOH at all eight markers (12, 24, 25). Sporadic and MEN1-related gastrinomas showed either LOH spanning chromosome 11q13 or retention with all eight markers (12, 25). Tumors with no detectable allelic loss and tumors with limited LOH were selected for further study with an additional 12 polymorphic markers. Six tumors were identified that provided potentially useful boundaries for the *MEN1* gene (Fig. 1). The minimal interval is located between markers *PYGM* and *D11S4936*, a region of approximately 300 kb. All six tumors showed LOH patterns consistent with a single locus for the *MEN1* gene. No boundaries were detected in any of the 188 tumors that did not coincide with the *PYGM-D11S4936* interval. Fig. 2 demonstrates a lung carcinoid (*Tumor 4*) from a MEN1 patient that places the gene distal to *PYGM*. The tumor shows retention at markers *D11S956*, *D11S1883*, and *PYGM* and shows LOH for the wild-type allele at markers *D11S1783*, *D11S4939*, *D11S4933*, and *D11S4908*. *INT2* shows retention, indicating that the allelic loss does not involve the entire long arm of chromosome 11. Fig. 2 also shows a sporadic gastrinoma (*Tumor 5*) that places the gene proximal to *D11S4936*. Markers *D11S956*, *D11S480*, *D11S599*, and *D11S4939* show LOH, and markers *D11S4936*, *D11S449*, *D11S4933*, and *D11S2072* show retention.

The reliability of the present approach is reflected in the frequency of wild-type LOH that was detected in the study. We were able to determine the disease allele in nine MEN1 families by analysis of constitutional DNA from multiple affected family members. Thirty-six tumors from members of these families were analyzed for LOH,

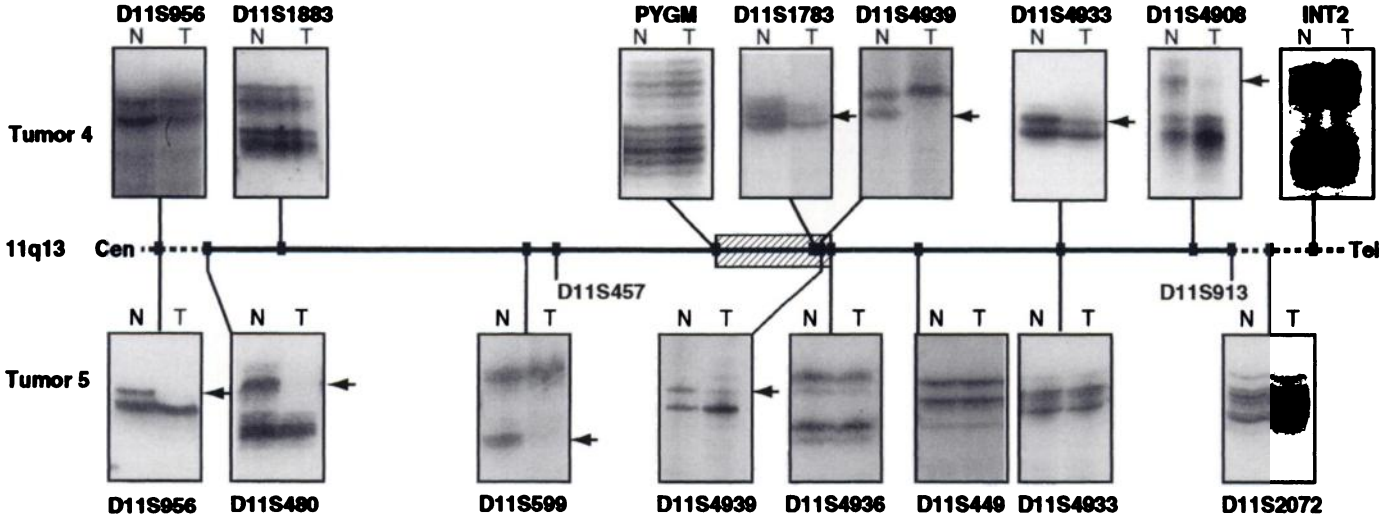

Fig. 2. Schematic diagram of chromosome 11q13. *Insets*, denaturing electrophoresis gel analysis of polymorphic markers for tumors 4 and 5. *N*, normal DNA, *T*, tumor DNA. Tumor 4 shows allelic retention at markers *D11S956*, *D11S1883*, *PYGM*, and *INT2*, and shows allelic loss at markers *D11S1783*, *D11S4939*, *D11S4933*, and *D11S4908* (*arrow*). The retention observed at *PYGM* represents the centromeric LOH boundary and thus is critical to the determination of the minimal gene interval. The upper *PYGM* allele in both the normal and tumor samples is of less intensity than the smaller lower allele, as is commonly observed with this marker, and is indicative of allelic retention in the tumor. The lower allele is wild type in this patient, thus LOH, if present, would result in loss of the lower band. Marker *D11S1783* migrates as a doublet with a more intense lower band and a lighter stutter band 2 bp higher in the gel. The two lower bands in the patients normal DNA sample, which are separated by 1 bp, represent the dominant bands of each of the patients' alleles. LOH in the tumor results in loss of the upper allele. Marker *D11S4933* does not seem to show clean LOH in the tumor. However, the presence of a light upper band that appears partially lost in the tumor sample actually represents a stutter band from the lower allele and is routinely observed at similar intensity in patients who are homozygous for the lower allele. Tumor 5 shows LOH at markers *D11S956*, *D11S480*, *D11S599*, and *D11S4939* and shows allelic retention at markers *D11S4936*, *D11S449*, *D11S4933*, and *INT2*.

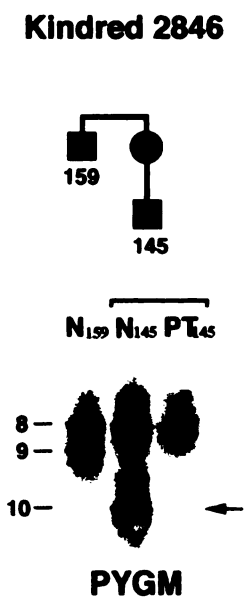

Fig. 3. Denaturing electrophoresis gel analysis of constitutional DNA (*N*) and parathyroid tumor DNA (*PT*) from two affected members from MEN1 kindred 2846. Patient 159 is the uncle of patient 145 and shows alleles 8 and 9 at marker *PYGM*. Patient 145 shares allele 8, indicating this is the allele carrying the *MEN1* disease gene. The parathyroid tumor shows loss of the wild-type allele. Similar results showing loss of the wild-type allele were obtained in 33 MEN1 tumors.

and 33 of these tumors showed allelic loss on chromosome 11q13. All tumors showed loss of the wild-type allele at every marker tested where LOH was present (as an example, see Fig. 3). Loss of the disease allele was never observed, supporting the notion that neuroendocrine tumor LOH analysis is a robust approach and has utility in defining boundaries for the location of the *MEN1* gene.

There are several caveats we considered when interpreting the present data. Of primary concern is artifactual assignment of allelic retention at a given marker in a tumor with LOH, thereby falsely excluding a portion of the candidate interval. This is particularly problematic with PCR-based analysis. For example, in our experience, if tumor samples contain substantial contaminant normal cells (>10%), then one is forced to consider LOH present based upon allelic imbalance. However, PCR primer sets often differentially amplify the contaminant normal allele, making interpretation of LOH difficult and potentially misleading. This difficulty is compounded when allele stutter bands and overlapping alleles are encountered. Careful microdissection of tumors and avoidance of normal endothelial cells when dissecting parathyroid lesions proved valuable to us in the firm assignment of LOH (12).

A secondary danger is artifactual assignment of LOH based on allelic dropout. This is a particular problem when DNA from formalin-fixed paraffin-embedded material is being analyzed. The PCR signal obtained at a polymorphic locus often shows less amplification of the larger allele due to the poor quality of the DNA. If the alleles in a given patient differ substantially in size, the effect can be pronounced and can mimic LOH even when a deletion is not present. Recovery of normal cell DNA from tissue sections is an important control to avoid this problem.

LOH analysis of parathyroid tumors can be problematic due to the presence of multiple independently arising tumor clones with different allelic loss profiles within a single gland (12). Homogenization of whole tumor specimens combines the separate clones and makes interpretation of LOH results difficult at markers where separate tumor clones do not share the same LOH pattern.

However, we believe that tumor LOH analysis can be reliable. Careful microdissection of samples, duplicate PCR reactions with template dilutions, and comparison of tumor results against normal cells procured from tissue sections are useful. The present study identified six tumors that provided boundaries for the *MEN1* gene. Four separate tumors showed *PYGM* as a proximal boundary that is unlikely to be due to background LOH. Interestingly, both of the cases that provided telomeric boundaries are sporadically arising tumors. The reliability of sporadic tumors in *MEN1* gene localization is less clear, but the fact that the entire data set, including MEN1 and sporadic tumors, shows an overlapping and internally consistent region of LOH adds confidence to the conclusion that this is the correct interval. Thus the data suggest that tumor LOH represents inactivation of the identical gene in both MEN1 and sporadic tumors, as predicted by the Knudson model (26), and point to the region between *PYGM* and *D11S4936* as the most likely interval harboring the *MEN1* gene.

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