

Familial Multiple Myeloma: a Family Study and Review of the Literature

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Background: The etiology of multiple myeloma (MM) remains obscure, although reports of familial clustering have implicated both a host susceptibility factor and environmental effects. Here we describe the medical histories of members of a family prone to MM. **Methods:** We developed a pedigree for an MM-prone family by using information obtained from a questionnaire. Protein immunoelectrophoresis of serum and urine from the proband and from 19 family members was performed to detect monoclonal immunoproteins. Peripheral blood obtained from the proband and from five relatives was subjected to standard cytogenetic studies to detect constitutional chromosomal abnormalities. Multi-fluor-fluorescence *in situ* hybridization (M-FISH) and standard FISH studies were performed on peripheral blood from the proband and from two other affected living relatives to determine their karyotypes and to detect clonal chromosomal abnormalities frequently seen in patients with MM. **Results:** Within this family, a sibship of seven included three individuals (including the proband) with histologically verified MM and two individuals with a monoclonal gammopathy of unknown significance (MGUS), as determined by immunoelectrophoresis of serum and urine. This family also had members with acute lymphocytic leukemia, malignant melanoma, and prostate cancer. In the family members tested, we detected no constitutional chromosomal abnormality. None of the three individuals analyzed by FISH had a deletion of the retinoblastoma (Rb-1) locus, which is frequently deleted in patients with MM, and only one (the proband) had a translocation involving chromosomes 11 and 14, a clonal abnormality commonly seen in MM. **Conclusion:** The study of familial MM may provide insights into the pathogenesis and, ultimately, the prevention of MM.

MM and related disorders. [J Natl Cancer Inst 2001;93:1479-83]

Multiple myeloma (MM), regardless of its initial response to therapy, is usually fatal (1). MM has a projected incidence of 13 600 new cases in the United States during the year 2000, which closely approximates its projected mortality of 11 200 (1). MM is a malignancy that involves both mature and immature plasma cells. The proliferation and accumulation of these cells, coupled with their overproduction of specific proteins, have an impact on the clinical manifestations of this disorder (2). Although the etiology of MM remains obscure, environmental factors, particularly radiation exposure among radiologists (3), have been implicated. Compared with other racial groups, African-Americans, especially males, have an increased frequency of MM (4). MM among married couples (5,6) and community clusters of MM (7,8) have also been described, suggesting the potential importance of environmental factors in the etiology of MM.

Reports of substantial familial clustering of MM (4,5,7,9-23) and one report of a pair of identical twins with MM (21) suggest that primary genetic factors may have a role in the etiology of MM. Here we describe an MM-prone family and discuss the clinical pathology and genetic features of the affected family members.

SUBJECTS AND METHODS

Family Study

This study was approved by the Institutional Review Board at Creighton University, Omaha, NE. It was initiated after the proband, the first family member identified, expressed concern to one of the authors (H. T. Lynch) about an excess of MM in her family and gave us permission to study the family. We sent a questionnaire to each of the proband's first- and second-degree relatives requesting a detailed genealogy and medical history, which included their history of cancer at all anatomic sites. We asked living family members with a history of any cancer or the legal next of kin of deceased family members with a history of any cancer to sign permission forms that allowed us to obtain the original medical and pathology documents and any available tissue specimens (slides or blocks) of the affected individuals. A hematopathologist (D. D. Weisenburger) reviewed the slides and tissue blocks.

On the basis of the information that we obtained from the questionnaires, we developed a working pedigree of the proband's family (Fig. 1). Twenty-five available family members (including spouses

session (i.e., a family information service) (24) that covered the natural history of MM, current knowledge about familial factors in this disease, and the aims and objectives of our study. Each individual in attendance was then told that we were interested in identifying a possible genetic basis for MM through studies of DNA obtained from samples of their peripheral blood.

The family members in attendance were told that they could decline participation in this study at any time without penalty. Those family members not in attendance were informed about our study by letter. They were advised that all findings would be held in strict medical confidence and that their identities would be protected if the results of the study were published in the future. Family members were also told that any findings with clinical translation to their benefit would be provided to them and, with their permission, to their family physicians. Genetic counseling was provided to each member of the family individually. The information provided by the genetic counselor was based on MM risk determined by the individual's position in the pedigree, with particular attention being paid to whether they had a first-degree relative with MM.

Immunofixation Electrophoresis of Urine and Serum

Urine was collected over a 24-hour period from 19 first-degree relatives of the proband. We also obtained peripheral blood samples by venipuncture from the same individuals; a portion of each of those samples was used to obtain serum, which was stored frozen at -70 °C. We used the Paragon Electrophoresis System (Beckman Diagnostic Systems, Brea, CA) and the manufacturer's recommended protocol to perform standard immunofixation electrophoresis to identify monoclonal immunoglobulins in the urine and serum samples.

Cytogenetic Studies

Peripheral blood cells were also used for standard cytogenetics studies. Phytohemagglutinin (PHA)-stimulated and unstimulated cell cultures were established from peripheral blood obtained from individuals III-1, III-4, III-5, III-8, III-9, and IV-3. High-resolution G-banding was performed on chromosome preparations from PHA-stimulated cultures to determine if there were any constitutional chromosomal abnormalities or rearrangements segregating with myeloma in this family. Unstimulated peripheral blood cultures were used to determine if there was an acquired clonal chromosomal abnormality.

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tures of the twelfth thoracic and the second lumbar vertebrae and diffuse, abnormal signals in the bone marrow that were consistent with MM. A bone marrow specimen from the sacrum of III-5 showed marked plasmacytosis. A final diagnosis of plasma cell myeloma with hypogammaglobulinemia was made when the urine immunoelectrophoresis revealed monoclonal kappa light chains. Individual III-6 sustained a pathologic fracture of the fourth lumbar vertebra at the age of 53 years. Smears of his bone marrow revealed that approximately 30% of the cells were plasma cells. Serum protein immunoelectrophoresis of this individual's serum detected an IgG-lambda monoclonal protein, which was consistent with MM.

We performed immunofixation electrophoresis on urine and serum samples obtained from 19 other members of this family; the results of these analyses are summarized in Table 1. With the exception of individuals III-1 and III-8, all family members tested had normal test results; i.e., we detected no monoclonal proteins in their urine or serum. Individual III-1 had small amounts of kappa proteins in his serum, whereas individual III-8 had small amounts of monoclonal IgG-lambda proteins in his serum. Neither III-1 nor III-8 displayed any evidence of MM during a thorough clinical and laboratory work-up by their personal physicians. On the basis of these results, we conclude that the appropriate diagnosis for these two individuals is monoclonal gammopathy of unknown significance (MGUS). In addition, we suggest that in-

dividuals III-1 and III-8, who are the brothers of the three siblings diagnosed with MM, may have an increased lifetime risk of developing MM and should be followed carefully in the future. Unfortunately, we cannot determine their absolute risk for MM.

Cytogenetics

Standard cytogenetic and FISH studies were performed. We observed no mitotic cells in unstimulated cell cultures established from peripheral blood samples obtained from individuals III-1, III-4, III-5, III-8, III-9, and IV-3. High-resolution chromosome analysis of the PHA-stimulated cell cultures established from the peripheral blood samples revealed that all but two of these individuals had the normal complement of chromosomes (i.e., 46,XX for females and 46,XY for males). The two exceptions were individual III-5, who had a very unusual polymorphism (variant) involving a very large satellite stalk on the short arm of chromosome 14, and individual III-8, who had the same polymorphism on both copies of chromosome 14. G-banding analysis at the 750-band level and M-FISH studies on individuals III-5, III-8, and IV-3 revealed no consistent constitutional chromosomal abnormality or polymorphism that might serve as a cytogenetic marker to follow the segregation of the MM phenotype in this family.

The retinoblastoma (Rb-1) locus at 13q14 is frequently deleted in individuals with MM (27). Therefore, we performed interphase FISH studies on unstimulated

cultures of peripheral blood from individuals III-5, III-8, and IV-3 by using the LSI D135319 probe, which detects 13q14. All three individuals had two copies of the 13q14 locus, thereby indicating that they did not have a deletion of the Rb-1 region. We also performed interphase FISH on these same individuals by using the LSI IgH/CCND1 probe, which detects t(11:14)(q13;q32), a translocation involving bands 11q13 and 14q32 that is also found frequently in individuals with MM (28,29). Individual III-5 had a translocation involving bands 11q13 and 14q32, but individuals III-8 and IV-3 did not.

Genetic Counseling

We advised individuals III-1 and III-8 of the abnormal electrophoretic findings and their long-term cancer risk implications. These individuals were made fully aware of their increased risk for MM. They were also advised that these findings, particularly in concert with the fact that three of their siblings had MM, warranted long-term follow-up. Specifically, we recommended that each of them have annual protein immunoelectrophoresis of their serum and a 24-hour urine sample to screen for myeloma protein as well as a bone marrow examination when clinically indicated, because of their perceived increased risk of developing MM.

DISCUSSION

This family contains a remarkable sibship, wherein three siblings developed MM and two others, III-1 and III-8, currently have MGUS. However, our genealogic investigations do not allow us to ascribe a mode of genetic transmission for MM in this family. The proband's father, who had skin carcinoma (type unknown) at age 42 years and prostate cancer at age 61 years and who died at age 84 years, did not have any type of hematologic cancer that we can determine. The proband's mother, who died at age 66 years, could conceivably have transmitted MM to her children but may not have lived long enough to develop the disease. This finding may be due to decreased penetrance of a deleterious gene. According to family history, the proband's maternal grandfather (I-2) had leukemia, but whether or not he had MM is unclear. His wife (I-1) died at age 35 years of an unknown cause. The proband's maternal aunt (II-6) died

Table 1. Serum and urine protein immunofixation electrophoresis test results for this kindred*

Individual†	Test (monoclonal proteins detected)		
	SIFE-1	SIFE-2	UIFE
III-1	A (IgA-kappa)	A (IgA-kappa)	N
III-4	N	ND	N
III-5	N	ND	NS‡
III-8	A (IgG-lambda)	A (IgG-lambda)	N
III-9	N	N	N
IV-2, 2 individuals§	N	ND	N
IV-3	N	N	N
IV-5, 5 individuals§	N	ND	N
IV-6, 6 individuals§	N	ND	N

*SIFE = serum immunofixation electrophoresis; UIFE = urine immunofixation electrophoresis; N = normal protein pattern; A = abnormal protein pattern; NS = no urine sample was received from that individual; ND = the test was not repeated; IgA = immunoglobulin A; IgG = immunoglobulin G.

†The number designation for each individual corresponds to the individual number designations on the pedigree in Fig. 1.

‡UIFE results were obtained from this individual's medical records.

Using both high-resolution chromosome analysis and M-FISH, we found no apparent constitutional chromosomal abnormalities or rearrangements that segregated with MM or with MGUS in this family. However, our observation that individual III-8 had two identical copies of a very unusual polymorphism on the short arm of chromosome 14 suggests that this individual's biologic parents may have had a common ancestor. Our inquiries into the possibility of consanguinity have produced no evidence of this to date. Alternatively, it is also possible that the homozygous appearance of the chromosome 14 polymorphism in individual III-8 represents uniparental disomy. Finally, we found that individual III-5 had a translocation involving bands 11q13 and 14q32, which is an acquired chromosomal abnormality commonly linked to MM. Although t(11:14)(q13;q32) is a recurrent clonal abnormality in MM, the prognostic significance is not yet clear.

The literature contains some reports of families with multiple cases of MM. In a review of 53 published families with MM in more than one family member, Roddie et al. (11) identified only three families with three affected siblings. In one of those three families, MM occurred in the three siblings over a 6-year period. Grosbois et al. (10) identified 15 families with multiple cases of MM; three of those families had members with MGUS. The cases of MM in 10 of those 15 families occurred in siblings whose mean age at diagnosis was similar to the mean age at which sporadic MM is diagnosed in the general population. It is interesting that the mean age at diagnosis in those 10 families decreased in successive generations, suggesting the genetic phenomenon of anticipation, which is the increase in the severity of symptoms of a genetic disease with an earlier age of onset in successive generations. Deshpande et al. (12) also found that the mean age at which MM is diagnosed in successive generations was lower for children than for parents, which also raises the possibility of anticipation in familial MM.

Family studies reveal that a subset of MM shows substantial familial clustering consonant with a hereditary etiology of MM. In their review of the pertinent literature, Shoenfeld et al. (19) analyzed 37 families with MM. It is interesting that the family members with MM showed no major differences with regard to sex, age,

cal and laboratory data, or prognosis compared with individuals with nonfamilial myeloma in the general population. The authors did observe an increased incidence of immunoglobulin abnormalities in healthy relatives of the patients manifesting MM, suggesting possible genetic susceptibility to MM. Meijers et al. (18) also identified a family with an increased incidence of immunoglobulin abnormalities in healthy members; in that family, three individuals died of MM and three manifested asymptomatic paraproteinemia.

Studies have also found MGUS occurring in families that also manifested MM. Horwitz et al. (20) described a family in which MM was present in three siblings, two of whom had a history of a monoclonal gammopathy. Their review of the literature suggested that some cases of MM may have a hereditary basis and that other family members may be at increased risk for developing the disease. They concluded that, while families exhibiting several individuals with benign (monoclonal) gammopathies may not be unusual, frank myeloma in three siblings appeared very rarely. Bizzaro and Pasini (16) studied a family in which five siblings had a monoclonal gammopathy. Two of the five siblings were diagnosed with MGUS, and one sister died of MM. There was, however, no association between the human leukocyte antigen haplotypes of these affected individuals and the presence of a monoclonal protein.

Individuals with familial MM, like those with the majority of hereditary cancer syndromes (30), appear to show susceptibility to other hematologic cancers as well as solid tumors. Eriksson and Hallberg (14) studied hematologic malignancies and different types of cancer in related individuals in Sweden. They found that, among 239 case subjects with myeloma and 220 control subjects, individuals who had first-degree relatives with hematologic malignancies, specifically MM, had an increased risk of MM themselves. They also observed an increased risk of MM for individuals whose first-degree relatives had had other types of tumors, especially if they occurred in the prostate or brain.

In the pedigree of the family we have described (Fig. 1), note that the proband's maternal aunt (II-6) had early-onset malignant melanoma, a disorder often associated with a mutation in the CDKN2A

et al. (15) have described a family in which a germline mutation of CDKN2A was present in four melanoma-affected individuals as well as in a fifth family member who had MM. Loss-of-heterozygosity studies performed on sorted bone marrow from the MM patient showed loss of the wild-type CDKN2A allele in the malignant plasma cells. Dilworth et al. (15) have suggested “. . . that germline mutations of CDKN2A may predispose individuals to a wider variety of malignancy than has been hitherto reported, but that the expression of these cancers may depend heavily on the genetic background of the patient.” Whether this CDKN2A mutation is present in our family has not yet been established; this issue will be addressed in our future studies of this family. However, the significance of melanoma and other cancers in our MM family must be viewed cautiously, given the fact that this is a single family and of limited size.

Racial differences in the incidence of MM could indicate cultural and/or inherited susceptibilities to MM. Brown et al. (33) conducted a population-based, case-control interview study of 361 white and 204 black individuals with MM to determine whether family history of cancer contributed to MM and what, if any, factors might explain the racial disparity of risk. For both racial groups, the risk of MM was statistically significantly higher among individuals who had a first-degree relative with MM than among those lacking a first-degree relative with MM. The risk of MM was also increased among those who had a family history of any hematolymphoproliferative cancer, particularly if the affected individual was a sibling of the person whose risk was being assessed. Brown et al. concluded that their study provided no evidence for differences in MM incidence rates according to race.

There are many limitations to our understanding of familial clustering of MM, in which a chance association must always be considered. For example, any estimate of the frequency of familial clustering of cancers such as MM could be substantially distorted by ascertainment bias. Additional biases that could lower the frequency estimates include the reduced penetrance of genes that cosegregate with the MM phenotype and the possible association of MM with a hereditary predisposition to other cancers, such as

ing familial clustering of MM is more likely to be observed in large families, in which genetic susceptibility to MM is present, than in small families with a similar genetic susceptibility, given a larger number of genetically informative individuals in the larger families. Such findings of strong familial clustering of MM are also more likely to be published than are occurrences in patients from small families, skewing the information in the literature.

The etiology of MM remains elusive. However, several studies (14,15,30) have suggested that individuals with familial MM may be susceptible to various other hematologic cancers as well as solid tumors. Although we identified cases of leukemia, prostate cancer, and malignant melanoma in the MM-prone family presented in this report, we believe that caution in the genetic interpretation of these cancer cases must be invoked, given the fact that this is a single family. Our findings that three of five siblings had a diagnosis of MM and two had MGUS appear to defy chance and suggest that an as-yet-unknown host susceptibility factor and/or interaction with common environmental exposures may be associated with these conditions. Although familial MM is rare, our experience indicates that the study of families that have a preponderance of any type of cancer, including those with MM, could be rewarded by insights into the pathogenesis and, ultimately, the control and prevention of the disease.

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NOTES

Supported by revenue from Nebraska cigarette taxes (LB595) awarded to Creighton University and to the University of Nebraska Medical Center by the Nebraska Department of Health and Human Services; by Public Health Service grants 1U01CA86389-01 and CA36727 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; and by the University of Nebraska Medical Center Human Genetics Laboratory Development Funds.

The pedigree in Fig. 1 was drawn by Tami Richardson-Nelson, Department of Preventive Medicine, Creighton University, Omaha, NE.

The contents of this report are solely the responsibility of the authors and do not necessarily represent the official views of the State of Nebraska or the Nebraska Department of Health and Human Services.

Manuscript received March 6, 2001; revised July 23, 2001; accepted July 30, 2001.