Research Vistas in the Multiple Endocrine Neoplasia Syndromes

John J. Mulvihill
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Progress in understanding the single gene, cytogenetic, and multifactorial traits that predispose to human cancer suggests possible new directions for research in the multiple endocrine neoplasia (MEN) syndromes. Among the other 200 or so monogenic disorders associated with human neoplasia, advances have come from further delineation of syndromes by various clinical specialists, the recognition of subtypes of syndromes previously thought to be homogeneous, the search for in vitro manifestations of the mutant gene in fibroblasts, and the establishment of cell, tissue and patient registries and of voluntary lay organizations to serve as advocates for the disease. With regard to cytogenetics, the findings of 20p- and chromosomal fragility demand clarification. Because the age at tumor development varies in MEN, the influence of environment on the mutant gene (ecogenetics) deserves emphasis in future studies. There is a need to achieve a consensus on clinical care, especially the indications and timing of surgery, and to measure the frequency of the disease in the general population.

The genetics of any disease, including neoplasia, can be considered in three parts: single gene disorders, cytogenetic anomalies, and polygenic or multifactorial (ecogenetic) traits. Research in these three areas of cancer genetics, in general, has met with successes and blind alleys that might guide further investigations, in particular, in the multiple endocrine neoplasia (MEN) syndromes.

**Single Gene Traits**

Among the 2,336 monogenic traits in McKusick’s sixth edition (1), there are some 200 traits that have benign or malignant neoplasia as a feature or complication (2). Hence, 9% of the known human genes influence the predisposition or resistance to neoplasia. For ease of memory, these 200 traits, including MEN, can be considered in five arbitrary categories: hereditary neoplasia, hamartomatous disorders, genodermatoses, chromosomal fragility diseases, and immunodeficiencies (3). The MEN syndromes could fit into the first and, especially in the case of MEN-2B or -3 (the multiple mucosal neuroma syndrome), into the second categories. What lessons do other disorders in these five categories have for studies of MEN?

**Polyposes of the colon: A prototype hereditary neoplasia**

Genetic heterogeneity refers to the existence of two or more fundamentally distinct entities within an apparently identical phenotype. Within the disorder generally called hereditary colorectal polyposis, there are up to ten syndromes delineated largely by the pattern of associated findings (4). Examples include familial polyposis coli, Gardner syndrome, and Turcot syndrome. In contrast to the ready recognition of genetic heterogeneity within the polyposes, there seems to be hesitation to distinguish variants of MEN-1 (Wermer syndrome), MEN-2 (Sipple syndrome), and MEN-2B or -3 (the multiple mucosal neuroma syndrome). In fact, many investigators stop with just MEN-1 and -2, presumably in an effort to lump together obviously similar disorders.

For other clusters of related conditions, clinical geneticists have traditionally preferred to split rather than to lump, and with profit. The hereditary anemias were split into hemoglobinopathies and enzyme defects, for example, and further divisions were recognized, to the point that even the alpha-thalassemias have numerous variants at the level of DNA. Mucopolysaccharidoses, Ehlers-Danlos syndrome, and osteogenesis imperfecta each have seven to ten types, for some of which the molecular defects are known (1). Taken to logical conclusion, such splitting makes every affected patient or family a unique MEN, owing to the particular genetic background. My hope in urging recognition of perhaps up to a dozen MEN syndromes is that refined clinical delineation of the constellation of disorders would speed discovery of the molecular basis.

An approach using multiple specialists may aid recognition of genetic heterogeneity. The Gardner syndrome, one of the genetic polyposes, is that rare medical syndrome named after a PhD geneticist. Dr. Eldon Gardner

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*Clinical Epidemiology Branch, National Cancer Institute, National Institutes of Health

Address reprint requests to Dr. Mulvihill, Clinical Genetics Section, Landow Building BC41, Clinical Epidemiology Branch, National Cancer Institute, NIH, Bethesda, MD 20205.
ascertained his first kindred because of familial colon cancer (5), then engaged various clinical specialists in clarifying the family’s health problems. Surgeons saw tumors in the colon and under the skin; radiologists, osteomases; dentists, jaw cysts (6). New features of even well-known Mendelian traits continue to be recognized. For example, a newly described peripheral retinal pigmented defect may be pathognomonic of the Gardner syndrome (7). It is not unreasonable to expect that Mendelian traits having multiple organ defects, even of late onset, might have some dysmorphic features; however, these might be subtle and require the attention of enthusiastic clinical specialists. Have MEN patients been overly sequestered in the endocrinology clinics? Would syndrome-oriented ophthalmologists, dysmorphologists, orthopedists, dentists, etc, be able to recognize new subtypes of MEN?

Another lesson has been derived from the polyposes in the extensive studies of the phenotypic manifestations of the mutant gene in tissue culture, especially fibroblasts (8). While some of the reported traits seem to represent cellular transformation, a few, notably ready transformability of fibroblasts exposed to Kirsten sarcoma virus (9), have been duplicated independently.

Tumorigenesis following exposure to the classic tumor promoter, phorbol ester, suggests that polyposis fibroblasts are already “initiated” (10). (Phorbol ester does not cause tumors in normal animals without prior exposure to an “initiator.”) That polyposis cells are already “initiated” may be a result of the deformed actin cables below the plasma membrane. This primary defect could be related to loss of growth inhibition by cell-cell contact, a typical feature of malignancy.

A relevant point for research in MEN is that fibroblasts from affected patients have the mutant gene and may deserve additional in vitro study even though defects of connective tissue are not recognized in these disorders. A prerequisite for such laboratory explorations is the easy availability of cell lines to laboratory scientists. Yet, the current catalogues of the two largest public repositories in the United States (11,12) list no stored fibroblasts from MEN patients. Furthermore, they include no tumor cell lines from medullary carcinoma of the thyroid and pheochromocytoma. Although the use of tumor cell lines is not the same as using direct tumor preparations, such studies are valuable to understanding tumor biology. For example, partly through investigations of newly established cell lines, small cell carcinoma of the lung has been found to have a distinct chromosomal deletion of 3p14-23, a peculiar pattern of cell products suggesting neural crest origins, and an astonishingly good response to multiple modality cancer therapy (13).

Finally, we have a problem of specimen resources. Techniques of modern molecular genetics require fresh or frozen tissue, and single laboratory investigators have difficulty obtaining appropriate samples without a centralized system of specimen collection, storage, and distribution.

At the level of the pedigree resources, experience with the polyposes shows the value of long-term registry of patients and kindreds. St Mark’s Registry, London, established in 1925, is the source of most of the information concerning natural history necessary for clinical counseling (14).

In summary, the lessons for MEN researchers derived from the experience with another hereditary neoplasm, the polyposis syndromes, are these:

1. recognition of genetic heterogeneity;
2. delineation of subtypes of the syndrome (in part by astute clinical specialists);
3. possibility of fruitful genetic studies with fibroblast tissue cultures;
4. need for the storage and distribution of necessary biologic specimens such as fibroblasts, tumor cell lines and fresh tumors;
5. continued need for registration of kindreds.

**Neurofibromatosis: A prototype hamartomatous disorder**

Several features of multiple neurofibromatosis (NF, von Recklinghausen disease) resemble MEN-2B or -3: pheochromocytoma, café-au-lait spots, cutaneous neural tumors, and the autosomal dominant inheritance pattern. Moreover, many of the research challenges in NF (15) apply to MEN as well:

1. Are these diseases truly neurocristopathies (abnormal derivations of the embryonic neural crest)? Bolande advanced the argument favoring this classification for NF with MEN (16), but Schimke does not consider the MEN syndromes as neurocristopathies (17).

2. What are the minimal diagnostic criteria? One hundred years after von Recklinghausen’s monograph, the bases for the diagnosis of NF remain debatable (18). It should not be discouraging that they are unsettled for MEN.

3. Where is the gene locus and what does the normal allele do? In 15 patients with NF, we observed no cytogenetic defects using an 850-band prophase technique on peripheral lymphocytes (19). The cosegregation of NF with two myotonic dystrophy families suggests linkage on chromosome 19 (20). Family studies with 32 standard markers suggest linkage to GC on chromosome 4p (21). As reported elsewhere in this issue, the search for the MEN gene(s) is focusing on 20p and 11p.
4. Is there a maternal effect? Some observers (22), but not all (23), report that offspring with NF are more severely affected when the disease is present in the mother rather than in the father. A factor that crosses the placenta is postulated as responsible. Since there are many obvious blood factors in MEN, a maternal effect should be sought. The occurrence of Hirschsprung disease in offspring of mothers (but not fathers) with MEN in one large Quebec pedigree could represent a similar maternal effect (24, 25).

Ataxia-telangiectasia: A prototype disorder with immune deficiency, radiosensitivity, chromosomal breakage

Ataxia-telangiectasia (AT), a single gene trait predisposing to malignancy, epitomizes much of modern cancer biology (26). Some features seen in MEN resemble what is known about AT.

AT patients have elevated serum levels of alpha-fetoprotein and carcinoembryonic antigen. The MEN syndromes have hormonally active substances for disease markers. In AT, immunoglobulins E and A in serum are reduced or nearly absent; although helper T-cells are defective, the exact immunologic defect is unclear. Similarly, immune functions in MEN merit analysis by current techniques.

AT manifests unusual sensitivity to ionizing radiation both in vivo and in vitro. Clinically, severe radiation toxicity may occur following customary doses of radiation given for lymphomas. Experimentally, the survival of colonies of AT fibroblasts following gamma-radiation is impaired in comparison to normal fibroblasts or even xeroderma pigmentosum cells, which are unusually sensitive to ultraviolet radiation.

Medullary thyroid carcinoma is not known to follow radiation exposure, but epidemiologic experience with this rare tumor is limited. Three patients were described at this conference who developed medullary thyroid carcinoma years following thyroid ablation by radioactive therapy. Clearly, the C-cells had escaped the lethal effects of radiation. Possibly they were damaged, but they survived radiation exposure later to manifest a somatic mutation that caused cancer, while the thyroid cells or more radiosensitive C-cells died. There is precedent for this hypothesis. A family studied at the National Institutes of Health had a variety of cancer types, including a boy with osteosarcoma, which arose in the field of radiation given for a malignant schwannoma; a second male family member who had occupational exposure to radionuclide production (27) developed polycythemia vera. In this kindred the fibroblasts were found to be unusually resistant to killing by radiation.

A final common feature of AT and MEN is the phenomenon of chromosomal fragility. In general, chromosome breakage can be constitutional as in the Fanconi and Bloom syndromes, or be acquired following environmental exposure, as in survivors of the atomic bombs in Japan. In AT, chromosome breakage is seen in vitro following radiation, but does not occur spontaneously. As reported in this issue (28,29) and elsewhere (30-32), excessive chromosome breakage has been seen in some MEN-2 patients but not all. If constitutional chromosome fragility is verified in MEN, testable hypotheses concerning the pathogenesis of the tumors are possible. If the breakage proves to be acquired, there is further reason to study radiosensitivity in MEN. In the lymphomas and leukemias that complicate AT, the nonrandom clonal abnormalities involve chromosome 14 and are almost exclusively translocations.

Apart from the possibility of fragility, the cardinal constitutional cytogenetic abnormality in MEN-2 seems to involve chromosome 20p (28).* This finding deserves major pursuit because of the obvious success in assigning two other single gene traits, retinoblastoma and Wilms tumor, to a chromosome locus and because of the opportunity to explore the mode of gene expression. All five disorders—MEN-1, -2A and -2B, retinoblastoma, and Wilms tumor—are autosomal dominant traits. All but MEN-1 have been associated with chromosomal deletions, reported by at least some laboratories. All can present as childhood tumors; most are associated with certain birth defects. These features raise the question of whether intense scrutiny of DNA restriction fragment length polymorphisms from tumors of MEN patients would yield results similar to those obtained for retinoblastoma and Wilms tumor (33, 34); hereditary neoplasms that are dominant at the level of the organism appear to be recessive at the level of the cell. Specifically, several different observations indicate that in those two embryonal tumors, the mutant gene is often present in a double dose. This is the functional definition of an autosomal recessive trait. By analogy, MEN tumors may also be expressed as recessive traits at the level of the cell.

In summary, investigations in MEN might follow leads more fully developed in studies of AT, immunodeficiency, chromosomal breakage, and sensitivity or resistance to radiation.

Ecogenetics (Multifactorial Inheritance)

Just as pharmacogenetics refers to inherited variation in response to drugs, ecogenetics refers to such variation in response to environmental agents in general (35). Geneticists tend to ignore the prominent role of the environment in genetic disorders. In some 13 human genetic traits, interaction of an environmental agent

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*Ed. note: This abnormality is discussed in more detail in the paper by Van Dyke, et al in this issue.
with a mutant gene causes tumor formation or, in the
case of AT, toxicity occurs in response to conventional
tumor therapy with radiation. Examples of ecogenetic
interactions include hemochromatosis, tyrosinemia,
skin cancer following solar radiation in albinos, and
hepatoma following androgen treatment in Fanconi
anemia patients.

What are the environmental influences on the expres­
sion of the MEN gene? The tumors are not congenital
but develop over time. Perhaps epidemiologists should
study patients with MEN, medullary thyroid carcinoma,
or pheochromocytoma in a case-control fashion to
determine whether environmental differences, in
medications, occupation, or associated diseases, for
example, correlated with early-age pheochromocytoma
in MEN-2 compared with late-age sporadic pheo­
chromocytoma. Why do some family members develop
pheochromocytoma first and others, medullary thyroid
carcinoma? Twin studies, a favorite tool of geneticists,
deserve consideration as a way to clarify environmental
influences and to strengthen the knowledge of the
spectrum of the diseases at the same time.

**MEN as a Public Health Concern**

Geneticists must remind health professionals and lay
persons that hereditary disease is treatable, and some
manifestations are preventable (36). The MEN syndromes
have earned a place on the short list of traits where
surgery is performed prophylactically to prevent cancer.
Thyroidectomy is recommended whenever the MEN-2B
or -3 phenotype is recognized to prevent inevitable
thyroid cancer. Similarly, in MEN-2, thyroidectomy is
indicated when plasma calcitonin levels suggest C-cell
hyperplasia. The timing of adrenalectomy before dem­
onstration of malignant pheochromocytoma remains a
matter of debate. In familial polyposis, colectomy
should be carried out before age 20 years to prevent an
inevitable cancer of the colon. Some women from fam­
ilies shown to have familial breast or ovarian cancer
have chosen surgery for prophylaxis. Health care scien­
tists should verify that MEN patients and their families
are offered appropriate surveillance and surgery.

**Improving Research Resources**

**Developing lay support**

Patients and their relatives should be regarded as a
potential research resource. Support for finding the
Huntington disease gene locus is attributed to an en­
thusiastic lay organization. Muscular dystrophy, cystic
fibrosis, Tay-Sachs disease, and neurofibromatosis have
special interest groups. National organizations of
patients and interested citizens distribute information
and publicity better than do clinicians and scientists,
provide a ready source of subjects for new clinical
research projects, and stimulate research finance by
funding pilot efforts which may qualify for larger grants.

**Collection and storage of specimens**

Certain research projects require obtaining biologic
specimens from human patients. Such specimens,
which must be properly collected from selected indi­
viduals, include serum, lymphocytes, and red blood
cells for genetic markers. Furthermore, some fibroblast
lines should be stored as renewable sources of cells and
DNA with mutant genes. Chromosome analysis of solid
tumors, which is done well in few laboratories, requires
fresh tumor specimens. Surgeons and pathologists
must be warned not to drop the entire operative speci­
men into fixative. Frozen tumor tissue is satisfactory for
studies of tumor DNA (eg, to look for oncogenes).
Finally, in those areas where cattle are known to have
multiple endocrine tumors, university veterinary clinics
should be enlisted to collect tumor specimens. Inves­
tigators of the human MEN syndromes have not yet fully
evaluated the analogous, if not homologous, traits
which occur in cattle in Michigan (37) and Indiana (38).

**Disease frequency**

There are no reliable estimates of the prevalence or
incidence of the MEN syndromes, and enumeration of
some of the component tumors is only slightly better.
The United States system of measuring cancer inci­
dence is the analysis of a 10% sample of the United
States population. In the mid 1970s, only four malignant
pheochromocytomas and 371 medullary carcinomas of
the thyroid were registered annually (39). (The MEN
syndromes are not recorded as single syndromes in the
Cancer Surveillance, Epidemiology and End Results or
SEER program, which almost exclusively registers neo­
plasmats that are coded as histologically malignant.)

In the worldwide standard for classifying diseases,
neoplasms are coded by organ site (40). Thus, medullary
carcinoma of the thyroid is grouped with all other his­
tologic types of thyroid malignancies. Not listed in this
index are “multiple endocrine neoplasm,” “multiple
mucosal neuroma syndrome,” or “Sipple syndrome.”
Wermer syndrome or MEN-1 is assigned to T258.0,
“polyglandular activity in multiple endocrine ade­
nomatosis,” which is not to be used as the primary
code for multiple endocrine adenomatosis. The latter
tumor is assigned T237.4, “neoplasm of uncertain be­
havior of other and unspecified endocrine glands,
including parathyroid and thyroid glands.” This four-digit
topology number is the code used in death certificates
and most hospital discharge summaries nationwide.
Finally, the index lists “adenomatosis, endocrine (mul­
tiple)” with the code M8360/1. This morphology code,
which is used by some cancer registries and most
pathology departments, fails to distinguish among
MEN-1, -2A and -2B or -3, or between familial and sporadic cases. Codes are changed periodically by a World Health Organization (WHO) committee, but only when asked to do so.

Conclusion

Progress in research of single gene, cytogenetic, and ecogenetic traits that predispose to human cancer suggest various directions for future investigations of MEN. The highest priorities are: 1) further syndrome delineation, 2) collection and availability of cellular and tissue specimens for laboratory research and genetic marker studies, 3) confirmation of the cytogenetic associations, and 4) encouragement of a lay organization on behalf of MEN.

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References

References