Genetic Studies of Multiple Endocrine Neoplasia Type 2 Syndromes: A Workshop Commentary

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Several papers presented at this workshop have emphasized the need for continuing studies of multiple endocrine neoplasia type 2 (MEN-2) at the basic science level. As yet, we do not know whether this hereditary cancer is determined by a deletion and/or a point mutation in a gene or genes. The MEN-2 syndromes have been classified clinically (1), but we still do not know whether the same genetic mechanism determines all the clinical subtypes, whether each of the subtypes is genetically unique, or whether genetic heterogeneity exists within the subtypes.

Using high resolution banding, Babu, et al (2) have demonstrated a deletion in four families with MEN-2A and in three of five families with MEN-2B (MEN-3). Technically, demonstration of the deletion has been extraordinarily difficult, and among samples from 14 patients and 11 controls, they made three "diagnostic errors" (in one patient and in two controls) at the first testing. All of their samples were studied in a double-blind fashion. The retesting of chromosomes from five individuals was somewhat selective. Repeated were samples from two controls in which the deletion was seen on the first but not on the second test; samples from two siblings with MEN-2B, in which one had the deletion and the other did not on first testing, whereas both had the deletion on the second test; and samples from one MEN-2A patient in which the deletion was seen in both tests. Van Dyke, et al (3) have presented additional evidence for the deletion in nine of 10 families with MEN-2A (90%) and in three of five families with MEN-2B (60%). Furthermore, they found no deletion in four unrelated patients with MEN-1 (the Wermer syndrome).

The deletion hypothesis is theoretically attractive because, for this dominantly inherited cancer, the first of the two hits (4) could be the deletion, and the second hit could lead to tumor formation in the thyroid or adrenal glands. The mechanism in MEN-2 might be analogous to the variable cytogenetic recognition of a constitutional deletion in lymphocytes of retinoblastoma patients with the loss of heterozygosity or, in other words, loss of the normal allele in their tumor cells (5). The hypothesis for MEN-2 can be extended to suggest that there are different-sized deletions, some of which cannot be recognized cytogenetically but may exist, nevertheless, as so elegantly proved for retinoblastoma by Cavenee, et al (5) using protein and DNA markers. If the same is true for MEN-2, this mechanism would account for the MEN-2 patients in whom Babu and Van Dyke did not recognize the deletion.

Furthermore, if the deletion data are correct, one has to postulate that the clinical subtypes MEN-2A and -2B are determined by a deletion at the same site on chromosome 20, although the deletion might vary in length. Van Dyke at this workshop has given us the opportunity to see the deletion first-hand. It is disturbing, however, that others cannot demonstrate the deletion (6-9). This might be explained by genetic heterogeneity or some unknown difference in the exacting technique. On the other hand, P. J. Goodfellow, et al at this workshop presented evidence (unpublished observations) that a DNA sequence that maps to the same chromosome band as the deletion and the gene for MEN-2A in one kindred are not linked to each other. This probe was assigned to the chromosome band p12 by "in situ" hybridization by two cytogeneticists independently (H-S. Wang, division of Medical Genetics, University of Manitoba, Winnipeg; and A. M. V. Duncan, Division of Medical Genetics, Queen's University, Kingston). Goodfellow, et al (at this meeting) had evidence that the locus for MEN-2 is not within three centiMorgans (cM) on either side of the DNA probe sequence using restriction fragment length polymorphisms and lod score analysis (10) in the Kingston kindred with MEN-2A (11), and subsequently not within 10 centiMorgans in two additional families (12). Since the DNA sequence maps to the same band as the deletion, it is now necessary to exclude linkage using samples from a family in which the putative deletion has been seen. The three families studied by Goodfellow, et al may be genetically different from those studied by Babu and Van Dyke. At present, Van Dyke is attempting to visualize the deletion in the Kingston family, and Goodfellow is searching for linkage data in the SLA family in the Babu, et al.
The physical length of 20p is estimated to be about 30 cm (13), and the p12 band is considerably less than one third of 20p. On the other hand, the genetic length as measured by chiasma frequency or linkage data may be considerably longer than the physical length, as has been shown for the distal long arm of chromosome X (14) and chromosome 1p (15). Even so, a linkage exclusion at 10 cm would suggest that the MEN-2 locus is not at 20p12, at least in some families.

Obviously, the way in which the gene or deletion determines the thyroid and adrenal tumors is the ultimate goal of mapping studies. The location of the gene may be seen as a stepping stone toward isolating the gene and understanding its expression. If not at 20p12, then where? We know several places where it is not. The shortest regions of overlap (SROs) are shown in the Figure for loci that are not within a 10 cm distance of the MEN-2 locus (16). These data are pooled from published studies (7,17-20) and from unpublished data (Forster-Gibson and Simpson; Kruger, et al; and Goodfellow, et al). Caution is necessary when interpreting combined data since we do not know if we are dealing with genetic heterogeneity between MEN-2 families. The exclusion data will tell us where not to look first for linkage. The Figure illustrates that a very small proportion (about 6%) of the autosomal genetic material has been excluded from having the MEN-2 locus using the criteria of a lod score of ≤ 2.0 when θ = 0.10, ie, the MEN-2 locus is not within 10 cm of the marker locus. Furthermore, only when the SRO for the marker locus is very small can one even consider that the locus for MEN-2 is excluded from that particular region of the chromosome. Most of the studies used conventional markers, but studies using DNA restriction fragment length polymorphisms are underway, as this workshop has indicated. If the 20p deletion hypothesis should later prove to be untenable, although the search will still be very wide (Figure), the number of polymorphisms available for linkage studies will increase exponentially.

Another direction in genetic studies is the search for evidence of increased chromosome instability as measured by growth inhibition, frequency of spontaneous or induced chromosomal aberrations or of sister chromatid exchanges. Data on chromosome instability presented at this workshop have been somewhat inconsistent, as have those in the literature. Hsu, et al (6), studying patients with MEN-2, and Gustavson, et al (8), studying both MEN-1 and MEN-2, found an increase in chromosomal aberrations in lymphocytes from patients as compared to controls. An increase in sister chromatid exchanges was not observed in either of the above studies or in data reported by Van Dyke, et al (3). In this workshop, Wurster-Hill had evidence for chromosome instability from increased chromosomal aberrations in lymphocytes from one MEN-2A family but not from another. Deed found an increase in neither spontaneous nor induced (X-ray) chromosomal aberrations in lymphocytes from patients with MEN-2B (presented at this workshop). Deed, however, observed a low mitotic index in a sample from a patient with sporadic medullary carcinoma of the thyroid. Such inconsistencies could be the result of genetic heterogeneity, and other studies are necessary to establish whether chromosome instability occurs in the MEN syndromes.

A comparison of karyotypes in peripheral lymphocytes and tumor cells is yet another obvious direction for research in the MEN-2 syndromes. Studies of the rare hereditary cancers such as retinoblastoma (RB) (5,21,22), Wilms' tumor (23-26), and MEN-2 may be models that eventually will help us to understand the common cancers. These three cancers have certain common characteristics: the tumors are tissue specific; they are derived from neural crest ectodermal cells; they are inherited in an autosomal dominant pattern; and they occur at an early age. Combined linkage and chromosomal studies have contributed to the understanding of the first two hereditary cancers. For example, in retinoblastoma the dominant RB gene was known to be linked to esterase D (a polymorphic red cell enzyme) (27), and the gene for this enzyme had been mapped to 13q14 (28). In some patients a constitutional deletion of 13q14 was seen in their lymphocytes. In the tumor cells from these patients at least a portion of 13q14 of the remaining normal chromosome 13 homolog was shown always to be lost in the tumor cells. In some cases a constitutional deletion was not observed, but a submicroscopic deletion in the tumor cells was implied by the lack of esterase D activity, loss of heterozygosity for the enzyme variant, or loss of a DNA marker mapped to the 13q14 site. It has been postulated that the tumor cells have lost the marker allele and the normal RB allele from the previously normal chromosome 13 homolog (5).

Although no definitive answers have yet come from genetic studies, the studies presented at this workshop and the opportunities for discussion have shown new directions and great enthusiasm for solving the genetic mechanism determining MEN-2.
The loci for genetic markers and their shortest regions of overlap (SROs) (16) for which linkage with the locus for MEN-2 has been excluded up to a recombination fraction (θ) of 0.10, i.e., when lod scores were = -2.0. Linkage data from published and unpublished studies were combined.

*INS and HBBC in this region each had a lod score of -1.0 for θ = 0.10 when tested against the MEN-2 locus (19).

**Exclusion of linkage between the pRI2.21 DNA sequence mapped to 20p12 (the site of the putative deletion) has been shown at θ = 0.03 and was reported at the workshop by Goodfellow, et al in one family and later in two additional families (12). This divergence from the 20p deletion data of Babu, Van Dyke, and Jackson (2) awaits explanation.
References

17. Jackson CE, Conneally PM, Sizemore GW, Tashjian AH. Possible linear order of genes for endocrine neoplasia type 2, the P red cell antigen and HLA on chromosome 6. Birth Defects 1976;12:159-64.