Published in final edited form as:

Cancer Genet Cytogenet. 1987 January ; 24(1): 129–135.

High Resolution Chromosome and DNA Analysis in Multiple Endocrine Neoplasia Type II Syndrome

Merlin G. Butler, David R. Repaske, Geeta M. Joseph, and John A. Phillips III

Division of Genetics, Department of Pediatrics, Vanderbilt University School of Medicine, Nashville, TN

Abstract

Multiple endocrine neoplasia type II (MEN-II or Sipple's syndrome) is an autosomal dominant disorder characterized by medullary thyroid cancers, pheochromocytomas, and parathyroid adenomas. A blind analysis of high resolution G-banded chromosomes was performed on blood specimens from eight MEN-II individuals from three unrelated families and six control subjects. Seven of eight MEN-II patients and one of six control subjects were determined to have a deletion at 20p12.2. These findings support the hypothesis that MEN-II patients have a 20p12.2 deletion ($\chi^2 = 6.99$; $p < 0.01$).

Genomic DNA from seven of the eight MEN-II patients was studied using the DNA probe, D20S5, localized by in situ hybridization to 20p12. The probe binding site is not deleted in some MEN-II patients, as demonstrated by the presence of two alleles detected as restriction fragment length polymorphisms. Thus, D20S5 does not hybridize to DNA sequences that are deleted based on cytogenetic analysis in MEN-II patients.

INTRODUCTION

Multiple endocrine neoplasia type II (MEN-II; also known as MEN type 2a or Sipple's syndrome) is an autosomal dominant disorder with high penetrance and variable expressivity [1–5]. MEN-II patients develop medullary thyroid carcinoma (nearly 100% of cases), pheochromocytoma (50% of cases), and parathyroid adenoma (30% of cases). An elevated calcitonin level (either basal or after provocation by Ca^{++} or pentagastrin infusion) may precede the development of frank medullary carcinoma of the thyroid (MCT) by as much as 20 years [6].

Several associated cytogenetic abnormalities have been reported since Sipple [7] first described the syndrome in 1961. Although Al-Saadi and Lieberman [8] reported a high incidence of aneuploidy in leukocyte preparations from three affected patients, these findings were not confirmed by others [9, 10]. Chromosome instability in patients with MEN-II, including chromatid and chromosome aberrations (e.g., breaks, gaps, chromatid exchanges, dicentric and ring chromosomes, and acentric fragments) not confined to a particular chromosome, has been reported [11–13]. In blind, age-matched studies of 14

individuals with MEN-2a and 2b, however, Babu et al. [14] and Van Dyke et al. [15] did not find an increased prevalence of chromosome or chromatid abnormalities.

Using high resolution G-banding techniques, Van Dyke et al. [16] reported a specific deletion of 20p12.2 from each of seven MEN-II patients. This was followed by a blind cytogenetic study of eight MEN-II patients from four kindreds with 11 controls [17]. A deletion localized to 20p12.2 was detected in all of eight MEN-II patients, but also in two of 11 controls. Other investigators [11, 12, 18, 19] have failed to detect this 20p12.2 deletion in a total of 12 MEN-II syndrome patients studied. Only two of these studies [11, 12] used high resolution G-banding techniques, however, and each of these studies involved only two patients.

Recently, Goodfellow et al. [20] reported a linkage study suggesting that the MEN-II locus does not segregate with a sequence assigned to 20p12. Using an anonymous chromosome #20 DNA probe, D20S5, assigned by in situ hybridization to 20p12, they excluded linkage with MEN-II at recombination fraction $\theta = 0.13$. These studies involved no cytogenetic analysis in MEN-II syndrome patients.

We report herein our combined high resolution chromosome findings and DNA analyses with the D20S5 probe in eight MEN-II patients from three unrelated families and six control subjects.

MATERIALS AND METHODS

Subjects and Clinical Evaluation

Eight affected subjects from three unrelated kindreds with MEN-II syndrome inherited in an autosomal dominant pattern and six unrelated control subjects (all of European ancestry) had grossly normal intelligence and were not dysmorphic. The eight MEN-II syndrome individuals (five female, three male) had an average age of 40 years (range, 25–58 years), and the six control subjects (three female, three male) had an average age of 45 years (range, 31–68 years). All MEN-II syndrome individuals in our study were found to have medullary carcinoma of the thyroid, three had a pheochromocytoma, and one had a parathyroid adenoma (Table 1).

High Resolution Chromosome Analysis

Chromosomes were studied by high resolution techniques, with a modification of actinomycin D pretreatment [21, 22]. Cells were grown for 66–68 hours in RPMI 1603 medium with actinomycin D (5 $\mu\text{g}/\text{ml}$, final concentration) for the last 45 minutes. Colcemid (0.06 $\mu\text{g}/\text{ml}$, final concentration) was added for the last 5–6 minutes before harvesting. Harvesting, slide preparation, and G-banding were routine.

Slides were coded and analyzed without knowledge of subject identity. Prometaphase and late prophase spreads were located and two or three cytogeneticists independently scored each pair of chromosome #20 for the 20p deletion. All scoring was performed through the microscope to maximize optical resolution, similar to the procedure described by Babu et al. [17]. Nine to 24 cells without overlap or twist at 20p (at the 700–1200 band level) were

identified and analyzed. After the cytogeneticists agreed that 20p was normal or deleted in the majority of cells, the code was broken.

Restriction Endonuclease Analysis

High molecular-weight DNA was isolated from peripheral leukocytes [23]. Aliquots of 5 µg genomic DNA from each subject was digested overnight with Msp I or Pvu II (Bethesda Research Labs and New England Biolabs) using conditions specified by the manufacturer. The resulting fragments were separated by electrophoresis in 1% agarose gels and transferred to Biodyne (Pall) or Gene Screen Plus (New England Nuclear) membranes by Southern blotting [24]. Clone D20S5 (gift of D. Shaw) contains an anonymous genomic sequence, which has been assigned to 20p12 by in situ hybridization [20], was used as a probe after nick translation with ³²P-dATP and ³²P-dCTP [25]. Hybridization and washing of filters and autoradiography were carried out using standard methods.

RESULTS

High Resolution Chromosome Analysis

Our blind study included coded blood samples and microscope slides from eight patients with MEN-II syndrome and six control subjects (Table 1). Seven MEN-II syndrome patients and one control subject were scored as being heterozygous for the 20p12.2 deletion (Table 2; Fig. 1). The correlation of the chromosome #20 cytogenetic status with the individual's disease state in 12 of 14 individuals in our blind study is statistically significant ($\chi^2 = 6.99$; $p < 0.01$).

Restriction Endonuclease Analysis

Digestion of human genomic DNA with Msp I or Pvu II yields restriction fragment length polymorphisms (RFLP) when hybridized with the D20S5 probe [20]. Following Msp I digestion, a constant 5.6 Kb fragment, as well as 3.8 Kb (the A1 allele) or 3.0 Kb fragments (the A2 allele) are detected. Following Pvu II digestion, fragments of 4.8 Kb, 3.2 Kb, and either 2.8 Kb (the B2 allele) or 1.9 Kb (the B1 allele) were detected.

Genomic DNA from seven of the eight MEN-II patients was studied using D20S5 (Table 3). Four patients had only the A2 and B1 alleles of the D20S5 RFLP following Msp I and Pvu II digestion, respectively, whereas, three others were heterozygous (i.e., had A2 and A1, or B1 and B2 alleles) following digestion with either endonuclease. DNA from three control subjects analyzed for D20S5 binding showed heterozygosity for the same RFLP following digestion with either endonuclease. Furthermore, analysis of inheritance of RFLP that bind D20S5 directly demonstrate that an affected patient transmits a normal sized B1 allele to her affected offspring (Fig. 2).

DISCUSSION

In this blind study we observed differences in 20p that were interpreted as an interstitial deletion in seven of eight patients with MEN-II syndrome, and in one of six control subjects. The single control subject judged to have the deletion is the nonconsanguineous wife of an

affected patient. Because her family history is negative for MEN-II she is unlikely to be an undiagnosed patient.

Our cytogenetic findings are similar to those reported by Babu et al. [17], in which an interstitial deletion of 20p12.2 was identified in eight of eight individuals with MEN-II syndrome and in two of 13 control subjects. Other investigators [11, 12, 18, 19] have failed to detect a 20p12.2 deletion in MEN-II syndrome patients, although their studies were apparently not blind and included only two patients per study. Because the apparent deletion is subtle it may be easily overlooked if the chromosomes are not G-banded or in prometaphase. For this reason, the use of blind studies and high resolution techniques are indicated. Using these methods, we found a statistically significant correlation between 20p12.2 deletion and the MEN-II syndrome.

In our DNA studies we did not observe decreased binding of the D20S5 probe to the DNA of any affected persons, as would have been expected with deletion of an allele from one chromosome #20. Furthermore, our finding of heterozygosity for the RFLP of affected persons indicates that the probe is hybridizing to two distinct alleles: one from each chromosome #20. The demonstration of transmission of a normal sized D20S5 allele from an affected mother to her affected offspring indicates that inheritance is not via a null allele. These results indicate that the sequences examined are not deleted on either chromosome #20 in MEN-II patients. These findings are consistent with those of Goodfellow et al. [20], which indicate that the D20S5 probe and the MEN-II locus are not closely linked. However, our confirmation of the finding of a 20p12.2 deletion in MEN-II subjects argues against their tentative conclusion that MEN-II locus does not lie within 20p12. The possibility exists that the probe hybridization site may not lie at 20p12.2. Exact assignment by in situ hybridization is difficult, and the localization data presented [20] is not inconsistent with hybridization to any point on the short arm of chromosome #20. Alternatively or additionally, there may be a region of high frequency recombination between the D20S5 site and the MEN-II locus. Furthermore, the possibility of genetic heterogeneity was also not ruled out with cytogenetic analysis of the families studied [20]. Finally, it is possible that the apparent deletion is simply a chromosomal variant or possibly a paracentric inversion that is in linkage disequilibrium with the MEN-II gene. However, its statistically significant association with MEN-II and the lack of known chromosomal variants in euchromatic regions argue against such a variant.

Our cytogenetic findings agree with those reported by Babu et al. [17] and Van Dyke et al. [16], suggesting that a chromosomal deletion at 20p12.2 occurs in MEN-II subjects. Our DNA study using the D20S5 probe excluded deletion of the probe binding sequences in three affected individuals (from two of the three families) who were heterozygous for RFLP. Thus, this probe can not be used as a simplified diagnostic method for identification of individuals heterozygous for MEN-II. Further investigation of the basis of apparent nonlinkage between MEN-II and D20S5 is indicated.

Acknowledgments

Supported in part by Vanderbilt University Research Council Grant and Biomedical Research Support Grant RR-05424 (M. G. B.), NIH Research Training in Diabetes and Endocrinology Grant 5T32AM07061 (D. R. R.), NIH Grant AM35592, and RCDA Grant AM01434 (J. A. P.).

The authors thank Judy Copeland for expert preparation of the manuscript.

References

1. Baylin SB. The multiple endocrine neoplasia syndromes: Implications for the study of inherited tumors. *Semin Oncol.* 1978; 5:35–45. [PubMed: 345454]
2. Schimke, RN. Tumors of the neural crest system In: Genetics of Human Cancer. Mulvihill, JJ, Miller, BW., Freymen, JR., editors. Raven; NY: 1977. p. 179–198.
3. Sipple JH. Sipple families. *Lancet.* 1977; i:939–940.
4. Lips, CJM. The multiple endocrine neoplasia syndrome type 2A (Sipple's syndrome) in a large family in the Netherlands. Drukkerij Teldermann; Arnhem: 1979.
5. Steiner AL, Goodman AD, Powers SR. Study of a kindred with pheochromocytoma, medullary thyroid carcinoma, hyperparathyroidism and Cushing's disease: Multiple endocrine neoplasia, type 2. *Medicine.* 1968; 47:371–409. [PubMed: 4386574]
6. Gagel RF, Jackson CE, Block MA, Feldman ZT, Reichlin S, Hamilton B, Tashjian AH Jr. Age-related probability of development of hereditary medullary thyroid carcinoma. *J Pediatr.* 1982; 101:941–46. [PubMed: 7143171]
7. Sipple JH. The association of pheochromocytoma with carcinoma of the thyroid gland. *Am J Med.* 1961; 31:163–166.
8. Al-Saadi A, Lieberman L. Familial medullary thyroid carcinoma. *Recent Prog Horm Res.* 1972; 28:446.
9. Melvin KEW, Toshjian AH, Miller HH. Studies in familial (medullary) thyroid carcinoma. *Recent Prog Horm Res.* 1972; 28:399–470. [PubMed: 4347303]
10. VanDyke DL, Jackson CE, Babu VR. Localization of autosomal dominant multiple endocrine neoplasia 2 syndrome (MEN2) to 20p12.2. *Cytogenet Cell Genet.* 1982; 32:324.
11. Hsu TC, Pathak S, Samaan N, Hickey RC. Chromosome instability in patients with medullary carcinoma of the thyroid. *J Am Med Assoc.* 1981; 246:2046–2048.
12. Gustavson KH, Jansson R, Oberg K. Chromosomal breakage in multiple endocrine adenomatosis (types I and II). *Clin Genet.* 1983; 23:143–149. [PubMed: 6132690]
13. Samaan NA, Hsu TC, Pathak S, Saad MF, Ordonex NG, Hickey RC. Chromosomal instability in medullary carcinoma of the thyroid. *World J Surg.* 1984; 8:487–492. [PubMed: 6148807]
14. Babu VR, VanDyke DL, Jackson CE, Mayeda K. Absence of increased chromosome instability in multiple endocrine neoplasia type 2 syndrome. *Am J Hum Genet.* 1983; 35:59A.
15. VanDyke DL, Babu VR, Jackson CE. Chromosomes in multiple endocrine neoplasia type 2 syndromes. *Henry Ford Hosp Med J.* 1984; 32:266–268. [PubMed: 6152464]
16. VanDyke DL, Jackson CE, Babu VR. Prometaphase chromosomes in cancer families: multiple endocrine neoplasia 2 syndrome and neurofibromatosis. *Clin Res.* 1981; 29:37A.
17. Babu VR, VanDyke DL, Jackson CE. Chromosome 20 deletion in human multiple endocrine neoplasia types 2A and 2B: A double-blind study. *Proc Natl Acad Sci USA.* 1984; 81:2525–2528. [PubMed: 6585814]
18. Emmertsen K, Lamm LU, Rasmussen KZ, Elbrand I, Hansen HH, Henningsen K, Jorgensen J, Petersen GB. Linkage and chromosome study of multiple endocrine neoplasia lia. *Cancer Genet Cytogenet.* 1983; 9:241–259.
19. Zatterale A, Stabile M, Nunziata V, DiGiovanni G, Vecchione R, Ventruto V. Multiple endocrine neoplasia type 2 (Sipple's syndrome): Clinical and cytogenetic analysis of a kindred. *J Med Genet.* 1984; 21:108–111. [PubMed: 6143828]

20. Goodfellow PJ, White BN, Holden JJA, Duncan AMV, Sears EVP, Wang H-S, Berlin L, Kidd KK, Simpson NE. Linkage analysis of a DNA marker localized to 20p12 and multiple endocrine neoplasia type 2A. *Am J Hum Genet.* 1985; 37:890–897. [PubMed: 2864854]
21. Yu RL, Aronson MM, Nichols WW. High-resolution bands in human fibroblast chromosomes induced by actinomycin D. *Cytogenet Cell Genet.* 1981; 31:111–114. [PubMed: 7307580]
22. Rybak J, Tharapel A, Robinett S, Garcia M, Mankinen C, Freeman M. A simple reproducible method for prometaphase chromosome analysis. *Hum Genet.* 1982; 60:328–333. [PubMed: 7106769]
23. Kunkel LM, Smith KD, Boyer SH, Borgaonkar DS, Wachtel SS, Miller OJ, Breg WR, Jones HW, Rary JM. Analysis of human Y chromosome specific reiterated DNA in chromosome variants. *Proc Natl Acad Sci USA.* 1977; 74:1245–1249.
24. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol.* 1975; 98:503–517. [PubMed: 1195397]
25. Maniatis T, Kee GS, Efstratiadis A, Kafatos FC. Amplification and characterization of a β -globin gene synthesized in vitro. *Cell.* 1976; 8:163. [PubMed: 61066]

Figure 1.

Chromosome #20 pairs from a patient with MEN-II with the normal homolog on the right, and bands 20p12.1 and 20p12.3 indicated by the arrowheads. Light staining 20p12.2 band is not evident in the deletion homolog (approximately 850 band stage).

Figure 2.

Autoradiogram of DNA from five members of MEN-II kindred 3 after digestion with Pvu II and hybridization with radiolabeled D20S5. Shading in pedigree indicates MEN-II.

Fragment size (in kilobases) is indicated at the right. BiB is heterozygous for the RFLP, whereas, BiA is homozygous (or haploid). However, BiE and JB each have the 2.8-Kb fragment, which they have received from BiB. They each have a 1.9-Kb fragment, that they must, therefore, have received from BiA. Thus, the probe hybridization site is not deleted in individuals with MEN-II.

Table 1

Clinical and cytogenetic findings of 14 individuals with and without MEN-II syndrome

Subject ^a	Sex/Age (yr)	Karyotype	Phenotype ^c
1-DM	F/35	20p –	MEN-II (MCT)
1-MM	F/46	20p –	MEN-II (MCT, P)
2-BD	F/36	20p –	MEN-II (MCT, PTA)
2-BB	F/46	20p –	MEN-II (MCT)
2-BC	M/38	20p –	MEN-II (MCT)
3-BiC	M/34	20p –	MEN-II (MCT, P)
3-JB	M/25	normal ^b	MEN-II (MCT)
3-BiA	F/58	20p –	MEN-II (MCT, P)
BE	M/38	normal	Control
BF	F/35	normal	Control
CP	F/31	normal	Control
BA	M/68	normal	Control
BiD	F/32	20p– ^b	Control
BiB	M/67	normal	Control

^aSubject initials preceded by kindred number^bBlind karyotype interpretation not consistent with phenotype^cMCT, medullary carcinoma of thyroid; P, pheochromocytoma; PTA, parathyroid adenoma.

Table 2

Summary of cytogenetic findings in blind study

Subject group	Chromosome #20 ^a	
	Normal	20p Deletion
MEN-II (<i>n</i> = 8)	1	7
Control (<i>n</i> = 6)	5	1

^aTwo-tailed χ^2 test with 1 *df* was 6.99 (*p* < 0.01).

Table 3

RFLP genotypes for probe D20S5

Subject	Phenotype	Msp I allele	Pvu II allele
1-MM	MEN-H	A2	B1
2-BD	MEN-II	A2	B1
2-BC	MEN-II	A1.A2	B1,B2
2-BB	MEN-II	A1,A2	B1,B2
3-BiC	MEN-II	A2	B1
3-JB	MEN-II	A1,A2	B1,B2
3-BiA	MEN-II	A2	B1
BE	Control	A1.A2	B1,B2
BA	Control	A1,A2	B1,B2
BiB	Control	A1,A2	B1,B2