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This report presents pairwise linkage results from our search for the locus of the gene (MEN2A) for the multiple endocrine neoplasia type 2A (MEN-2A) syndrome in one large kindred (the N kindred), clearly segregating for an autosomal dominant form. About 25% of the autosomal genome is excluded when these new results are combined with those we have published previously. The genetic markers employed are distributed across at least 19 of the 22 autosomes. Seven genetic markers whose chromosomal locations are not yet established have also been studied. (Henry Ford Hosp Med J 1987;35:164-7)

Multiple endocrine neoplasia type 2A (MEN-2A) is a syndrome characterized by medullary thyroid carcinoma (MTC), pheochromocytoma, and hyperparathyroidism. It is inherited in an autosomal dominant manner with almost complete penetrance by the age of 40 (1). The etiology of the disorder is unknown and no strongly supported hypotheses exist. Although screening tests permit early detection of the tumors, these tests cannot detect carriers of the gene until pathogenetic changes occur. Thus, the disorder is an excellent candidate for a linkage study. Identification of a closely-linked genetic marker would identify those family members actually carrying the MEN-2A gene (MEN2A) and would consequently improve screening and genetic counseling. In addition, location of MEN2A would permit studies, including cloning of the gene, which may clarify the nature of tumorigenesis in this disorder.

Linkage studies to date have failed to identify a marker linked to the MEN2A locus. Simpson (2) has reviewed several of the early studies. Slightly positive results obtained for some loci in some studies have been offset by negative results for these same loci in other studies. However, the possibility of genetic heterogeneity must be considered; abnormal alleles at different loci may be the cause of MEN-2A in different families. This difficulty may be circumvented by studying single large families since within any such family affected individuals will represent the same genetic etiology.

The advent of large numbers of DNA markers now makes it certain that we can find the MEN2A locus if we look hard enough. The first DNA marker was identified in 1978 (3). In 1980 Botstein et al (4) coined the term RFLP for restriction fragment length polymorphisms and discussed the value of RFLPs in mapping the genome and locating genes for inherited disorders such as MEN-2A. Of the more than 1,000 RFLPs known (5), over 400 have been individually described and cataloged (6). Their value has been amply demonstrated by the location of the gene for Huntington's disease (7) and Duchenne muscular dystrophy (8), among a growing list.

Our recent pairwise linkage studies (9-12) used only one large family, the N kindred, in which MEN-2A is inherited in a classic autosomal dominant pattern. Another recent study (13) used this same family and one other large family. These studies excluded linkage of MEN2A with several classical markers (10), some DNA markers from chromosome 11 (9), and some assorted DNA markers (11-16).

Methods

Laboratory methods

Lymphoblastoid cell lines were established on 52 individuals in the N kindred. The full kindred was illustrated in Kruger et al (10). Standard methods for establishing cell lines, extracting DNA, and typing for RFLPs were used as described in previous publications (9,12,13,15). The RFLPs that we studied are shown in Table 1 (7,14,17-43), which also indicates the individuals who have been kind enough to share their probes with our group.

Linkage methods

Pairwise linkage analyses were carried out as described in Kruger et al (10) and Kidd et al (12). Age-dependent penetrances similar to those employed with LIPED were incorporated into the four-point analysis. The multipoint linkage results were generated using a version of the LINKMAP program of Lathrop et al (44), which was adapted for use on a Cray X-MP computer.

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Table 1
RFLPs Studied

Locus	Location	Probe	Source	Reference for RFLP
AT3	1q23-q25	ATIII	S. Orkin	(17)
AMY1	1p21	pEB-8	M. Meisler	(18)
REN	1p21-qter	HREN	J. Chirgwin	(19)
NGFB	1p22.1	NGF	J. Darby	(20)
D2S6	2q32-q36	pXG18	P. Szabo	(21)
POMC	2p23	pL26	S. Cohen	(22)
CRYG	2q33-q35	p5G1	L.-C. Tsui	(23)
D3S5	3q21-qter	DR2	A. Driesel	(24)
TFRC	3q26.2-qter	pCPTr1	McClelland/Ruddle	Unpublished data
D4S1	4pter-q26	p4c3.6/1.2	Gilliam/Williamson	Davies HGM7, unpublished data
D4S14	4q26-qter	M6/2.5	R. Williamson	(25)
D4S35	4pter-q12	G9-20	Gilliam/Gusella	HGM7, unpublished data
FGB	4q26-q28	FbgB	D. Chung	(26)
D4S10	4p16	pK082/G8	J. Gusella	(7)
ADH2	4q21-q25	pADH36	M. Smith	(27)
ADH3	4q21-q25	pADH73	M. Smith	(27)
MT2P1	4p11-q21	pHM6	L.-C. Tsui	(28)
D6Z1	6	p308	Jabs/Migeon	(29)
D7S8	7q22	pJ3.11	R. Williamson	(30)
CA2	8	H25-3.8	R. Tashian	(31)
Anonymous	10	p9-12-A	McDermid/Simpson	Unpublished data
D11S16	11	pL32-1	L. Cavalli-Sforza	(32)
CALC1	11p14-q15	pTT42	B. Nelkin	(33)
CAT	11	ScaI-SnaICAT	R. Gravel	(34)
D13S7	13q12	pHU26	T. Dryja	(35)
D13S1	13q12-q14	p7F12	R. White	(36)
D13S2	13q22	p9D11	R. White	(36)
D13S5	13q12-q22	pHUB8	T. Dryja	(35)
D13S6	13q13	pHU10	T. Dryja	(35)
D13S3	13q22-qter	p9A7	R. White	(36)
APRT	16q22	M13-APRT	I. Stambrook	(37)
D17Z1	17cen	p17H5	H. Willard	(38)
TK1	17q21-q22	TkHC9	P. Lin	(39)
D19S11	19p	p13-1-82	M. Litt	(40)
D20S5	20p12	pRI2.21	D. Shaw	(14)
D21S1	21q11.2-q21	pPW228C	P. Watkins	(41)
D21S8	21q21	pPW245D	P. Watkins	(41)
D21S11	21q11.2-q21	pPW236B	P. Watkins	(41)
Anonymous	21	pG95-11d11	N. Simpson	Goodfellow, unpublished data
D22S1	22q.11.2-q13	pMS3-18	R. White	(42)
D22S9	22q11	p22/34	A. Duncan	(43)
Anonymous	22	p22/13	A. Duncan	Unpublished data
Anonymous	?	LDR93	A. Roses	Unpublished data
Anonymous	1?	p308, 4.0	E. Jabs	(29)
Anonymous	1?	p308, 2.8	E. Jabs	(29)

Discussion

Some previously reported lod scores have been revised since they were first published. Additional individuals have become affected with MEN-2A since the earlier findings, and some younger members of the family have been added to the study. However, the lod scores have changed little after incorporating these new pieces of information. In general, the portion of the autosomes excluded from linkage with MEN2A remains the same.

The new pairwise linkage results are reported in Table 2, and new multipoint data on chromosome 11 are displayed in the Figure. Pooling our previously published findings as well as those shown in Table 2 and the Figure, while taking into account the linkage relationships present among various markers, leads to the conclusion that about one fourth of the human autosomes has been excluded from linkage with MEN2A.*

This multipoint analysis in the region of chromosome 11 spanned by calcitonin (CALC1), D11S16, and catalase (CAT) excludes the MEN2A locus from about 40 centiMorgans (cM) on the proximal (centromeric) side of CALC1. Previous analyses (9) excluded the region on the distal side of CALC1 out past the HRAS1 locus.

*Editors' note: See later reference to chromosome 10 linkage provided in the guest editorial, p 93.

Table 2
Lod Scores for MEN2A*

CHR	Marker	Recombination Fraction (Male Theta = Female Theta) 0.000	0.001	0.050	0.100	0.150	0.200	0.300	0.350	0.400
1	AMY	−1.20	−1.15	−0.34	−0.12	−0.03	0.02	0.02	0.01	0.00
1	REN	−7.27	−4.96	−1.70	−1.04	−0.66	−0.41	−0.14	−0.07	−0.03
1	AT3	−8.61	−8.05	−3.00	−1.84	−1.18	−0.75	−0.26	−0.13	−0.06
1	DR10	−7.46	−6.82	−1.17	−0.07	0.42	0.63	0.62	0.48	0.28
1?	p308, 4.0	0.05	0.05	0.05	0.05	0.04	0.04	0.03	0.02	0.01
1?	p308, 2.8	0.06	0.06	0.05	0.05	0.04	0.04	0.03	0.02	0.01
2	CRYG	−2.97	−2.37	−0.64	−0.30	−0.13	−0.03	0.04	0.05	0.04
2	D2S6	−5.48	−4.87	−1.76	−1.05	−0.67	−0.43	−0.17	−0.10	−0.05
3	D3S5	−7.47	−5.19	−1.74	−0.93	−0.51	−0.27	−0.05	−0.02	−0.00
4	D4S10	−9.75	−7.72	−2.77	−1.85	−1.32	−0.96	−0.47	−0.30	−0.18
4	D4S35	−11.42	−9.45	−4.96	−3.24	−2.24	−1.57	−0.74	−0.47	−0.26
4	MT2P1	−8.13	−5.48	−2.00	−1.23	−0.79	−0.50	−0.18	−0.10	−0.05
4	D4S1	−4.09	−3.86	−1.36	−0.78	−0.47	−0.28	−0.07	−0.03	−0.00
4	ADH3	−4.32	−4.06	−1.95	−1.32	−0.94	−0.68	−0.34	−0.23	−0.14
4	ADH2	−5.64	−4.66	−1.38	−0.69	−0.32	−0.10	0.07	0.07	0.05
4	FGB	−0.30	−0.30	−0.18	−0.10	−0.05	−0.02	0.02	0.02	0.02
4	D4S14	−5.47	−3.02	−0.47	−0.11	−0.01	−0.01	−0.12	−0.15	−0.14
6	D6Z1	−2.82	−2.16	−0.70	−0.50	−0.40	−0.32	−0.21	−0.15	−0.10
7	D7S8	−5.03	−4.65	−1.93	−1.41	−1.12	−0.91	−0.56	−0.41	−0.26
10	p9-12-A	−5.96	−4.26	−1.14	−0.66	−0.42	−0.29	−0.14	−0.09	−0.05
†11	CALC1	−3.55	−2.57	−0.66	−0.35	−0.23	−0.17	−0.12	−0.10	−0.07
†11	D11S16	−4.62	−3.72	−0.87	−0.44	−0.25	−0.16	−0.08	−0.06	−0.04
†11	CAT	−7.62	−6.79	−2.51	−1.55	−1.01	−0.66	−0.25	−0.14	−0.06
13	D13S2	−6.97	−6.41	−3.20	−2.11	−1.51	−1.11	−0.59	−0.40	−0.24
13	D13S3	−2.61	−0.70	0.69	0.69	na	0.44	0.19	na	0.04
13	D13S5	−2.53	−2.23	−0.69	−0.32	−0.12	−0.00	0.07	0.05	0.02
13	D13S6	−8.54	−6.68	−2.19	−1.16	−0.62	−0.31	−0.02	0.03	0.04
13	D13S7	−6.48	−4.93	−1.32	−0.64	−0.30	−0.12	0.04	0.05	0.04
13	D13S1	−10.05	−6.74	−2.00	−1.10	−0.64	−0.36	−0.09	−0.04	−0.02
17	D17Z1	−8.53	−8.21	−4.43	−2.63	−1.68	−1.09	−0.47	−0.30	−0.19
17	TK1	−2.55	−2.22	−0.07	0.37	0.52	0.53	0.36	0.24	0.13
†19	D19S11	−7.57	−7.15	−3.16	−2.18	−1.60	−1.18	−0.59	−0.37	−0.20
20	D20S4	−6.83	−6.15	−3.23	−2.15	−1.53	−1.12	−0.56	−0.37	−0.21
20	D20S5	−7.85	na	−0.95	−0.16	0.15	0.27	0.24	0.17	0.10
20	D20S6	−2.97	−1.68	−0.16	0.00	0.05	0.07	0.06	0.04	0.02
†21	D21S8	−3.56	−2.48	−0.81	−0.51	−0.34	−0.23	−0.09	−0.05	−0.03
†21	D21S1	0.13	0.12	0.10	0.07	0.05	0.04	0.02	0.02	0.01
†21	D21S11	0.13	0.14	0.17	0.17	0.14	0.11	0.04	0.02	0.00
†21	"pG95-11d11"	−5.66	−5.24	−1.81	−1.02	−0.60	−0.35	−0.11	−0.07	−0.04
†22	D22S9	−9.28	−6.78	−2.33	−1.59	−1.17	−0.85	−0.39	−0.23	−0.11
†22	"p22/13"	−7.23	−5.51	−1.65	−0.91	−0.51	−0.27	−0.04	−0.00	0.01
†?	"LDR93"	−7.08	−6.52	−2.02	−0.82	−0.22	0.10	0.28	0.24	0.15

*New pairwise lod scores (LIPED) screening for MEN2A locus in kindred N (92 cases). All analyses employed straight line age correction. Lod scores marked with a dagger are based on 69 of the 92 cases. AMY haplotype combines AMY1 and AMY2.
CHR = chromosome.

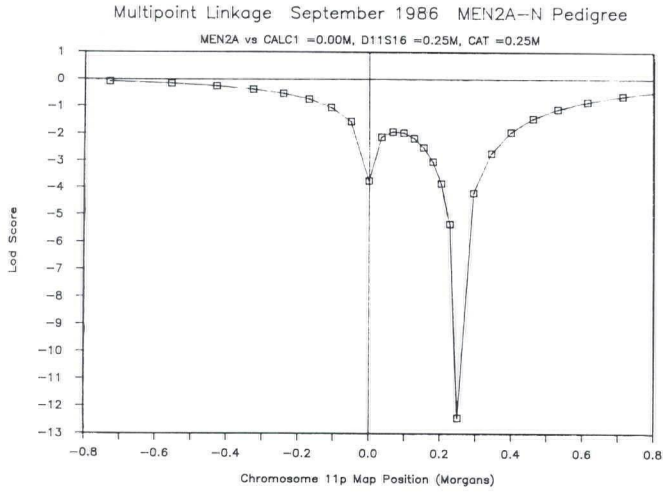

Figure (left)—September 1986 multipoint analysis excluding MEN2A from chromosome 11 region encompassed by CALC1, D11S16, and CAT.

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References

1. Gagel RF, Jackson CE, Block MA, et al. Age-related probability of development of hereditary medullary thyroid carcinoma. J Pediatr 1982;101:941-6.

2. Simpson NE. Genetic studies of multiple endocrine neoplasia type 2 syndromes: A workshop commentary. Henry Ford Hosp Med J 1984;32:273-6.

3. Kan YW, Dozy AM. Polymorphism of DNA sequence adjacent to human β-globin structural gene: Relationship to sickle mutation. Proc Natl Acad Sci USA 1978;75:5631-5.

4. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 1980;32:314-31.

5. Willard HF, Skolnick MH, Pearson PL, Mandel J-L. Report of the committee on human gene mapping by recombinant DNA techniques. HGM8: Eighth International Human Gene Mapping Workshop. Cytogenet Cell Genet 1985; 40:360-489.

6. Kidd KK, Willard HF, Pearson PL, et al. Human DNA restriction fragment length polymorphisms (RFLPs). In: O'Brien SJ, ed. Genetic Maps. Cold Spring Harbor: Cold Spring Harbor Laboratory, 1987;4:610-41.

7. Gusella JF, Wexler NS, Conneally PM, et al. A polymorphic DNA marker genetically linked to Huntington's disease. Nature 1983;306:234-8.

8. Davies KE, Pearson PL, Harper PS, et al. Linkage analysis of Duchenne muscular dystrophy using DNA sequences flanking the locus on the short arm of the human X chromosome. HGM7: Seventh International Workshop on Human Gene Mapping. Cytogenet Cell Genet 1984;37:446-7.

9. Kidd KK, Kruger SD, Gerhard DS, Kidd JR, Housman D, Gertner JM. Linkage data excluding a locus for multiple endocrine neoplasia type 2 syndromes from the distal part of the short arm of chromosome 11. Henry Ford Hosp Med J 1984;32:262-5.

10. Kruger SD, Gertner JM, Sparkes RS, et al. Linkage analyses of multiple endocrine neoplasia, type 2 (MEN-2) with 23 classical genetic polymorphisms. Hum Hered 1986;36:6-11.

11. Kidd KK, Kidd JR, Castiglione CM, et al. Progress toward resolving the possible linkage of multiple endocrine neoplasia type 2A to haptoglobin and group-specific loci: Use of restriction fragment length polymorphisms extends exclusion region. Genet Epidemiol 1986;3:195-200.

12. Kidd KK, Kidd JR, Castiglione C, et al. Linkage analyses of multiple endocrine neoplasia, type 2 (MEN-2A) with 20 DNA polymorphisms: 5% of the genome excluded. Hum Hered 1986;36:243-9.

13. Goodfellow PJ, White BN, Holden JJA, et al. Linkage analysis of a DNA marker localized to 20p12 and multiple endocrine neoplasia type 2A. Am J Hum Genet 1985;37:890-7.

14. Goodfellow PJ, White BN, Duncan AMV, et al. Towards a linkage map of chromosome 20. Cytogenet Cell Genet 1985;40:645.

15. Farrer LA, Goodfellow PJ, LaMarche CM, et al. An efficient strategy for gene mapping using multipoint linkage analysis: Exclusion of the multiple endocrine neoplasia 2A (MEN2A) locus from chromosome 13. Am J Hum Genet 1987;40:329-37.

16. Farrer LA, Goodfellow PJ, White BN, et al. Linkage analysis of multiple endocrine neoplasia type 2A (MEN-2A) and three DNA markers on chromosome 20: Evidence against synteny. Cancer Genet Cell Genet 1987;27:327-34.

17. Prochownik EJ, Markham AF, Orkin SH. Isolation of a cDNA clone for human antithrombin III. J Biol Chem 1983;258:8389-94.

18. Gumucio DL, Meisler MH, Kidd JR. Detection of two RFLPs near the human salivary amylase gene on the short arm of human chromosome 1 (abstract). Am J Hum Genet 1985;37:A155.

19. Chirgwin JM, Schaefer IM, Rotwein PS, et al. Human renin gene is on chromosome 1. Somatic Cell Molec Genet 1984;10:415-21.

20. Darby JK, Kidd JR, Pakstis AJ, et al. Linkage relationships of the gene for the β subunit of nerve growth factor (NGFB) with other chromosome 1 marker loci. Cytogenet Cell Genet 1985;39:158-60.

21. Davatelis G, Szabo P, Siniscalco M. A common RFLP on human chromosome 2. Cytogenet Cell Genet 1985;40:612.

22. Feder J, Migone N, Chang ACY, et al. A DNA polymorphism in close physical linkage with the proopiomelanocortin gene. Am J Hum Genet 1983; 35:1090-6.

23. Tsui L-C, Brietman ML, Meakin SO, et al. Localization of the human gamma-crystallin gene cluster (CRYG) to the long arm of chromosome 2, region q33-q35. Cytogenet Cell Genet 1985;40:763-4.

24. Driesel AJ, Cleef S, Breilman D, et al. A human arbitrary DNA-fragment detects RFLPs on chromosome 3q21-qter. Cytogenet Cell Genet 1985;40:620.

25. Scambler P, Robbins T, Gilliam C, et al. Linkage studies between polymorphic markers on chromosome 4 and cystic fibrosis. Hum Genet 1985; 69:250-4.

26. Murray JC, Buetow K, Chung D, Aschbacher A. Linkage disequilibrium of RFLPs at the beta fibrinogen (FGB) and gamma fibrinogen (FGG) loci on chromosome 4. Cytogenet Cell Genet 1985;40:707-8.

27. Smith M, Duester G, Carlock L, Wasmuth J. Assignment of ADH1, ADH2, and ADH3 genes (class 1 ADH) to human chromosome 4q21-4q25, through use of DNA probes. Cytogenet Cell Genet 1985;40:748.

28. Schmidt CJ, Hamer DH, McBride OW. Chromosomal location of human metallothionein genes: Implications for Menkes' disease. Science 1984; 224:1104-6.

29. Jabs EW, Meyers DA, Bias WB. Linkage studies of polymorphic, repeated DNA sequences in centromeric regions of human chromosomes. Am J Hum Genet 1986;38:297-308.

30. Bartels I, Grzeschik K-H, Cooper DN, Schmidtke J. Regional assignment of six cloned DNA sequences and restriction fragment length polymorphisms (RFLPs) on human chromosome 7. Cytogenet Cell Genet 1985;40:577.

31. Lee BL, Venta PJ, Tashian RE. DNA polymorphism in the 5′ flanking region of the human carbonic anhydrase II gene on chromosome 8. Hum Genet 1985;69:337-9.

32. Feder J, Yen L, Wijsman E, et al. A systematic approach for detecting high-frequency restriction fragment length polymorphisms using large genomic probes. Am J Hum Genet 1985;37:635-49.

33. Nelkin BD, Rosenfeld KI, de Bustros A, Leong SS, Roos BA, Baylin SB. Structure and expression of a gene encoding human calcitonin and calcitonin gene related peptide. Biochem Biophys Res Commun 1984;123:648-55.

34. Quan F, Lorneluk RG, MacLeod HL, et al. An RFLP associated with the human catalase gene. Nucleic Acids Res 1985;13:8288.

35. Dryja TP, Rapaport JM, Weichselbaum R, Bruns GAP. Chromosome 13 restriction fragment length polymorphisms. Hum Genet 1984;65:320-4.

36. Cavenee W, Leach R, Mohandas T, Pearson P, White R. Isolation and regional localization of DNA segments revealing polymorphic loci from human chromosome 13. Am J Hum Genet 1984;36:10-24.

37. Stambrook PJ, Dush M, Trill JJ, Tischfield JA. Cloning of a functional human adenine phosphoribosyl transferase (APRT) gene: Identification of a restriction fragment length polymorphism and preliminary analysis of DNAs from APRT-deficient families and cell mutants. Somatic Cell Molec Genet 1984;10:359-67.

38. Willard HF, Waye JS, Schwartz C, Skolnick M, Phillips JA. Chromosome-specific human alpha satellite DNA: Isolation and characterization of a polymorphic repeated DNA family from chromosome 17 (D17Z1). Cytogenet Cell Genet 1985;40:778-9.

39. Murphy PD, Kidd JR, Castiglione CM, et al. A frequent polymorphism for the cytosolic thymidine kinase gene, TK1, (17q21-q22) detected by the enzyme TaqI. Nucleic Acids Res 1986;14:4381.

40. Bufton L, Bruns G, Magenis RE, et al. Four RFLPs revealed by probes from a single cosmid map to chromosome 19. Cytogenet Cell Genet 1985; 40:596.

41. Watkins P, Tanzi R, Landes G, Tricoli JV, Shows TB, Gusella JF. DNA segments for human chromosome 21. Cytogenet Cell Genet 1984;37:602.

42. Schafer M, White R. Three random loci in the human genome with base pair change polymorphisms. Cytogenet Cell Genet 1982;32:314-5.

43. McDermid H, Duncan AMV, Brasch K, et al. Molecular analysis of the supernumerary chromosome in Cat Eye syndrome. Cytogenet Cell Genet 1985;40:695-6.

44. Lathrop GM, Lalouel J-M, Julier C, Ott J. Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 1984;81:3443-6.