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Studies of Multiple Endocrine Neoplasia Type 2A Syndrome: Linkage Analyses and Comparison of Constitutional and Tumor Genotypes

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Linkage analyses were carried out in nine Japanese kindreds with multiple endocrine neoplasia type 2A (MEN-2A) using polymorphic classical markers and DNA markers. We excluded close linkage of the MEN-2A gene (MEN2A) locus with Gm, JK, PGM1, and a DNA segment, D20S5, which is assigned to band 12 of the short arm of chromosome 20 (20p12.2). Assuming that MEN2A is recessive at the cellular level as in retinoblastoma (RB) and Wilms' tumor (WT), comparison of constitutional and tumor genotypes may be useful in the search for the MEN2A locus. When DNA samples from 12 patients with medullary thyroid carcinoma (MTC) were compared with 15 polymorphic DNA markers including two assigned to chromosome 20, the results were negative. Both the negative linkage data and the failure to find loss of heterozygosity in MTC with chromosome 20 probes suggest that MEN2A may not be at 20p12.2, which was previously suggested as the site of an inherited chromosomal deletion in MEN-2A. (Henry Ford Hosp Med J 1987;35:157-60)

Multiple endocrine neoplasia type 2A (MEN-2A) is inherited in an autosomal dominant fashion. Babu and colleagues (1,2) reported a minute constitutional interstitial deletion in the short arm of chromosome 20, del (20) (p12.2, p12.2) and suggested that this might be the site of the MEN-2A gene (MEN2A). Other research groups (3-6), however, failed to demonstrate the deletion, and Goodfellow et al (7) excluded close linkage between the MEN2A locus and the D20S5 locus assigned to the region of 20p12.2.

Two possible explanations for the discrepancy between the cytogenetic findings by Babu et al (1) and the negative results of linkage analysis might be genetic heterogeneity of the MEN-2A syndrome or high levels of meiotic recombination between the two loci, MEN2A and D20S5. To investigate this further, we studied Japanese kindreds with MEN-2A using two strategies, ie, linkage analysis with the probe pR12.21 for the D20S5 locus, and comparison of constitutional and tumor genotypes with probes on chromosome 20. The results obtained with other genetic markers are also presented.

Materials and Methods

DNA samples

Heparinized blood samples were obtained from 123 individuals belonging to 17 Japanese MEN-2A kindreds, of which 60 individuals were known to be affected. Aliquots of blood were used for analyses of classical markers such as blood groups or enzyme polymorphisms, transformation by the Epstein-Barr virus to establish lymphoblastoid cell lines, and direct extraction of DNA (8).

Genetic markers

In each member of the kindred, 26 classical markers were determined using the standard method by one of the authors (HM). These were nine blood groups: ABO, MNSs, P, Rh, Kell, Kidd (JK), Duffy (FY), Lutheran, and Diego; six plasma proteins: HP, Tf, GC, Gm, Km, and PI; and 11 enzymes: ACP1, PGM1, ADA, PGD, ESD, GPT, GOT, PHI, LDH, UMPK, and GLO. Each member was also typed with polymorphic DNA markers by Southern blot analyses (8).

Tumor tissues

Fresh medullary thyroid carcinoma (MTC) tumor tissues were frozen immediately at the time of surgery and kept at −80°C until DNA extraction.

Probes

Probes to detect DNA polymorphisms were: phT-B3 (CALC1), pTBB-2 (HRAS1), ADJ762 (D11S12), p9F11 (D12S4), p7F12 (D13S1), p9D11 (D13S2), p1E8 (D13S4), pHUB8 (D13S5), pMS1-14 (D15S1), C-H800 (GH1), 12-62 (D18S1), pMS1-27 (D20S4), pR12.21 (D20S5), and pMS3-18 (D22S1). These probes were kindly provided by other researchers. In addition, we used seven probes generated in our laboratory (8,9): 0S-4 (D18S5) and six unassigned polymorphic DNA segments (0S-1, 0S-2, 0S-5, 0S-6, 0S-8, and 0S-9).

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Linkage analysis

Nine of 17 kindreds collected contained at least one potentially informative nuclear family, designated by letters A to I (Table 1). Individuals were classified as "affected" if they had been operated on for any component of MEN-2A or had high basal and/or stimulated levels of plasma calcitonin. Those under 15 years of age were excluded. Lod scores for various values of recombinant fraction (θ) were calculated (10).

Results

Linkage analyses with classical markers

Table 1 summarizes lod scores for 11 classical markers. Close linkages were excluded for Gm and JK at $\theta = 0.10$, and for PGM1 at $\theta = 0.05$.

Linkage analyses with polymorphic DNA markers

Table 2 summarizes lod scores for 11 polymorphic DNA markers. Linkage with D20S5 was excluded at $\theta = 0.10$. All nuclear families informative for D20S5 had at least one recombinant individual. None of the probes showed evidence of close linkage with MEN2A, although somewhat positive scores were obtained with our probe 0S-2 (an unassigned DNA segment).

Comparison of constitutional and tumor genotypes

DNA samples from 12 patients with MTC, eight hereditary and four sporadic, were examined with 15 polymorphic DNA probes. A total of 48 tumor/blood comparisons were possible and in each case the tumor DNAs retained heterozygosity (Table 3).

Discussion

Cytogenetic studies are important in the search for the locus of the gene responsible for a hereditary disease, because abnormalities such as deletion or translocation often provide evidence for the location of the gene. For the MEN-2A syndrome, however, cytogenetic data are not conclusive (1-6). The alternative technique is linkage analysis. Many classical genetic markers have been excluded from linkage with MEN2A (4,11-14), and we have excluded close linkage with Gm, JK, and PGM1 in Japanese kindreds. The exclusion of close linkage with Gm is consistent with the findings of Emmertsen et al (4) and Simpson (12), while exclusion of JK and PGM1 is consistent with the results of Kruger et al (14).

When we used restriction fragment length polymorphisms (RFLPs), our result for D20S5 agreed with that of Goodfellow et

Table 1
Lod Scores for MEN2A with the 11 Classical Genetic Polymorphisms

	θ Values					Remarks*
	0.05	0.10	0.20	0.30	0.40	Kindreds
ACP1	−1.442	−0.888	−0.388	−0.152	−0.036	A (2,1) (1,1)
ADA	−1.185	−0.673	−0.254	−0.087	−0.018	B (4,2)
ESD	−1.442	−0.887	−0.388	−0.151	−0.035	B (3,2)
Gm	−4.181	−2.187	−0.633	−0.117	0.002	A (2,1) (1,1), B (2,1) (5,1), C (2,0), D (2,0) (1,1), E (4,2) (1,1)
GOT	0.258	0.215	0.134	0.064	0.017	D (2,0)
GPT	−1.184	−0.673	−0.254	−0.088	−0.019	C (2,1), E (2,1) (2,0)
HP	−0.188	0.021	0.124	0.094	0.031	A (3,0), B (1,1)
JK	−5.048	−3.106	−1.358	−0.530	−0.124	B (2,1) (3,3), D (1,1), E (3,2)
MNSs	−1.442	−0.888	−0.388	−0.152	−0.036	A (2,1) (1,1)
PGM1	−2.833	−1.575	−0.568	−0.186	−0.037	B (4,2), D (1,1), E (4,2) (2,0)
PI	−0.628	−0.168	0.129	0.146	0.058	B (2,1) (5,1)

*Lod scores were calculated from kindreds indicated here. Figures in the parentheses indicate the number of nonrecombinant, recombinant offspring in each nuclear family.

Table 2
Lod Scores for MEN2A with the DNA Polymorphisms

		θ Values					Remarks†
	Chromosome*	0.05	0.10	0.20	0.30	0.40	Kindreds
CALC1	(11)	0.258	0.215	0.134	0.064	0.017	E (2,0)
D12S4	(12)	−0.463	−0.229	−0.060	−0.012	−0.001	A (2,1), D (2,0)
D13S1	(13)	−1.442	−0.888	−0.388	−0.152	−0.036	A (1,1), D (1,1)
D13S2	(13)	−0.206	−0.014	0.074	0.053	0.016	B (3,1), D (2,0)
GH	(17)	0.258	0.215	0.134	0.064	0.017	D (2,0)
D18S5	(18)	−0.205	−0.014	0.074	0.052	0.016	A (2,0), D (2,0), F (1,1)
D20S5	(20)	−3.605	−2.220	−0.970	−0.380	−0.090	A (2,1), B (2,1), D (1,1), F (1,1), G (2,1)
OS-1	(?)	−1.442	−0.888	−0.388	−0.152	−0.036	A (1,1), D (1,1)
OS-2	(?)	0.140	0.472	0.516	0.316	0.096	A (3,0) (2,0), B (3,0) (1,1), D (2,0), G (2,1)
OS-5	(?)	−1.389	−0.687	−0.180	−0.036	−0.003	A (1,1) (2,1), D (2,0), E (1,1), H (2,0), I (2,0)
OS-6	(?)	−0.463	−0.229	−0.060	−0.012	−0.001	F (2,0), I (2,1)

*The chromosome to which the DNA segment has been assigned.
†See Table 1.

al (7). This indicates no close linkage of this locus with MEN2A not only in Caucasian but also in Japanese families. Moreover, we observed at least one recombinant person in each of the five nuclear families informative for D20S5. Thus it is unlikely that genetic heterogeneity is masking a close linkage between D20S5 and MEN2A in a few families.

MEN-2A has features in common with familial retinoblastoma (RB) and Wilms' tumor (WT). The pattern of inheritance is autosomal dominant, the tumors are usually multicentric and bilateral, and pheochromocytomas are associated with MTC in MEN-2A as osteosarcomas are associated with RB. Therefore, it is reasonable to assume that the tumors in MEN-2A might develop through the same genetic mechanisms as in RB or WT.

The gene responsible for RB was found to be recessive at the cellular level, ie, the elimination of normal activities of both maternal and paternal alleles at the disease locus seemed necessary for tumorigenesis (15). This theory was induced from the observation of frequent loss of heterozygosity in the tumor DNA of the constitutionally heterozygous patients with sporadic or hereditary retinoblastoma. Polymorphic DNA probes on chromosome 13 were used to study the genotypes, because the RB gene had been assigned to the long arm of chromosome 13 (13q14) cytogenetically (16). Loss of heterozygosity for chromosome 13 probes was also demonstrated in osteosarcomas with or without RB (17,18). Similar results were also reported for sporadic WT (19-22) and sporadic or hereditary acoustic neuromas (AN) (23), with probes on chromosome 11 and 22, respectively.

These findings can be assembled into the following hypotheses: 1) autosomal dominant hereditary tumors result from loss of both copies of a gene at a single locus by either structural changes or deletion; 2) the same gene is involved in both hereditary and sporadic tumors; and 3) the second tumor, which is often associated with a hereditary tumor such as osteosarcoma in RB, rhabdomyosarcoma in WT, or meningioma in AN, arises through the same mechanism involving the same or adjacent gene as for the first tumor.

If these hypotheses are applicable to MEN-2A, the comparison of constitutional and tumor genotypes may provide a useful clue to the chromosomal location of the MEN-2A gene. For this reason we searched for loss of constitutional heterozygosity in DNA from 12 MTC tumors but without positive results so far (Table 3). It is noteworthy that probes on the chromosome 20 did not detect loss of heterozygosity in the tumor DNA in any of the seven informative cases (Table 3). From these findings combined with the data from linkage analysis, we conclude that it is unlikely that MEN2A is on the short arm of chromosome 20.

Wurster-Hill et al (24) reported that the loss or structural alteration of a chromosome 22 was the only change common to each of three MTC specimens. Comparison of constitutional and tumor genotypes in MTC and/or pheochromocytoma as well as linkage analysis of MEN-2A kindreds should be carried out using probes on chromosome 22.

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Table 3
Comparison of Constitutional and Tumor Genotypes*

Patient	HRAS1 11	CALC1 11	D11S12 11	D13S4 13	D13S5 13	D15S1 15	D18S1 18	D18S5 18	D20S4 20	D20S5 20	D22S1 22	OS-1 ?	OS-2 ?	OS-8 ?	OS-9 ?
F1-1	12	—	—		—	—	—	—	—	12	—	—		—	—
-2	12	—	—		—	—	12	—	—	—	—	—	—	12	
-3	12	—	—	—	—	12	—	—	12	12	—	—	12	12	12
-4	12	—	—		12	12	—	—	—	—	—	—	—	—	12
F2-1	—	12	—		—	12	12	—	—	—		—	12	12	—
F3-1		12						—				—	12		
F4-1	—	—	—		—	12	12	—	12	12	—	12	12	—	—
F5-1	12		—	12		12	—				—	—	—		
S1	12	12	—		—	—	12	—	12	—	—	12	—	12	12
S2	—	—	—	—	—			12	—	12	12	—	—	12	12
S3	—		—			12	—				12				
S4	12		—		—		12				—				

*'12' indicates that the patient was constitutionally heterozygous for the marker, and the heterozygosity was retained in the tumor DNA. '—' indicates that the patient was constitutionally homozygous and not informative. The absence of an entry indicates that this marker was not tested.

Note: In the table headings, the top line indicates the marker, and the bottom line indicates the chromosome, ie, HRAS1 = the marker, and 11 = the chromosome number.

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