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Cytogenetic Studies of Individuals from Four Kindreds with Multiple Endocrine Neoplasia Type II Syndrome

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Abstract

Multiple endocrine neoplasia type II (MEN-II) syndrome is an autosomal dominant condition characterized by medullary carcinoma of the thyroid, pheochromocytoma, and parathyroid adenoma. A cytogenetic investigation was conducted on 13 MEN-II syndrome patients from four unrelated kindreds and 13 age-matched control subjects for chromosome instability and the chromosome 20 deletion reported in MEN-II syndrome. A significant increase ($p < 0.05$) was found in the total number of chromatid and chromosome aberrations in MEN-II cells (12.3%) compared with control cells (6.9%) grown at 96 hours in mitomycin C (20 ng/ml, final concentration). The major difference between the two groups was in chromatid, and not chromosome, aberrations. There was no difference between MEN-II and control individuals in fragile site expression, the number of sister chromatid exchanges or cell kinetics.

A blind analysis of high-resolution G-banded chromosomes was performed on blood specimens from 13 MEN-II and seven control individuals. Twelve of 13 MEN-II patients and one of seven control subjects were scored as having a 20p12.2 deletion ($\chi^2 = 12.6$; $p < 0.001$). Additional research is needed to determine if this cytogenetic finding is due to a chromosome deletion, inversion, or polymorphism.

INTRODUCTION

Multiple endocrine neoplasia type II or IIA (MEN-II or IIA) syndrome is an autosomal dominant condition with high penetrance and variable expressivity [1–5]. Affected individuals develop medullary thyroid carcinomas and may develop pheochromocytomas and parathyroid adenomas. Recently, a probable small deletion of the short arm of chromosome 20 was reported [6–8]. Increased chromosome breakage has also been observed in this syndrome [9–12]. Herein, we report a follow-up study on high-resolution chromosomes and chromosome breakage analysis with mitomycin C, folate deficient culture medium, and sister chromatid exchanges (SCE) of 13 individuals from four unrelated kindreds with MEN-II syndrome and 13 age-matched control subjects.

MATERIALS AND METHODS

Our study consisted of 13 patients with MEN-II syndrome (seven males and six females with ages ranging 25–58 years, average 39 years) from four unrelated kindreds and control subjects matched for age. Approximately 10 ml of peripheral blood was obtained from each patient and an age-matched control subject. None of the patients or control subjects had a history of chemotherapy or radiation exposure and each denied recent illnesses. Chi-square and t-tests were used throughout the study for statistical analyses.

Mitomycin C

Age-matched blood samples from seven MEN-II patients and seven controls were grown in RPMI 1640 medium with 20 ng/ml mitomycin C (MMC) for 48 hours, and blood from 12 patients and 12 controls were also grown for 96 hours at 37°C in the same culture conditions. Thirty minutes before harvest, colcemid (0.2 µg/ml, final concentration) was added. Conventional air-dried slides were prepared and stained with Giemsa. Approximately 100 metaphase cells were scored at 96 hours and 50 cells from the 48-hour cultures. Only diploid cells (44 chromosomes) were analyzed for chromosome breakage. The following aberrations were observed: a) chromatid type, including chromatid breaks, gaps, and exchange figures (e.g., triradials and quadriradials); and b) chromosome type (dicentrics, rings, double minutes, fragments, and markers). Cells with tetraploidy, micronuclei, and endoreduplication were also recorded.

Folate-Deficient Culture Conditions

Age-matched blood samples from 13 MEN-II patients and 13 controls were cultured for 96 hours at 37°C in folate-deficient medium 199. Colcemid (0.15 µg/ml) was added 30–45 minutes before harvesting. Conventional harvest methods were used and air-dried slides were stained with Giemsa. All fragile sites, as well as chromosome and chromatid aberrations, were recorded from 100 metaphases. If fragile sites were observed the chromosomes were then destained and banded with the trypsin–Giemsa technique for chromosome identification.

Sister Chromatid Exchanges

Sister chromatid exchanges (SCE) were analyzed from 12 MEN-II patients and 12 controls matched for age. Blood was obtained and cultured with 20 µM 5-bromodeoxyuridine (BrdU) for 72 hours. The chromosomes were stained with the FPG technique. Cells were analyzed and the percentage of first, second, and third divisions were recorded. The number of SCE from 30 analyzable metaphases were recorded, with special attention to the number of SCE seen in the F group chromosomes. The average number of exchanges per cell was recorded and the replicative index was calculated from the percentage of cells in first, second, and third divisions [13].

High-Resolution Chromosomes

Chromosomes were studied by high-resolution with a modification of the actino-mycin D pretreatment procedure [8]. Cells were grown for 66–68 hours in RPMI 1640 medium with actinomycin D (5 µg/ml, final concentration) for the last 45 minutes. Colcemid (0.06 µm/ml,

final concentration) was added for the last 5–6 minutes before harvesting. Harvesting, slide preparation, and G-banding were routine. This report is an extension of an earlier blind high-resolution chromosome analysis of eight MEN-II patients and six controls [8].

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 chromosome 20 pair for the 20p deletion. All scoring was done through the microscope to maximize optical resolution, similar to the procedure described by Babu et al. [7] and Butler et al. [8]. Nine to 24 cells without overlap or twist of the short arm of chromosome 20 at the 700–1200 band level were identified and analyzed. After agreement among the cytogeneticists that the 20p was normal or appeared deleted in the majority of cells, the code was broken.

RESULTS

Mitomycin C

Table 1 includes the individual data and Table 2 summarizes the results of the MMC studies from 12 patients with MEN-II syndrome and 12 age-matched control subjects grown in 20 ng/ml MMC for 96 hours. The average frequency and standard deviation for total chromosome and chromatid aberrations per 100 cells was 6.9 ± 3.1 for the controls and 12.3 ± 7.8 for the MEN-II patients. The average frequency and standard deviation for chromatid aberrations was 5.8 ± 2.4 for the control subjects and 10.8 ± 6.7 for the MEN-II patients. Therefore, among the MEN-II patients the frequency was significantly higher ($p < 0.05$) for total and chromatid aberrations, but not for chromosome aberrations.

Table 3 includes the individual data and Table 4 summarizes the MMC studies from seven patients with MEN-II and seven age-matched controls grown in 20 ng/ml MMC for 48 hours. The average frequency and standard deviation for total chromosome and chromatid aberrations per 50 cells was 3.4 ± 1.6 for the controls and 7.7 ± 8.6 for the MEN-II patients. Therefore, there was no significant difference in the number of aberrations observed at 48 hours between the controls and MEN-II patients.

Folate-Deficient Culture Conditions

Table 5 includes the individual data and Table 6 summarizes the folate-deficient cultured lymphocyte studies on 13 MEN-II patients and 13 age-matched controls. The average frequency of total chromosome and chromatid aberrations per 100 cells was 6.2 for the controls and 4.9 for the MEN-II patients. Therefore, there was no significant difference in the number of aberrations between the two groups and no increased number of fragile sites in the MEN-II patients compared with controls.

Sister Chromatid Exchanges

Table 7 summarizes the sister chromatid exchange data from 12 MEN-II patients and 12 age-matched controls. The average SCE frequency and standard deviation for the control and MEN-II patients was 9.1 ± 1.9 and 9.2 ± 1.7 , respectively. Therefore, there was no significant difference in the number of SCE observed between the two groups. There were

also no significant differences in the percentage of cells observed in first, second, or third divisions, as determined by the replicative index between the two groups. There also was no excess number of SCE observed in the F group chromosomes.

High-Resolution Chromosomes

A blind analysis of high-resolution G-banded chromosomes was performed on blood specimens from 13 MEN-II patients and seven controls (Table 8). Twelve of 13 MEN-II patients and one of seven controls were scored as having the 20p12.2 deletion (Fig. 1, $\chi^2 = 12.58$, $P < 0.001$) previously reported by Babu et al. [7] and Butler et al. [8].

DISCUSSIONS

Our cytogenetic investigations suggest that chromosome instability and a minor deletion of 20p in the majority of MEN-II syndrome patients are features of the MEN-II syndrome. The chromosome breakage data are similar to those previously reported [9–12], although other investigators have not confirmed chromosome instability in their MEN-II patients [14, 15].

Our chromosome breakage data with 20 ng/ml of MMC at 96 hours, but not 48 hours, showed an increased number of total aberrations, particularly of the chromatid type in the MEN-II patients compared with age-matched controls. A preponderance of chromatid aberrations in our study was in agreement with the patients studied by Gustavson et al. [10], although Hsu et al. [9] reported a preponderance of chromosome type aberrations in MEN-II patients compared with their controls. Chromosome breakage data from our folate-deficient cultured lymphocytes and frequency of SCE were also in agreement with Gustavson et al. [10] in that no significant differences were found between MEN-II patients and controls. There also was no difference in cell kinetics as determined by the replicative index between the MEN-II and control groups.

Chromosome instability is associated with certain autosomal recessive disorders such as Bloom's syndrome, Fanconi's anemia, and ataxia telangiectasia, with specific defects in DNA synthesis and/or repair. Although MEN-II is inherited in an autosomal dominant fashion, this condition shares the propensity of malignancy with the cancer-prone autosomal recessive chromosome breakage disorders. Our chromosome breakage data would indicate that chromosome instability is a feature of MEN-II syndrome. This chromosome instability along with high-resolution analysis of chromosome 20 imply that a presymptomatic test is possible to identify those family members at risk for MEN-II syndrome. Preliminary chromosome data from two MEN-II patients and two controls grown in 50 ng/ml MMC for 48 hours showed a higher number of aberrations compared with 20 ng/ml MMC cultures. Therefore, research is in progress to determine if higher concentrations of MMC will induce more breakage and be more useful as a screening tool for identifying MEN-II syndrome patients.

In an earlier study we reported an interstitial deletion of 20p12.2 in seven of eight MEN-II patients and one of six controls [8]. We have now extended our blind high-resolution chromosome study to include 13 MEN-II patients and seven controls. Twelve of 13 MEN-II patients and one of seven controls were interpreted to have the 20p12.2 deletion. Our

expanded study confirmed the earlier reports of an interstitial deletion of the short arm of chromosome 20; however, other investigators have not confirmed the deletion 20 [9, 10, 16, 17]. These studies were apparently not blind, and included only two patients per study. Because the apparent deletion is subtle it may be easily overlooked if high-resolution methods are not used in a blind fashion. It is possible that the apparent deletion is simply due to homolog discordance, a chromosomal variant, or possibly a paracentric inversion, which is in linkage disequilibrium with the MEN-II gene. DNA studies with the D20S5 probe, which has been assigned to the short arm of chromosome 20, have not indicated a deletion or linkage between MEN-II and the D20S5 probe [8, 18]. Further investigation with more patients is needed to understand the basis of apparent nonlinkage between MEN-II and D20S5, as well as to confirm the chromosome deletion in the MEN-II syndrome.

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Figure 1.

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

Chromosome aberrations in cultured lymphocytes treated with 20 ng/ml MMC for 96 hours

Table 1

Case	Sex/Age (yrs)	Diagnosis	Number of chromosome aberrations	Number of chromatid aberrations	Cells with aberrations (%) ^a	Number of cells examined
DM	F/35	MEN-II	0	17	17	100
PN	F/32	MEN-II	0	6	6	100
RN	M/38	MEN-II	3	6	9	100
MIN	M/36	MEN-II	0	9	9	100
HB	M/39	MEN-II	2	2	4	100
BT	M/38	MEN-II	0	19	19	100
BB	F/46	MEN-II	0	5	5	102
BC	M/39	MEN-II	2	11	13	102
BD	F/37	MEN-II	7	17	24	101
BiA	F/58	MEN-II	0	3	3	99
BiC	M/35	MEN-II	1	12	13	104
BiJ	M/25	MEN-II	4	22	26	100
BJ	F/31	Control	0	5	5	101
BM	F/39	Control	0	9	9	100
LR	F/25	Control	0	3	3	100
CP	F/31	Control	0	8	8	100
BE	F/38	Control	2	2	4	103
BF	F/34	Control	3	7	10	100
BiB	M/67	Control	1	5	6	80
BiD	F/32	Control	4	9	13	101
RH	M/41	Control	1	7	8	100
NP	F/34	Control	1	3	4	100
GK	M/27	Control	0	4	4	100
DR	M/34	Control	2	7	9	100

^aSignificant difference ($p < 0.05$; matched t -test)

Table 2

Percentage of cells with aberrations treated with 20 ng/ml MMC for 96 hours

Subject group	Chromatid and chromosome aberrations	Chromatid aberrations	Chromosome aberrations
MEN-II (<i>n</i> = 12)	12.3	10.8	1.6
Control (<i>n</i> = 12)	6.9	5.8	1.2
<i>t</i> -test	2.47 ^{<i>a</i>}	2.73 ^{<i>a</i>}	1.0 ^{<i>b</i>}

^{*a*} *p* < 0.05 (matched two-tailed *t*-test).^{*b*} Not significant (*p* > 0.05).

Table 3
Chromosome aberrations in cultured lymphocytes treated with 20 ng/ml MMC for 48 hours

Case	Sex/Age (yrs)	Diagnosis	Number of chromosome aberrations	Number of chromatid aberrations	Cells with aberrations	Cells with aberrations (%)*	Number of cells examined
DM	F/35	MEN-II	1	3		16	23
PN	F/32	MEN-II	0	1		2	50
RN	M/38	MEN-II	0	2		4	50
MIN	M/36	MEN-II	0	5		10	50
HB	M/39	MEN-II	0	0		0	50
BT	M/38	MEN-II	0	0		0	50
MM	F/46	MEN-II	2	9		22	50
BJ	F/31	Control	0	1		2	50
BM	F/39	Control	1	0		2	50
LR	F/25	Control	0	3		4	70
CP	F/31	Control	0	3		6	50
BF	F/34	Control	0	1		2	51
RH	M/41	Control	0	2		4	50
ML	F/36	Control	0	1		2	50

*No significant difference ($p > 0.05$; matched t -test)

Table 4

Percentage of cells with aberrations treated with 20 ng/ml MMC for 48 hours

Subject group	Chromatid and chromosome aberrations	Chromatid aberrations	Chromosome aberrations
MEN-II ($n = 7$)	7.7	6.6	1.1
Control ($n = 7$)	3.4	3.1	0.3
<i>t</i> -test	1.04 ^a	1.05 ^a	0.8 ^a

^aNot significant ($p > 0.05$).

Table 5

Chromosome aberrations in folate-deficient cultured lymphocytes for 96 hours

Case	Sex/Age (yrs)	Diagnosis	Number of chromosome aberrations	Number of chromatid aberrations	Cells with aberrations	Cells with aberrations (%) ^a	Number of cells examined
BiA	F/58	MEN-II	0		1	1	103
BiC	M/35	MEN-II	0		5	5	103
JB	M/25	MEN-II	0		11	11	104
BC	M/39	MEN-II	0		4	4	105
BB	F/46	MEN-II	0		3	3	104
BD	F/37	MEN-II	0		5	5	101
DM	F/35	MEN-II	1		17	18	100
MM	F/46	MEN-II	0		1	2	40
RN	M/38	MEN-II	0		1	1	100
PN	F/34	MEN-II	0		0	0	100
MN	M/36	MEN-II	0		1	1	100
BT	M/38	MEN-II	2		5	7	100
HB	M/39	MEN-II	0		6	6	100
BF	F/34	Control	0		34 ^b	34 ^b	100
BE	F/38	Control	0		14	14	104
CP	F/31	Control	1		12	13	104
BM	F/39	Control	0		2	2	101
LR	F/25	Control	0		2	2	100
DR	M/34	Control	0		3	3	100
JD	F/40	Control	0		3	3	100
LK	F/30	Control	0		0	0	100
TK	F/22	Control	0		4	3	120
BiB	M/67	Control	0		4	4	109
BU	M/35	Control	2		3	5	100
BJ	F/31	Control	0		2	2	100
BiD	F/32	Control	1		6	7	103

^aNo significant difference in frequency of aberrations between MEN-II and age-matched control subjects; *t*-test = 0.42 (*p* > 0.05).^bControl subject with a 20p fragile site accounting for 22 cells.

Table 6

Percentage of cells with aberrations in folate-deficient cultured lymphocytes

Subject group	Chromatid and chromosome aberrations ^a	Chromatid aberrations ^a	Chromosome aberrations ^a
MEN-II (<i>n</i> = 13)	4.9	4.6	0.3
Control (<i>n</i> = 13)	6.2	5.2	1.0

^aNo significant difference in aberrations between MEN-II patients and controls.

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Table 7

Frequency of SCE from control and MEN-II subjects

Subject group	Number	Age		Sex M/F	Average number of SCE (group mean $\pm$ SD range)			Total number of cells analyzed	Average replicative index
		Mean	Range		Per cell	Per individual			
MEN-II	12	38.9	25–58	7/6	9.2 $\pm$ 1.7	7.8–13.7	369	1.77	
Control	12	34.3	20–45	5/7	9.1 $\pm$ 1.9	5.9–12.4	363	1.82	

Table 8

Summary of high-resolution chromosome findings in blind study

Subject group	Chromosome 20 ^a	
	Normal	20p deletion
MEN-II (<i>n</i> = 13)	1	12
Control (<i>n</i> = 7)	6	1

^aTwo-tailed χ^2 test with 1 df was 12.58 ($p < 0.001$).