Am J Hum Genet 35:919–931, 1983

Inherited Susceptibility to Retrovirus-Induced Transformation of Gardner Syndrome Cells

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SUMMARY

Skin fibroblasts from patients with Gardner syndrome (GS), those with familial polyposis coli (FPC), and spouse or unrelated controls were karyotyped and tested for various growth properties including susceptibility to transformation by viral or chemical agents. Our results indicated that based on the higher susceptibility to retrovirus-induced transformation and chromosomal aneuploidy, the GS and FPC cells could be distinguished from those of the general population with more than 70% accuracy. However, much work is in order before any biological assay can be used for clinical diagnosis of GS or FPC patients.

INTRODUCTION

The Gardner syndrome (GS) is a dominantly inherited genetic disease that manifests a range of benign soft tissue and bone tumors to multiple intestinal adenomas predisposed to malignancy [1]. Familial polyposis coli (FPC) also shows an autosomal dominant inheritance pattern for multiple adenomas in the large intestine [2–4]. Although the early symptoms of FPC are not well defined, soft tissue and other benign tumors in childhood or adolescence signal the beginning of GS followed by adenomas in the colorectal region. Moreover, management of these diseases depends entirely on an early diagnosis, preventive surgery, and genetic counseling for the family members. Results of this study and others indicate that GS and FPC may be the same genetic disease controlled by the same or similar pleiotropic gene(s).

Received December 10, 1982; revised February 15, 1983.

This investigation was supported by grant CA-21623 from the U.S. Public Health Service awarded by the National Cancer Institute, Department of Health and Human Services.

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 0002-9297/83/3505-0012$02.00

In the past decade, several assay procedures were developed for possible identification of the high-risk members of hereditary cancer families [5–23]. Therefore, in 1980, a workshop sponsored by the Biological Carcinogenesis Branch of the National Cancer Institute (NCI) was held to review and discuss the merits and deficiencies of the individual assay systems. Emphasis was placed on determining the parameters for the validity and reproducibility of various tests. Since a number of in vitro studies previously indicated that skin fibroblasts can be used to distinguish individuals with GS from those of the general population [10–22], it was specifically recommended that a "double-blind" study in separate laboratories may lead to a better evaluation of some of these tests. Because of an early onset of the extracolorectal symptoms and the appearance of colon polyps between the ages of 11–18, it is important to predict and identify children who may have inherited the GS or FPC gene. One of us (E. J. G.) therefore suggested the use of cells from affected members of kindred 109, which has been under surveillance for more than 30 years, together with fibroblasts or lymphocytes from unaffected related or unrelated people as controls. Dr. R. Moon of Salt Lake City, Utah, was appointed to code the test cells and distribute comparable subcultures to the separate laboratories of Rasheed (University of Southern California [USC]), Rhim (NCI), and Gardner (Utah State University [USU]). Here we describe the results of such a study and confirm previous findings on the higher susceptibility of GS cells to murine sarcoma virus-induced transformation [13, 15]. Our studies also indicate that the GS cells can be identified with more than 70% accuracy in the retrovirus transformation assay only when the cells show comparable growth rate and the assay conditions are kept constant.

MATERIALS AND METHODS

Forty-four fibroblast cultures were derived from upper-arm normal skin biopsies of the clinically diagnosed patients with GS kindred no. 109 [1], FPC, and unaffected, unrelated, or related kindred members who had missed the dominant gene for GS or FPC. As controls, we also included cells from individuals with other diseases such as xeroderma pigmentosum and Fanconi anemia and four cell strains derived from patients with solitary adenomas but who were free of classical GS or FPC symptoms. Cells were distributed to the three laboratories (USC, NCI, and USU) at comparable passage levels, where they were maintained in minimum essential medium with 10% fetal bovine serum (FBS) supplemented with 2 mM glutamine (maintenance medium). Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Plating Efficiency

Seventeen randomly selected cell cultures were tested for plating efficiency in media containing 1%, 5%, and 10% FBS. Plating efficiency of each of these cultures was also tested in the presence or absence of various concentrations of 12-O-tetradecanoyl phorobol-13-acetate (TPA) (Consolidated, Midland, Brewster, N.Y.) in 0.01% dimethylsulfoxide (DMSO) (table 1).

Triplicate sets of each culture (p7–p12) were plated with 500 viable cells (trypan blue dye excluded) per 60-mm dish in media with or without appropriate dilutions of TPA. Each cell strain was treated with media containing 1%, 5%, and 10% FBS with or without the varying concentrations of TPA ranging from 0.001 μg/ml to 1 μg/ml (more than 3,000 dishes). In addition, cells were also plated in a dilution of .002 μg (2 ng)/ml TPA because this concentration was previously shown to give the optimum plating efficiency that dis-

TABLE 1

PLATING EFFICIENCY IN THE PRESENCE OR ABSENCE OF TPA

Cells	Untreated	1.0 μg/ml	0.1 μg/ml	0.01 μg/ml	0.001 μg/ml	0.002 μg/ml
GS:						
109-IV-22	35	22	30	34	13	21
V-28	27.5	17	15	15	18	21
-34*	48	22	35	46	15	18.5
-62*	55.6	49.6	49.4	46.5	23.9	33.7
-63*	46	45	39	36	21	15.8
-84*	20	19.6	12.7	15.7	13.7	13.7
Average	38.68	29.2	30.18	32.2	17.4	20.62
FPC:						
267-IV-2*	27	21	15	14	NT†	NT
-3*	43	18	22.2	25.4	17.2	17.6
Average	35	19.5	18.6	19.7	17.2	17.6
Controls:						
109-IV-33*	30.8	21.6	29	28	17	17.2
109-V-29*	29.1	20.5	24.2	22.2	22.8	23.2
237-I	27.3	14.5	20.9	17.8	14.9	13.6
239-I*	29	16	15	13	NT	NT
213-I	9.1	6.8	6.9	9	3.8	5.1
3190	25.9	15.2	17.4	16.3	NT	NT
Average	25.2	15.77	18.9	17.72	14.63	14.78

NOTE: Duplicate or triplicate cultures of each cell strain were separately treated in 1%, 5%, and 10% fetal bovine serum (FBS) in the presence or absence of various concentrations of TPA as described in MATERIALS AND METHODS. Although the percent plating efficiency of cells grown in 10% FBS without TPA was slightly higher than in TPA-containing media, fewer cells plated in 1% and 5% serum. The results are therefore given only for varying concentrations of TPA in 10% FBS-containing media and represent percent (%) plating efficiency of two dishes in each dilution.

*These cells were plated in the maintenance medium (1%–10% FBS) and after 24 hrs treated with various concentrations of TPA as indicated in MATERIALS AND METHODS.

†Not tested.

tinguished GS fibroblasts from those of other cells [16]. Cultures were left undisturbed for 9 days, and plating efficiency was determined on Giemsa-stained cells. In a repeat assay, 500 viable cells per 60-mm dish were separately plated in maintenance media containing 1%, 5%, and 10% FBS without TPA, and after 24 hrs, fluid was removed and duplicate sets of each culture were treated by the same media containing various concentrations of TPA ranging from 0.001 μg/ml to 1 μg/ml as described above.

Transformation Assays

A large virus pool was made of the Kirsten strain of murine sarcoma virus (KiMSV) genome pseudotyped with the baboon endogenous virus (BaEV) as a helper component [KiMSV (BaEV)] [15]. This stock virus showed a titer of $10^{5.6}$ focus-forming units (FFU/ml) on the normal rat embryo kidney (NRK) cells that are most susceptible to focus induction by these viruses. For transformation assays, human skin fibroblasts (p7–p15) were plated at a density of 5×10^5 cells per 60-mm dish in maintenance media containing 4 μg/ml polybrene, and after 24 hrs, medium was removed and two dishes each were exposed to 0.5 ml of threefold dilutions of the stock virus ranging from 10^0 to 10^{-4}. Cells were incubated for 1 hr with occasional shaking for even spread of the virus, and fresh medium was added after this period. Thereafter, the medium was changed every 2 days and cells observed for morphological alterations. Foci of transformed cells were counted on live, unstained cultures under the phase contrast lens on the seventh and again on the tenth or twelfth day after infection.

Chromosome Analysis

Short-term cultures of lymphocytes and fibroblasts were tested for possible chromosomal abnormalities. Upper-arm skin biopsies were cultured in McCoy's 5A medium supplemented with 2 mM glutamine and 20% FBS, which upon subculture was reduced to 15% FBS. Peripheral blood lymphocytes were stimulated with phytohemagglutinin (1.2 μg/ml) and grown for 72 hrs in RPMI-1640 media containing 2 mM glutamine and 20% FBS. Chromosomes were harvested and processed for trypsin-Giemsa-banding according to described procedures [1, 24, 25]. At least one or two standard control cultures were tested simultaneously with kindred 109 cells or those from unrelated affected individuals.

RESULTS

Forty-four coded samples of fibroblast cultures were grown and tested for murine sarcoma virus-induced transformation at two separate laboratories (USC and NCI), and plating efficiency in the presence or absence of TPA was carried out at USC. Results were sent directly to E. J. G. at USU for decoding of the samples with appropriate diagnosis and correlation with chromosome analysis performed at his laboratory.

Morphology and Behavior of Cells Used in Various Assays

The cell morphology of all cultures tested for retrovirus-induced transformation or plating efficiency in TPA-containing media was fibroblastic. In general, cells derived from GS or FPC patients were fibroblastic and could not be distinguished morphologically or by any other in vitro growth parameter from those established from their spouses, or from unrelated or unaffected control individuals. Although some of the GS and FPC cells showed propensity to grow in dense multiple layers, all cultures were contact inhibited, and none formed colonies in 0.3% soft agar or in 0.8% methylcellulose.

Plating Efficiency in Different Concentrations of Serum

Seventeen randomly selected cell strains were tested for plating efficiency in media containing 1%, 5%, and 10% FBS. After trypan blue dye exclusion, duplicate or triplicate sets of each culture were plated separately at a density of 500 cells per 60-mm dish in various serum concentrations and left undisturbed for 8–9 days. In general, the plating efficiency (PE) of the GS and FPC cells ranged between 20%–55% (average 36%) in 10% FBS, and the fibroblasts derived from spouse or healthy unrelated individuals showed 9%–30% (average 25%) PE in the same serum concentration. Of the seven GS and three FPC cells tested, one GS culture did not grow well and showed a PE of 5%. This culture was excluded from the calculations of average PE of GS cells (table 1). Similarly, two of the control cells that showed 0% and 2% PE in 10% FBS were also not included while estimating average PE for spouse and unrelated healthy individuals (table 1). An averge of about 0.4% to 4% cells plated in 1% FBS, whereas approximately 1.5 to threefold and three- to fivefold higher plating efficiencies were observed in 5% and 10% FBS, respectively, in all groups of cultures tested. Most of the control cells plated in 1% or 5% FBS did not form colonies. However, small colonies of 5–30 cells and 10–70 cells were discernible in GS or FPC cultures plated in the presence of 1% and 5% serum concentrations, respectively.

Plating Efficiency in the Presence of 12-O-Tetradecanoyl Phorobol-13-acetate (TPA)

Plating efficiencies of six GS, two FPC, and six control cultures were tested in various concentrations of serum and TPA (table 1). Four serial 10-fold dilutions of TPA ranging from 1 μg/ml to 0.001 μg/ml and an additional dilution of 0.002 μg/ml were tested. Cells were treated with each of the five TPA dilutions separately made in media containing 1%, 5%, and 10% FBS; control untreated cultures were plated in media without TPA but in the corresponding serum dilutions (i.e., 1%, 5%, and 10%). Plating efficiency was determined by two methods: (1) cells were plated in media containing various concentrations of TPA and serum as indicated above and were left undisturbed for 8–9 days prior to staining with Giemsa, and (2) cells were plated in maintenance medium, and after 24 hrs, medium was removed and fresh media containing various concentrations of TPA and serum were added as described above and left undisturbed until stained.

The average plating efficiencies of cells treated with 1 μg/ml, 0.1 μg/ml, or 0.01 μg/ml concentrations of TPA were similar but those that were plated in 0.001 μg and 0.002 μg/ml dilutions showed somewhat lower PE than that observed in cultures plated in higher concentrations of TPA (table 1). Compared to the untreated cells, all TPA-treated cultures of the same cell strain regardless of their origin or plating methods gave approximately 10%–20% lower PE. However, both the treated and untreated GS cultures exhibited slightly higher (6%–11%) PE in 1 μg/ml, 0.1 μg/ml, and 0.001 μg/ml concentrations of TPA than that obtained by similarly treated or untreated cells from spouse or unrelated healthy individuals. The PE of FPC cells in various concentrations of TPA was closer to the GS cells than that obtained for the normal healthy controls.

Retrovirus-induced Transformation

We tested fibroblasts from 12 GS, four FPC, four undiagnosed, and 13 spouse or unrelated healthy individuals for susceptibility to transformation by Kirsten strain of murine sarcoma virus pseudotype of baboon endogenous virus [KiMSV (BaEV)]. On the basis of our previous observations [15], serial threeefold dilutions of virus ranging from 10^0 to 10^{-4} were tested on duplicate sets of each culture. Transformation was assessed by morphological alterations of elongated, spindle-shaped fibroblasts to slightly enlarged, highly refractile, rounded or epithelioid cells. Unlike the murine cells transformed by the same virus, the human cells did not form compact foci of fast-growing rounded cells. Instead, the foci in human cells were arrayed as "schools of fish" throughout the monolayer cultures [13, 15]. Because it was difficult to obtain an accurate count in cells exposed to higher virus dilutions or those that had a preponderance of singly dispersed transformed cells, these cultures were simply scored as "positive." However, we counted the foci in cells exposed to higher dilutions (10^{-2} to 10^{-4}) or those showing fewer foci (75 or less per dish) with distinct boundaries between them. Taken together, the highest number of foci were induced in the GS cells and the least number of cells transformed in cultures derived from normal related or unrelated individuals. Eight of the 10 cultures tested induced transformed foci at 10^{-2} to 10^{-3} dilutions of virus, indicating higher susceptibility to transformation

than control cells, most of which transformed only at 10^0 or 10^{-1} (table 2). Also, cultures derived from duplicate biopsies of four of the eight GS patients were transformed up to $10^{-2.5}$ or 10^{-3} dilutions. However, some discordance was observed. Of the 10 GS cell strains tested, two (109-V-63 and -84) did not grow well in one laboratory (S. R.) and transformed only to 10^{-1} dilution, but one of these two cultures (109-V-63) tested in another laboratory (J. R.) transformed up to 10^{-3} dilution. In a repeat experiment, one of the GS cultures that had transformed previously to $10^{-2.5}$ showed foci up to $10^{-1.5}$ dilution only. Moreover, one of the control cultures (not GS) consistently transformed by the virus dilution of 10^{-2}.

The four FPC cultures were transformed by the same virus dilutions as those from the GS patients with the exception of one of three cultures tested in another laboratory that transformed only to 10^{-1} (table 2). Furthermore, three cultures from patients with uncertain diagnoses also induced similar levels of transformation as developed by the GS cells, although some variations in transformation titers were seen in results obtained in the two laboratories (table 2). These patients were referred to the Utah clinic by their physicians from other parts of the country because of the presence of solitary colon polyps or adenomas. However, no family history of colon cancer or presence of soft tissue, bone tumors, or multiple adenomas typical of GS or FPC syndromes was reported in these patients.

Chromosome Analysis

Both peripheral blood lymphocytes and fibroblasts were tested for chromosome numbers by trypsin-Giemsa-banding techniques. Our result of the 50 cell counts in each preparation indicated near diploidy or aneuploidy of chromosomes (i.e., presence of more or less than 46 chromosomes) in 14%–32% of the lymphocyte samples from the eight GS patients, in 32% of one FPC, in 2%–6% of cells from the five spouse or other controls, and in 19% of the cells from one patient with a solitary polyp (uncertain diagnosis, table 2). We also tested chromosomes of seven fibroblast cultures from GS and FPC patients, 10 normal individuals, and one culture from an undiagnosed individual with solitary adenoma (table 2). All cultures from GS exhibited aneuploidy ranging from 29% to 38%, one FPC cell strain showed 37%, another culture from a patient with solitary adenoma showed 34% instable chromosomes, and only five of the 10 cell strains tested from controls showed 3%–7% aneuploidy. However, no consistent pattern of aneuploidy was seen in GS or FPC cells. Chromosome instability was also reflected in structural aberrations such as deletions, inversions, or translocations in patients with both GS and FPC. Twelve structural aberrations were observed in 100 karyotypes from seven GS and two FPC patients compared with none in 100 karyotypes from controls.

DISCUSSION

We confirmed previous findings of our laboratories and others that the murine sarcoma virus transformation assay can distinguish skin fibroblasts from patients with GS or FPC from similar cultures of unaffected related or unrelated individuals [13, 15, 20, 21]. Furthermore, we extended these findings by correlating the susceptibility to KiMSV (BaEV) transformation with chromosomal instability

TABLE 2

SUSCEPTIBILITY TO VIRAL TRANSFORMATION AND CHROMOSOME ANALYSES

Cells‡	Age	TRANSFORMATION BY KiMSV (BaEV)*		% ANEUPLOIDY†	
		Rasheed	Rhim	Lymphocyte	Fibroblast
Gardner syndrome:					
109-IV-20	39	2§	NT‖	25	NT
-22	28	2.5	3	NT	NT
V-20	19	3	3	32	33
-21	17	NT	NT	NT	29
-28	21	2	1	32	30
-30	17	2.5	0	30	30
-31	13	NT	NT	23	NT
-33	8	2	3	16	NT
-34	6	3	2	18	NT
-62	16	2.5	2	15	35
-63	14	1#	3	14	NT
-84	25	1#	NT	30	38
Familial polyposis coli:					
267-IV-2	21	2.5	3	NT	NT
-3	19	3	2	NT	NT
386 -1	53	2	1	32	37
401-III-1	27	3	NT	NT	NT
Uncertain diagnosis:					
268-IV-8	29	NT	NT	NT	34
273-III-4	36	1	3	19	NT
358 -1	54	2.5	0	NT	NT
359 -1	70	3	2	NT	NT
Unaffected controls:					
109-IV-9	48	NT	NT	5	6
-33	45	1	1	0	NT
-29	19	NT	NT	6	NT
V-32	11	NT	NT	6	NT
V-105	40	NT	NT	5	NT
267-IV-1	23	0	1	NT	NT
269 -1	64	1	NT	NT	NT
349 -1	39	1	NT	4	NT
365 -1	25	1	1	5	NT
370 -1	23	1	0	2	3
390 -1	16	1	1	NT	NT
401-III-2	26	1	0	2	0
422 -1	25	1	0	5	0
239 -1	32	2	2	3	NT
133-IV-9	64	NT	NT	0	NT
-96	25	NT	NT	0	0
-65	25	NT	NT	NT	7
C 3	35	NT	NT	NT	4
C 4	67	NT	NT	0	0
C 6	28	NT	NT	7	6
C 7	30	NT	NT	1	0
C 8**	55	1	NT	NT	NT
C 9**	62	0	NT	NT	NT
C 10	61	0	NT	NT	NT

*Cells were plated in media containing 4 μg/ml polybrene and exposed to serial threefold dilutions of Kirsten strain of mouse sarcoma virus pseudotyped with baboon endogenous virus [KiMSV (BaEV)]. Foci of transformed cells were counted after 5–7 and again at 10–12 days under a phase contrast microscope.

†Chromosomes were karyotyped using standard methods [17, 23, 24].

‡Of the 44 cell strains tested, 12 GS cultures were from kindred 109, four from FPC, and 24 from spouse and unrelated controls, and four patients did not show a familial history of GS or FPC (uncertain diagnosis).

§Nos. represent reciprocal of the end-point log-dilutions of the virus that induced foci of transformed cells.

‖NT = not tested.

#The low titers may be due to slow growth of these cells.

**C8 are cells from patients with Fanconi anemia and C9 cells are from patients with xeroderma pigmentosum.

(aneuploidy) in the fibroblasts and in lymphocyte cultures of the same patients. About 80% of both FPC and GS cells were transformed between 10^{-2} and 10^{-3} of virus dilutions in repeat assays performed in two separate laboratories. According to this assay, cells from four patients with uncertain diagnoses can also be grouped with either GS or FPC rather than with the "controls" (table 2). Although some discrepancies were noted in matching the retrovirus-induced transformation with the pedigree analyses (fig. 1 and table 2), the important observation is that 12/13 controls tested in the double-blind assay did not transform beyond 10^{0} or 10^{-1} virus dilution and only three GS or FPC cultures gave ambiguous results in the two laboratories.

The adenomas that predispose to cancer in FPC and GS seem to be identical. Furthermore, the similarity of GS and FPC cells in susceptibility to transformation by KiMSV also indicates a similar gene inheritance pattern for both diseases. This is particularly important because on the basis of KiMSV transformation assays, one of us (S. R.) was unable to distinguish skin fibroblasts of healthy individuals from those with Huntington disease, another disorder due to an autosomal dominant gene transmission [26]. Since these data were obtained without prior knowledge of the cell origin, it indicates that the retrovirus transformation test is reliable, provided the culture conditions are similar and the subculture passages of cells are comparable. We previously showed that the cells from two other GS or FPC pedigrees also differed in the susceptibility to transformation by a hybrid DNA virus Adeno-12 SV40 [15, 20]. Two GS cultures from kindred 109 were also exposed to this virus and found to transform within 10 days of exposure as compared to the 4–5 weeks for transformation of a control culture (S. Rasheed, unpublished results, 1982).

Loss of anchorage dependence and a differential sensitivity of the human skin fibroblasts to TPA were reported by Kopelovich et al. [16, 19]. We therefore tested PE of six GS, two FPC, and six control cultures on duplicate or triplicate sets with varying concentrations of serum (1%, 5%, and 10%) in five different dilutions of TPA as described in MATERIALS AND METHODS. This enormous experiment was performed under extremely controlled conditions by the use of the same stocks of TPA, media, serum, and dishes in each test. The PE of the cells in the presence or absence of TPA did not give clearcut results although the GS and FPC cells showed slightly higher PE in 10% FBS medium without TPA when compared to the cells derived from healthy individuals. Relative to this basic difference in the PE of cells, the TPA-treated cultures of all groups in the same concentrations of FBS exhibited somewhat lower PE than observed in the untreated groups. The TPA concentrations of 1 ng and 2 ng/ml showed slightly lower PE in all groups of cells than seen in cells treated with higher concentrations of TPA. Recently, Antecol and Mukherjee [27] tested effects of TPA on cells from various genetic disorders including FPC. These authors concluded that TPA treatment did not transform cells but rather induced similar changes on cellular morphology, growth rate, saturation density, epidermal growth factor binding, and cytoskeleton in fibroblasts from normal and FPC syndrome. Thus, the results of our present study on PE and growth rate are in agreement with observations made by these authors [27].

In contrast to the 100- to 1,000-fold increased KiMSV-induced transformation of GS or FPC fibroblasts as compared to the spouse or unrelated healthy individuals, the PE in the presence or absence of TPA shows only insignificant differences. Because of too many variables in this assay, it does not yield reproducible results of sufficient magnitude for a diagnostic test.

Kopelovich et al. [18] also showed that the tumor promoter TPA preferentially transformed normal skin fibroblasts from patients with adenomatosis of the colon and rectum but not those that were derived from healthy individuals. However, we were unable to transform two GS cultures treated separately with 1 μg/ml and 0.1 μg/ml concentrations of TPA consecutively for 6–8 weeks. Although more rounded, refractile cells were present in TPA-treated cultures compared to the untreated, none of these "anchorage independent" cells gave rise to colonies in soft agar (0.3%) nor did they continue to express the rounded phenotypes after one subculture without TPA. Moreover, one of these TPA-treated cultures inoculated subcutaneously in an athymic nude mouse did not produce a tumor.

Chemical transformation of cultured skin fibroblasts from individuals with adenomatosis of colon and rectum has also been reported [18, 22, 28–30]. As one of us (S. R.) reported [20], cells treated with *N*-methyl-*N*-nitroso-nitroguanidine (MNNG) or 4-nitroquinoline 1-oxide (4NQO) in the presence or absence of 0.1 μg/ml of TPA showed phenotypic changes in the cell morphology. However, this rounded or cuboid morphology was not a permanent feature of the chemically treated cells which reverted back to fibroblastic morphology when the drug was removed. Moreover, these cells did not form colonies in 0.3% soft agar, 0.8% methylcellulose, or induce tumors in athymic nude mice. Furthermore, the cellular heterogeneity and variations in the in vitro culture conditions of this laborious test make difficult the standardization of these procedures.

The chromosome analysis conducted on the same cells that were used for the viral transformation showed high levels of chromosome instability (aneuploidy and/or structural aberrations) for GS and FPC cells as compared with the controls. Although these abnormalities were observed, even in children without adenomas of the colorectum or other extracolorectal manifestations, no consistent pattern of chromosomal instability was established for a clinical diagnosis of these individuals.

The mechanism of transformation is not well understood. However, it is well known that retroviruses transform cells after random integration of their genes into the host cell chromosomes. Recently, a gene that is preferentially expressed in human colon carcinoma (not GS or FPC) has been shown to be very similar if not identical to a rat transforming gene c-*kis* [31–34]. This rat-transforming gene is the same as that present in the KiMSV genome (v-*kis*) [32, 33]. It is possible that a retrovirus-related cellular transforming gene similar to c-*kis* is also activated in GS or FPC cells, and perhaps a preferential integration site of KiMSV in the GS cellular DNA makes these cells more susceptible than the normal cells. Alternately, the integration of KiMSV (BaEV) in GS or FPC DNA may affect the *cis*-acting control elements in the cellular or viral DNA in such a manner that the rate of viral transcription is enhanced and more of GS or FPC cells are transformed than those from other individuals. Molecular analyses of the cellular genes expressed

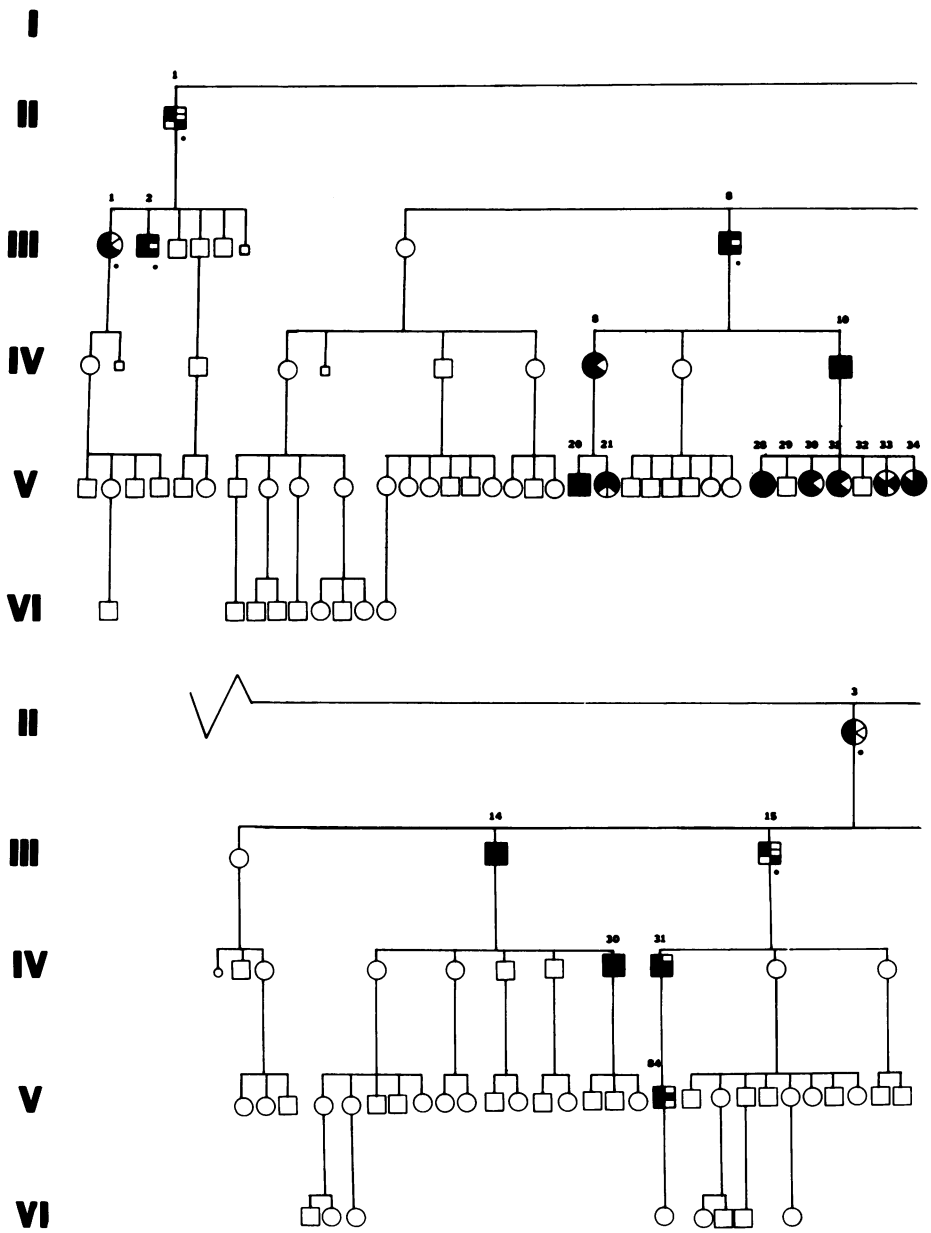

FIG. 1.—Genealogy of kindred 109 (continued on next page)

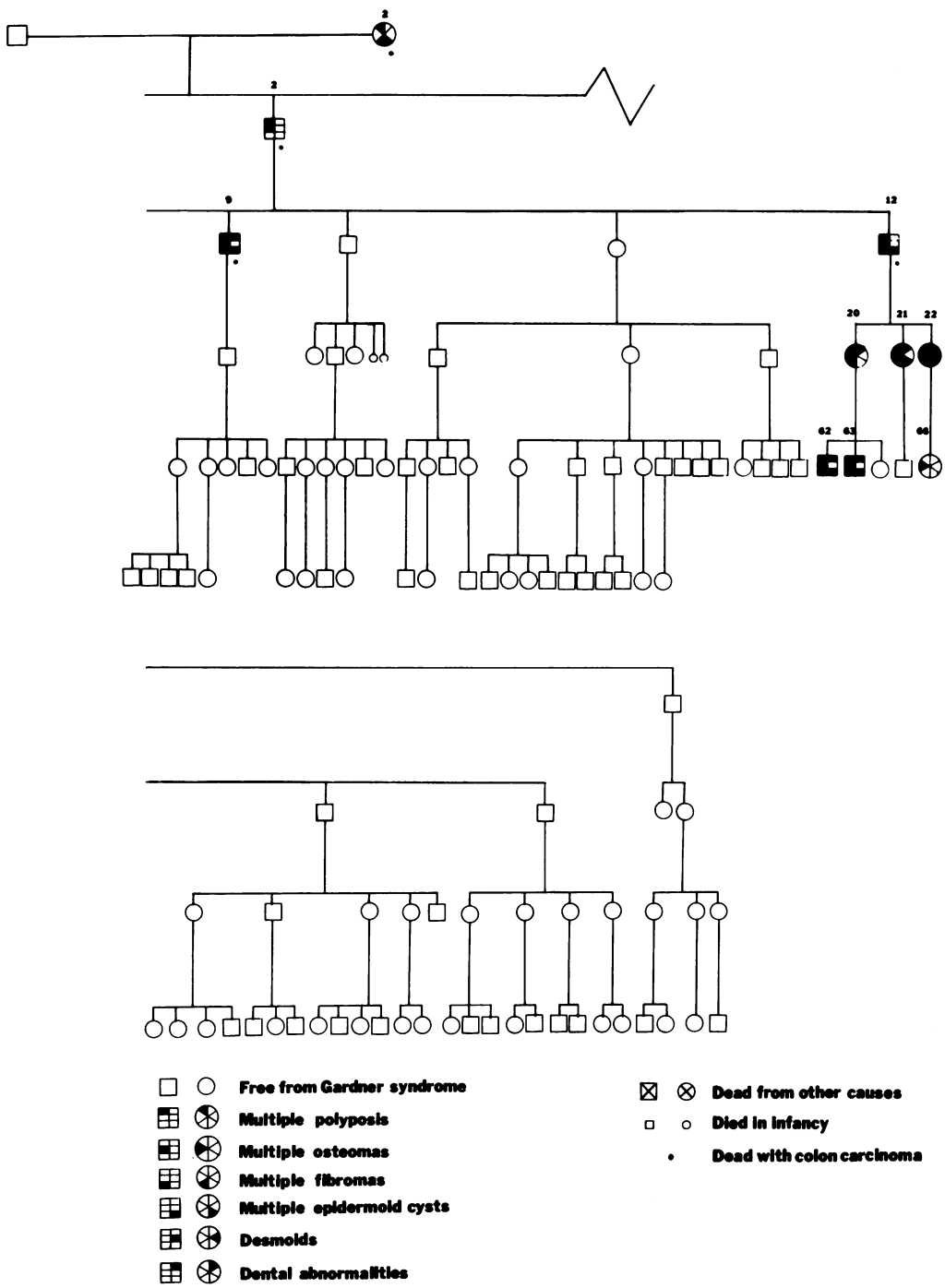

FIG. 1.—Continued from previous page

in the GS and FPC cells will help in a better understanding of the oncogenic process and protein(s) expressed in these tumors.

ACKNOWLEDGMENTS

We thank Geraldine Trail for excellent technical help and Marylynn Dispenza for preparing the manuscript.

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