Teratogenesis, Carcinogenesis, and Mutagenesis 22:31–40 (2002)

Chromosomal Fragile Sites and Relationship Between Genetic Predisposition to Small Cell Lung Cancer

M. Karadağ,[1] B. Tunca,[2] G. Çeçener,[3] Ü. Egeli,[2] N. Özyardimci,[1] E. Ege,[1] and O. Gözü[1]

[1]*Department of Chest Diseases, Faculty of Science, University of Uludag, Bursa, Turkey*

[2]*Department of Medical Biology and Genetics, Faculty of Medicine, Faculty of Science, University of Uludag, Bursa, Turkey*

[3]*Department of Molecular Biology, Faculty of Science, University of Uludag, Bursa, Turkey*

Fragile sites are non-staining gaps and breaks on mammalian chromosomes. Several investigators have pointed out that these sites may act as factors that predispose to specific chromosomal rearrangements that are present in some cancer cases. The expression of common fragile sites induced by aphidicolin (Apc) was evaluated on prometaphase chromosomes obtained from the peripheral blood lymphocytes of 15 patients with lung cancer, 20 of their clinically healthy family members, and 20 age-matched normal controls. As a result of cytogenetic evaluation carried out by the High Resolution Banding (HRB) technique, 1q21, 2q33, 3p14, 7q32, 13q13, 16q23, 17q21, and 22q12 are defined as fragile sites in patients and relatives. The rate of total fragile sites and 2q33, 3p14, and 16q23 are statistically significant in both patients and relatives when compared with the control group. Therefore, our results showed that common fragile sites might be unstable factors in the human genome and they can be used as suitable markers for genetic predisposition to lung cancer. *Teratogenesis Carcinog. Mutagen. 22:31–40, 2002.*

Key words: small cell lung cancer; chromosome aberration; common fragile sites; genetic predisposition; peripheric blood lymphocyte cultures

INTRODUCTION

Chromosomal fragile sites are regions susceptible to breakage under specific culture conditions. Antifolates, fluorodeoxyuridine (FdU), methotrexate (MTX), and

*Correspondence to: Dr. Ünal Egeli, Department of Medical Biology and Genetics, Faculty of Medicine, Faculty of Science, University of Uludag, Bursa, Turkey. E-mail:egeli@uludag.edu.tr

Aphidicolin (Apc) DNA polymerase inhibitors induce the expression of fragile sites during prometaphase and metaphase as nonstaining gaps or breaks, usually involving both chromatids [1,2].

Recently, several investigators and our study revealed that there are great individual differences in the expression of fragile sites and that this phenomenon is more frequent in normal cells of patients with certain types of neoplasms [1,3–5], and sometimes in their first-degree family individuals [6–12]. However, some scientists obtained negative results [13–15]. Therefore, the role of the carcinogenesis process at these sites is not known yet.

In the present study, the expression of fragile sites in lymphocytes of patients with Small Cell Lung Cancer (SCLC) and first-degree relatives was investigated and compared with the control group. Patients and relatives exhibited an increased sensitivity to fragile site induction by Apc.

MATERIAL AND METHODS

The present study has been carried out on 15 patients with SCLC, 20 of them being their first-degree relatives and on 20 normal healthy persons (Tables 1–3). Fifteen normal controls were selected from persons without a familial cancer history. In selecting the subjects, we ascertained that they had neither received X-irradiation nor suffered from a viral infection, and they had not taken medications or any drugs and environmental agents within the last 3 months.

The peripheral blood samples taken from patients, relatives and normal controls were cultured in RPMI 1640 medium containing 15% fetal bovine serum, 6 μg/ml phytohemagglutinin L, 0.5 mg/ml L-glutamine, and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin) for 72 h at 37°C. Apc (0.2 μM) was added to the medium 24 h before the harvest for fragile site expression. Ethidium bromide (1 μg/ml) and colchicine (1 μg/ml) were added to the medium 2 and 1 h before the harvest, respectively. Chromosome preparations were made according to a routine method. Three slides were prepared for each subject. The slides were stained with a Giemsa solution. The structural chromosome aberrations in 50 prometaphases belonging to each subject were counted by means of a light microscope (Zeiss axioplan) blindly. Subsequent by the prometaphases with breaks on the slides were destained with methanol (Merck, Darmstadt, Germany). Then, High Resolution Banding (HRB) was performed to determine the exact locations of gaps and breaks. A site was considered fragile if it appeared one or more times in cells analyzed for each subject and for at least three of all groups. As statistical analysis nonparametric Mann-Whitney test was used for the comparison of the frequency of chromosome aberrations and fragile sites in patients with SCLC, their relatives, and the control groups. Differences with a $P<0.05$ were accepted as statistically significant.

RESULTS

The cytogenetic and statistical evaluation of the results related to patients with SCLC, their relatives, and the control group are presented in Tables 1–5. The chromosomal aberration rates per cell (including gaps and breaks) were determined to be 0.34 for the patients, 0.29 for relatives, and 0.06 for the control group (Tables 1–3 and Fig. 1). When the rates of chromosomal aberrations of patients and their rela-

TABLE I. Characteristics and Chromosome Aberrations of Patients With SCLC

Subject Number	Sex	Age (years)	Smoking habit (packet/day)	Classification of clinicradyology	Metaphase number	Abberrant cell	Gap +break/cell
1	M	52	2	Common	30	0.00	0.00
2	M	61	—	Common	30	0.30	0.27
3	M	63	—	Common	50	0.58	1.22
4	M	71	0.5	Common	40	0.25	0.33
5	M	62	1	Common	40	0.10	0.13
6	M	70	1	Common	50	0.32	0.34
7	M	49	0.25	Uncommon	30	0.00	0.00
8	M	50	2	Uncommon	40	0.23	0.23
9	M	51	—	Common	30	0.13	0.13
10	M	57	1	Uncommon	50	0.22	0.24
11	M	44	2	Common	30	0.17	0.23
12	M	64	1	Common	40	0.25	0.28
13	M	62	1	Common	50	0.43	0.73
14	M	54	1	Common	50	0.24	0.54
15	M	66	—	Common	50	0.36	0.48
Mean ± SD						0.239 ± 0.153*	0.343 ± 0.310*

*Statistically significant at $P < 0.0005$ when compared with control group.

tives were compared with the control group, they were found to be statistically significant ($P < 0.0005$, $P < 0.0001$, respectively). However, they were insignificant when the patients with cancer were compared to their relatives ($P > 0.05$). Chromosomal localizations of gap, break and fragile sites are shown in Figure 2. We determined aphidicolin-type common fragile site by using our criteria. These sites are the

TABLE II. Characteristics and Chromosome Aberrations of Relatives of Patients With SCLC

Subject Number	Sex	Age (years)	Relationships with patient	Smoking habit (packet/day)	Metaphase number	Abberrant cell	Gap +break/cell
1	F	42	Brother	—	50	0.16	0.24
2	M	22	Father	0.25	50	0.06	0.06
3	M	41	Father	1	30	0.13	0.13
4	M	34	Father	1	40	0.20	0.28
5	M	37	Father	—	30	0.10	0.10
6	M	52	Father	0.75	40	0.18	0.18
7	M	46	Father	—	50	0.14	0.22
8	M	48	Father	1	40	0.68	0.83
9	M	35	Father	0.5	50	0.16	0.20
10	F	38	Father	—	50	0.32	0.42
11	M	35	Father	0.75	50	0.26	0.26
12	M	44	Father	1.5	40	0.20	0.38
13	M	40	Father	—	30	0.00	0.00
14	M	25	Father	0.5	30	0.26	0.23
15	F	20	Father	—	50	0.32	0.46
16	M	18	Father	—	50	0.20	0.32
17	F	41	Father	—	50	0.26	0.34
18	M	31	Father	—	50	0.20	0.26
19	M	20	Father	—	50	0.34	0.50
20	M	21	Father	—	50	0.24	0.32
Mean ± SD						0.22** ± 0.14	0.29** ± 0.18

TABLE III. Characteristics and Chromosome Aberrations of Controls

Subject Number	Sex	Age (years)	Smoking habit (packet/day)	Metaphase number	Abberrant cell	Gap +break/cell
1	F	53	—	30	0.06	0.07
2	F	40	1.5	50	0	0
3	F	42	—	30	0.10	0.07
4	F	47	—	30	0.03	0.03
5	M	70	—	30	0.10	0.13
6	F	35	1	50	0	0
7	F	40	—	30	0	0
8	M	29	1	50	0	0
9	F	73	—	30	0.07	0.07
10	M	47	—	30	0	0
11	F	33	—	30	0	0
12	F	46	—	30	0.03	0.03
13	M	31	1	50	0	0
14	F	39	—	50	0.02	0.02
15	M	39	1	30	0.03	0.03
16	F	47	2	30	0.17	0.23
17	M	35	2	50	0.12	0.16
18	M	25	1	50	0.04	0.02
19	M	38	1	30	0.23	0.27
20	F	48	—	30	0.13	0.13
Mean ± SD		42.85 ± 12.09	0.58 ± 0.71		0.056 ± 0.066	0.063 ± 0.081

following: 1q21, 2q33, 3p14, 7q32, 13q13, 16q23, 17q21, and 22q12 (Table 4, Fig. 3). Total fragile site rates were defined to be 0.192 for the patients with SCLC, 0.120 for the first-degree relatives, and 0.006 for the healthy control group. When the rates of total fragile sites and 2q33, 3p14, and 16q23 fragile sites of patients and their relatives were compared with the control group, they were found to be statistically significant (Table 4). However, they were insignificant when the patients were compared to their relatives.

DISCUSSION

In our study, chromosomal instability was relatively increased in induced lymphocyte cultures by Apc of patients with SCLC and their first-degree relatives. Cancer has been observed unequally in human population because within the population there are some groups more susceptible to environmental carcinogens than others. The host factors that predispose certain individuals to cancer can be investigated at several different levels [16]. Hsu et al. have laid out a hypothesis with regard to mutagen sensitivity testing that can serve as an assay for determining differences in genetic susceptibility [17,18]. Mutagen sensitivity, which is the increased expression of genetic defect, may render one person more prone to develop cancer than another [19]. Thus, these opinions help us to understand better the relationship between chromosome fragility and mutagen hypersensitivity.

In our study, the fra(3)(p14) expression was observed most frequently (Table 4). Various studies have demonstrated that chromosome 3p allelic losses occur in many forms of cancers including lung [20–26]. Recently, a chromosome 3p14.2 gene sensitive to carcinogens called FHIT was discovered and predisposed as a candidate

TABLE IV. Comparison of Fragile Sites Between Patients With SCLC, Their Relatives, and Control Groups

Groups	1q21	2q33	3p14	7q32	13q13	16q23	17q21	22q12	Total
Patients	0.01±0.021	0.0222±0.0311	0.0479±0.018	0.007±0.01279	0.007±0.01279	0.01587±0.02128	0.01153±0.02658	0.01508±0.03868	0.192±0.26
Relatives	0.006±0.0108	0.0130±0.0208	0.05775±0.03811	0.00615±0.01383	0.0050±0.011	0.0153±0.0193	0.0095±0.0283	0.0073±0.01517	0.12±0.078
Controls	0.002±0.0062	0.001±0.0045	0.001±0.0045	0.00±0.00	0.00±0.00	0.001±0.0045	0.00±0.00	0.00±0.00	0.006±0.013
Comparatives									
Patients and control	$P>0.05$	$P<0.01$	$P<0.001$	$P>0.05$	$P>0.05$	$P<0.005$	$P>0.05$	$P>0.05$	$P<0.0001$
Relatives and control	$P>0.05$	$P<0.05$	$P<0.0001$	$P>0.05$	$P>0.05$	$P<0.005$	$P>0.05$	$P>0.05$	$P<0.0001$
Patients and relatives	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$	$P>0.05$

TABLE V. Comparison of Totally Chromosomal Abnormalities and Fragile Sites Between Smokers in Patients, Relatives, and Control Groups

	Mean ± SD		
Groups	Aberrant cell	Gap + break/cell	Total fragile sites
Patients	0.20 ± 0.13	0.28 ± 0.21	0.087 ± 0.071
Relatives	0.24 ± 0.18	0.28 ± 0.22	0.113 ± 0.087
Control	0.04 ± 0.06	0.08 ± 0.11	0.011 ± 0.018
Compared	*P*		
groups	Aberrant cell	Gap + break/cell	Total fragile sites
Patients-control	$P < 0.05$	$P < 0.05$	$P < 0.01$
Relatives-control	$P < 0.005$	$P < 0.05$	$P < 0.001$
Patients-relatives	$P > 0.05$	$P > 0.05$	$P > 0.05$

tumor suppressor gene for lung, colorectal, and other cancers [27,28]. Therefore, this site was considered one of the primary sites for all human cancers. Restriction fragment length polymorphism (RFLP) analysis of several tumor tissues indicates that all of these tumor tissues may have a deletion at this site [29].

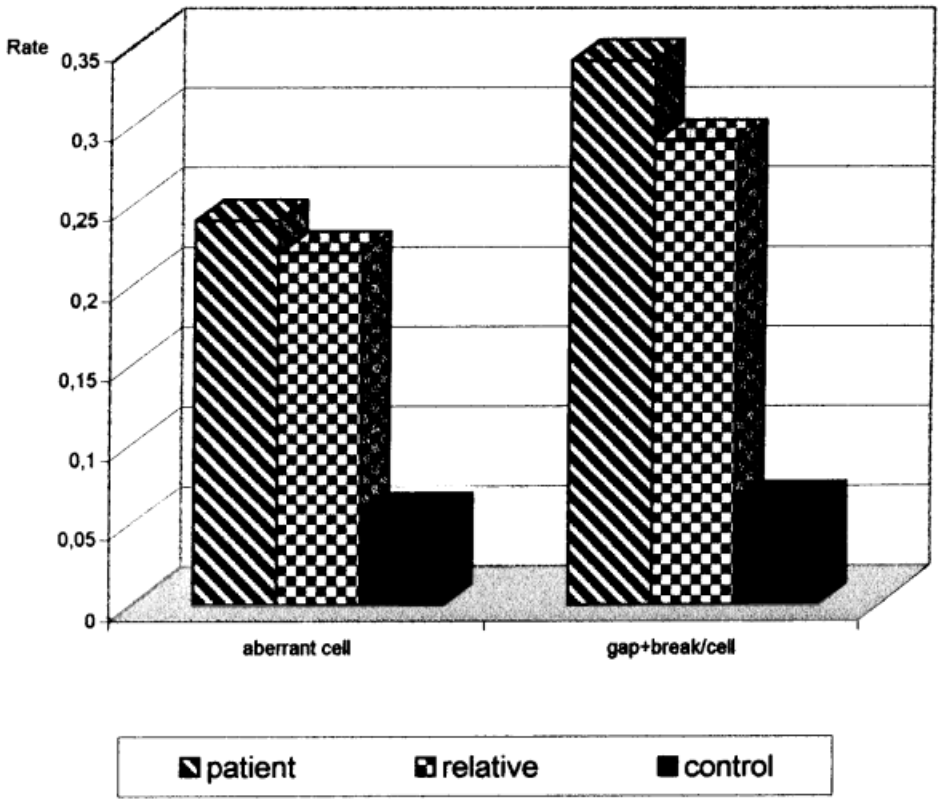

Fig. 1. Comparison of aberrant cell and gap + break/cell (%).

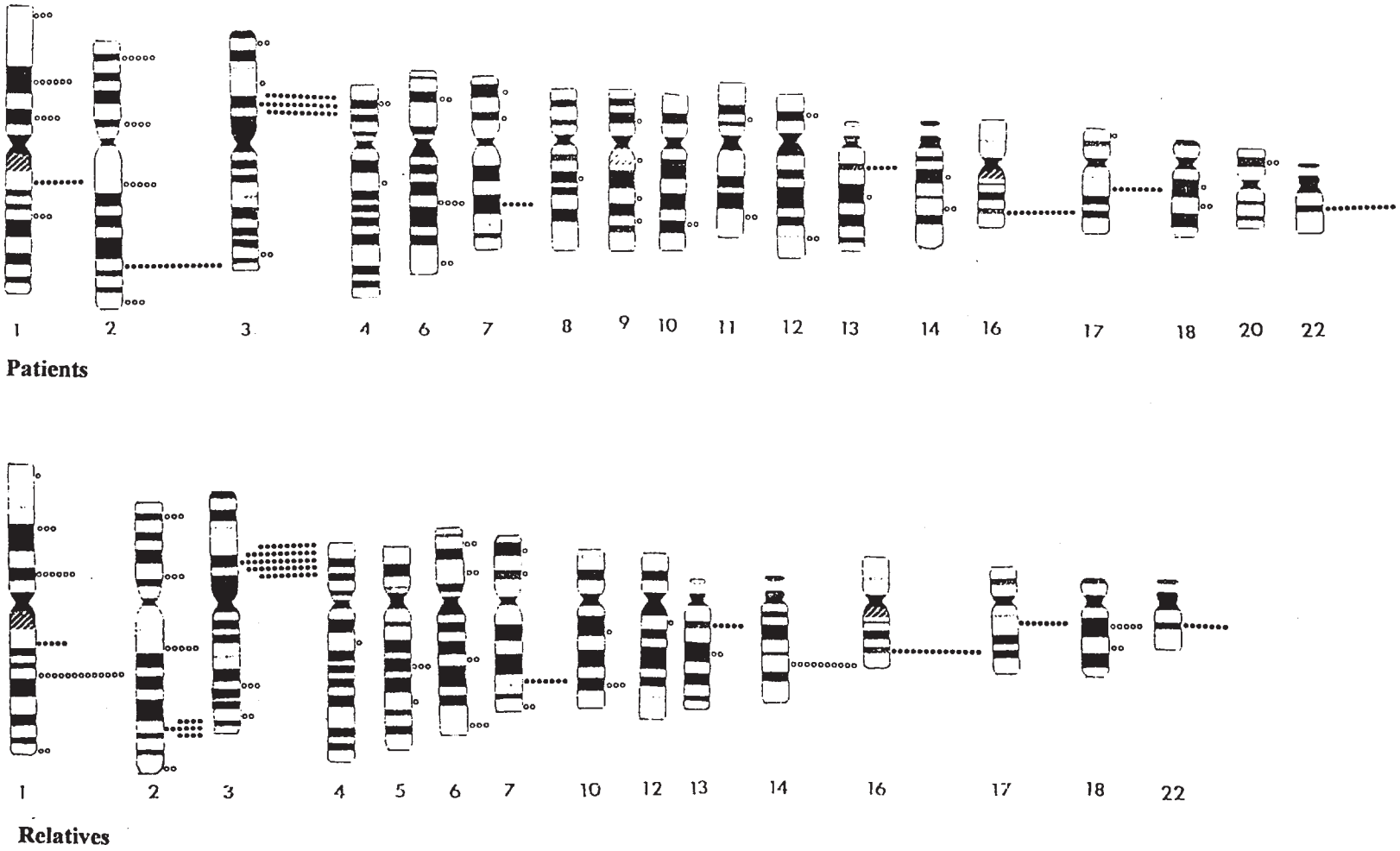

Fig. 2. Localization of gap and break points (white space) observed in patients with lung cancer and relatives. Dark space, accepted fragile sites of gap or break points.

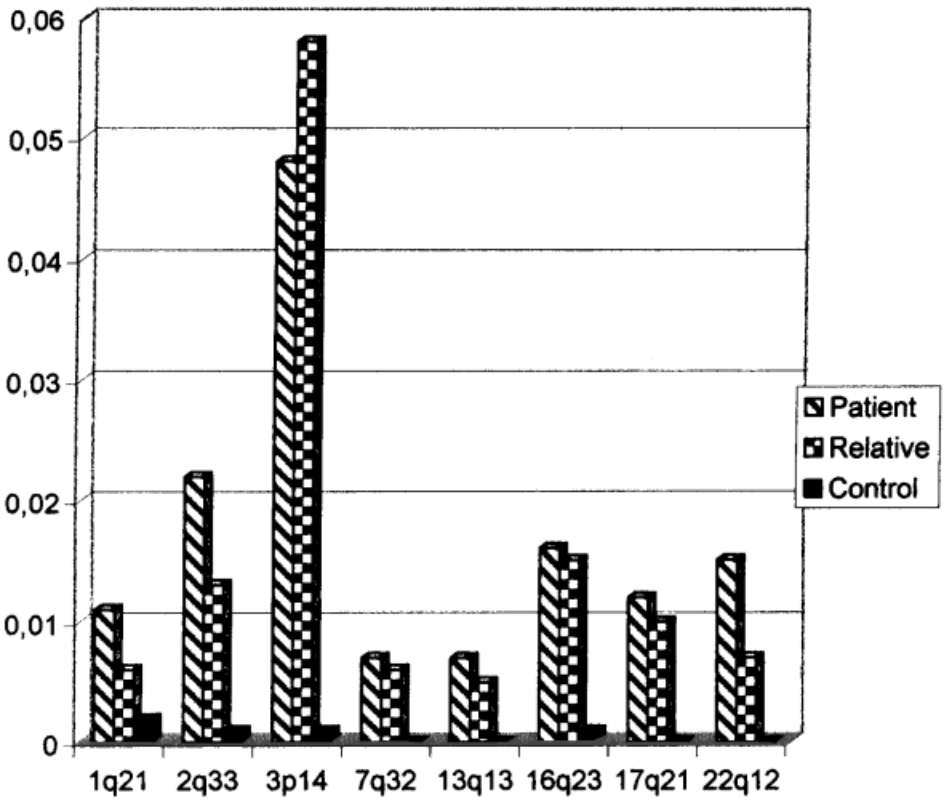

Fig. 3. Frequency of fragile sites in three groups.

As we also had defined in our previous studies, it has been well established that smoking played a major role in the development of lung and head and neck cancers [8,12]. Smoking causes chromosomal damage and fragile site expression. In our study 73%%of patients, 45% of relatives, and 45% of controls were smokers (Tables 1–3). When the rates of total fragile sites in smoking patients and their relatives were compared with the smoking control individuals, results were found to be statistically significant (Table 5). However, the findings were insignificant when the patients were compared with their relatives (Table 5). Especially, the expression of 3p14 was observed to be very high in patients and relatives according to the control (Fig. 3). Tobacco smoke contains several carcinogens such as benzo(a)pyrene (BP), benzo(a)pyrene diol epoxide (BPDE, the metabolic product of BP), dimethylsulphate, and diethylnitrousamine. These carcinogens attack especially 3p14 and 3p21 sites [30,31]. In this situation, we have also believed that mutagene-induced chromosome aberrations were not random and might reflect the inherited genetic susceptibility of specific loci to damage by several chemical carcinogens. The short arm of chromosome 3 may be a hot spot for such damage. Therefore, deletion of 3p may be a particularly useful genetic marker for genetic predisposition to lung cancers.

Clearing up the mystery of fragile sites may be helpful in the determination of genetic susceptibility to cancer, in the elucidating of biological mechanism of cancer

formation and in protection from cancer. However, the studies of this type must be supported with molecular genetic studies of genes related to cancer formation such as oncogene, tumor suppressor gene, and mismatch repair genes.

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