[CANCER RESEARCH 60, 1619–1625, March 15, 2000]

Chromosomal Aberrations in Lymphocytes Predict Human Cancer Independently of Exposure to Carcinogens[1]

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ABSTRACT

An increased risk of cancer in healthy individuals with high levels of chromosomal aberrations (CAs) in peripheral blood lymphocytes has been described in recent epidemiological studies. This association did not appear to be modified by sex, age, country, or time since CA test, whereas the role played by exposure to carcinogens is still uncertain because of the requisite information concerning occupation and lifestyle was lacking. We evaluated in the present study whether CAs predicted cancer because they were the result of past exposure to carcinogens or because they were an intermediate end point in the pathway leading to disease. A nested case-control study was performed on 93 incident cancer cases and 62 deceased cancer cases coming from two prospective cohort studies performed in Nordic countries (Denmark, Finland, Norway, and Sweden) and Italy. For each case, four controls matched by country, sex, year of birth, and year of CA test were randomly selected. Occupational exposure and smoking habit were assessed by a collaborative group of occupational hygienists. Logistic regression models indicated a statistically significant increase in risk for subjects with a high level of CAs compared to those with a low level in the Nordic cohort (odds ratio, 2.35; 95% confidence interval, 1.31–4.23) and in the Italian cohort (odds ratio, 2.66; 95% confidence interval, 1.26–5.62). These estimates were not affected by the inclusion of occupational exposure level and smoking habit in the regression model. The risk for high *versus* low levels of CAs was similar in subjects heavily exposed to carcinogens and in those who had never, to their knowledge, been exposed to any major carcinogenic agent during their lifetime, supporting the idea that chromosome damage itself is involved in the pathway to cancer. The results have important ramifications for the understanding of the role played by sporadic chromosome damage for the origin of neoplasia-associated CAs.

INTRODUCTION

The possible use of biomarkers representing intermediate steps in the pathway from exposure to disease to estimate the risk of cancer in human populations has gained increasing attention. The approach offers practical advantages, such as the smaller size and lower costs of studies and the potential for early detection of risk in exposed populations (1, 2). CAs[3] are among the biomarkers most commonly considered for such a purpose (3, 4).

A relationship between chromosomal damage and cancer development has been suggested since the beginning of the 20th century (5), but only since 1960 have extensive data been gathered on the frequency of CAs in PBLs of humans exposed to known or suspected genotoxic carcinogens. The idea of a causal association between CAs and cancer risk is based on the concept that genetic damage in lymphocytes reflects similar damage in cells undergoing carcinogenesis. However, only recently have prospective cohort studies performed in the Nordic countries (Denmark, Finland, Norway, and Sweden) and in Italy shown that CA frequency measured in PBLs of healthy individuals is predictive of cancer risk (6–8). Despite the marked interlaboratory variability and the different outcomes investigated (cancer incidence and cancer mortality), results were consistent. Subjects with the highest level of CAs showed an incidence ratio of 2.08 (95% CI, 1.26–3.40) and a mortality ratio of 2.56 (95% CI, 1.35–4.86) in the Nordic countries and Italy, respectively, compared with subjects with the lowest level. These findings did not appear to be modified by sex, age, country, or time since CA test (6–8). The contribution of exposure to carcinogens was not evaluated in those studies because of the requisite information concerning occupation and lifestyle was lacking. Therefore, it was impossible to ascertain whether CAs predicted cancer because they were the result of past exposure to carcinogens or because they were an intermediate end point in the pathway leading to disease, perhaps reflecting inherent genetic susceptibility. This is a crucial issue; the demonstration of a causal role for chromosomal damage in carcinogenesis has important and obvious implications for the study of mechanisms, as well as for public health purposes. To test whether CAs are associated with cancer risk, we conducted a case-control study nested within the two above-mentioned cohorts. The nested design allowed restricting the assessment of carcinogen exposure to cancer cases identified during the follow-up of the cohorts and, likewise, to a sample of subjects who were free of cancer during the period when the cancer cases occurred.

Exposure to carcinogens could modify the prediction of cancer risk based on CA frequency in different ways. Three models seem most plausible: (*a*) exposures early in life induce persistent chromosome damage or genomic instability, causing an increase in cancer incidence; (*b*) recent exposures induce CAs in the target organs (and in PBLs) and increase the risk of cancer; and (*c*) exposures close to cancer onset may modify the effect of earlier events, such as CAs. The role of exposure was evaluated by assessing exposure to occupational carcinogens and cigarette smoke in each of these three periods, as illustrated in Fig. 1.

Received 8/27/99; accepted 1/19/00.

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[1] This study was supported by the European Union Biomed 2 Program (Contract BMH4-CT96–0874), the Swedish Medical Research Council, the Swedish Cancer Society, the Swedish Council for Work Life Research, the Academy of Finland, the Associazione Italiana per la Ricerca sul Cancro, the Italian Ministry of Health, and the Italian Ministry of the University and of Scientific and Technological Research. Other members of European Study Group on Cytogenetic Biomarkers and Health include Anton Brøgger, Norwegian Radium Hospital, Oslo, Norway; Benkt Högstedt, Department of Occupational Medicine, Central Hospital, Halmstad, Sweden; Christina Reuterwall, National Institute of Work Life, Solna, Sweden; Bo Lambert, Department of Biosciences, CNT/Novum, Karolinska Institute, Stockholm, Sweden; Felix Mitelman, Department of Clinical Genetics, Lund University, Sweden; Ingrid Nordenson, National Institute of Work Life, Umeå, Sweden; Sisko Salomaa, Finnish Centre for Radiation and Nuclear Safety, Helsinki, Finland; and Staffan Skerfving, Department of Occupational and Environmental Medicine, Lund University, Sweden.

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[3] The abbreviations used are: CA, chromosomal aberration; OR, odds ratio; CI, confidence interval; PBL, peripheral blood lymphocyte.

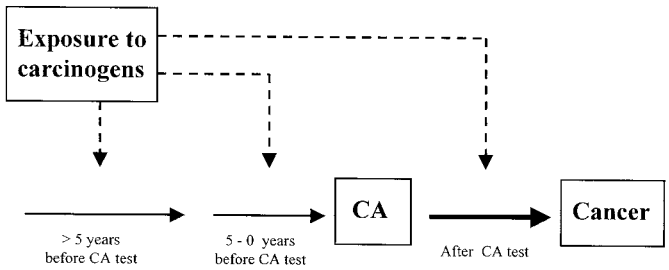

Fig. 1. Occupational exposure to carcinogens and smoking habit were separately assessed for each of the three periods. The aim of this process was to evaluate different hypotheses for interaction among exposure to carcinogens, CA frequency, and cancer risk.

SUBJECTS AND METHODS

Cohorts and Cytogenetic Methods. A database was established of 3541 subjects (1968 from the Nordic countries and 1573 from Italy) examined for CAs in PBLs at adult age in 10 Nordic and 10 Italian laboratories from 1965 to 1988 (9). For each subject, a personal identification code and the year and result of the CA test (total percentage of aberrant cells) were registered. CAs were classified according to the International System for Human Cytogenetic Nomenclature (10), and at least 100 metaphases from each individual were scored. Gaps were not counted. To standardize for interlaboratory variation, the CA scores were trichotomized within each laboratory into three levels, low (1st to 33rd percentiles), medium (34th to 66th percentiles), and high (67th to 100th percentiles). The CA data based on 48- and 72-h culture times were trichotomized separately. More details on the cohort studies and the laboratory protocols can be found in previously published reports (6–9).

Cases and Controls. In the Nordic cohorts, information on malignant tumors diagnosed from the date of CA testing until the end of 1993 (Denmark), 1994 (Sweden and Norway), or 1995 (Finland) was obtained from national cancer registries. Overall, the Nordic cohorts comprised 93 incident cancer cases [2 more Swedish cases were included than in the cohort analysis (8) due to an additional year of follow-up]. In the Italian cohort, the specific causes of death until April 30, 1996, were obtained from the municipality of residence. In total, the Italian cohort comprised 62 deceased cancer cases (two cases included in the cohort analysis were excluded because their tumors had been diagnosed before the date of CA testing). The distribution of subjects in the whole database by major cancer site and by the other variables considered in the analysis is given in Table 1.

The study protocol was approved by ethics committees and legal authorities in all participating countries.

For each case, our aim was to select four controls from the cohort (11). Controls were retrospectively and randomly selected from the subjects in the cohort who were at risk for the outcome event in the calendar year of each case occurrence (12). Controls were matched with their corresponding case by country (only the Nordic data), sex, year of birth, and year of CA test. The maximum differences permitted between the matched subjects' year of birth and year of CA test were ±15 and ±5 years, respectively. Thus, the matched sets generally consisted of one case and four controls, except for eight Nordic and three Italian sets, which had three controls.

Ascertainment of Occupational Exposure and Smoking Habits. Occupational hygienists performed partly structured telephone interviews with cases and controls or, if deceased, with next-of-kin (widows, widowers, children, parents, or siblings). Exposure measurements performed for the original cytogenetic studies or for other purposes were used. Other information sources included contacts with companies at which the subjects had been employed, former co-workers, company records, and medical records. In some instances, when the medical or company records were reliable and covered the whole occupational history, the subjects were not interviewed. Exposure data for 4 cases and 10 controls in the Nordic countries and for 2 cases and 20 controls in Italy were missing or were too poor for exposure classification. Subjects who had quit smoking were classified as nonsmokers when the available data implied that the subject had quit smoking cigarettes more than 5 years before the CA test year. Eight cases and 13 controls in the Nordic countries and 10 cases and 30 controls in Italy lacked smoking data.

Occupational Exposure Matrix. Scientific publications and other information on all original cytogenetic studies from which the cohorts were recruited were scrutinized for occupational exposures of potential interest (6, 7, 13). A matrix comprising 22 categorized exposure indices was constructed (Table 2). Exposure assessment was semiquantitative except for three categories classified as exposed *versus* nonexposed.

The exposures in the matrix were ultimately classified into three groups to optimize statistical analysis (Table 2). Group A: exposures evaluated in the original cytogenetic study to agents classified by the IARC as human carcinogens (class 1); Group B: exposures to other IARC class 1 agents not described in the original papers and revealed by the exposure assessment process, and Group C: all other agents in the matrix.

Occupational exposure was assessed annually starting from the year the subject left school until the end of follow up. Only exposure periods exceeding 1 year were assessed.

Table 1 *Basic description of the case-control data from Nordic countries (cancer incidence data) and Italy (cancer mortality data)*

		Nordic countries	Italy
Variable	Category	No. of cases + controls	No. of cases + controls
Tumor diagnosis/cause of death	Lung/larynx	11	24
	Bladder	5	0
	Lymphohematopoietic	9	5
	Other sites	68	33
CA frequency	Low	24 + 121	15 + 93
	Medium	22 + 135	20 + 81
	High	47 + 107	27 + 71
Country	Denmark	2 + 8	
	Finland	26 + 102	
	Norway	30 + 115	
	Sweden	35 + 138	
Sex	Female	24 + 92	10 + 39
	Male	69 + 271	52 + 206
Year of CA test	1965–1975	11 + 41	27 + 105
	1976–1980	46 + 181	25 + 96
	1981–1985	25 + 112	8 + 37
	1986–1990	11 + 29	2 + 7
Age at CA test (yr)	23–39	17 + 71	4 + 17
	40–49	22 + 87	22 + 103
	50–59	35 + 132	33 + 120
	60–85	19 + 73	3 + 5
Time since CA test (yr)	<1–4	27 + 103	5 + 18
	5–7	19 + 89	11 + 43
	8–10	16 + 62	10 + 47
	11–15	26 + 96	20 + 72
	16–31	5 + 13	16 + 65

Table 2 *Distribution of cases and controls in Nordic countries and Italy with respect to exposure levels for different occupational agents (see "Subjects and Methods" for group definition)*

Occupational agents	Nonexposed[a]		Low exposure		Intermediate exposure		High exposure	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Group A								
Anticancer agents[b]								
Nordic countries	88	347	0	4	2	2	3	10
Italy	62	243	0	0	0	1	0	1
Asbestos[c]								
Nordic countries	80	323	11	33	2	7	0	0
Italy	57	237	3	7	1	0	1	1
Benzene[c]								
Nordic countries	90	345	2	15	1	3	0	0
Italy	60	230	0	3	1	0	1	12
Cadmium[c]								
Nordic countries	92	361	1	2	0	0	0	0
Italy	59	236	1	4	1	2	1	3
Chromium (VI)[c]								
Nordic countries	89	349	1	0	2	7	1	7
Italy	54	232	8	13	0	0	0	0
Ethylene oxide[c]								
Nordic countries	91	252	0	1	1	3	1	7
Italy	62	244	0	0	0	0	0	1
Ionizing radiation[b]								
Nordic countries	88	355	5	7	0	1	0	0
Italy	46	160	10	34	2	16	2	14
Nickel[c]								
Nordic countries	85	338	1	3	3	8	4	14
Italy	62	245	0	0	0	0	0	0
PAH[b]								
Nordic countries	88	331	3	20	2	10	0	0
Italy	59	229	3	10	0	5	0	1
Rubber chemicals[b]								
Nordic countries	86	345	1	5			6	13
Italy	61	244	0	0			1	1
Vinyl chloride[c]								
Nordic countries	87	337	0	2	0	0	6	24
Italy	62	245	0	0	0	0	0	0
Group B								
Other IARCI agents[d]								
Nordic countries	89	356	4	5				
Italy	54	208	7	36				
Group C								
Aromatic hydrocarbons[c,e]								
Nordic countries	76	296	12	43	2	12	3	10
Italy	58	229	1	5	3	11	0	0
Formaldehyde[c]								
Nordic countries	88	345	2	7			3	11
Italy	62	241	0	4			0	0
Lead[f]								
Nordic countries	88	352	2	6	1	1	1	4
Italy	52	192	0	0	1	4	9	49
MMMF[b,g]								
Nordic countries	87	331	6	32				
Italy	62	245	0	0				
Organic solvents[c,h]								
Nordic countries	69	262	19	68	1	10	4	16
Italy	56	208	2	12	4	13	0	12
Pesticides[b,i]								
Nordic countries	93	357	0	1	0	0	0	6
Italy	62	245	0	0	0	0	0	0
Propylene oxide[c]								
Nordic countries	91	359	0	0	2	3	0	1
Italy	62	245	0	0	0	0	0	0
Styrene[c]								
Nordic countries	86	331	3	6	2	19	2	7
Italy	62	245	0	0	0	0	0	0
Tannery[b]								
Nordic countries	93	363	0	0				
Italy	61	244	1	1				
Welding fumes[b]								
Stainless steel								
Nordic countries	89	350	1	0	2	6	1	7
Italy	62	245	0	0	0	0	0	0

[a] The number of subjects classified as nonexposed for all agents during the period was 32 cases and 134 controls for the Nordic countries and 16 cases and 37 controls for Italy. Four cases (3 from Italy) and 29 controls (22 from Italy) who were assigned uncertain exposure are not shown in the table.
[b] The cutoff limits were based on work activities.
[c] The cutoff limits were based on 8-h airborne time-weighted exposure levels.
[d] Classified as exposed *versus* nonexposed.
[e] Aromatic hydrocarbon solvents not including benzene or styrene.
[f] The cutoff limits were based on blood level.
[g] Man-made mineral fibers.
[h] Organic solvents not including aromatic hydrocarbons. The cutoff limits for different solvents were based on the percentage of threshold limit values.
[i] Fungicides, herbicides, or insecticides.

Table 3 *Total cancer predictivity of chromosomal aberrations in case-control data from Nordic countries (cancer incidence cases) and Italy (cancer mortality cases)*

Country	CA frequency[a]	OR_{crude} (95% CI)	$OR_{adjusted}$ (95% CI)[b]
Nordic countries	Low[c]	1.00	1.00
	Medium	0.82 (0.44–1.54)	0.88 (0.46–1.66)
	High	2.22 (1.27–3.86)	2.35 (1.31–4.23)
Italy	Low[c]	1.00	1.00
	Medium	1.53 (0.74–3.18)	1.73 (0.80–3.69)
	High	2.36 (1.17–4.76)	2.66 (1.26–5.62)

[a] The numbers of cases and controls are given in Table 1.
[b] OR adjusted for matching factors.
[c] Reference category.

For subjects classified as nonexposed, an accuracy assessment of their exposure information was performed. Subjects for whom occupational exposure to any agent in the matrix was unlikely, either because of the nature of their reported job or because information available totally excluded any such exposure, were classified as high-accuracy nonexposed. The rest of the subjects, for whom exposure had been possible although not likely, were classified as moderate-accuracy nonexposed.

Quality Control of Occupational Exposure Assessment. After the first national exposure assessments, a description of working histories, including types and levels of exposures, was sent to an independent occupational hygienist. Based on those descriptions, the hygienist selected 27 histories to be used in the harmonization procedure (5% of the whole study group). Discrepancies were discussed at a meeting, and consensus on all assessments was reached, which resulted in a harmonization of the exposure categorization. The final exposure assessments were thereafter performed on a national basis. At the end of the assessment, a quality control round testing interassessor repeatability was carried out. The round included 55 (10%) randomly selected subjects evenly distributed among the countries. For the occupational exposure classification presented in Table 4, three different classifications were possible. All five occupational hygienists were in agreement for 33 subjects (60%), and four of five were in agreement for 13 subjects (23.6%). For the remaining nine subjects (16.4%), a lower degree of agreement was reached, although for eight of nine subjects, the disagreement was restricted to contiguous exposure classifications.

Statistical Methods. Associations between total cancer incidence and mortality, CAs at test, and occupational exposure and smoking habits were modeled by means of conditional logistic regression (14), whereby the matching factors [*i.e.*, country (only the Nordic data), sex, year of birth, and CA test] were controlled in the analyses. The ORs between the medium and low groups and between the high and low CA groups were estimated. This measure can be interpreted as the total cancer incidence or mortality ratio between the compared CA groups (15). The main purpose of the statistical analysis was to examine whether stratification of data by occupational exposure or smoking habit generated different stratum-specific ORs. Homogeneity of stratum-specific ORs was tested by the likelihood ratio test, comparing models without and with the relevant interaction term (14). We also performed restricted analyses based on matched sets in which the case had occurred within a limited time period after the date of CA testing, as well as on matched sets in which the case tumors belonged to a certain category of diagnosis in the three time periods described in Fig. 1. The threshold of 5 years before CA test, which discriminates recent from past exposures, was chosen in consideration of the half-life of PBLs (16). Moreover, subgroup analyses among nonexposed subjects and among nonsmokers were performed by means of unconditional logistic regression.

RESULTS

Cancer Predictivity of CAs. The adjusted ORs for developing cancer for subjects with a high level of CAs indicated a statistically significant increase in risk compared to those with a low level of CAs (Table 3). These estimates did not differ substantially from the crude ORs and were not significantly modified by country (only considered for the Nordic countries), sex, age at test, or time since test (all $P > 0.11$; likelihood ratio test).

Impact of Occupational Exposure on the Cancer Predictivity of CAs. The ORs associated with CA frequency reported in Table 3 did not change noticeably, when occupational exposure level was included in the conditional logistic regression models. Table 4 shows the data for the period of 5 years or less before CA test; results from the other two periods were similar.

No marked confounding by occupational exposure was found when the analysis was restricted to cases (and their controls) occurring within a limited period of time after the CA test (7 and 10 years, median values for Nordic countries and Italy, respectively).

An informative data subset is represented by those subjects classified as nonexposed during the whole assessment period. Crude ORs of 1.22 (0.30–5.22) for the medium *versus* low CA group and 2.86 (0.85–9.66) for the high *versus* low CA group were found for Nordic countries (18 cases and 80 controls). The estimates were not noticeably changed when adjustment was made for the matching factors and the accuracy scoring of the nonexposed subjects. For Italy, the corresponding subgroup analysis could not be performed due to the small number of cases ($n = 4$) and controls ($n = 20$).

Table 5 shows the maximum occupational exposure levels of the cases and controls assessed for different time periods relative to the time of CA testing. For most individuals, the exposure classification remained the same during all three time periods. Regression models considering changes of exposure status between time periods or based on alternative definitions of exposure (duration of occupational exposure and different time periods relative to the CA test) were performed. None of these models revealed any evidence of modification or confounding effect.

Table 4 *Effect of occupational carcinogen exposure on the total cancer predictivity of chromosomal aberrations in case-control data from Nordic countries (cancer incidence data) and Italy (cancer mortality data)*

		Occupational exposure[a]						
		No exposure		Groups A (low exposure), B, and C		Group A (intermediate or high exposure)		Total
Country	CA frequency	$n_{ca} + n_{co}$[b]	OR[c]	$n_{ca} + n_{co}$	OR[c]	$n_{ca} + n_{co}$	OR[c]	$OR_{adjusted}$ (95% CI)[d]
Nordic countries	Low[c]	11 + 56	1.00	7 + 41	1.00	5 + 23	1.00	1.00
	Medium	7 + 38	0.94	5 + 54	0.54	8 + 36	1.02	0.84 (0.44–1.61)
	High	14 + 41	1.74	16 + 35	2.68	16 + 29	2.53	2.29 (1.28–4.11)
Italy	Low[e]	5 + 14	1.00	6 + 46	1.00	2 + 16	1.00	1.00
	Medium	4 + 14	0.80	13 + 46	2.17	3 + 19	1.26	1.66 (0.76–3.61)
	High	7 + 9	2.18	15 + 37	3.11	5 + 25	1.67	2.76 (1.28–5.91)

[a] In the 5 years before CA test. See Table 2 for definition of the occupational exposure levels.
[b] Number of cases + number of controls.
[c] Crude OR.
[d] OR adjusted for matching factors and smoking habit.
[e] Reference category.

Table 5 *Maximum occupational carcinogen exposure levels at different time periods for cases and controls from Nordic countries and Italy*

Time period with respect to CA test	Exposure level[a]	No. of cases + controls ≤5 yr before CA test: No exposure	Groups A (low exposure), B, and C	Group A (intermediate or high exposure)	Missing exposure data
Nordic countries[b]					
>5 yr before	No exposure	18 + 83	2 + 7	2 + 4	0 + 0
	Other exposure	8 + 29	21 + 90	1 + 8	0 + 0
	Medium or high exposure	4 + 13	5 + 32	25 + 76	0 + 0
	Missing exposure data	2 + 10	0 + 1	1 + 0	4 + 10
After	No exposure	32 + 126	4 + 5	3 + 13	1 + 1
	Other exposure	0 + 2	22 + 118	3 + 11	0 + 2
	Medium or high exposure	0 + 2	1 + 6	23 + 61	0 + 0
	Missing exposure data	0 + 5	1 + 1	0 + 3	3 + 7
Italy[c]					
>5 yr before	No exposure	6 + 14	1 + 8	1 + 6	0 + 4
	Other exposure	2 + 5	26 + 87	2 + 4	0 + 0
	Medium or high exposure	7 + 16	7 + 32	7 + 49	0 + 0
	Missing exposure data	1 + 2	0 + 2	0 + 0	2 + 16
After	No exposure	13 + 31	13 + 51	3 + 23	0 + 4
	Other exposure	0 + 0	18 + 65	1 + 6	0 + 0
	Medium or high exposure	0 + 0	0 + 1	2 + 24	0 + 0
	Missing exposure data	3 + 6	3 + 12	4 + 6	2 + 16

[a] See Table 2 for definition of the occupational exposure levels.
[b] Ninety-three incident cancer cases and 363 controls were assessed.
[c] Sixty-two deceased cancer cases and 245 controls were assessed.

Effect of Cigarette Smoking on the Cancer Predictivity of CAs. The association between CA frequency and cancer risk did not change markedly for the Nordic countries or for Italy when cigarette smoking status assessed for the time period ≤5 years before CA testing was included in the conditional logistic regression models (Table 6).

For Italy, the relatively large number of deaths from respiratory tract cancer (codes 161–162 according to 7th International Classification of Diseases), *i.e.*, 5, 7, and 11 in the low, medium, and high CA groups, respectively, allowed restriction of the analysis to this site. When smoking was factored in, the ORs changed from 1.80 (CI, 0.50–6.51) to 1.36 (CI, 0.33–5.70) for medium *versus* low CA group and from 2.75 (CI, 0.87–8.62) to 2.40 (CI, 0.67–8.56) for high *versus* low CA group. In the Nordic countries, there were too few respiratory tract cancer cases (two, one, and eight cases in the low, medium, and high CA groups, respectively) to allow such a subgroup analysis.

The crude ORs within each stratum of the cigarette smoking variable revealed no obvious modification of the total cancer predictivity of the CA biomarker ($P = 0.6$ for the Nordic countries; $P = 1.0$ for Italy; likelihood ratio test; Table 6). The group of Nordic subjects (28 cases and 134 controls) classified as nonsmokers during the whole assessment period (including non-pipe- and non-cigar-smokers) showed crude ORs of 1.28 (CI, 0.47–3.49) for medium *versus* low CA group and 2.52 (CI, 0.92–6.94) for high *versus* low CA group. These estimates were not markedly changed when adjustment was made for the matching factors. For Italy, the corresponding subgroup analysis was not performed because of the small number of cases classified as nonsmokers during the whole assessment period (8 cases and 63 controls).

Finally, ORs adjusted for matching factors and both occupational exposure and smoking habit were 0.86 (CI, 0.45–1.66) for medium *versus* low CA group and 2.25 (CI, 1.24–4.09) for high *versus* low CA group for the Nordic countries and 1.59 (CI, 0.72–3.48) and 2.56 (CI, 1.18–5.56), respectively, for Italy.

DISCUSSION

The results of the case-control study show CAs to be a valid and reliable predictor of cancer occurrence irrespective of extent of occupational exposure to carcinogens and cigarette smoking. These data strengthen and extend the findings of previous cohort analyses (8), supporting the role of CAs as a biomarker of cancer risk and not only of exposure.

The potential effect of occupational exposure on the association between CA frequency and cancer risk was estimated separately for each of the three time periods (Fig. 1). The overlapping results of all regression models made unlikely the hypothesis that the cancer predictivity of CAs would differ with extent or intensity of exposure. This conclusion was upheld by the subgroup analysis of subjects who had never to their knowledge been exposed to any occupational carcinogen during their lifetime. The ORs of cancer associated with

Table 6 *Effect of cigarette smoking on the total cancer predictivity of chromosomal aberrations in case-control data from Nordic countries (cancer incidence data) and Italy (cancer mortality data)*

Country	CA frequency	Cigarette smoking status[a]: Nonsmoker $n_{ca} + n_{co}$[b]	Nonsmoker OR[c]	Smoker $n_{ca} + n_{co}$	Smoker OR[c]	Total $OR_{adjusted}$ (95% CI)[d]
Nordic countries	Low[e]	15 + 85	1.00	8 + 31	1.00	1.00
	Medium	13 + 73	1.01	6 + 59	0.39	0.88 (0.46–1.67)
	High	18 + 48	2.13	25 + 54	1.79	2.33 (1.28–4.24)
Italy	Low[e]	2 + 29	1.00	8 + 46	1.00	1.00
	Medium	3 + 28	1.55	14 + 46	1.75	1.64 (0.76–3.56)
	High	6 + 28	3.11	19 + 38	2.88	2.54 (1.18–5.54)

[a] In the 5 years before CA test.
[b] Number of cases + number of controls.
[c] Crude OR.
[d] OR adjusted for matching factors and occupational exposure.
[e] Reference category.

high CA frequencies in this subgroup were close to those observed among the carcinogen-exposed subjects.

Like occupational exposure, smoking habit showed no evidence of being a confounder or a modifier of the association between CAs and cancer risk. This finding, too, was upheld by the restricted analyses performed in the subgroup of those who had never smoked (possible only in the Nordic cohort).

Due to limited numbers, diagnosis-specific analyses could only be performed for respiratory tract cancer within the Italian cohort (23 cases). Even within this group of smoking-associated cancer cases, smoking habits did not appear to modify CA predictivity.

It should be noted that in the present study, we evaluated the effect of carcinogen exposure on the association between cancer risk and level of CAs. The ORs shown in the tables estimate relative risks between groups, and ORs between different groups cannot be directly compared. No inference is made about the absolute risk of cancer, which might be higher in smokers as well as in subjects occupationally exposed to carcinogens.

The present results, as well as those of the cohort analysis (8), gave no evidence that the prediction of cancer risk by CA frequency is dependent on the time interval after CA testing. Different hypotheses have been suggested to interpret this finding. Exposure may have continued after the CA test for a large portion of the exposed subjects, or different induction-latency periods for different cancers and exposures may account for the lack of modifying effect of time since test. Both of these hypotheses are based on the role of carcinogen exposure, which was shown not to affect the CA-cancer association. The results may be interpreted instead as evidence of proneness to develop cancer due to individual susceptibility factors, such as genetic instability or DNA repair deficiencies.

Our finding that the association between CAs and cancer was not attributable to exposure to carcinogens is consistent with the idea of increased CA frequency as a cancer-prone state. The results are provocative and have obvious ramifications for the understanding of the role played by high levels of chromosome damage in the origin of neoplasia-associated chromosome aberrations. The presence of recurrent and specific chromosome abnormalities, which characterize all neoplastic disorders that have been studied sufficiently to permit conclusions (17), provides strong evidence that they have a causal role in carcinogenesis. The mechanisms involved are unknown, but at least one breakage event is a prerequisite.

The present study no doubt underestimates the true frequency of chromosome breakage because the results are based on conventional cytogenetic analyses of unbanded metaphase preparations of PBLs. Future studies of interphase cells by fluorescent *in situ* hybridization and spectral karyotyping (18) should clarify the association between the frequency and type of chromosome damage in PBLs and other, perhaps more relevant, cell types and tumor-associated chromosome aberrations.

An increasing amount of data has shown that some individual characteristics associated with cancer risk, such as differences in metabolizing enzymes or DNA repair capacity, also have an effect on CA occurrence (16, 19–25). These findings raise the obvious question of whether the association between CAs and cancer risk depends on individual metabolism and DNA repair capability, so that CAs would better predict cancer risk in people with as yet unknown predisposing genes. Some polymorphisms of carcinogen-metabolizing enzymes, such as *N*-acetyltransferase 2, also appear to affect baseline CAs, which may reflect interaction with genotoxic exposures common to most people or involvement of such enzymes in metabolism affecting CAs (23). CA levels may also be affected by nutritional conditions such as folate deficiency (26). The role of genetic and other individual factors remains open, even if the framework of this study could be used to test the hypothesis.

The generalization of our results to all carcinogen exposures depends on how complete our exposure survey was. Because tobacco smoke is one of the most important carcinogens and occupational carcinogen exposures are usually higher than nonoccupational, we believe that substantial exposure was taken into account. Probably the most important exposures that we could not evaluate were those from food and beverages, a limitation that should be kept in mind.

Some factors limiting the use of CAs as a marker of cancer risk should be considered, such as the relatively low increase in risk or the impossibility of controlling subsequent steps of carcinogenesis that might modify the validity of the prediction. The evidence of a causal association between chromosomal damage and cancer occurrence, however, is well substantiated by theoretical and epidemiological data, and preventive policies and measures are always recommended when an increase of CAs is found in a group of exposed subjects.

In conclusion, the results of this study contribute to the validation of CAs as an intermediate end point in carcinogenesis. Occupational exposures and smoking did not modify the association between CA level and cancer. Although genotoxic carcinogens induce CAs and cancer, the cancer predictivity of high CA rate does not require such exposure. Individual characteristics not identified in the present study are probably behind the CA-cancer risk association, and may include polymorphisms of genes involved in carcinogen metabolism and DNA repair, genetic instability, and nutritional status.

ACKNOWLEDGMENTS

We are indebted to Aage Andersen, Cecilia Lando, Zoli Mikoczy, Anneli Ojajärvi, Harri Paakulainen, Eero Pukkala, and Hans Storm for epidemiological assistance and to Marina Buratti, Per Sundell, Jan Rosemberg, Carita Lindholm, and Felix Bernal for valuable help in the exposure assessment. Paola Bigatti, Laura Lamberti, Lucia Migliore, Isabella Sbrana, Riccardo Crebelli, Francesca Degrassi, Rosario Casalone, Rossana Pasquini, Angelo Abbondandolo, Marcella De Ferrari, and Claudia Bolognesi contributed data to the Italian cohort.

REFERENCES

1. Schatzkin, A., Freedman, L. S., Schiffman, M. H., and Dawsey, S. M. Validation of intermediate end points in cancer research. J. Natl. Cancer Inst., *82:* 1746–1752, 1990.
2. McMichael, A., and Hall, J. The use of biological markers as predictive early-outcome measures in epidemiological research. *In:* P. Toniolo, P. Boffetta, D. E. G. Shuker, N. Rothman, B. Hulka, and N. Pearce (eds.), Application of Biomarkers in Cancer Epidemiology, pp. 281–289. IARC Scientific Publ. No. 142. Lyon, France: IARC, 1997.
3. Tucker, J. D., Eastmond, D. A., and Littlefield, L. G. Cytogenetic end-points as biological dosimeters and predictors of risk in epidemiological studies. *In:* P. Toniolo, P. Boffetta, D. E. G. Shuker, N. Rothman, B. Hulka, and N. Pearce (eds.), Application of Biomarkers in Cancer Epidemiology, pp. 185–200. IARC Scientific Publ. No. 142. Lyon, France: IARC, 1997.
4. Liou, S-H., Lung, J-C., Chen, Y-H., Yang, T., Hsieh, L-L., Chen, C-J., and Wu, T-N. Increased chromosome-type aberration frequencies as biomarkers of cancer risk in a Blackfoot endemic area. Cancer Res., *59:* 1481–1484, 1999.
5. Boveri, T. Zur Frage der Entstehung Maligner Tumoren, pp. 1–64. Jena, Germany: Gustav Fisher, 1914.
6. Hagmar, L., Brøgger, A., Hansteen, I-L., Heim, S., Högstedt, B., Knudsen, L., Lambert, B., Linnainmaa, K., Mitelman, F., Nordenson, I., Reuterwall, C., Salomaa, S., Skerfving, S., and Sorsa, M. Cancer risk in humans predicted by increased levels of chromosome aberrations in lymphocytes: Nordic Study Group on the Health Risk of Chromosome Damage. Cancer Res., *54:* 2919–2922, 1994.
7. Bonassi, S., Abbondandolo, A., Camurri, L., Dal Pra', L., De Ferrari, M., Degrassi, F., Forni, A., Lamberti, L., Lando, C., Padovani, P., Sbrana, I., Vecchio, D., and Puntoni, R. Are chromosome aberrations in circulating lymphocytes predictive of a future cancer onset in humans? Preliminary results of an Italian cohort study. Cancer Genet. Cytogenet., *79:* 133–135, 1995.
8. Hagmar, L., Bonassi, S., Strömberg, U., Brøgger, A., Knudsen, L., Norppa, H., and Reuterwall, C. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). Cancer Res., *58:* 4117–4121, 1998.

9. Hagmar, L., Bonassi, S., Strömberg, U., Micoczy, Z., Lando, C., Hansteen, I-L., Huici Montagud, A., Knudsen, L., Norppa, H., Reuterwall, C., Tinnemberg, H., Brøgger, A., Forni, A., Högsted, B., Lambert, B., Mitelman, F., Nordenson, I., Salomaa, S., and Skerfving, S. Cancer predictive value of cytogenetic markers used in occupational health surveillance programs: a report from an ongoing study by the European Study Group on Cytogenetic Biomarkers and Health. Mutat. Res., *405:* 171–178, 1998.
10. International System for Human Cytogenetic Nomenclature. An International System for Human Cytogenetic Nomenclature. Birth Defects, *21:* 1–118, 1985.
11. Wacholder, S., Silverman, D. T., McLaughlin, J. K., and Mandel, J. S. Selection of controls in case-control studies. III. Design options. Am. J. Epidemiol., *135:* 1042–1050, 1992.
12. Wacholder, S., McLaughlin, J. K., Silverman, D. T., and Mandel, J. S. Selection of controls in case-control studies. I. Principles. Am. J. Epidemiol., *135:* 1019–1028, 1992.
13. Nordic Study Group on the Health Risk of Chromosome Damage. A Nordic data base on somatic chromosome damage in humans. Mutat. Res., *241:* 325–337, 1991.
14. Hosmer, D. W., and Lemeshow, S. Applied Logistic Regression. New York: John Wiley & Sons, 1989.
15. Rothman, K. J. Modern Epidemiology, Boston. Little & Brown, 1986.
16. Bogen, K. T. Reassessment of human peripheral T-lymphocyte lifespan deduced from cytogenetic and cytotoxic effects of radiation. Int. J. Radiat. Biol., *64:* 195–204, 1993.
17. Mitelman, F., Mertens, F., and Johansson, B. A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. Nat. Genet., *15:* 417–474, 1997.
18. Ried, T., Liyanage, M., du Manoir, S., Heselmeyer, K., Auer, G., Macville, M., and Schrök, E. Tumor cytogenetics revisited: comparative genomic hybridization and spectral karyotyping. J. Mol. Med., *75:* 801–814, 1997.
19. Sorsa, M., Osterman-Golkar, S., Peltonen, K., Saarikoski, S. T., and Šrám, R. Assessment of exposure to butadiene in the process industry. Toxicology, *113:* 77–83, 1996.
20. el-Zein, R., Conforti-Froes, N., and Au, W. W. Interactions between genetic predisposition and environmental toxicants for development of lung cancer. Environ. Mol. Mutagen., *30:* 196–204, 1997.
21. Norppa, H. Cytogenetic markers of susceptibility: influence of polymorphic carcinogen-metabolizing enzymes. Environ. Health Perspect., *105* (Suppl. 4)*:* 829–835, 1997.
22. Šrám, R. J. Effect of Glutathione S-transferase M1 polymorphisms on biomarkers of exposure and effects. Environ. Health Perspect., *106* (Suppl. 1)*:* 231–239, 1998.
23. Knudsen, L. E., Norppa, H., Gamborg, M. O., Nielsen, P. S., Okkels, H., Soll-Johanning, H., Raffn, E., Järventaus, H., and Autrup, H. Chromosomal aberrations induced by urban air pollution in humans: influence of DNA repair and polymorphisms of glutathione S-transferase M1 and N-acetyltransferase 2. Cancer Epidemiol. Biomarkers Prev., *8:* 303–310, 1999.
24. Tlsty, T. D., Briot, A., Gualberto, A., Hall., I., Hess., S., Hixon, M., Kuppuswamy, D., Romanov, S., Sage. M., and White, A. Genomic instability and cancer. Mutat. Res., *337:* 1–7, 1995.
25. Lengauer, C., Kinzler, K. W., and Vogelstein, B. Genetic instabilities in human cancers. Nature (Lond.), *396:* 643–649, 1998.
26. Blount, B. C., Mack, M. M., Wehr, C. M., MacGregor, J. T., Hiatt, R. A., Wang, G., Wickramasinghe, S. N., Everson, R. B., and Ames, B. N. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implication for cancer and neuronal damage. Proc. Natl. Acad. Sci. USA, *94:* 3290–3295, 1997.

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Cancer Res 2000;60:1619-1625.