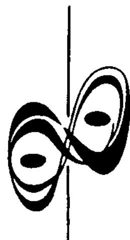
Acta Genet Med Gemellol 34:153-165 (1985)
© 1985 by The Mendel Institute, Rome



Received 25 February 1985
Final 26 July 1985

A Study of Multiple Biological Markers in Twins

H. Anton-Guirgis[1], B.D. Culver[1], T. Kurosaki[1], R. Elston[2]

[1] *Department of Community & Environmental Medicine, University of California, Irvine;*
[2] *Department of Biometry and Genetics, Louisiana State University Medical Center, New Orleans*

Abstract. Genetic and environmental influences on the phenotypic expressions of several biological markers were studied in 18 monozygous (MZ) and 8 dizygous (DZ) twin pairs. Zygosity was determined using ABO, Rh, and HLA. The biomarkers studied included: T & B lymphocytes, suppressor and helper T lymphocytes (T_γ, T_μ), T cell (PHA) mitogen activation (MA), serum immunoglobulins (IgA, IgM, and IgG), plasma carcinoembryonic antigen (CEA), aryl-hydrocarbon hydroxylase (AHH) and sister chromatid exchange (SCE) in lymphocytes. Temporal variation of markers over a 6-month period was not significant. The mean absolute differences between levels from first and second blood draws were less than one standard deviation. Variability associated with age was not significant. Females had higher levels of T lymphocytes than males. A gender related association was observed for the IgM immunoglobulin test: females had a higher mean level of IgM. Smoking was found to influence the levels of SCE, T helper lymphocytes and mitogen activation. The variability of these biomarkers within and between twin pairs was quantified. Immunoglobulin levels, particularly that of IgM, showed statistically greater similarity within MZ twins than within DZ twins. Several other markers suggested heritability.

Key words: Cancer; T & B lymphocytes; Mitogen activation; Serum immunoglobulins; Plasma carcinoembryonic antigen; Aryl-hydrocarbon hydroxylase; Sister Chromatid Exchange

1. INTRODUCTION

Immunologic, cytogenetic and biochemical markers are being increasingly studied to determine their value in the early detection of malignant disease [10,17,21,27,29,30].

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

Markers have been extensively studied in experimental animals [9,11,23,28] to predict cancer susceptibility.

The clinical use of biomarkers in cancer has, for the most part, been limited to the follow-up of cancer patients [19,22]. Knowledge about the behavior of biomarkers is not adequate for the identification of cancer susceptible individuals, nor for the early detection of this disease. Prior to such use of biomarkers, there must be an understanding of their behavior in the normal population. We have studied several biomarkers, previously found to be associated with cancer [5-7,16,18,25,32], in healthy identical and non-identical twins, in an attempt to quantitate genetic versus environmental influences on their phenotypic expression.

The biomarkers studied include the following: T and B lymphocyte subpopulations, lymphocyte-mitogen activation (MA), serum immunoglobulins (IgG, IgM and IgA), plasma carcinoembryonic antigen (CEA), aryl-hydrocarbon hydroxylase inducibility in lymphocytes (AHH), and sister chromatid exchange (SCE).

By estimating the relative importance of genetic and environmental influences, the results of the present study will strengthen the foundation upon which future studies of the applicability of these biomarkers can be based.

2. MATERIALS AND METHODS

2.1 Subjects Studied

A sample of twins was recruited from Southern California Mothers of Twins Clubs. Interested persons completed a questionnaire which included basic demographic data, smoking and drinking history, environmental exposure assessment, personal medical history, and medical history of first- and second-degree relatives. An initial blood sample was taken, and approximately six months later a second was drawn. Zygosity was determined by testing the first blood samples for ABO blood groups, Rh system and histocompatibility antigens (HLA). The HLA antigens (92 possible antigens) were determined serologically using specific cytotoxic antibodies and MLC reaction for detection of loci A, B, C, D and DR.

2.1.1 Questionnaire data

The sample consisted of 26 pairs of twins (18 MZ and 8 DZ). There were 17 males and 35 females. The mean age was 28.4 yr, the median age 22.5 yr, and the standard deviation was 6.12 yr. In Table 1, the age distribution is shown by zygosity and gender. All subjects have completed high school and about half of the subjects also have some college education. Seventeen are presently students, 12 are employed in the service occupations, 7 are professionals, 5 work in the major industries, 4 work in clerical sales, 4 are employed in other occupational categories, 1 is unemployed, and information was not obtained on 2 persons.

Table 2 summarizes the subjects' exposure to various environmental agents and their health status. Over two-thirds of the subjects have reported no history of any serious medical condition which required hospitalization or continued medical attention, such as heart disease, diseases of the circulatory system, stroke, hypertension, diabetes, liver disease, cancer, chronic respiratory disease, ulcers, rheumatic fever or arthritis. Not all study participants filled out the entire questionnaire, thus there are missing data in many of the distributions.

2.1.2 Family History of Cancer

The frequency of cancer in 25 of the 26 families of these twins is shown in Table 3 (first- and second-degree relatives are included). We can divide the number of occurrences of cancer by the total number of individuals in a family (sum of the numbers of brothers, sisters, maternal aunts and uncles, paternal aunts and uncles, two parents, two maternal grandparents, and two paternal grandparents) to obtain a relative frequency of cancer cases. The frequency of cancer in the twin families ranged from 0 to 34%; with 32% having more than 15% of their family members with history of cancer.

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

Table 1. Age Distribution by Zygosity and Gender

Age	MZ		DZ		
	MM	FF	MM	FF	MF
<20	3	4	1	0	1
20 - 29	2	2	1	2.	2
30 - 39	0	4	0	1	0
⩾40	0	3	0	0	0
TOTAL	5	13	2	3	3

Table 2. Distribution of Host Factors in Study Subjects

	Yes	No
Ever smoked cigarettes	16	36
Current cigarette smokers	6	37*
Industrial chemical exposure	7	43
Garden, chemical and fertilizer exposure	5	45
Personal history of cancer	1	49
Chronic chest condition	11	39
Seasonal allergy	5	45

* In addition there were nine former smokers

Table 3. Frequency of Cancer in First and Second Degree Family Members of the Twins Studied

Number of cases of cancer per family	No. of families	Frequency (%)
0	5	20
1	9	36
2	4	16
3	4	16
4	3	12
Relative frequency of cancer	No. of families	Frequency (%)
0.00 - 0.04	6	24
0.05 - 0.14	11	44
0.15 - 0.24	4	16
0.25 - 0.34	4	16

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

2.2 Laboratory Methods

2.2.1 Separation of Lymphocytes

Whole blood diluted with RPMI 1640 culture medium was layered on a ficoll-hypaque gradient in glass test tubes. The test tubes were centrifuged; the lymphocyte layer was collected and washed in Hank's balanced salt solution before total lymphocyte count was taken.

2.2.2 Enumeration of T and B Lymphocytes

T cells were enumerated by the E Rosette method. Lymphocytes were incubated overnight at 4°C with washed sheep red blood cells. Sixteen hours later the cell pellet was gently resuspended, a drop of toluidine blue stain was added, and two wet mounted slides were prepared per sample. A total of 100 lymphocytes were counted from each slide and the number of rosettes (a lymphocyte with 3 or more sheep red blood cells adhered) was recorded as a percentage of total lymphocyte count.

B cells were quantitated by the EAC rosette method. In this method lymphocytes were mixed with sheep red blood cells which had been sensitized with hemolysin and mouse serum. This mixture was incubated in a 37°C water bath for 30 minutes. Slides were prepared and rosettes were counted in the same manner as the E rosettes.

2.2.3 Methods for Supressor T Cells (T_γ) and Helper T Cells (T_μ)

The first step was to isolate the T lymphocytes by an E Rosette method (described above). The resuspended lymphocyte and sheep red blood cell mixture was layered on a ficoll-hypaque gradient and centrifuged. Rosetted T cells formed a pellet and the remaining supernatant and lymphocytes were aspirated. The red cells were lysed and the remaining T lymphocytes were divided for the T_γ and T_μ tests. An aliquot of washed ox red blood cells was pretreated by incubation with rabbit anti-ox IgG and another incubated with rabbit anti-ox IgM. An aliquot of IgG-ox red blood cells was added to one half of the T lymphocyte sample and IgM-ox red blood cells were added to the other half. Cell suspensions were mixed, centrifuged and incubated at 4°C for two hours. Cell pellets were gently resuspended, a drop of toluidine blue stain was added and both chambers of a hemocytometer slide were filled with cell suspension. A total of 100 T lymphocytes was counted in each chamber and rosettes were recorded as a percentage of the total T lymphocyte count.

2.2.4 Sister Chromatid Exchange

Lymphocytes were cultured in medium containing 20% fetal calf serum, 2% phytohemagglutinin and bromodeoxyuridine at a final concentration of 5 μg/ml. Cells were incubated in test tubes at a concentration of 0.5 x 10^6 cells /ml in a humid 37°C, 5% CO_2 incubator for 72 hours. Cultures were done in duplicate. Two hours prior to cell harvest, colcemid was added to cultures at a final concentration of 5μg/ml. At the end of 72 hours, cultures were centrifuged and KCl solution (0.07 M) was added for a period of 7 minutes after which the cells were fixed by adding a fixative (3 parts methanol: 1 part glacial acetic acid). The fixing procedure was repeated two more times and then slides were prepared by dropping a drop of cells in fix solution on a clean microscope slide. Slides were allowed to air dry for at least 24 hours before staining. They were stained in Hoechst stain for 10 minutes, covered with Sorensen's buffer and exposed to fluorescent light for three hours, incubated at 60°C in standard sodium citrate for 30 minutes and finally stained in 3% Giemsa solution.

2.2.5 Immunoglobulins

The immunoglobulins (IgG, IgA, and IgM) were quantitated by radial immunodiffusion using plates manufactured by Behring Diagnostics, Dept. of Hoechst Pharmaceuticals, Inc., Somerville, New Jersey. Serum samples were delivered into 5 1 wells in agar plates containing goat and anti-human serum specific for IgG, IgA or IgM. Precipitin rings were measured after a period allowing for maximum diffusion (24-28 hr). A standard curve was constructed by plotting squared diameters of precipitin rings of the standard serum dilutions. The concentrations of the sample immunoglobulins were read from the standard curve.

2.2.6 Mitogen Activation

Lymphocytes from peripheral blood were cultured in 20% fetal bovine serum and 80% RPMI using phytohemagglutinin (0.5%, 1% or 2%). Cells were incubated in microtiter plates in quadruplicates at a concentration of 1 x 10^5 cells/well in a humid, 37°C, 5% CO_2 incubator for 72 hours with a 6-hour pulse with ^{3}H-thymidine at 66 hours. Cells were harvested on a MASH II unit (Multiple Automated Sample Harvestor) using glass fiber filters. Filters were allowed to dry overnight; individual

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

discs were placed in scintillation vials with 3 ml of ACS (Aqueous Counting Scintillant) and counted for 2 minutes each in a Tracor scintillation counter. Quadruplicate cpm's were averaged and dpm's were calculated and recorded for all variables.

2.2.7 Aryl-hydrocarbon Hydroxylase (AHH) Activity

A radiometric assay for the quantitation of the rate of metabolism of ^{3}H-benzo(a)pyrene (BP) by mitogen activated and carcinogen-induced human lymphocytes was used [13]. This assay consists of a 72-hour mitogen activation using PHA followed by a 24-hour induction using BP and then pulsing with ^{3}H-BP and measurement of the rate of production of metabolites of ^{3}H-BP. The water soluble metabolites are extracted and quantitated every 2 hours (0, 2, 4, 6, 8 hours after the addition of ^{3}H-BP). AHH activity was calculated using a computer estimation of the cell count at each of the 2-hour intervals at which we quantitate the amount of water soluble metabolites of ^{3}H-BP. AHH activity was expressed as that activity per cell per hour.

2.3 Statistical Methods

Data were computerized and analyzed with standard statistical packages using Sigma 7 and VAX computers. Student t-test and Wilcoxon Rank sum test comparisons of means (medians), were performed after examining the results of equal-variance tests. All quoted significance levels are nominal ones for the particular test performed: no allowance was made for multiple tests. A nominal significance level greater than 0.05 is recorded as non-significant (NS). Assessment of heritability was accomplished by a comparison of DZ and MZ intraclass correlation coefficients. A test based on the Fisher Z-transformation was performed to determine if the intraclass correlation coefficients were different in MZ and DZ twins. Although some investigators assess the influence of heredity by the heritability index which compares the intrapair variances of DZ and MZ twins [24] or by twice the difference between the two intraclass correlation coefficients, these measures are very sensitive to differences in the total variance between DZ and MZ twins [8].

3. RESULTS

Temporal variability of biomarkers was tested using a paired t-test analysis between the results from the first and second blood draws. For biomarkers where temporal variation was *not* statistically significant and results were *not* available from the first blood draw, results from the second blood draw were utilized. For all other cases, tests and estimates were based on data from the first blood draw *only.*

The distributions of the blood markers are described in Table 4. The mean, standard deviation, skewness and kurtosis (coefficient of kurtosis minus 3) are listed for each marker. In Table 4 not all biomarkers are normally distributed. Significant skewness is present at $P < 0.05$ in WBC, % B cells, CEA, IgM, MA and AHH. Kurtosis is significant at $P < 0.05$ in WBC, MA and SCE.

As presented in Table 5, there is no significant difference in WBC, % T lymphocytes, suppressor T cells, AHH, CEA, IgM and IgG between the first and second blood draws. Significant differences are present between first and second blood draws in %B lymphocytes, helper T cells, IgA and mitogen activation.

Possible differences between males and females in the biomarkers studied were tested using t-tests and Wilcoxon rank sum tests; only t-test results are presented in Table 6, since both methods yielded essentially the same results. For markers in which the test for equal variance was significant, a separate variances t-test was performed. Significant increases in % T lymphocytes, and IgM in females are observed.

The effect of age on the biomarkers studied was tested using a correlation coefficient (Table 7). None of the correlation coefficients was significant at the 0.05 level. Significantly higher values were found in T-helper (T_{μ}) and sister chromatid exchange (SCE) and lower values were found for the mitogen activation index (MA) in former and current

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

Table 4. Distribution of Blood Markers

Biomarker	N	Mean	SD	Skewness		Kurtosis	
WBC $10^3/mm^3$	41	7.15	2.8	1.68	$P<0.001$	2.44	$P<0.001$
% T-cells	51	66.07	13.1	−0.18	ns	−0.45	ns
% B-cells	49	22.60	11.2	0.99	$P<0.003$	0.45	ns
T_γ%	47	23.21	10.7	0.02	ns	−1.38	ns
T_μ%	37	43.27	13.1	0.27	ns	−0.43	ns
CEA ng/ml	51	2.65	1.8	0.96	$P<0.005$	0.85	ns
IgA mg/100 ml	47	192.51	55.2	0.30	ns	−0.32	ns
IgM mg/100 ml	51	144.94	62.2	1.05	$P<0.001$	1.08	ns
IgG mg/100 ml	51	1325.29	231.6	−0.60	ns	−0.34	ns
MA index	49	1.73	1.5	2.72	$P<0.001$	7.25	$P<0.001$
SCE (number per metaphase)	27	7.75	1.8	0.92	ns	2.94	$P<0.010$
AHH activity	45	0.94	0.9	1.36	$P<0.001$	1.03	ns

Table 5. Temporal Variability of Biomarkers

Biomarker	N. tested	First draw Mean ± SD	N. tested	Second draw Mean ± SD	Paired t-test
WBC $10^3/mm^3$	35	6.76 ± 2.3	41	7.15 ± 2.8	ns
% T-cells	51	66.07 ± 13.1	43	69.89 ± 7.8	ns
% B-cells	49	22.06 ± 11.2	43	30.92 ± 12.8	$P<0.02$
T_γ%	37	22.11 ± 10.5	41	26.76 ± 10.7	ns
T_μ%	37	43.27 ± 13.1	41	35.49 ± 12.2	$P<0.01$
CEA ng/ml	32	2.14 ± 1.5	40	3.14 ± 2.2	ns
IgA mg/100 ml	47	192.51 ± 55.2	42	177.81 ± 52.5	$P<0.004$
IgM mg/100 ml	47	145.45 ± 60.3	42	140.81 ± 54.3	ns
IgG mg/100 ml	47	1332.34 ± 223.9	42	1301.91 ± 250.7	ns
M.A. index	49	1.73 ± 1.5	43	7.00 ± 9.0	$P<0.0005$
SCE* (number per metaphase)	27	7.75 ± 1.8			
AHH activity	35	0.86 ± 0.7	28	1.74 ± 3.6	ns

* SCE's were determined on a single blood draw only.

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

Table 6. Biomarker Differences Between Males and Females

Biomarker	Male Mean ± SD	(N)	Female Mean ± SD	(N)	Male vs Female
WBC 10/mm	6.77 ± 2.7	(15)	7.05 ± 2.3	(31)	ns
% T-cells	59.63 ± 7.6	(17)	69.29 ± 14.1	(34)	P < 0.003*
% B-cells	24.82 ± 13.1	(17)	21.42 ± 10.0	(32)	ns
T_γ %	25.60 ± 11.1	(15)	22.09 ± 10.5	(32)	ns
T_μ %	39.92 ± 14.1	(13)	45.08 ± 12.4	(24)	ns
CEA ng/ml	3.32 ± 1.9	(17)	2.31 ± 1.7	(34)	ns
IgA mg/100 ml	181.33 ± 51.4	(15)	197.75 ± 56.8	(32)	ns
IgM mg/100 ml	114.82 ± 29.6	(17)	160.00 ± 68.9	(34)	P < 0.002*
IgG mg/100 ml	1383.53 ± 220.8	(17)	1296.18 ± 234.6	(34)	ns
MA index	1.79 ± 2.2	(17)	1.70 ± 1.0	(32)	ns*
SCE (number per metaphase)	7.90 ± 1.2	(17)	7.66 ± 2.2	(10)	ns
AHH activity	0.66 ± 0.5	(14)	1.07 ± 1.1	(31)	ns*

* Separate variance t-test.

Table 7. Effect of Age on Biomarkers

Biomarker	Correlation coefficient*
WBC $10^3/mm^3$	0.14
% T-cells	0.24
% B-cells	–0.15
T_γ %	0.05
T_μ %	–0.19
CEA ng/ml	0.26
IgA mg/100 ml	–0.26
IgM mg/100 ml	0.08
IgG mg/100 ml	0.14
MA index	0.04
SCE (number per metaphase)	0.04
AHH activity	0.08

* None of the correlation coefficients are significant at the 0.05 level.

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

smokers when compared with nonsmokers (Table 8). There were no significant differences between smokers and nonsmokers in the other biomarkers studied. t-tests (including separate variance t-tests) and Wilcoxon rank sum tests were performed. Basically, results of these tests were the same.

The intraclass correlation model was utilized to evaluate heritability of blood markers (Table 9). For markers having statistically significant skewness or kurtosis (distributions of biomarkers are shown in Table 3), data were transformed by taking logarithms. For % B cells, CEA, IgM, and AHH activity skewness and/or kurtosis were reduced on taking logarithms; hence, the intraclass correlation coefficient analyses were performed on the transformed data. The intraclass correlation coefficients are not significantly different between the MZ and DZ twins except for Log(IgM). However, the intraclass correlations are consistently higher in MZ than in DZ twins, except for levels of Log(B lymphocytes), T helper and T suppressor cells and sister chromatid exchange.

4. DISCUSSION

4.1 Humoral Immune Measurements — Immunoglobulins and CEA

The distributions of IgA, IgM, and IgG were tested for skewness and kurtosis without regard to zygosity. IgM was found to have significant skewness but neither IgA nor IgG were significant with regard to these distribution factors. Females were found to have significantly higher levels of IgM than males. This result is similar to that of Mandeville et al [17]. Intraclass correlations for IgA, IgM, and IgG were all higher for MZ twins but only in the case of Log (IgM) was the difference in the correlations statistically significant. These findings support the concept that a genetic component may be responsible for the phenotypic expression of serum immunoglobulins. Our findings are similar to those of other investigators [17]. The distribution of CEA in our sample showed significant skewness but no kurtosis. The intraclass correlation was moderately high ($r = 0.81$) in MZ pairs and low ($r = -0.003$) in DZ pairs. The intrapair variance ratio (22.0) is high and suggests a genetic influence in the distribution of CEA (Table 10).

4.2 Cellular Immune Measurements

Total T-lymphocytes, B-lymphocytes, T_γ and T_μ-lymphocytes were tested for skewness and kurtosis. Only the distribution of B-cells was found to be significantly skewed. There was no kurtosis in the distributions of any of the cell subpopulations tested. Based on the intrapair variance, none of the lymphocyte subpopulations were influenced by a strong genetic component.

The distribution of mitogen activation values was significantly and positively skewed. Although the intraclass correlations for DZ and MZ twins were close and not significantly different, the intrapair variance ratio was very large (75.00) (Table 10).

4.3 Sister Chromatid Exchange

Intraclass correlation of SCE was slightly but not significantly higher in DZ than MZ twins. Other investigators [2,26,31] have also failed to find a genetic influence in the frequency of SCE. The intrapair variance ratio between DZ and MZ pairs was 94.65 (Table 10). These findings do not strongly support the case for genetic influence.

There was no association between the frequency of SCE and age in the present study. Several other studies have also failed to find an association with age [4,12,14]. However, Waksvik and associates [31] in a study of 21 like-sexed twin pairs, 11 MZ and 10 DZ, did

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

Table 8. Effect of Smoking on Blood Markers

Biomarker	Smoking Ever vs Never	Ever smoked Mean ± SD	(N)	Never smoked Mean ± SD	(N)
WBC $10^3/mm^3$	ns	7.67 ± 3.2	(13)	6.68 ± 2.1	(33)
% T-cell	ns	64.38 ± 16.4	(15)	66.77 ± 11.7	(36)
% B-cells	ns	21.60 ± 8.8	(15)	23.04 ± 12.2	(34)
T_γ %	ns	26.13 ± 10.5	(15)	21.84 ± 10.7	(32)
T_μ %	$P < 0.029$	53.83 ± 16.5	(6)	41.23 ± 11.5	(31)
CEA ng/ml	ns	3.26 ± 2.3	(15)	2.39 ± 1.5	(36)
IgA mg/100 ml	ns	173.87 ± 55.9	(15)	201.25 ± 53.4	(32)
IgM mg/100 ml	ns	124.40 ± 52.7	(15)	153.50 ± 64.5	(36)
IgG mg/100 ml	ns	1265.33 ± 242.7	(15)	1350.28 ± 225.6	(36)
MA index	$P < 0.030$*	1.25 ± 0.1	(13)	1.90 ± 1.7	(36)
SCE (number per metaphase)	$P < 0.010$*	9.18 ± 2.2	(8)	7.14 ± 1.3	(19)
AHH Activity	ns	0.85 ± 0.8	(15)	0.99 ± 1.0	(30)

* Separate variances test.

Table 9. The Intraclass Correlation Coefficient Model

Biomarker	Intraclass correlation		Comparison of intraclass correlation coefficients
	MZ twins	DZ twins	
WBC $10^3/mm^3$	0.59	0.54	ns
% T-cells	0.47	0.22	ns
Log (% B-cells)	0.45	0.54	ns
T_γ %	0.72	0.79	ns
T_μ %	0.48	0.56	ns
Log (CEA ng/ml)	0.81	–0.003	ns
IgA mg/100 ml	0.49	0.44	ns
Log (IgM mg/100 ml)	0.95	0.46	$P < 0.0214$
IgG mg/100 ml	0.77	0.75	ns
MA index	0.82	0.71	ns
SCE (number per metaphase)	0.44	0.66	ns
Log (AHH activity)	0.85	0.59	ns.

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

Table 10. Summary of Findings of Biological Markers

Biomarkers	WBC	% T-cells	% B-cells	% $T\gamma$	% $T\mu$	CEA	IgA	IgM	IgG	Mitogen activation	SCE	Induced AHH activity
Skewness	sig	ns	sig	ns	ns	sig	ns	sig	ns	sig	ns	sig
Kurtosis	sig	ns	ns	ns	ns	ns	ns	ns	ns	sig	sig	ns
Temporal variability	ns	ns	sig	ns	sig	ns	sig	ns	ns	sig	–	ns
Sex differences	ns	sig	ns	ns	ns	ns	ns	sig	ns	ns	ns	ns
Age	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Smoking	ns	ns	ns	ns	sig	ns	ns	ns	ns	sig	sig	ns
Intraclass correlation MZ	.59	.47	.45	.72	.48	.81	.49	.95	.77	.82	.44	.85
Intraclass correlation DZ	.54	.22	.54	.79	.56	−0.003	.44	.46	.75	.71	.66	.59
Comparison of intraclass correlations	ns	ns	ns	ns	ns	ns	ns	sig	ns	ns	ns	ns
Intrapair variance ratio	2.12	1.67	0.40	1.59	0.29	22.0	1.73	7.69	3.06	75.00	94.65	0.83

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

Table 11. SCE Exchanges in Chromosome Groups: Comparison of Smokers and Nonsmokers

Chromosome group	Smokers Mean ± SD	Nonsmokers Mean ± SD	t value	t-test significance level
A1	0.94 ± .3	0.60 ± .2	3.40	0.001
A2	0.81 ± .4	0.80 ± .3	0.07	ns
A3	0.73 ± .3	0.51 ± .3	1.83	0.05
A	2.48 ± .8	1.92 ± .5	2.33	0.01
B	1.53 ± .4	0.97 ± .4	3.50	0.001
C	3.61 ± .9	3.18 ± .8	1.19	ns
D	0.88 ± .9	0.65 ± .2	1.00	ns
E	0.45 ± .2	0.33 ± .2	1.50	ns
F	0.11 ± .1	0.05 ± .1	2.00	0.05
G	0.06 ± .1	0.02 ± .0	2.00	0.05
TOTAL	9.18 ± 2.2	7.14 ± 1.3	3.04	0.01

find a significant difference in SCE frequency when their subjects were divided into 9 pairs older than 56 and 12 pairs younger than 40.

Our data show no SCE frequency difference between males and females. Similar results have been found by other investigators [12,14,31]. The frequency of SCE per chromosome was found by us as well as others [14,20] to be proportional to the length of the chromosome.

Smoking is positively associated with frequency of SCE per metaphase in the present study as well as those of others [13-15,31]. A new finding from our study is the specificity of the association of smoking on certain groups of chromosomes. Chromosome group data are summarized in Table 11. In smokers, SCE were significantly elevated in chromosome groups A-1, A-3, A, B, F and G. Other chromosome groups (A-2, C, D, and E) were also elevated in smokers but not to a statistically significant level.

4.4 AHH

The difference in the intralcass correlation of AHH inducibility between MZ and DZ twins was not significant. This does not confirm previous reports [1,3]. Also, it must be noted that the intrapair variance ratio of 0.83 indicates a minimal genetic influence. This discrepancy might be due to the large variability in AHH levels of inducibility.

4.5 Data Set

Because of the limited sample size for this study, results will need to be replicated. Recruiting a large sample of twins and performing the biomarkers carried out in this study are difficult tasks. The data for the 26 twin pairs of this study will be made available to researchers who wish to pool these data with data from similar studies.

5. CONCLUSION

Statistical tests suggest a genetic influence on immunoglobulins and CEA. There was no suggestion of genetic influence of SCE, mitogen activation and lymphocyte subpopulations. A noteworthy finding was the specificity of smoking on particular chromosome groups. These results need to be replicated in view of the limited size of the sample.

Acknowledgements. This research was supported by ONR research contract No. N00014-80-C-0571, and USPHS research grant GM 28356 from the National Institute of General Medical Sciences.

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

REFERENCES

1. Atlas SA, Vesell ES, Nebert DW (1976): Genetic control of interindividual variations in the inducibility of aryl hydrocarbon hydroxylase in cultured human lymphocytes. Cancer Res 36: 4619-4630.
2. Cheng W, Mulvihill JJ, Green MH, Pickle LW, Tsai S, Whang-Peng J (1979): Sister chromatid exchanges and chromosomes in chronic myelogenous leukemia and cancer families. Int J Cancer 23:8-13.
3. Coomes ML, Mason WA, Muijsson IE, Cantrell ET, Anderson DE, Busbee DL (1976): Aryl hydrocarbon hydroxylase and $^{16}\alpha$-hydroxylase in cultured human lymphocyte. Biochem Genet 14:671-685.
4. Crossen PE, Drets ME, Arrighi FE, Johnston DA (1977): Analysis of the frequency and distribution of sister chromatid exchanges in human lymphocytes. Hum Genet 35:345-352.
5. Gross RL, Latty A, Williams EA, Newberne PM (1975): Abnormal spontaneous rosette formation and rosette inhibition in lung carcinoma. N Engl J Med 292:439-443.
6. Guirgis HA, Lynch HT, Mate T, Harris RE, Wells I, Caha L, Anderson J, Maloney K, Rankin L (1976): Aryl-hydrocarbon hydroxylase activity in lymphocytes from lung cancer patients and normal controls. Autorensonderdrucke Oncol 33:105-109.
7. Guirgis HA, Lynch HT, Harris RE, Vandevoorde JP (1978): Genetic and communicable effects on carcinoembryonic antigen expressivity in the cancer family syndrome. Cancer Res 35:2523-2528.
8. Haseman JK, Elston RC (1970): The estimation of genetic variance from twin data. Behav Genet 1:11-19.
9. Hellstrom I, Hellstrom KE, Sjogren HO, Warner GA (1971): Demonstration of cell-mediated immunity to human neoplasms of various histological types. Int J Cancer 7:1-16.
10. Heyderman E, Chapman DV, Richardson TC (1982): Biological markers in lung cancer: an immunocytochemical approach. Cancer Detect Prev 5:427-449.
11. Kouri RE, Billups LH, Rude TH, Whitmire CE, Sass B, Henry CJ (1980): Correlation of inducibility of aryl hydrocarbon hydroxylase with susceptibility to 3-methylcholanthrene-induced lung cancers. Cancer Lett 9:277-284.
12. Lambert B, Ringborg U, Harper E, Lundblad A (1978): Sister chromatid exchanges in lymphocyte cultures of patients receiving chemotherapy for malignant disorders. Cancer Treat Rep 62:1413-1419.
13. Lambert B, Lindblad A, Nordenskjold M, Werelius B (1978): Increased frequency of sister chromatid exchanges in cigarette smokers. Hereditas 88:147-149.
14. Livingston GK, Cannon LA, Bishop DT, Johnson P, Fineman RM (1983): Sister chromatid exchange: variation by age, sex, smoking, and breast cancer status. Cancer Genet Cytogenet 9:289-299.
15. Livingston GK, Fineman RM (1983): Correlation of human lymphocyte SCE frequency with smoking history. Mutat Res 119:59-64.
16. MacSween JM, Eastwood SL (1980): Immunoglobulins associated with human tumors in vivo: IgG concentrations in eluates of colonic carcinomas. Br J Cancer 42:503-509.
17. Mandeville R, Lamoureux G, Lesault-Poisson S, Poisson R (1982): Biological markers and breast cancer, a multiparametric study; depressed immune competence. Cancer 50:1280-1288.
18. McLemore TL, Martin RR, Wray NP, Cantrell ET, Busbee DL (1978): Dissociation between aryl hydrocarbon hydroxylase activity in cultured pulmonary macrophages and blood lymphocytes from lung cancer patients. Cancer Res 38:3805-3811.
19. Minton JP, James KK, Hurtubise PE, Rinker L, Joyce S, Martin Jr EW (1978): The use of serial carcinoembryonic antigen determinations to predict recurrence of carcinoma of the colon and the time for a second-look operation. Surg Gynecol Obstet 147:208-210.
20. Morgan WF, Crossen PE (1977): The frequency and distribution of sister chromatid exchanges in human chromosomes. Hum Genet 38:271-278.
21. Murphy GP (1981): The diagnosis and detection of urosential cancers. Cancer 47:1193-1199.
22. Neri A, Brusiatelli M, Ozser-Topuz U, Astaldi G (1981): Reduced natural killer cell activity in non-Hodgkin's lymphoma. Boll-Ist-Sieroter-Milan 60:394-407.
23. Old LJ, Boyse EA (1966): Specific antigens of tumors and leukemias of experimental animals. Med Clin N Am 50:901-912.

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press

24. Osborne RH, DeGoerge FV (1959): Genetic Basis of Morphological Variation. Cambridge Mass.: Harvard University Press.
25. Otter M, Palmer CG, Baehner RL (1979): Sister chromatid exchange in lymphocytes from patients with acute lymphoblastic leukemia. Hum Genet 52:185-192.
26. Pederson C, Ohah E, Merrild U (1979): Sister chromatid exchanges in cultured peripheral lymphocytes from twins. Hum Genet 52:281-294.
27. Rasmuson T, Bjork GR, Damber L, Holm SE, Jacobsson L, Jeppsson A, Littbrand B, Stigbrand T, Westman G (1983): Evaluation of carcinoembryonic antigen, tissue polypeptide antigen, placental alkaline phosphatase, and modified nucleosides as biological markers in malignant lymphomas. Recent Results. Cancer Res 84:331-343.
28. Scornik JC, Klein PA (1978): Antibody-dependent lysis of tumor cells in vivo. 1. Early lysis of tumor cells. J Natl Cancer Inst 61:1143-1148.
29. Thyss A, Milano G, Caldani C, Lesbats G, Schneider M, Lalanne CM (1982): Polyamines as biological markers in malignant lymphomas. Eur J Cancer Clin Oncol 18:611-616.
30. Waalkes TP, Rosenhein NB, Shaper JH, Ettinser DS, Woo KB, Paone JF, Gehrke CW (1982): A feasibility study in the development of biological markers for ovarian cancer. J Surg Oncol 21:207-214.
31. Waksvik H, Magnus P, Berg K (1981): Effects of age, sex and genes on sister chromatid exchange. Clin Genet 20:449-454.
32. Williams RR, McIntire KR, Waldmann TA, Feinleib M, Go VLW, Kannel WB, Dawber TR, Castelli WP, McNamara PM (1977): Tumor-associated antigen levels (carcinoembryonic antigen, human chorionic gonadotropin, and alphafetroprotein) antedating the diagnosis of cancer in the Framingham study. J Natl Cancer Inst 58:1547-1551.

Correspondence: Dr. Hoda Anton-Guirgis, Department of Community and Environmental Medicine, University of California, Irvine, CA 92717, USA.

https://doi.org/10.1017/S0001566000004670 Published online by Cambridge University Press