Genetic and Communicable Effects on Carcinoembryonic Antigen Expressivity in the Cancer Family Syndrome

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ABSTRACT

Plasma carcinoembryonic antigen (CEA) levels were studied in 272 members of six pedigrees manifesting the cancer family syndrome and in 191 normal controls. The CEA distributions per se were transformed to square root CEA (\sqrt{CEA}) as a correction for skewness and kurtosis. Duration of smoking and age showed a positive correlation with the level of \sqrt{CEA} in both family members and controls. Distributional features of \sqrt{CEA} among members of the cancer family syndrome pedigrees and the independently sampled controls of the same smoking status did not differ significantly. However, when pedigree data were classified by closeness of relationship to cancer patients there was a significant linear increase in mean \sqrt{CEA} with increasing genetic cancer risk. Surprisingly, unrelated spouses had mean levels of \sqrt{CEA} similar to that for the corresponding risk class of their direct-line mates, and the intraclass correlations of \sqrt{CEA} between direct-line relatives and their spouses approached significance when both spouses were concordant for smoking status.

These results suggest the existence of a genetic-connubial effect on plasma CEA levels, presumably due to a common environmental agent acting in concert with the degree of genetic predisposition to cancer. An integrative target cell theory is proposed to explain CEA expressivity in this syndrome. The model involves the interaction of the cancer family syndrome genotype with physical factors (cigarette smoking) and a communicable agent (oncogenic virus).

INTRODUCTION

CEA has been identified as a putative colon cancer marker by Gold et al. (12–14). However, the clinical application of CEA as a cancer marker suffers from its relatively nonspecific association with a variety of inflammatory diseases, malignant neoplasms in addition to colon cancer, and environmental factors including cigarette smoking (8, 21, 25, 29, 30). Elevated CEA values have also been found to cluster in pedigrees prone to retinoblastoma (8), in patients with medullary thyroid carcinoma (18), and in the cancer family syndrome (15, 22).

The purposes of this study were: (a) to test the potential of CEA as a discriminant of genetic cancer risk in pedigrees with the cancer family syndrome; (b) to quantitate the role of age and duration of cigarette smoking in CEA expression; and (c) to test for connubial effects on CEA in this hereditary cancer syndrome.

MATERIALS AND METHODS

Clinical Resource. During a 5-year period (1972 to 1976), plasma samples were collected from 186 relatives and 84 spouses of 6 cancer-prone kindreds manifesting the cancer family syndrome (23). This disorder is characterized by predisposition to adenocarcinomas, particularly of the colon and endometrium; early onset; excess of multiple primary cancer; and an autosomal dominant mode of genetic transmission of cancer. An independent comparison group was comprised of 191 normal individuals sampled during the same time period. Age, sex, smoking status, and duration of smoking (years) were recorded for all individuals at the time of sampling.

Laboratory Determinations. Plasma samples were analyzed for CEA by Hoffmann-LaRoche Laboratories, Nutley, N. J., throughout the study. All CEA assays were performed with the radioimmunoassay methods of Hansen et al. (16). CEA values were expressed as ng CEA per ml plasma.

Statistical Analysis. Distributions of CEA for smokers and nonsmokers were tested for normality by Komolgorov-Smirnov tests and the standard measures of skewness and kurtosis (31, 32, 34). The CEA values per se were distributed in Poisson fashion as indicated by significant skewness and similarity between the sample mean and variance of each group. Consequently, all results were transformed by taking the square root of CEA values to make the mean and variance independent and to stabilize the variance for tests of significance (32, 34).

Mean comparisons were made between males and females of the same smoking status and between smokers and nonsmokers with the use of standard t tests (31). These comparisons revealed no significant sex differences but a significantly higher mean \sqrt{CEA} in smokers. Consequently, classification for smoking status was retained in subsequent analyses, while that for sex was ignored.

Pedigree data were utilized to test the potential of CEA as a discriminant of cancer risk. Direct-line relatives of the 6 cancer family syndrome pedigrees were classified in accordance with the schematic diagram shown in Chart 1. Risk Class 1 consists of cancer survivors, Risk Class 2 consists of unaffected individuals at relatively high cancer risk (1 or more first-degree relatives with a positive cancer history), and Risk Class 3 consists of unaffected individuals at relatively low cancer risk (no first-degree relatives with...
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Chart 1. Pedigree classification according to genetic cancer risk status.

Cancer-prone families and comparison groups are shown in Chart 2. The √CEA distributions were approximately normal, as evidenced by nonsignificant skewness and kurtosis and the empiric agreement with the theoretical normal cumulative distributions. Kolmogorov-Smirnov tests of normality were applied to each of the 4 √CEA sampling distributions; none showed significant lack of fit (lowest value exceeded 0.25).

Overall, smokers had a significantly higher average level of √CEA ($p < 0.01$) than did nonsmokers. However, family members show no difference in mean √CEA when compared to the control groups of the same smoking status.

Pertinent simple correlations involving √CEA, age, and duration of smoking are given in Table 1. The level of √CEA showed highly significant positive correlation with age and duration of smoking among smokers and with age among nonsmoking family members. Among nonsmoking control subjects the correlation between √CEA and age was not significantly different from 0.

Based upon linear regression √CEA increases 0.39 unit for each 10 years of smoking among family members ($b = 0.039; p < 0.01$) and 0.32 unit among smoking controls ($b = 0.032; p < 0.01$). Among nonsmokers the increase in √CEA with age is less than one-third of that of smokers ($b = 0.009; p < 0.01$ for family members. $b = 0.003; p < 0.33$ for controls).

Results of the analyses of covariance with pedigree classification for risk status are shown in Table 2. Separate analyses are given for smokers and nonsmokers. In both analyses there was a linear trend in √CEA with changing risk status after adjustment for duration of smoking and age.

Among smokers the partition of variance attributable to linear regression on duration of smoking and age was highly significant. Separate regressions among cells of the 2-way table were not found to be heterogeneous; and therefore a single regression model was used. Notably, the variation among risk classes after adjustment for the 2 concomitant variables is approaching significance; and, more importantly, there is a significant linear decline in √CEA with decreasing risk status.

After correction for the effects of duration of smoking and age, the average decline in √CEA with each increment decrease in risk is reflected by the regression coefficient estimated from the partial sum of squares due to the linear effect of risk class in the analysis. In √CEA units the estimated regression coefficient is $b = 0.2$ ($S.E. = 0.09; p < 0.03$). None of the other partitions of variance (namely, relatives versus spouses or interaction) approached statistical significance.

Among nonsmokers the partition of variance attributable to linear and quadratic regression of √CEA on age was highly significant. Again, a single regression model was utilized due to negligible heterogeneity of regressions among cells. The only other mean square in the analysis that approaches significance arises from the linear effect of risk status on √CEA ($p < 0.07$). The regression coefficient estimated from the partial sum of squares due to the linear effect of risk classes is $b = 0.12$ ($S.E. = 0.065; p < 0.07$).

Adjusted means of √CEA for relatives and spouses with classification for cancer risk status and smoking are given.
Table 1

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Mean square</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear regression on duration of smoking and age</td>
<td>2</td>
<td>3.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Risk classes</td>
<td>2</td>
<td>0.60</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>Risk classes linear</td>
<td>1</td>
<td>0.98</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Risk classes nonlinear</td>
<td>1</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Relatives versus spouses</td>
<td>1</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>2</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>90</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear and quadratic regression on age</td>
<td>2</td>
<td>2.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Risk classes</td>
<td>2</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Risk classes linear</td>
<td>1</td>
<td>0.72</td>
<td>&lt;0.07</td>
</tr>
<tr>
<td>Risk classes nonlinear</td>
<td>1</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Relatives versus spouses</td>
<td>1</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>2</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>173</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

a Statistical significance of the sample correlation coefficient at the 0.01 level of probability.

Table 2

Results of analyses of covariance in Table 3. Overall means for each risk class are shown. All estimates are corrected for age and duration of smoking; i.e., each was obtained by taking the difference between the unadjusted cell mean and the estimated effects of the concomitant variables (age and duration of smoking) based upon linear regression. The data of Table 3 reflect an increasing trend in the mean $\sqrt{\text{CEA}}$ with increasing cancer risk. A surprising result is that $\sqrt{\text{CEA}}$ levels of spouses show the same trend as their direct-genetic-line mates (spouses were classified according to the genetic risk status of their mates rather than their own genetic lineage). Among both direct-line relatives and their spouses, there is a consistent linear decline in $\sqrt{\text{CEA}}$ with declining risk status. Progressing from high to low risk, the magnitude of the risk class effects in $\sqrt{\text{CEA}}$ units is roughly 0.4 in smokers and 0.2 in nonsmokers.

Table 4 shows the spouse-pair data on CEA with classification for concordance and discordance in smoking status. In 2 classes concordant for smoking (Classes 1 and 2), mean $\sqrt{\text{CEA}}$ levels were similar for direct-line relatives and spouses, and the intraclass correlations approach significance. In Class 3, nonsmoking relatives and their smoking spouses have a similar mean $\sqrt{\text{CEA}}$, and the intraclass correlation is on par with that of the concordant classes ($r_i = 0.36$). These first 3 classes have a pooled intraclass correlation of 0.33, $p < 0.025$. In Class 4, smoking relatives have a significantly higher mean $\sqrt{\text{CEA}}$ than do their non-smoking spouses (1.60 versus 1.13, $p < 0.01$), and the intraclass correlation is negligible.

DISCUSSION

Interest in CEA and other oncodevelopmental proteins...
has been intense during the last decade. This fetal antigen has shown potential as a marker for endodermal cancer, particularly cancer of the colon (13, 14, 17, 20).

The distributions of CEA per se were highly skewed, with the mean similar to the variance. Poisson-like distributional features were apparent not only among family members of the cancer-prone pedigrees but also in the independently sampled control population. These features may thus be typical of the distribution of CEA in the general population. To make the distributions approximately normal, we elected to use a square root transformation.

In previous studies of CEA, data have typically been expressed qualitatively rather than in a quantitative manner (i.e., utilizing 2.5 ng/ml as the point of truncation between normal and elevated levels). An arbitrary truncation of this sort ignores the shape of the distribution; does not allow adjustment for concomitant variables such as age and/or duration of smoking; and, most importantly, leads to a variable false-positive and false-negative error rate. Because of these limitations we have treated CEA as a quantitative variable.

One of the objectives of this study was to compare CEA in family members of pedigrees manifesting the cancer family syndrome with that of an age-matched cohort. The mean √\(\text{CEA}\) did not differ between family members and controls of the same smoking status. Furthermore, the rates of change in √\(\text{CEA}\) with increasing age and duration of smoking were similar in both cohorts. Duration of smoking significantly elevated CEA levels over time. For example, based on linear regression a 20-year-old family member is expected to have a √\(\text{CEA}\) of 1.26 (CEA = 1.6 ng/ml); if that individual then becomes a smoker, his √\(\text{CEA}\) by age 40 would be expected to increase to 2.04 (CEA = 4.2 ng/ml).

There was a significant effect of genotype on CEA expression in the cancer family syndrome pedigrees studied. This is evidenced by the linear increase in mean √\(\text{CEA}\) with increasing genetic cancer risk (Table 3). Interestingly, the adjusted mean difference in √\(\text{CEA}\) between the high- and low-genetic-risk classes is about the same as the increase due to 10 years of smoking exposure. Although these results reflect statistically significant effects of smoking and genetic-risk classes on CEA expressivity, these changes are small, and single observations may have limited diagnostic or prognostic implication for the individual patient.

Results in high-risk relatives and their spouses concordant for smoking status support the existence of a connubial effect in the families studied (Tables 3 and 4). However, the issue is exceedingly complex. For example, the data on discordant spouse-pairs suggest that CEA levels in direct-line relatives are influenced by the smoking status of the spouse, but the converse association does not hold. Apparently, CEA expressivity is less likely to be influenced by exogenous smoking factors in the absence of genetic cancer susceptibility.

There has been an increasing interest in an "infectious" etiology of cancer in specific malignant lesions, i.e., Burkitt's lymphoma (27), nasopharyngeal carcinoma (6), Hodgkin's disease (36), osteogenic sarcoma (26), leukemia (35), and uterine cervical and prostatic carcinoma (19, 30). These communicable implications are supported by geographic, racial, familial, connubial, and time-space clustering data (3, 5, 29, 35, 36).

### Table 3

Adjusted cell means of √\(\text{CEA}\) among relatives and spouses according to risk class

Means are adjusted for linear effects of duration of smoking and age in smokers and linear and quadratic effects of age in nonsmokers.

<table>
<thead>
<tr>
<th>Risk class&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Relatives</th>
<th>Spouses</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>√(\text{CEA})</td>
<td>√(\text{CEA})</td>
<td>√(\text{CEA})</td>
</tr>
<tr>
<td></td>
<td>(\bar{x})</td>
<td>(s^2)</td>
<td>(n)</td>
</tr>
<tr>
<td>Smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 1</td>
<td>1.53</td>
<td>0.10</td>
<td>12</td>
</tr>
<tr>
<td>Class 2</td>
<td>1.52</td>
<td>0.07</td>
<td>35</td>
</tr>
<tr>
<td>Class 3</td>
<td>1.23</td>
<td>0.08</td>
<td>13</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class 1</td>
<td>1.24</td>
<td>0.21</td>
<td>14</td>
</tr>
<tr>
<td>Class 2</td>
<td>1.15</td>
<td>0.07</td>
<td>43</td>
</tr>
<tr>
<td>Class 3</td>
<td>1.04</td>
<td>0.05</td>
<td>71</td>
</tr>
</tbody>
</table>

<sup>a</sup> Class 1 contains cancer patients and their spouses, Class 2 contains first-degree relatives of cancer patients and their spouses, and Class 3 contains more distant relatives of cancer patients and their spouses.

### Table 4

CEA in spouse pairs of cancer family syndrome pedigrees with classification for concordance-discordance for smoking status

<table>
<thead>
<tr>
<th>Class</th>
<th>Smoking classification</th>
<th>No. of spouse pairs</th>
<th>Mean age of class (yr)</th>
<th>Mean √(\text{CEA})</th>
<th>(t) test of difference ((p))</th>
<th>Intraclass correlation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Both nonsmokers</td>
<td>23</td>
<td>46.5</td>
<td>1.10</td>
<td>&lt;0.86</td>
<td>0.29</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>2</td>
<td>Both smokers</td>
<td>11</td>
<td>44.7</td>
<td>1.63</td>
<td>&lt;0.34</td>
<td>0.34</td>
<td>&lt;0.14</td>
</tr>
<tr>
<td>3</td>
<td>Relative nonsmoker</td>
<td>11</td>
<td>46.9</td>
<td>1.44</td>
<td>&lt;0.59</td>
<td>0.36</td>
<td>&lt;0.11</td>
</tr>
<tr>
<td>4</td>
<td>Relative smoker</td>
<td>16</td>
<td>46.7</td>
<td>1.60</td>
<td>&lt;0.01</td>
<td>0.05</td>
<td>&lt;0.50</td>
</tr>
</tbody>
</table>

<sup>a</sup> Pooled correlation, Classes 1, 2, and 3: \(r = 0.33\) (<0.025).

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Recent evidence supporting communicable effects of CEA has been obtained by Gitnick and Molnar (11). These authors observed that, after transfusion of blood units containing <2.5 ng CEA, nearly 50% of the recipients manifested persistent CEA elevation (3.3 to 21.5 ng/ml over a 6- to 10-month period).

Our data reflect an intricate chain of events involving genetics, communicability, and physical interacting phenomena. These targeted factors appear to interact differentially at the molecular-cellular level to cause CEA variation. A schematic diagram of our theoretical explanation for these phenomena is given in Chart 3. In the model the highly specific genotype of the cancer family syndrome, a communicable agent (oncogenic virus?) that may be capable of causing cellular mutagenesis and/or carcinogenesis and that might conceivably be the agent in the transfer of CEA, and a physical factor (cigarette smoking) are crucial for explicable theory.

**Communicable Factors (Oncogenic Virus?).** The proposed model has support from several lines of evidence in biology and clinical genetics. At the human level Almond (1) identified gene specificity for the range of host susceptibility in the influenza virus. Similar phenomena have been prodigiously documented at the infrahuman level (24). With respect to the latter, it has been suggested that specific disease processes including cancer might result from the interaction of endogenous (host genetic) and exogenous (acquired carcinogen-induced) stimulation of oncogenes (24).

Assuming the communicable agent to be ubiquitous and considering the long latent period between infection and cancer occurrence in genetically susceptible subjects, it becomes exceedingly difficult to answer the following questions: (a) is the alleged oncogenic virus an etiological cofactor in the neoplastic process; and/or (b) does it have a secondary role, as an agent for the transfer of CEA to spouses?

**Physical Factors.** A physical factor, namely an inhaled carcinogen (cigarette smoke), also appears to be integral to our model, although several questions arise relevant to its etiological significance in this particular system. We have identified a significant influence of cigarette smoke on CEA expression. However, the answers to the following questions are not clear: (a) does cigarette smoke have a primary role in induction of neoplasia and thereby a secondary role in CEA elevation; (b) does cigarette smoke act as a promoter in neoplasia and/or CEA elevation; or (c) does cigarette smoke in some unknown manner influence other mechanisms, such as immunosuppression, with subsequent CEA elevation?

**Nature-Nurture Issues.** The essence of the proposed model pertains to the nature-nurture issue, a phenomenon clearly evident in favism (33), a disorder in which predisposed individuals harbor a deficiency of glucose-6-phosphate dehydrogenase, which, when coupled with fava bean ingestion, causes a severe form of recurrent hemolytic anemia. In the more recently identified Wernicke-Korsakoff syndrome (4), a specific gene-determined abnormality (transketolase enzyme deficiency) may be triggered by environmental events (chronic alcoholism) leading to an exquisite thiamine deficiency, which is then reflected by the neurological sequelae of this syndrome.

**Gene Derepression.** The components of the proposed model require further explanation at the molecular level. Such a link has been espoused by Anderson (2). This author proposes that certain oncogenic viruses may incorporate embryonic genes, which could then infect cells of other hosts, inducing malignant transformation and possibly synthesis of embryonic fetal antigens. This process could conceivably be influenced by the host’s genetic susceptibility to cancer and/or environmental interacting exposures.

**Teratogenesis, Carcinogenesis, and Tissue Susceptibility.** Evidence from laboratory animals (7) and from man (10) suggests that chemicals known to be carcinogenic affect only a small percentage of fetuses exposed at the critical time of development. These observations suggest that certain prenatally determined diseases result from the influence of multiple environmental agents and their complex interaction, in concert with susceptibility factors in the host. Indeed, familial aggregation of certain malformations in offspring of women receiving teratogens, as seen in the case of genetic susceptibility to the effects of trimethadione (an anticonvulsant), supports the familial susceptibility-ter-
atogenicity hypothesis (9). Again, these lines of evidence are supportive of our integrative target theory.

Our clinical model, namely the cancer family syndrome, is a highly selected one, and in this setting our observations have led us to the construction of the proposed integrative target theory. However, this must be interpreted cautiously since it is based upon results and conclusions from a single hereditary cancer syndrome. Genotype-environmental interaction in our theory has been emphasized unidirectionally, with cancer as an end point. However, our model may also hold for so-called cancer reversibility with genotype (28) and/or immune competence playing an integral role.

It would seem plausible that in other cancer genetic models certain of these studies may or may not be supportive of our theory, since particular vital components, including the genotype, will vary. Nevertheless, search for corroboration or refutation of our theory must be made in the cancer family syndrome, as well as in other familial-hereditary precancerous disorders, so that its potential for elucidation of etiology and carcinogenesis might be maximally exploited.

ACKNOWLEDGMENTS

We wish to acknowledge with gratitude the dedicated assistance of Jane F. Lynch, R.N., Kathy Maloney, R.N., and Laurie Carmody, R.N., for their help in the collection of samples; Mary Bourque, secretary, for her assistance in the preparation of this manuscript; and Betty Walker, M.S., and Kathleen Carney for their assistance in the data processing.

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