ras Oncogene Activation and Occupational Exposures in Acute Myeloid Leukemia

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Background: Epidemiologic studies of acute myeloid leukemias **(AMLs)** show small increases in risk of disease associated with certain occupations and chemical exposures. *Purpose*: This study was designed to determine whether the presence of mutationally activated ras oncogenes in **AML** are associated with occupational and chemical exposures. *Methods*: We interviewed 62 patients with newly diagnosed **AML** (or their next-of-kin), all of whom were enrolled in a national multicenter clinical trial, and 630 healthy control subjects. DNA extracted from patients' pretreatment bone marrow samples was amplified by using the polymerase chain reaction and probed with allele-specific oligonucleotides for activating point mutations at the 12th, 13th, and 61st codons of three proto-oncogenes: H-ras (also known as HRAS), K-ras (also known as KRAS2), and N-ras (also known as NRAS). Re***sults:*** Patients with ras mutation-positive **AML** had a higher frequency (six of **10** patients) of working 5 or more years in an a priori high-risk occupation than did patients with ras mutation-negative **AML** (eight of ***52;*** odds ratio **[OR]**= 6.8; 95% confidence interval [CI] = 1.3-36). Patients with ras mutation-positive **AML** were more likely than patients with ras mutation-negative **AML** to have breathed chemical vapor on the job (OR = ***9.1; 95%*** CI = 1.3-64) or to have had skin contact with chemicals (OR = 6.9; 95% CI = 1.3-37). When ras-positive patients were compared with healthy control subjects, the ORs for occupation and occupational exposures remained elevated, while patients with ras mutation-negative **AML** showed no

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increased risk when compared with control subjects. *Conclusion*: Activation of ras prolo-oncogenes may identify an etiologic subgroup of AML caused by occupation and chemical exposure. *Implication*: Disease etiology may be better understood if epidemiologic measures of exposure ure integrated with molecular assays of the genetic defects responsible for cancer initiation and promotion. [J Natl Cancer Inst 84:1626–1632, 19921

Prior case-control studies of leukemia have demonstrated only a slight increase in risk of disease for persons with occupational or chemical exposures. Except for special groups exposed to high levels of benzene or radiation, the reported risks associated with occupation and chemicals have generally been less than twofold (*1*), making these exposures of questionable etiologic significance. Since acute myeloid leukemia (AML) appears to be a heterogeneous disease at the molecular and cytogenetic level, it is possible that certain environmental agents might be linked to molecular subtypes. If so, the association between an exposure and a specific subtype of AML could be strong, while the association between that same exposure and all types of AML might be weak.

Animal studies (2) suggest that mutation of ras family proto-oncogenes, which creates activated ras oncogenes, is frequently an initiating, or at least an early, event in some chemically induced tumors. The frequency of such mutationally activated ras genes is much higher in some chemically induced rodent tumors compared with that in spontaneous tumors (*3*), and the pattern of mutational activation can show chemical specificity (*3,4*). In human tumors, a relationship between oncogene activation and environmental exposures has not been reported except for limited studies of lung cancer and smoking (*5,6*) and another of melanoma of the skin and sunlight exposure (*7*).

Mutationally activated ras family oncogenes occur in 15%-30% of patients with AML and commonly occur in myelodysplastic syndrome, a preneoplastic precursor of AML (8). Myelodysplastic syndrome may be more likely to progress to AML in patients who have activated ras oncogenes (8). A variety of chemical agents have been identified as risk factors for ieukemia, although, with the exception of benzene, the associations have not been strong (*1,9*). This study was designed to test whether AML patients with mutationally activated ras oncogenes are more likely to have a history of occupational exposure to chemicals.

Methods

Study 'Population

The study took place in collaboration with Cancer and Leukemia Group B (CALGB), a multi-institutional cooperative cancer treatment group. The study population consisted of patients who were jointly enrolled in two independent CALGB investigations: a case-control study of risk factors for acute leukemias in adults with 625 case patients (CALGB protocol 8661) and a study of the prognostic significance of oncogene activation in 99 patients (CALGB protocol 8765). Sixty-two patients were jointly enrolled in both studies and thus had both detailed exposure information and oncogene analysis. Patients were enrolled between January 30, 1986, and January 31, 1989. Written informed consent was obtained from the patients at the time of enrollment.

Interviewers contacted patients initially in the hospital within a day or two of diagnosis and either completed the telephone interview at that time or arranged an appointment for a later interview. For the few patients who were too ill to participate or who died shortly after diagnosis, telephone interviews were carried out with next-of-kin.

With the structured questionnaire used in the interview, we obtained data on demographics, medical history, medication use, family medical history, smoking, occupational history, hobbies, residential history, and environmental exposures. We requested a lifetime occupational history and asked, for each job, whether the patient had been exposed to six broad classes of chemicals, radiation, or other agents associated with cancer risk. In addition, patients were asked specifically whether they had been employed in one or more of 32 occupations (Table 1) previously reported to be associated with increased leukemia risk (*1,9*). Within this set of 32 a priori "at-risk" occupations, we also identified a subset of 22 "high-risk" occupations in which, based on previous industrial hygiene review, either the frequency or intensity of chemical exposure was considered to be greater. We also obtained information

Table 1. A priori at-risk occupations

Occupation	High-risk occupation*
Artistic painting	+
Auto mechanics or repair	+
Automobile or heavy equipment manufacturing	+
Beautician, barber, or cosmetologist	
Biological or medical laboratory technician	
Chemical manufacturing	+
Dry cleaning	+
Dye manufacturing	+
Electrician	+
Electronic industry or manufacturing	+
Farming	
Funeral director or embalmer	
Furniture manufacturing, repair, or refinishing	+
Gasoline station attendant	+
Landscaping or gardening	
Leather/shoe industry or shoe repair	+
Munitions/explosives manufacturing	+
Nuclear power industry	+
Nursing	
Other professional arts	
Other health professions with patient care	
Paint manufacturing or application	+
Paper, pulp, saw mill, or lumbering	+
Pest extermination	+
Petroleum industry or manufacturing	+
Pharmaceutical worker	
Plastics manufacturing	+
Printing	+
Rubber industry	+
Textile manufacturing	+
Truck, bus, or taxi driver	
X-ray technician	+

*+ = a priori high-risk occupations with increased frequency or intensity of chemical or radiation exposure.

on exposure to a series of specific chemicals, but the number of reported exposures to individual agents was too small to permit analysis.

Patients were registered to treatment protocols on the basis of clinical and laboratory data available at the time of presentation; their tumors were later classified histopathologically using the French, American, and British (FAB) classification (*10*) following central review **of** their slides. Bone marrow samples obtained at the time of hospital admission were frozen for later oncogene analysis.

As part of the larger case-control study (CALGB protocol 8661), disease-free control subjects were selected from the general population by a two-stage random telephone sampling procedure. Control subjects were frequency matched to case patients by age (10-year age intervals), race (White/non-White), sex, and region of residence (six regions in the United States plus Canada). Control subjects and case patients completed the same questionnaire; the questionnaire was administered over the telephone by trained study interviewers. A total of 630 control subjects completed an interview. We used all of the available control subjects and adjusted for appropriate demographic factors in the analysis.

Statistical Analysis

Associations between disease and exposure were assessed by calculating the odds ratio (OR) and ***95%*** confidence intervals (CIs). The OR is the odds **of** exposure in one group divided by the odds of exposure in a referent group. When there were other variables that needed to be controlled for in the analysis, an adjusted OR was calculated on the basis of Mantel-Haenszel's formula (*11*). This adjusted OR is a weighted average of the stratum-specific **ORs.** Chi-squared statistics can be used to test whether the ORs and adjusted ORs differ from their null value of unity. Comparisons were made between ras mutation-positive (ras-positive) patients and ras mutation-negative (ras-negative) patients and between these two groups and healthy control subjects.

When healthy control subjects are the referent group and it is thus assumed that disease is rare, the OR closely approximates relative risk (RR). The RR is the ratio of the probability of disease given exposure to the probability of disease given no exposure. This ratio cannot be estimated directly because of our study design (*11*).

We used Student's *t* test to assess differences in coptinuous variables between groups and the Wilcoxon-Mann-Whitney test to compare medians. The latter test is simply a Student's *t* test performed on the ranks of the continuous variables and is especially useful for small samples (*12*).

Polymerase Chain Reaction

DNA from bone marrow cells was extracted using standard procedures (*13*). The polymerase chain reaction (PCR) was performed as described previously *(14).* Briefly, 200-500 ng of high-molecular-weight DNA was used to amplify the areas surrounding codons 12, 13, and **61** of three proto-oncogenes: N-ras (also known as NRAS), **K-ras** (also known as KRAS2), and H-ras (also known as HRAS), respectively. The primers for the PCR (final concentration, 0.5 μM) were from Clontech (Palo Alto, Calif.). The PCR was performed for **35** cycles in an automatic thermocycler (Perkin-Elmer Corp., Norwalk, Conn.) in a 100-μL solution containing the following: 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, **1.5** m*M* $MgCl_2$, 0.001%(w/vol) gelatin (Sigma Chemical Co.. St. Louis, Mo.), 200 μM of each of the four deoxynucleoside triphosphates (pH 7.0: Boehringer Mannheim Biochemicals, Indianapolis, Ind.), and 2 **U** AmpliTaq polymerase (Cetus **Corp..** Emeryville, Calif.). In the first cycle, denaturing was performed at **94** "C for **5** minutes, annealing at 55 "C **for** 1 minute, and extension at 72 °C for 1 minute. In all other cycles, denaturing time was 1 minute. Otherwise, cycle times and temperatures were similar to those used in the first cycle. After the PCR was completed, 5-10 μL of the reaction mixture was electrophoresed on a 3% NuSieve (FMC Corp., Rockland, Me.) and 1% agarose gel to ascertain the effectiveness of the PCR amplification.

Oligonucleotide Hybridization

The remaining **90-95 μL** of the PCR solution was denatured with 150 μL of **0.4 N** NaOH, heated to **95** "C for 2 minutes, neutralized with 200 μL of 2 *M* Tris (pH 7.5). and slotted on nylon filter paper (Hybond N; Amersham Corp.. Arlington Heights, Ill.) according to the manufacturer's instructions. DNA was cross-linked using UV light. **The** oligonucleotide probes covering all possible mutations and the wild-type sequence were from Clontech. For initial screening, a mixture of **3-6** specific oligonucleotides (each probe, 7 pmol) was end-labeled with **T4** polynucleotide kinase and **[λ-^{32}P]adenosine** triphosphate (DuPont NEN Research Products, Boston, Mass.) (*14*).

The filters were then hybridized overnight in **5X** (i.e., five times the standard concentration) sodium chloride–sodium phosphate ethylenediamine tetraacetic acid, 5X Denhardt's solution, 100 m*M* sodium pyrophosphate, and 0.5% sodium dodecyl sulfate and washed with 3 *M* tetramethylammonium chloride. The filters were exposed for one to several hours to an XOMAT film (Kodak. Rochester, N.Y.) at –70 "C using intensifying screens. To confirm the specific mutation, we reamplified the DNA samples from ras-positive case patients and analyzed them by oligonucleotide hybridization with individual oligonucleotide probes after Southern blot transfer. For the Southern blot transfer, 7 μL of the PCR solution was electrophoresed in a 3% NuSieve and 1% agarose gel. and **the** transfer was performed in a vacuumblotter (Hoefer, San Francisco, Calif.) onto Hybond-N membranes. After the transfer was completed, DNA was cross-linked by using UV light and hybridized with individual mutation-specific oligonucleotide probes (*14,15*).

Two ras-positive samples were selected for further confirmation using direct sequencing of the PCR products as described previously *(14).* In both cases, the allele-specific oligonucleotide hybridization analysis concurred with the direct sequencing results (data not shown). The sensitivity of these two procedures for detecting a ras mutation in the **presence** of normal cells is one mutant to 10 normal cells when

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allele-specific oligonucleotide hybridization is used and one mutant to four normal cells when direct sequencing is used (14,16).

Results

Characteristics of AML Patients With Activated ras Oncogenes

Of the 62 patients in this study, 10 (16%) were positive for activated K- or N-ras genes at codon 12, 13, or 61 (Table 2). Three patients had activation of both K- and N-ras genes. None had activated H-ras. Non-Whites were more likely to have ras activation than Whites (OR = 9.4; 95% CI = 2.0-44); five (50%) of 10 non-Whites and five (10%) of 52 Whites had activated ras genes. Among the non-White group, two of five Blacks, two of four American Indians, and the only Asian American in the study had activated ras genes. Patients with ras-positive tumors were older (mean age at diagnosis, 52.4 yrars) than those with ras-negative tumors (mean age at diagnosis, 44.5 years), although this difference is not statistically significant (P = .17). Median ages (56.5 versus 42 years, respectively) also did not differ significantly (P = .17). There were slight, but not statistically significant, gender differences in the frequency of ras activation (13% for females; 19% for males; P = .73). Of the ras-positive tumors, 40% were FAB M4, whereas only 17% of those that were ras negative were M4. This difference was not statistically significant (OR = 3.2; 95% CI = 0.7-14). There was no association between ras activation and smoking (data not shown).

Association Between Occupational Exposure and ras Activation

After adjusting for age and race (White/non-White), we found that ras-positive patients were more likely than ras-negative patients to have been employed in an a priori at-risk occupation (Tables 1, 3). Crude and adjusted ORs are similar in all analyses; only the adjusted ORs are presented. When we classified only those patients who had worked 5 or more years as exposed, the OR increased from 2.2 to 4.1 (95% CI = 0.7-23). The OR increased further to 6.8 (95% CI = 1.3-36) for 5 or more years of employment in a priori high-risk occupations (Table 3). Six of 10 ras-positive patients worked in an a priori high-risk occupation for 5 or more years, compared with only eight of 52 ras-negative patients.

After adjusting for age and race, we found that, compared with healthy control subjects, ras-positive patients were four times as likely (95% CI = 1.1-15) to report 5 or more years in an at-risk occupation and 5.9 times as likely (95% CI = 1.7-20) to report 5 or more years in a high-risk occupation. The employment histories of ras-negative patients and control subjects did not differ from each other: For 5 or more years' employment in an at-risk occupation, the adjusted OR was 0.9 (95% CI = 0.5-1.5), and in the high-risk occupations, it was 0.6 (95% CI = 0.3-1.4).

Patients with ras-positive leukemia were particularly more likely than ras-negative patients to report skin contact with chemicals (OR = 6.9; 95% CI = 1.3-37), breathing chemical vapors (OR = 9.1; 95% CI = 1.3-64), and exposure to dusty conditions (OR = 6.3; 95% CI = 1.1-35) while working (Table 4). The adjusted OR for exposure to solvents and degreasers (OR = 3.2; 95% CI = 0.5-20) was also elevated in ras-positive patients compared with that in ras-negative patients. The two remaining categories of occupational exposure, non-ionizing radiation and ionizing radiation, had too few exposed ras-positive patients to permit meaningful analysis (none and one, respectively).

When ras-positive patients were compared with healthy control subjects, the ORs for all exposures remained greater than one, although only in the case of exposure to chemicals

Table 2. Characteristics of AML patients with activated ras genes

Age, y	Sex	Race*	FAB	Gene	Codon	Mutation?	At-risk occupation
24	Male	W	M4	K-ras N-ras	61 61	CAA→CAT CAA—AAA	Auto mechanic$
31	Male	AI	MA	K-ras	12	GGT→GTT	
35	Female	W	M5A	N-ras	61	CAA→AAA	Paper mill‡ and nuclear power industry$
46	Male	A	M2	N-ras	13	GGT→GAT	Other health profession
55	Male	B	M2	K-ras	12	GGT→GAT	
58	Female	W	M6	N-ras	12	GGT→GAT	Electronics industry$
59	Male	W	M4	K-ras N-ras	12 61	GGT→GAT CAA→CGA	
65	Female	W	M4	N-ras	12	GGT→AGT	Textile industry$ and nursing
75	Female	AI	M2	K-ras N-ras	12 12	GGT→GAT GGT→GAT	Textile industry,$ rubber industry,$ and auto manufacturing$
76	Male	B	Unclassified§	K-ras	13	GGC→GAC	Furniture manufacturing.$ paint manufacturing,‡ and truck driver

*W = White; AI = American Indian; B = Black: and A = Asian.
†Because three individuals had two ras mutations concurrently, the number of mutations exceeds the number of case patients
‡A priori high-risk occupations.
§Unclassifiable acute leukemia.

Tnhle 3. Association between occupational risk category and ras activation in AML patients

Risk category	% exposed (No.) ras+ (N = 10)	ras- (N = 52)	Control subjects (N = 630)	Adjusted OR (95% CI)* ras+ versus ras–	ras+ versus control subjects	ras– versus control subjects
Ever exposcdt versus never	70 (7)	56 (29)	59 (373)	2.2 (0.4.-12)	1.9 (0.5-7.6)	0.9 (0.5-1.5)
⩾5 y exposed‡ versus <5 y	70 (7)	37 (19)	39 (247)	4.I (0.7-23)	4.0 (1.1-15)	0.9 (0.5-1.5)
⩾5 y high risk§ versus <5 y	60 (6)	15 (8)	22 (137)	6.8 (1.3-36)	5.9 (1.7-20)	0.6 (0.3-1.4)

*Adjusted for age and race.
†Ever worked in an at-risk occupation.
‡Worked ⩾5 years in an at-risk occupation.
§Worked ⩾5 years in a high-risk occupation.

Table 4. Association between general occupational exposures and ras activation in AML patients

Exposure	% exposed (No.) ras+ (N = 10)	ras– (N = 52)	Control subjects (N = 630)	Adjusted OR (95% CI)* ras+ versus ras–	ras+ versus control subjects	ras+ versus control subjects, protective clothing reclassified	ras– versus control subjects
Chemicals on skin	60 (6)	29 (15)	33 (207)	6.9 (1.3-37)	4.5 (1.3-16)	7.2 (2.2-24)	0.8 (0.4-1.4)
Chemical vapor	60 (6)	35 (18)	40 (249)	9.1 (1.3-64)	3.0 (0.8-11)	5.4 (1.5-19)	0.8 (0.4-15)
Dusty conditions	70 (7)	44 (23)	50 (316)	6.3 (1.1-35)	2.9 (0.7-12)	4.2 (I.1-17)	0.8 (0.5-1.5)
Solvents and degreasers	50 (5)	38 (20)	40 (255)	3.2 (0.5-20)	1.6 (0.5-5.9)	2.5 (0.7-9.0)	0.9 (0.5-1.5)

*Adjusted for age and race.

on the skin (OR = 4.5; 95% CI = 1.3-16) did the 95% CI exclude one (Table 4).

The use of protective masks and clothing by some workers could lead to misclassification of exposure status; workers who were exposed to an agent but who wore protective clothing would be biologically unexposed. Because such uncorrected misclassification could artificially lower risk estimates, we reclassified as unexposed those subjects who reported wearing masks or other protective garments. In every instance, the risk estimates for ras-positive leukemia associated with individual exposures increased (Table 4). In contrast to ras-positive patients, ras-negative patients did not differ from control subjects with regard to occupational exposures (Table 4). Taking into account protective clothing did not change this result (data not shown).

Specificity of ras Mutations

The most common activating mutation was a G to A transition, which accounted for eight of 13 mutations (Table 2). Because three individuals had two ras mutations concurrently, the number of mutations exceeds the number of ras-positive case patients. The first patient with two mutations had two G to A mutations, the second had a G to A and an A to G mutation, and the third had an A to T and a C to A mutation. After we excluded the case patient with both a G to A and an A to G mutation, we found that six of six case patients with G to A mutations were older than 45 years of age, while three of three case patients with other mutations (A to T, C to A, or G to T) were 35 years old or younger. The mean age of patients with G to A mutations (62.6 years; SD, 12) was significantly different ($P = .003$) from that of patients with other mutations (30.0 years; SD, 5.6). Median ages (61.5 versus 31 years) also differed significantly ($P < .05$). There were no associations between occupational exposure and the specific ras proto-oncogene activated, codon mutated, or base pair change.

Discussion

We found that patients with ras mutation-positive AML had higher rates of occupational exposure to chemicals than patients with ras mutation-negative disease. Of those exhibiting activated ras genes, 60% worked 5 or more years in a priori high-risk occupations, whereas only 15% of patients with ras mutation-negative AML had this same exposure. The link between chemical exposure and ras-positive AML is reinforced by evidence of increasing risk with duration of employmenl and likelihood of solvent exposure and is supported by the results for specific categories of occupational exposures. The apparent association with occupational exposure is unlikely to be the result of recall bias, since both ras-positive and ras-negative case patients have disease.

Although the ras-positive versus ras-negative comparisons can identify exposure differences, it is the comparison of these two groups with healthy control subjects that allows us to estimate the **RR** of exposure. In our data, the **ORs** for comparisons involving ras-positive patients versus healthy control subjects were elevated and similar to the **ORs** found for comparisons involving ras-positive patients versus ras-negative patients. In contrast, ras-negative patients had the same frequency of exposure as healthy control subjects. This result is consistent among the many different exposures we examined and offers additional evidence that occupational exposure to chemicals is strongly linked to ras-positive AML. It is interesting to note that, had we combined ras-positive and ras-negative patients and compared the entire group with healthy control subjects, there would have been no apparent increase in risk of disease associated with chemical or occupational exposures. **For** our two strongest ras-positive associations, the OR was reduced to **1.02** for 5 or more years in a high-risk occupation and to 1.05 for exposure to chemicals on the skin when we compared all combined case patients with control subjects.

Although recall bias is an unlikely explanation for our results, it is possible that there is some random misclassification of exposure status. Data were obtained by questionnaire and were not verified. Random misclassification errors usually (but not always) tend to bias the ORs toward the null. Our conclusions are based primarily on associations between ras activation and employment in specified jobs. Specific jobs are easily recalled, whereas occupational exposures are more difficult to recall. In addition, the associations between the categories of occupational exposures are not independent, so that people who reported exposure in one category also tended to report exposure in other categories.

Because the frequency of ras activation differed with age and race, we stratified by age and race to evaluate whether these factors explained the observed associations with occupational exposures. The associations between ras activation and employment in high-risk occupations were not affected in either case. Conversely, stratifying on occupational exposures did not change the age or race associations. Thus, within the limits of the small sample, it appears that these factors are independently associated with ras activation.

Mutationally activated ras family oncogenes (N-ras, K-ras, and H-ras) have been reported in **15%-30%** of patients with primary AML, making aberrant ras genes the most common molecular genetic abnormality in this form of acute leukemia (*17*). Since ras mutations are found in preleukemic disorders (18) and in affected pluripotent stem cells (*19*), it is likely that mutant ras genes have a role in the initiation of some AMLs. Considerable experimental evidence exists showing that specific carcinogens can cause a variety of cancers through the induction of point mutations in ras *(20)*.

Our overall frequency of ras mutations **(16%)** is somewhat lower than in previously published series (*21-23*), where the frequency is usually between **20%** and 30%. This difference could be due in part to the young age of our patient population; among patients under age **55,** four (10%) of **41** had activated ras, whereas in those aged **55** or older, six **(29%)** of **21** had activated ras. Alternatively, earlier studies may have elevated frequencies because they included AML patients with histories of antecedent hematologic disorders or used detection systems that can identify mutations which exist in only a tiny fraction of blast cells (*17*). In the larger group of **99** patients enrolled in the oncogene prognosis study (but for whom we do not have exposure data on all subjects), a similar frequency of ras mutations has been detected **(18%),** indicating that sampling bias did not occur.

We found significant associations with other common demographic parameters not previously noted. First, our analysis revealed a higher frequency of ras activation in non-Whites than in Whites, although the number of non-Whites in our study is small. Other published studies have not specified the race of ras-positive and ras-negative case patients. Second, we found an increased frequency of ras mutation in older patients, although this finding was not statistically significant, and one study which reported age data for both ras-positive and ras-negative patients did not show any difference *(21)*. Finally, including double ras mutations, eight of **13** activating mutations in N- and K-ras genes were ***G*** to A transitions. The predominance of this specific point mutation has been reported by other investigators (*21,24-28*); however. our data also revealed that patients with ***G to*** **A** mutations were statistically significantly older than those with other mutations. While this finding has not been previously noted, our review of other published studies with age data for ras-positive case patients shows similar trends, with ***G*** to **A** mutations more common in older patients (*21,24,26-29*), although childhood AML includes both ***G*** to A and other mutations (*29,30*). The reason for the predominance of the ***G*** to A mutation in older age groups remains unclear, but it may reflect subtle differences in age-related exposure, accumulation of DNA damage, or reduction in DNA repair.

There was no evidence of an association between occupation or occupational exposures and the specific ras gene activated, codon mutated, or base pair substitution. This finding is not surprising, given the small number of ras-positive patients in our study, the variety of occupations these patients held, and the large number of potential exposures within each occupation. A larger follow-on study may have sufficient ras-positive case patients and highly specific exposure information, which would enable us to explore associations between particular agents and specific patterns of DNA damage.

AML with activated ras oncogenes may represent a pathogenetically distinct subset of AML that is strongly associated with chemical exposure. The risk estimates that we describe for occupation and chemical exposure are larger than those found in other epidemiologic studies that do not separate AML by ras oncogene status. While the estimates of these risks may be uncertain because of small sample size, the fact that such consistent associations can be detected with so few case patients illustrates the value of using oncogene activation to define subclasses of disease. Such studies linking epidemiologic measures of exposure with molecular assays of the genetic defects responsible for cancer initiation and promotion may provide a more integrated understanding of disease etiology.

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