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Agricultural Pesticide Use and Risk of t(14;18)-Defined Subtypes of Non-Hodgkin Lymphoma

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Pesticides have been specifically associated with the t(14;18)(q32;q21) chromosomal translocation. To investigate whether the association between pesticides and risk of non-Hodgkin lymphoma (NHL) differs for molecular subtypes of NHL defined by t(14;18) status, we obtained 175 tumor blocks from case subjects in a population-based case-control study conducted in Nebraska between 1983 and 1986. The t(14;18) was determined by interphase fluorescence in situ hybridization in 172 of 175 tumor blocks. We compared exposures to insecticides, herbicides, fungicides, and fumigants in 65 t(14;18)-positive and 107 t(14;18)-negative case subjects with those among 1432 control subjects. Multivariate polytomous logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). Compared with farmers who never used pesticides, the risk of t(14;18)-positive NHL was significantly elevated among farmers who used animal insecticides (OR = 2.6; 95% CI, 1.0-6.9), crop insecticides (OR = 3.0; 95% CI, 1.1-8.2), herbicides (OR = 2.9; 95% CI, 1.1-7.9), and fumigants (OR = 5.0; 95% CI, 1.7-14.5). None of these pesticides were associated with t(14;18)-negative NHL. The risk of t(14;18)-positive NHL associated with insecticides and herbicides increased with longer duration of use. We conclude that insecticides, herbicides, and fumigants were associated with risk of t(14;18)-positive NHL but not t(14;18)-negative NHL. These results suggest that defining subsets of NHL according to t(14;18) status is a useful approach for etiologic research. (Blood. 2006; 108:1363-1369)

Introduction

Agricultural pesticide use has been repeatedly, but not consistently, associated with risk of non-Hodgkin lymphoma (NHL).[1-4] This inconsistency across studies could be because NHL is a heterogeneous group of malignancies that vary in cause,[3,5] and the distribution of various NHL subtypes differs across studies. There is evidence that associations between pesticides and some NHL histologic subtypes may be stronger than associations between the same risk factors and NHL in the aggregate.[6-9] Although the new World Health Organization (WHO) classification of NHL might be useful for etiologic research,[10] many WHO-defined subtypes are heterogeneous at the molecular level.[11] It has been suggested that epidemiologic studies should work toward using molecular classification to better define homogeneous disease entities.[12]

Approximately 60% of NHL in North America is either follicular NHL or diffuse large B-cell NHL,[13] and about 70% to 90% of follicular NHL, and 20% to 30% of diffuse large B-cell NHL have a t(14;18)(q32;q21) chromosomal translocation.[10,14] A study by Schroeder et al[15] found that the risk of NHL associated with farming and exposure to dieldrin, lindane, atrazine, or fungicides was limited only to case subjects with the t(14;18) translocation as assessed by polymerase chain reaction (PCR) analysis.[15] Those researchers suggested that NHL subtypes defined by t(14;18) status might have greater etiologic specificity than NHL in aggregate.

To further evaluate this hypothesis in the general population, we investigated the association of agricultural pesticide use with risk of t(14;18)-defined subsets of NHL in a population-based, case-control study conducted in Nebraska during 1983 to 1986. We determined t(14;18) status by using the fluorescence in situ hybridization (FISH) technique, which is considered the "gold standard" for detecting specific chromosomal abnormalities. We hypothesized that pesticides may act on a t(14;18)-dependent pathway, resulting in stronger associations with t(14;18)-positive than t(14;18)-negative NHL.

Patients, materials, and methods

Study population

The study population and methods have been reported in detail elsewhere.[6,16] Briefly, cases of NHL, Hodgkin disease, multiple myeloma, and chronic lymphocytic leukemia among white men and women, aged 21 years

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or older, residing in 66 counties in eastern Nebraska, and diagnosed between July 1, 1983, and June 30, 1986, were identified through the Nebraska Lymphoma Study Group and area hospitals. The current study included only cases of NHL. All cases underwent pathology review, and only histologically confirmed cases were included. Of the 426 eligible case subjects with NHL, 385 participated (men = 201; women = 184) for a response rate of 90%.

Control subjects without hematopoietic cancer were randomly selected from the same 66-county area by 3:1 frequency matching by sex, vital status, and age (5-year age groups) to the combined age distribution of the 4 cancer case types. Control subjects for living case subjects younger than age 65 years were randomly selected by 2-stage, random digit dialing, as described by Waksberg.[17] For living case subjects aged 65 years or older, control subjects were a simple random sample from the records of the Health Care Financing Administration (Medicare). Control subjects for deceased case subjects were selected from the Nebraska state mortality files by using the additional matching factor of year of death. Persons with an underlying cause of death of NHL, Hodgkin disease, multiple myeloma, leukemia, malignancy of unknown site, aplastic anemia, suicide, homicide, or legal intervention were excluded as control subjects. Of the 1655 eligible control subjects, 1432 participated (men = 725; women = 707) for a response rate of 87%.

Exposure assessment

Telephone interviews were conducted directly with the subjects, or with their next-of-kin if the subjects were deceased or incapacitated (40.5% of case subjects and 43.6% of control subjects). The interview included questions on specific herbicides and insecticides used; the application method used most often; use of protective equipment; duration of time wearing work clothes after handling pesticides; animals raised; and the use of fungicides, rodenticides, fumigants, wood preservatives, and fertilizers. For each specific herbicide and insecticide, information was obtained on the years of use, the average annual number of days of use on crops or animals, and the average annual number of days the pesticides were personally handled. Participants were also asked to provide information on demographics, anthropometric measures, occupational history, residential and water supply history, tobacco use, hair coloring products use, medical history, and a 31-item food-frequency questionnaire.

Ascertainment of the t(14;18)(q32;q21) by FISH analysis

All procedures were performed in accordance with a protocol approved by human investigation committees at Northwestern University and University of Nebraska Medical Center. A total of 175 paraffin-embedded tumor blocks were obtained through the statewide Nebraska Lymphoma Registry and Tissue Bank from the 385 NHL case subjects (45.5%) in the original case-control study. We evaluated the potential for selection bias by comparing the distribution of risk factors between case subjects with NHL with available tumor blocks and those whose tumor blocks could not be retrieved. Our findings indicate that selection bias is unlikely because the availability of tumor blocks did not differ by pesticide exposures, and it is unlikely that the t(14;18) status is related to tumor block availability.

Tissue microarrays (TMAs) were prepared from archival, paraffin-embedded tissue from all case subjects as follows. After examining the hematoxylin and eosin–stained sections to define the diagnostic areas, 4 representative 0.6-mm cores were obtained from each case subject and inserted in a grid pattern into a recipient paraffin block using a tissue arrayer (Beecher Instruments, Silver Spring, MD). Then, 4-μm TMA sections were cut and mounted on positively charged slides and pretreated before hybridization. The pretreatment protocol included dewaxing in HEM-D (Scientific Safety Solvents, Keller, TX) followed by dehydration in 95% ethanol, treatment with 0.2N HCl for 20 minutes, rinse in distilled, deionized water, and incubation in a sodium thiocyanate solution at 80°C for 15 minutes. After rinsing again in distilled, deionized water, the sections were digested with a protease solution at 37°C for 10 minutes, rinsed in distilled, deionized water, and sequentially dehydrated in 70%, 85%, and 95% ethanol. We used the commercially available LSI IGH/BCL2 dual-color, dual-fusion probe to define the t(14;18) rearrangement, and the centromeric enumeration probe of chromosome 18 to define the number of this chromosome present in a cell (Abbott-Vysis Inc, Downers Grove, IL). The probe mixture was placed on the tissue sections, covered with a coverslip, and sealed. Co-denaturation of probes and target DNA at 75°C for 5 minutes was followed by overnight hybridization at 37°C by using an automated hybridization chamber (HYBrite; Abbott-Vysis). The slides were then washed in 2 × standard saline citrate (SSC)/0.1% Nonidet P-40 (NP-40), pH 7.0, at 73°C for 2 minutes, followed by a room temperature rinse in 2 times SSC/0.1% NP-40, pH 7.0, for 2 minutes. Nuclei were counterstained with 4,6-diamidino-2-phenylindole at a concentration of 125 ng/mL in antifade solution, and analysis was performed on an Olympus BX51 microscope (Olympus, Mellville, NY) equipped with appropriate filters. Images were captured with CytoVision image capture software (Applied Imaging, Santa Clara, CA).

In the event of failure of hybridization or equivocal results in some case subjects, tissue cores from those case subjects were individually processed in a microtube; nuclei were isolated, placed on defined areas on a slide, and pretreated; and FISH was performed.[18] Briefly, the tissue cores were dewaxed 3 times in 100 μL xylene for 10 minutes, each followed by rehydration in 100 μL of an ethanol series (95%, 70%, and 50%) for 2 minutes each. Subsequent to enzymatic extraction using proteinase K solution, the tissue was minced and washed twice in phosphate-buffered saline. The cells were then centrifuged, and the nuclear pellets were placed on a defined area of a slide. The pretreatment protocol was similar to that used for the paraffin-embedded tissue with minor modification. The protocol did not include the dewaxing by HEM-D, and the 0.2N HCl treatment was 10 minutes. The denaturation, hybridization, and postwash procedures were also similar; however, the co-denaturation temperature was 72°C for 1 minute, and the first postwash treatment included 0.4 × SSC/0.3% NP-40, pH 7.0, at 73°C for 2 minutes.

The FISH probes used in the present study are approved by the US FDA as analytic specific reagents. The upper limit for false-positive results is 5%, and any specimen below this control limit was not considered positive for the t(14;18). Blind replicates of approximately 5% of the specimens were analyzed for quality control. In addition, classic cytogenetic analysis was performed in 5% of the samples. The t(14;18) status determined by FISH was validated by these 2 quality control measures. FISH analyses were performed blinded, with the unique identifiers unknown to the laboratory personnel involved in the analyses. A minimum of 100 interphase nuclei were independently examined by 2 individuals, a cytotechnologist, and an expert cytogeneticist (B.J.D.), for the presence of the t(14;18), as well as for the number of chromosome 18s. The agreement between the readers was 100%.

Data analysis

Subjects who had never lived or worked on a farm as an adult were defined as nonfarmers. Nonfarmers are commonly used as the referent group in epidemiologic studies of farming and NHL.[4,8,15,19] However, this approach has a limitation because nonfarmers may be exposed to pesticides through home or garden use.[20] Nonfarmers also have different lifestyles than farmers and, consequently, have different patterns of chronic disease than farmers.[21] Thus, to minimize potential misclassification of the referent group, we used farmers who had never used any pesticides (ie, insecticides, herbicides, fungicides, rodenticides, and fumigants) or fertilizers when they lived or worked on the farm as the reference population. The duration of insecticide and herbicide use was categorized into 2 groups ($<$ median and $\geq$ median) based on the distribution among control subjects who used insecticides and herbicides, respectively.

The odds ratio (OR) and the 95% confidence interval (CI) for each NHL subtype defined by t(14;18) status were derived from multivariate polytomous logistic regression with a 3-level nominal response variable (control subjects, t(14;18)-positive NHL, and t(14;18)-negative NHL), and the logit estimator always compared t(14;18)-defined NHL subtypes with control subjects. This allowed the comparison of ORs for t(14;18)-positive NHL versus t(14;18)-negative NHL. The reported *P* values are 2-sided. Potential confounders included in the models were dummy variables for age (20-44, 45-64, 65-74, $\geq$ 75 years), type of respondent (direct or proxy interview), and sex, because control subjects were frequency matched by these

variables to case subjects in the original case-control study. Other potential confounders were considered based on prior knowledge of risk factors for NHL, as well as change-in-estimate criteria.[22] A family history of cancer (yes or no) was included in the model. Factors such as tobacco use, marital status, and use of hair dyes were not included in the final models because they did not change the risk estimate by more than 10%. Although the approximate strength of the associations was stronger among men than women, the direction was similar. Therefore, we combined men and women in this report. We report point estimates only when there are at least 4 exposed case subjects in the subset of NHL defined by t(14;18) status. Because of high collinearity among pesticide groups, we did not mutually adjust for multiple pesticide exposures in our analyses. Information on HIV infection was not available. However, it is unlikely that HIV infection was a significant confounder or risk factor for NHL in the present study given the time period (ie, early to mid-1980s), location (ie, a Midwestern state where HIV infection and AIDS were not common), and age of the participants (ie, 88% of the case subjects and 87% of the control subjects were older than 50 years). Analyses were conducted using PROC CATMOD of the SAS statistical software package, version 9.1 (SAS Institute, Cary, NC).

Results

Characteristics of the study subjects and possible risk factors for case subjects with t(14;18)-positive NHL and with t(14;18)-negative NHL are shown in Table 1. A total of 175 tumor blocks were obtained from 385 case subjects (45.5%) in the case-control study, and t(14;18) status was determined in 172 (98.3%) of the 175 case subjects. Of these 172 case subjects, 65 case subjects (37.8%) were positive for the t(14;18), and 107 case subjects (62.2%) were negative. Control subjects, t(14;18)-positive case subjects, and t(14;18)-negative case subjects were similar with respect to age, respondent status, and education (data not shown). There was a higher proportion of women among the t(14;18)-negative case subjects than among the control subjects. Compared with control subjects, t(14;18)-positive case subjects were less likely to have used tobacco products, whereas t(14;18)-negative case subjects were more likely to have used hair dyes. In comparison with control subjects, both t(14;18)-positive and t(14;18)-negative case subjects were more likely to have first-degree relatives with hematopoietic cancer. The t(14;18)-positive case subjects were also more likely to have first-degree relatives with cancers other than hematopoietic cancer. The proportions of the t(14;18) across different histologic subtypes was consistent with previous reports.[14]

Table 1. Characteristics of control subjects and case subjects with t(14;18)-positive and t(14;18)-negative non-Hodgkin lymphoma (NHL)

Characteristic	Control subjects, no. (%)	Case subjects	
		t(14;18)-positive, no. (%)	t(14;18)-negative, no. (%)
Sex			
Male	725 (50.6)	33 (50.8)	43 (40.2)
Female	707 (49.4)	32 (49.2)	64 (59.8)
Respondent status			
Self	808 (56.4)	40 (61.5)	60 (56.1)
Proxy	624 (43.6)	25 (38.5)	47 (43.9)
Used tobacco products			
Never	677 (47.3)	38 (58.5)	51 (47.7)
Ever	749 (52.3)	27 (41.5)	56 (52.3)
Used hair dye			
Never	976 (71.1)	48 (73.9)	70 (65.4)
Ever	386 (28.1)	17 (26.2)	37 (34.6)
Family history of cancer			
No	908 (64.6)	32 (50.8)	62 (59.6)
Nonhematopoietic cancer	419 (29.8)	25 (39.7)	31 (29.8)
Hematopoietic cancer	78 (5.6)	6 (9.5)	11 (10.6)
WHO-defined histologic types			
Follicular lymphoma	NA	42	21
Diffuse large B-cell lymphoma	NA	21	44
Small lymphocytic lymphoma	NA	1	17
Other*	NA	1	25

For control subjects, n = 1432; for t(14;18)-positive case subjects, n = 65; for t(14;18)-negative case subjects, n = 107 (numbers in table may not sum to overall totals because of missing data).

NA indicates not applicable.

*Other includes Burkitt-like NHL (n = 1), lymphoplasmacytic NHL (n = 2), mantle cell NHL (n = 4), marginal zone NHL (n = 4), plasmacytoma (n = 2), unclassifiable B-cell NHL (n = 1), and T-cell NHL (n = 12).

Compared with farmers who never used agricultural pesticides, nonfarmers appeared to have a slightly elevated risk of t(14;18)-positive NHL but not t(14;18)-negative NHL (Table 2). For farmers who used pesticides, exposures to animal insecticides, crop insecticides, herbicides, and fumigants were associated with a significantly higher risk of t(14;18)-positive NHL but not t(14;18)-negative NHL (P for difference $< .05$). The risk of t(14;18)-positive NHL associated with animal insecticides, crop insecticides, and herbicides increased with longer duration of use. The OR from use of rodenticides was also elevated among t(14;18)-positive case subjects but not significantly so. There was no association of various farming activities, including handling chemically treated fence posts or wood, growing corn, or raising cattle with risk of either t(14;18)-positive or t(14;18)-negative NHL (data not shown).

The associations of various chemical classes of insecticides with risk of NHL according to t(14;18) status is shown in Table 3. Compared with farmers who never used pesticides, the risk of t(14;18)-positive NHL was significantly elevated among farmers who reported using organochlorines, carbamates, organophosphates, or pyrethroids. In contrast, there was no excess risk of t(14;18)-negative NHL among farmers who used these chemical classes of insecticides. The association of these chemical classes of insecticides differed significantly between t(14;18)-positive NHL and t(14;18)-negative NHL (P for difference $< .05$), except organochlorines for which P for difference $= .06$. Farmers who did not use a particular chemical class of insecticides, but who most likely used other pesticides, had small, nonsignificant increased risk of t(14;18)-positive NHL compared with farmers who never used any pesticides. When nonfarmers were used as the reference population, the ORs for t(14;18)-positive NHL became smaller but remained statistically significant, whereas the ORs for t(14;18)-negative NHL were essentially unchanged (data not shown).

The associations between chemical classes of herbicides and risk of NHL also differed according to t(14;18) status (Table 4). Compared with farmers who never used pesticides, farmers who used phenoxyacetic acids, triazines, amides, benzoic acids, and carbamates had an elevated risk of t(14;18)-positive NHL, but the farmers who did not report using these chemicals also had elevated risks for t(14;18)-positive NHL. There was no association between any of these chemical classes of herbicides and risk of t(14;18)-negative NHL. Overall, the association of various chemical classes of herbicides differed between t(14;18)-positive NHL and t(14;18)-negative NHL but not significantly so except for phenoxyacetic acids. Again, ORs for t(14;18)-positive NHL obtained using nonfarmers as the reference population were smaller than those estimated using nonexposed farmers, whereas the ORs for t(14;18)-negative NHL were essentially the same irrespective of type of referent (data not shown).

Table 2. The association of non-Hodgkin lymphoma (NHL) with agricultural pesticides and farming activities according to t(14;18) status

		t(14;18)-positive NHL vs control subjects		t(14;18)-negative NHL vs control subjects		
Farming status	Control subjects*	Case subjects†	OR (95% CI)‡	Case subjects§	OR (95% CI)‡	*P* for difference‖
Nonfarmers	419	19	2.0 (0.7-5.5)	31	0.7 (0.4-1.3)	.09
Farmers						
Never used pesticides	229	5	1.0 (referent)	23	1.0 (referent)	NA
Used pesticides	730	37	2.1 (0.8-5.4)	48	0.7 (0.4-1.2)	.04
Animal insecticides	417	27	2.6 (1.0-6.9)	27	0.7 (0.4-1.3)	.02
Less than 11 y	104	5	2.0 (0.5-7.0)	8	0.9 (0.4-2.0)	.3
11 y or more	107	9	3.2 (1.0-10.1)	9	0.9 (0.4-2.1)	.08
Missing	206	13	2.8 (1.0-7.9)	10	0.5 (0.2-1.1)	.01
Crop insecticides	301	22	3.0 (1.1-8.2)	21	0.7 (0.4-1.4)	.02
Less than 12 y	82	4	2.1 (0.5-8.1)	7	0.9 (0.4-2.3)	.3
12 y or more	87	9	3.8 (1.2-12.2)	6	0.8 (0.3-2.0)	.03
Missing	132	9	3.0 (1.0-9.3)	8	0.6 (0.2-1.4)	.02
Herbicides	352	25	2.9 (1.1-7.9)	22	0.7 (0.3-1.2)	.01
Less than 17 y	104	7	2.9 (0.9-9.4)	8	0.8 (0.3-2.0)	.09
17 y or more	109	9	3.2 (1.0-10.1)	8	0.8 (0.3-1.9)	.06
Missing	139	9	3.0 (1.0-9.1)	6	0.4 (0.1-1.1)	.007
Fungicides	59	2	NR¶	3	NR¶	NA
Rodentcides	523	28	2.2 (0.8-5.8)	39	0.8 (0.5-1.4)	.07
Fumigants	112	14	5.0 (1.7-14.5)	9	0.8 (0.3-1.8)	.006

NA indicates not applicable; NR, not reported.

*Numbers may not sum to 1432 because of missing data.

†Numbers may not sum to 65 because of missing data.

‡Odds ratios (ORs) and confidence intervals (CIs) adjusted for age (4 levels), sex, type of respondent (direct or proxy interview), and family history of cancer (yes or no).

§Numbers may not sum to 107 because of missing data.

‖*P* for difference was computed from polytomous logistic regression by testing the difference of regression coefficients of the usage of pesticides corresponding to t(14;18)-positive versus control subjects and t(14;18)-negative versus control subjects.

¶Not reported because there were fewer than 4 exposed case subjects.

Discussion

In this population-based, case-control study, we defined subsets of NHL according to their t(14;18) status and found that exposures to insecticides, herbicides, and fumigants were significantly associated with risk of t(14;18)-positive NHL but not t(14;18)-negative NHL. The risk increased with longer duration of insecticide and herbicide use. The positive associations of insecticides and herbicides with t(14;18)-positive NHL could not be attributed to any particular chemical class of pesticides, because excess risks were associated with all classes. Nonfarmers also have an elevated risk of t(14;18)-positive NHL but not t(14;18)-negative NHL, in comparison with farmers who never used pesticides. The ORs for

Table 3. The association of non-Hodgkin lymphoma (NHL) among farmers with chemical classes of insecticides according to t(14;18) status

		t(14;18)-positive NHL vs control subjects		t(14;18)-negative NHL vs control subjects		
Insecticide use	Control subjects	Case subjects	OR (95% CI)*	Case subjects	OR (95% CI)*	*P* for difference†
Never used pesticides	229	5	1.0 (referent)	23	1.0 (referent)	—
Organochlorines						
No‡	190	6	1.1 (0.3-3.9)	11	0.6 (0.3-1.3)	.4
Yes	164	17	3.8 (1.3-10.9)	18	1.2 (0.6-2.4)	.06
Carbamates						
No‡	251	12	1.8 (0.6-5.4)	18	0.8 (0.4-1.5)	.2
Yes	73	11	5.1 (1.6-16.0)	8	1.1 (0.5-2.8)	.04
Organophosphates						
No‡	209	7	1.2 (0.4-4.2)	12	0.6 (0.3-1.3)	.3
Yes	128	17	4.6 (1.6-13.2)	15	1.2 (0.6-2.5)	.04
Pyrethroids						
No‡	269	14	1.9 (0.7-5.6)	23	0.9 (0.5-1.7)	.2
Yes	55	8	4.9 (1.5-16.6)	3	NR§	NA

NR indicates not reported; NA, not applicable.

*Odds ratios (ORs) and confidence intervals (CIs), adjusted for age (4 levels), sex, type of respondent (direct or proxy interview), and family history of cancer (yes or no).

†*P* for difference was computed from polytomous logistic regression by testing the difference of regression coefficients of the usage of pesticides corresponding to t(14;18)-positive versus control subjects and t(14;18)-negative versus control subjects.

‡Did not use the specific chemical class of insecticides but may still have used other herbicides or chemical classes of insecticides.

§Not reported because there were fewer than 4 exposed case subjects.

Table 4. The association of non-Hodgkin lymphoma (NHL) among farmers associated with chemical classes of herbicides according to t(14;18) status

		t(14;18)-positive NHL vs control subjects		t(14;18)-negative NHL vs control subjects		
Herbicide use	Control subjects	Case subjects	OR (95% CI)*	Case subjects	OR (95% CI)*	*P* for difference†
Never used pesticides	229	5	1.0 (referent)	23	1.0 (referent)	NA
Phenoxyacetic acids						
No‡	31	4	5.0 (1.2-20.8)	4	1.5 (0.5-4.8)	.2
Yes	180	14	2.9 (1.0-8.4)	14	0.8 (0.4-1.6)	.04
Triazines						
No‡	88	6	2.8 (0.8-9.5)	7	0.9 (0.4-2.1)	.1
Yes	114	11	3.4 (1.1-10.4)	11	1.0 (0.4-2.2)	.07
Amides						
No‡	103	7	2.8 (0.8-9.1)	8	0.8 (0.4-2.0)	.1
Yes	99	10	3.5 (1.1-11.0)	10	1.0 (0.4-2.3)	.08
Benzoic acids						
No‡	143	11	3.0 (1.0-9.0)	12	0.9 (0.4-1.9)	.07
Yes	58	6	3.5 (1.0-13.0)	6	1.0 (0.4-2.9)	.1
Carbamates						
No‡	151	12	2.9 (1.0-8.7)	11	0.8 (0.4-1.7)	.05
Yes	50	5	3.9 (1.0-14.5)	7	1.4 (0.5-3.8)	.2
Dinitroanilines						
No‡	141	13	3.7 (1.3-10.8)	11	0.9 (0.4-1.9)	.03
Yes	62	4	1.8 (0.4-7.9)	7	1.0 (0.4-2.6)	.5

NA indicates not applicable.

*Odds ratios (ORs) and confidence intervals (CIs), adjusted for age (4 levels), sex, type of respondent (direct or proxy interview), and family history of cancer (yes or no).

†*P* for difference was computed from polytomous logistic regression by testing the difference of regression coefficients of the usage of pesticides corresponding to t(14;18)-positive versus control subjects and t(14;18)-negative versus control subjects.

‡Did not use the specific chemical class of herbicides but may still have used insecticides or other chemical classes of herbicides.

t(14;18)-positive NHL were larger when nonexposed farmers were used as the referent group as compared with using nonfarmers as the referents, whereas the ORs for t(14;18)-negative NHL were essentially the same irrespective of the referent group.

Our findings of larger ORs for t(14;18)-positive NHL associated with agricultural pesticide use compared with t(14;18)-negative NHL is consistent with the findings reported by Schroeder et al.[15] The ORs for t(14;18)-positive NHL observed in the current study are also stronger than associations between the same risk factors and NHL in aggregate that were previously reported in the parent case-control study.[6,16] Overall, these findings support the hypothesis that the association between agricultural pesticide use and risk of NHL is better defined according to t(14;18) status because cases with the t(14;18) are a more homogeneous molecular group than NHL cases as a whole. This approach is consistent with findings for colon cancer, breast cancer, and leukemia in which specific molecularly defined subsets of diseases are associated with particular exposures.[23,24]

In our study, the use of insecticides and herbicides was associated with a 2.6- to 3-fold higher risk of t(14;18)-positive NHL. These results are consistent with findings from previous studies in which pesticides were associated specifically with follicular NHL,[3,9] which is usually positive for the t(14;18). Farmers who are exposed to pesticides have an increased prevalence of the t(14;18) during the high pesticide use period.[25] In addition, several studies have found that use of pesticides was more common among t(14;18)-positive individuals.[26-29] In the only other population-based study that evaluated the associations of pesticides with NHL according to t(14;18) status,[15] the aggregate exposure to insecticides was weakly associated with risk of t(14;18)-positive NHL (OR = 1.3; 95% CI, 0.8-2.0). However, exposures to organochlorines were associated with t(14;18)-positive NHL, particularly dieldrin (OR = 3.7; 95% CI, 1.9-7.0), toxaphene (OR = 3.0; 95% CI, 1.5-6.1), and lindane (OR = 2.3; 95% CI, 1.3-3.9). In the current study, risk of t(14;18)-positive NHL was also elevated among farmers using dieldrin (OR = 2.4; 95% CI, 0.8-7.9), toxaphene (OR = 3.2; 95% CI, 0.8-12.5), and lindane (OR = 3.5; 95% CI, 1.4-8.4), compared with nonfarmers.

We also found that farmers who used fumigants had a 5-fold higher risk of t(14;18)-positive NHL in comparison with farmers who never used pesticides, whereas no such association was found for t(14;18)-negative NHL. Few epidemiologic studies have assessed the association of NHL with fumigant pesticides. A 4-fold excess of NHL among flour mill employees was reported in a nested case-control study within a cohort mortality study of 22 938 grain millers who were exposed to fungicides and fumigant pesticides.[30] Garry et al[26,27] reported that fumigant applicators had a significant increase in double-strand DNA breaks at chromosomes 18q21 and 14q32, the same regions involved in the t(14;18), in peripheral blood lymphocytes as compared with control subjects. However, Schroeder et al[15] reported little association of fumigants with t(14;18)-positive NHL (OR = 1.2; 95% CI, 0.7-2.0) or t(14;18)-negative NHL (OR = 1.0; 95% CI, 0.7-1.5).

Our findings support the hypothesis that pesticides might contribute to the development of NHL through pathways involving the t(14;18). A higher prevalence of the t(14;18) has been reported among individuals occupationally exposed to pesticides.[25,26,28,29,31] Although most pesticides are thought to be nongenotoxic,[32] some pesticides have been shown to cause cytogenetic damage in laboratory assays, animal studies, or in humans.[33-37] Alternatively, pesticides may cause a second or later hit in lymphoid cells with the t(14;18), which ultimately leads to the occurrence of NHL. The t(14;18) joins the *BCL2* gene on chromosome 18 to the immunoglobulin heavy chain gene on chromosome 14, resulting in an inhibition of programmed cell death through overexpression of the *BCL2* gene.[14,38] The presence of the t(14;18) is not sufficient for the

development of NHL because it can also be found in cells that are not neoplastic.[39] However, the prolongation of survival of affected B cells could allow further accumulation of DNA damage and, consequently, development of B-cell NHL.[40] In individuals with the t(14;18), pesticides might give rise to additional changes necessary for the development of NHL. However, the nature of the effects of pesticide use and the t(14;18) in malignant transformation of lymphoid tissue remains unclear. This issue could be addressed in longitudinal cohort studies with repeated measure of both pesticide exposures and t(14;18) status. Finally, lack of variability among pesticide classes (eg, insecticides, herbicides, and fumigants were all associated with the risk of t(14;18)-positive NHL) suggests that identical chemicals within these pesticide classes or unknown factors related to pesticide use might be important.

Our findings of greater risk for t(14;18)-positive NHL associated with pesticides are consistent with those reported by Schroeder et al,[15] although there is discrepancy for some individual associations. This could be due to the use of different reference groups. We used farmers who never used pesticides as the referent group, whereas the study by Schroeder et al[15] used nonfarmers and farmers without exposures as the referent group. The ORs from use of pesticides were attenuated when the same definition for the referent group[15] was applied to our data. In addition, different laboratory methods were used in determining the t(14;18) status. The current study used the FISH technique, which is considered the "gold standard" assay for detecting the t(14;18) in tissue specimens, whereas the study by Schroeder et al[15] used PCR analysis with primers that have been reported to miss approximately 20% to 40% of t(14;18) breakpoints.[41]

Although misclassification of agricultural pesticide use is possible because study subjects were unlikely to recall previous pesticide exposure with absolute accuracy, it is unlikely that exposure misclassification, recall bias, or interviewer bias are differentially associated with the t(14;18) status. However, our findings should be interpreted cautiously because the sample size is small and risk estimates might be imprecise. In our study, information on agricultural pesticide use was obtained from proxies in about 43% of subjects. This is a concern because proxies cannot provide information on pesticide use as accurately as the farmers themselves.[42] When analysis was limited to direct respondents, the ORs were, in general, smaller than those obtained using all respondents. Nevertheless, the pattern of stronger associations with t(14;18)-positive than t(14;18)-negative NHL remained, suggesting that proxy data might account for only a portion of the observed association. Farmers may use several pesticides.[43] However, we were unable to adjust one pesticide for another because of small sample size. This would be valuable in future studies with larger sample sizes. Analysis was conducted to test an a priori hypothesis; thus, no adjustment for multiple testing was made. Consequently, individual statistically significant association should be interpreted with caution. Finally, tumor blocks were available for only 45.5% of the case subjects in the original case-control study, thus limiting study power. However, there is little evidence for selection bias because the availability of tumor blocks did not differ by pesticide exposures.

In summary, the present study found that exposures to insecticides, herbicides, and fumigants were significantly associated with a higher risk of t(14;18)-positive NHL but not t(14;18)-negative NHL. These findings support our hypothesis that the cause of t(14;18)-positive NHL differs from that of t(14;18)-negative NHL, and defining subsets of NHL according to t(14;18) status appears to be a useful approach in epidemiologic studies. Future studies should apply this approach in evaluating other risk factors for NHL.

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