Letters to the Editor

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Anticipation in Familial Chronic Lymphocytic Leukemia

To the Editor:

The term "anticipation" in genetic diseases refers to earlier age at onset and/or increased severity in successive generations. For some neurodegenerative diseases, anticipation results from expansion of unstable trinucleotide repeats in successive generations (La Spada 1997). Epidemiological studies have demonstrated a significant familial effect for leukemia (Goldgar et al. 1994) and chronic lymphocytic leukemia (CLL) in particular (Cartwright et al. 1987; Linet et al. 1989). Families with multiple affected individuals are rare in population studies but may be more common in clinical samples (Cuttner 1992). The mode of inheritance is unknown for leukemia, although it has been hypothesized that in pedigrees with multiple affecteds the disease is due to a single autosomal dominant gene (Horwitz 1997). Evidence for anticipation in familial leukemia has been reported by Horwitz et al. (1996), on the basis of a literature review of published pedigrees with acute myelogenous leukemia (AML) and CLL. The average difference, in age of onset of CLL, between two generations in seven pedigrees (17 individuals), was 15 years, although the mean parent-offspring difference was 21 years. Yuille et al. (1998) have recently confirmed this finding, in 10 families with two generations affected with CLL (mostly parent-offspring pairs) systematically ascertained from a patient registry. They found the age of onset difference between generations to be 22 years. Given that there is a molecular basis for anticipation in some diseases, it is important to determine if there is anticipation in CLL. As with other diseases, anticipation in CLL could be due to a number of well-known sampling biases (reviewed by McInnis 1996), such as the tendency to select early-onset probands, parents with late onset, and families with simultaneous onset of disease in parents and offspring, or other biases that cause a truncation of the sample of families (Hodge and Wickramartne 1995; Fraser 1997). Another bias particular to CLL may arise from the fact that individuals are often diagnosed on the basis of routine blood tests when they are asymptomatic, and they may remain asymptomatic for a number of years. It is conceivable that an anticipation phenomenon could be attributable to changes in medical practice over time, such that, because of the greater routine use of clinical tests, individuals in the younger generations are being diagnosed earlier. Data collected by the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) program during the past 20 years have shown little secular change in the incidence of CLL (SEER). In fact, recent studies that have reported an increased incidence of CLL find the increase to be limited to *older* individuals (Call et al. 1994; Rozman et al. 1997). We have been studying familial CLL for a number of years and have analyzed age at onset in pedigrees with at least two generations affected. We find evidence for anticipation in these families, even when stage at diagnosis and other potential sampling biases are taken into account.

Since 1974, we have ascertained and collected clinical data on 27 families, each of which has two or more confirmed cases of CLL (see Caporaso et al. 1991). Thirteen of these families have cases of CLL in two generations; the remainder have affected siblings and/or cousins. We have complete age-at-onset data for 32 individuals from the 13 two-generation families. One family has missing age-at-onset data on an affected parent. In the majority of cases, age at onset was determined on the basis of medical chart review, but in a few cases we relied on personal reports. Insufficient information was available to determine the disease stage for each individual, by use of either the Rai or Binet staging methods (reviewed in Dighiero and Binet 1996), but, whenever possible, we determined whether the individual was "symptomatic" or "asymptomatic" when diagnosed. We classified as "symptomatic" individuals showing signs or symptoms attributed to CLL, including lymphadenopathy, splenomegaly, anemia, and thrombocytopenia. Individuals were classified as "asymptomatic" if they presented only with a peripheral-blood absolute lymphocyte count (ALC) $\geq 5.0 \times 10^9$/liter (Zwiebel and Cheson 1998). For those individuals diagnosed asymptomatically, we also noted the age at which they became symptomatic, when this information was available in the medical chart. Individuals were classified into two gen-

Table 1

Age at Onset and Year of Diagnosis in 13 Families Investigated for Anticipation

Family and Relationship to Proband	Generation	Age at Diagnosis (years)	Symptomatic at Diagnosis?[a]	Year at Diagnosis
1:				
Father	1	69	yes	1954
Proband (female)	2	47	yes	1972
Brother	2	49	no	1971
Brother	2	47	yes	1968
Sister	2	57[b]	no	1974
2:				
Father	1	75[c]	yes	1967
Uncle	1	63[c]	yes	1955
Proband (male)	2	56	?	1978
3:				
Uncle	1	70	?	1960
Proband (male)	2	50	no	1977
First cousin (female)	2	52	no	1978
4:				
Proband (male)	1	49	?	1984
Nephew	2	53	?	1988
5:				
Mother	1	82	yes	1980
Proband (female)	2	64	?	1985
6:				
Father	0[d]	79	?	1965
Proband (female)	1	74	no	1990
Son	2	55	yes	1991
7:				
Aunt	1	58	?	1961
Proband (male)	2	44	yes	1986
8:				
Mother	1	83	no	1978
Proband (male)	2	55	yes	1983
9:				
Mother	1	32	yes	1961
Proband (female)	2	37	yes	1993
Brother	2	42[b]	yes	1995
10:				
Mother	1	58	?	1965
Proband (female)	2	52	?	1991
11:				
Father	1	76	no	1997
Proband (male)	2	51	no	1997
12:				
Uncle	1	77	?	1984
Proband (male)	2	65	no	1996
Brother	2	50	no	1994
13:				
Mother	1	?	?	?
Proband (female)	2	35	yes	1990

[a] A question mark (?) indicates that data were not available.
[b] Individual sought diagnosis because of family history.
[c] Age at diagnosis is age at death.
[d] Not included in analysis.

erations, on the basis of their position in the pedigree. Generation 2 was defined as the youngest generation affected; the generation preceding them was considered to be generation 1. All affected individuals in each family were included in the analysis. There was one family with three generations affected; the individual in the oldest generation was not included in the analysis. The age-at-onset data for all individuals are displayed in table 1. In 11 of the 13 families, the proband was in the younger generation.

We found that the average age at onset in generation 1 is 66.7 years (SD 14.6) and that that in generation 2 is 50.7 (SD 7.8). These onset ages are similar to those reported by Horwitz et al. (1996). We used survival analysis to plot the age-at-onset distribution for each generation, using the Kaplan-Meier method as implemented in the SAS Lifetest procedure (Allison 1995). The results are displayed in figure 1. The difference between the two generations is highly significant, whether based on the log-rank test ($P = .0001$) or the Wilcoxon test ($P = .0009$).

We examined the generational differences after taking into account a number of possible biases. In this small sample, there were no significant age-at-onset differences between males and females, and each generation had an approximately equal proportion of males and females. We scored the individuals for whether they were symptomatic, asymptomatic, or unknown at diagnosis. The proportion of symptomatic individuals did not differ by generation (5/13 [.38] in generation 1 and 8/19 [.42] in generation 2; see table 1). In addition—after family 9, in which all members had unusually early ages at onset, was excluded—there was no difference, between symptomatic and asymptomatic individuals. There were two individuals in the sample who sought diagnosis because of their family history. These two individuals, indicated in table 1, had ages at onset of 57 and 42 years, so eliminating them would not change the findings. In order to analyze our data in the most conservative way possible, we increased the age at onset for five individuals (generation 2) who were asymptomatic at diagnosis but whose age at onset of clinical symptoms could be determined. In addition, there were two individuals from generation 1 who were diagnosed on the basis of death certificates and who were assigned onset ages equal to their ages at death. We lowered these individuals' ages at onset by 7 years, since this is the median time between age at onset of symptoms and death (Zwiebel and Cheson 1998). Even under these conservative assumptions, the age at onset in the second generation was significantly lower than that in the first generation (log-rank test $P = .0002$). It should also be noted that, even though we did not analyze all three generations in the three-generation family, the proband's father (generation 0) had a later age at onset than did either the proband or her son. We also considered the possible bias due to preferential ascertainment of families with simultaneous onset in two generations. In 5 of 12 families for which complete data were available, individuals in both generations were diagnosed within a 5-year period (table 1). In the seven other families, the average difference, in calendar year of diagnosis, between generations was 21 years, and the average difference, in age at onset, between generations was 13 years. Thus, our finding of anticipation is not being driven by the presence of families with simultaneous onset in two generations.

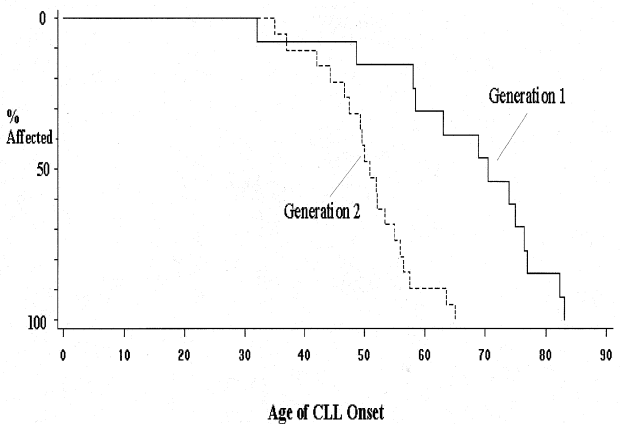

Figure 1 Age at onset of CLL, in two generations

We find that, between the two generations in the families we studied, the average decrease in age at onset of CLL was 16 years. One could argue that we have preferentially ascertained early-onset probands. The average age at onset in all 27 of the families that we studied is 54.9 years for probands only and 59.6 years for all affecteds. The average age at onset of CLL has been reported to be ~70 in some population-based samples (Travis et al. 1992; Hjalmar et al. 1996) but ~62–65 in other studies (Radovanic et al. 1994; Rozman et al. 1997). Thus, probands from the multiplex families that we studied have a somewhat decreased age at onset, compared with that in cases from the population. However, for this analysis, we included all affected individuals in a family, which decreases any effect due to earlier onset in the index case. We cannot completely rule out the possibility that anticipation in these families is due to a cohort effect, since, in all of the families that we studied, birth cohort is confounded with generation. As is to be expected from the way in which pedigrees are usually ascertained in genetic studies, the second generation of individuals are from cohorts born more recently than those of the first generation. However, a cohort effect seems unlikely, given the lack of secular trends in CLL, as mentioned above. The age at onset of CLL is also sufficiently late in life that it would not affect fertility, and we are not likely to have missed families with early onset in parents and later onset in offspring. Horwitz et al. (1996) suggested that the very large difference (21 years) that they found between parents and offspring in published families was also consistent with the effect of some common environmental exposure. There is no well-established environmental risk factor for CLL, although susceptibility to some environmental risk factor might exist in subjects with some unidentified

genetic susceptibility factor. Although we cannot rule out such an effect, we find a substantial but smaller age-at-onset decrease between generations. We also find the decrease to be present in families that are not correlated for year of diagnosis, arguing against a purely environmental explanation.

In conclusion, we report significant evidence for anticipation in familial CLL and have ruled out various biases that could account for the finding. Therefore, we plan to look for expanded trinucleotide repeats in candidate genes in families showing anticipation.

LYNN R. GOLDIN,[1] MARIA SGAMBATI,[1] GERALD E. MARTI,[2] LAURA FONTAINE,[3] NAOKO ISHIBE,[1] AND NEIL CAPORASO[1]

[1]*Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD;* [2]*Center for Biologics Evaluation and Research, Food and Drug Administration; and* [3]*Westat Research, Inc., Rockville, MD*

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Address for correspondence and reprints: Dr. Lynn R. Goldin, Genetic Epidemiology Branch, DCEG, NCI 6120 Executive Boulevard, Room 7008, MSC 7236, Bethesda, MD 20892-7236. E-mail: goldinl@exchange.nih.gov

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