

## Clinical Characteristics of Familial B-CLL in the National Cancer Institute Familial Registry

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In an ongoing study, families with two or more living cases of B-CLL in first-degree relatives have been recruited through physician and self-referral. Since 1967, 28 kindreds with 73 cases of B-CLL have been enrolled within the National Cancer Institute (NCI) Familial B-CLL Registry. Medical, clinical, and demographic information have been obtained from private physicians, patient interview, hospital records, and death certificates. We used SEER Registry data to compare characteristics of sporadic B-CLL to familial B-CLL. The mean age at diagnosis was approximately 10 years younger among familial cases ( $57.9 \pm 12.1$ ) than that observed in sporadic cases ( $70.1 \pm 11.9$ ). A higher percentage of second primary tumors among familial CLL cases compared to reports in sporadic was also observed (16% vs. 8.8%). However, the transformation rate to non-Hodgkin's lymphoma does not appear to be different from that reported for sporadic cases. In conclusion, we observed some differences between familial and sporadic cases; whether any of these characteristics affect survival time or severity of disease is unknown. The study of families with multiple B-CLL cases will aid in delineating the genes and environmental factors that may play a role in the development of both forms of B-CLL.

**Keywords:** pedigrees, familial, B-CLL, sporadic, descriptive, clinical

### INTRODUCTION

B-cell chronic lymphocytic leukemia (B-CLL) is a neoplastic disease characterized by the accumulation of small, mature-appearing lymphocytes in the blood, bone marrow and lymphoid tissues [1;2]. It is the most common adult leukemia in Western countries, accounting for 30% of all leukemia cases [3]. Cancer registries indicate a 10-fold variation in international

incidence rates, with the lowest rates observed in Asian populations and the highest among people of European descent [4]. Data from the United States Surveillance, Epidemiology and End Results (SEER 1973-95) Registry estimate U.S. incidence to be 3.1/100,000 with a median age of diagnosis of 70 years. The clinical picture of B-CLL is extremely variable with some patients having very indolent disease with survival greater than 10 years, while others

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have rapidly progressive disease that is poorly responsive to treatment [1].

Although advanced age, Caucasian ancestry, and family history of hematologic malignancies [5] are recognized risk factors, [6–8] the etiology of B-CLL is unknown. Environmental and occupational exposures, such as pesticides [9], magnetic fields [10], farming and animal breeding [11], and viruses [12;13], have received attention as possible etiologic agents of B-CLL; however, associations with these exposures have been weak and inconsistent.

In contrast, familial clusters of B-CLL are consistently observed [14–21]. A comprehensive study by Gunz *et al* [20] found that first-degree relatives with leukemia were much more frequent in families of patients with B-CLL than in families with other types of leukemia.

We describe a longitudinal study of B-CLL families that have been recruited by the National Cancer Institute (NCI) between 1967 and 1999 and examine differences between familial and sporadic B-CLL. Although numerous case reports of familial CLL exist in the literature, we believe this is the first report of a series of CLL families followed over time.

## MATERIALS AND METHODS

### Study Population

Families with two or more living cases of B-CLL in first-degree relatives in one or more generations were recruited either by physician or self-referral. Our recruitment at the National Institutes of Health (NIH) has been ongoing from July 1967. We discuss and analyze here 28 families with 73 affected individuals enrolled in the study of familial B-CLL. Relatives greater than second-degree in relation to the proband have been enrolled on occasion; however, only affected individuals and their first-degree relatives ( $n=499$ ) have been included in this report since data may be less reliable and less complete for more distant relatives.

All subjects signed informed consent in order to participate; medical records were obtained and biological specimens donated. Subjects also responded to a family studies questionnaire and were offered the opportunity for clinical evaluation and referral. The family studies questionnaire is a 19 item self-administered booklet with information on the respondent's demographics, gender, race, national origin, marital status, religion, individual medical history including cancer diagnoses and medical diagnoses on first and second-degree relatives.

### Data collection

Medical and clinical information was obtained from private physicians, hospital records, death certificates, and in most cases, a clinic visit to NIH. With the exception of one case, all cases had an existing diagnosis of B-CLL prior to referral to the NIH. All B-CLL cases were confirmed by one or more of the following methods: physician's report, pathology report, NIH slide review, death certificate, self-report or report by personal relation. Second malignancies and transformations were confirmed by obtaining a pathology or a physician's report or conducting NIH slide review. For those cases that we examined, routine clinical studies conducted on the first clinic visit included: complete blood counts, automated chemistries, quantitative serum immunoglobulins, urinalysis, chest x-ray, and electrocardiogram. Ultrasound or computed tomography (CT) imaging of the abdomen was often done to assess hepatosplenomegaly. Further clinical studies were obtained through follow-up visits on a subset of individuals. Cryopreserved biospecimens (lymphocytes, EBV transformed cells, buccal smears as a source of non-tumor DNA) have also been collected on 45 (61.6%) affected individuals and in many cases on unaffected members of a given kindred.

### Pedigree definition

In order to facilitate discussion of relatedness between family members, all bloodline members will

be defined from the designated proband in each family. Individuals included for analysis of other cancers in the family were first degree relatives of a case.

### Staging Definition

The modified Rai Staging system [22] was used to classify stage at diagnosis. When staging information was not explicitly stated, stage at diagnosis was retrospectively determined using all available clinical information (WBC, hemoglobin or hematocrit, platelet count, absolute lymphocyte count, presence of lymphadenopathy, or hepatosplenomegaly at diagnosis). Individuals for whom stage could not be determined were excluded from all stage analyses.

### Statistical Analysis

Information on age at diagnosis, race/ethnicity, transformation rate, and gender in sporadic B-CLL cases was obtained from the Surveillance, Epidemiology, and End Results (SEER) Program Public-Use CD-ROM (1973–1995) available from the National Cancer Institute using SEER-Stat [23]. Means and frequencies for continuous and categorical variables were performed with the SAS software package (SAS Institute Inc., Cary, NC).

## RESULTS

### Characteristics of Study Subjects

This paper describes 28 kindreds within the NCI B-CLL Family Registry. Each kindred has two or more affected individuals (range: 2 – 5 cases per family) resulting in a total of 73 cases of B-CLL among the kindreds. The majority of these families ( $n=21$ ) were recruited between 1990–1999. More specifically, the diagnosis of CLL was established on 27 (37%) cases by flow cytometry data analysis done on peripheral blood at the NIH. In all of these cases, the flow markers were consistent with B-CLL (CD5, CD19, CD20, CD23 positive). Another 7 cases (10%) had a lymph node biopsy or peripheral blood film

reviewed at the NIH; 21 (29%) had documentation by an outside physician report; 5 (7%) by death certificate or autopsy, and 4 (5%) by outside pathology report. Among the other 9 cases, diagnosis was obtained from self-report or report by a relative. In these subjects, further verification is either pending or unobtainable (subjects have died, hospital or physician records are lost or unattainable, or in some cases, individuals elected not to further participate in the study). Initial diagnostic information for staging purposes was available on 43 cases.

In two cases a presumptive diagnosis of B-CLL was made based on clinical symptoms, peripheral blood findings, bone marrow biopsy and lymph node biopsy, although the total absolute lymphocyte count (ALC) did not exceed  $5 \times 10^9$  / Liter. These may represent cases of small lymphocytic lymphoma (SLL) or B-CLL cases with low lymphocyte counts (CLL-LLC) [24].

All affected subjects enrolled were of Caucasian-descent. Forty-two of the 73 affected individuals were men (57.5%) and 31 were women (42.5%), resulting in a male to female ratio of approximately 1.35:1. This ratio is similar to the ratio observed in sporadic CLL from the SEER registry (1.40:1). Average age at onset in this study population was 57.9 ( $\pm 12.1$ ) years, compared with 70.1 ( $\pm 11.9$ ) years in sporadic cases. A greater percentage of cases were diagnosed  $\leq 50$  years of age (31.3%) compared to sporadic cases (5.7%) ( $p=0.001$ ). Among 43 cases with stage at diagnosis information, 19 (44.2%) were low stage, 22 (51.2%) were intermediate stage, and 2 (4.7%) were high stage. Table I summarizes these findings.

### Pedigree Descriptions

We did not observe any obvious single pattern of inheritance in our kindreds. For the purposes of description and discussion, we categorize our 28 pedigrees into 3 groups: 1) families with affected siblings only, 2) families with affected parent-offspring relationships only and 3) families with combinations of both the above or other type of relationships of the individuals affected with B-CLL. Representative pedigrees are shown in Figure 1.

TABLE I Descriptive characteristics of familial B-CLL cases (n=73) and sporadic B-CLL cases (SEER)

Variable	NIH Registry Mean ( $\pm$ SD)	SEER Registry Mean ( $\pm$ SD)
Age at diagnosis <sup>a</sup>	57.9 ( $\pm$ 12.1)	70.1 ( $\pm$ 11.9) <sup>b</sup>
Male	57.4 ( $\pm$ 10.9)	68.7 ( $\pm$ 11.7)
Female	58.7 ( $\pm$ 14.0)	72.1 ( $\pm$ 11.8)
Percentage cases diagnosed $\leq$ 50 years of age <sup>c</sup>	31.3%	5.7%
Gender		
Male	42 (57.5%)	10,594 (58.4%)
Female	31 (42.5%)	7,558 (41.6%)
Stage at diagnosis <sup>d</sup>		
Low (0)	19 (44.2%)	NA <sup>e</sup>
Intermediate (I/II)	22 (51.2%)	NA
High (III/IV)	2 (4.7%)	NA

a. Six cases were missing information on "age at diagnosis".

b. Excluded individuals who were diagnosed before the age of 25

c.  $p$ -value = 0.001

d. Modified Rai Staging system [22]; 30 cases missing information on stage at diagnosis.

e. Stage at diagnosis information unavailable in SEER Registry.

The most common pattern of affected family members was families with only affected siblings. Twelve families with a total of 72 siblings had 26 cases of B-CLL. The proportion of affected siblings is 36% (26 of 72) among this category of families. Of these 12 families, 10 had a pair of siblings affected and 2 had multiple siblings affected. Two families had affected females only, 4 families had affected males only and 6 families had a mix of affected males and females (with an overall total of 14 male cases and 12 female cases). There was one set of male monozygotic twins who were diagnosed with B-CLL within one year of each other. Mean age of onset for this category was 61.5 ( $\pm$ 11.9).

The second category includes multigeneration families with affected parent-offspring relationships only. In this parent-offspring category there were 9 families with 24 cases of B-CLL. There was one 3 generation family with an affected grandfather, mother and son; the remaining families had two generations affected. In these kindreds, the gender of the affected parent was twice as likely to be female rather than male. Counting the three-generation family as two parent-offspring pairs, seven affected mother-offspring pairs and three affected father-offspring pairs were observed. The mean age at diagnosis for this category

was 56.5 ( $\pm$ 17.3), with a mean age of 70.0 ( $\pm$ 16.9) for the parents and 50.5 ( $\pm$ 10.3) for the offspring.

The third category was a combination of the first two categories or other types of relationships among the individuals affected with B-CLL. This includes families with a parent-offspring affected plus another relative (e.g. nephew) or an affected sib-pair and another relative (e.g. uncle). Six of the seven families had at least two B-CLL cases that were first-degree relatives and the other cases in the kindreds were a mix of first and second-degree relatives in each family. Within these kindreds there were 4 parent-offspring cases: 3 father-offspring pairs and 1 mother-offspring pair. The other family had three cases of B-CLL, all of which were related as second or third-degree relatives. Seven families with 23 cases (32%) fit this category and the mean age at diagnosis was 56.5 years ( $\pm$ 9.0).

### Solid Tumors in B-CLL cases

Fourteen (19%) B-CLL cases had other solid malignancies (excluding basal cell carcinoma) that were diagnosed either prior to, simultaneously or following the diagnosis of B-CLL (Table II). The most common second tumors were carcinoma of the bladder (n=3)

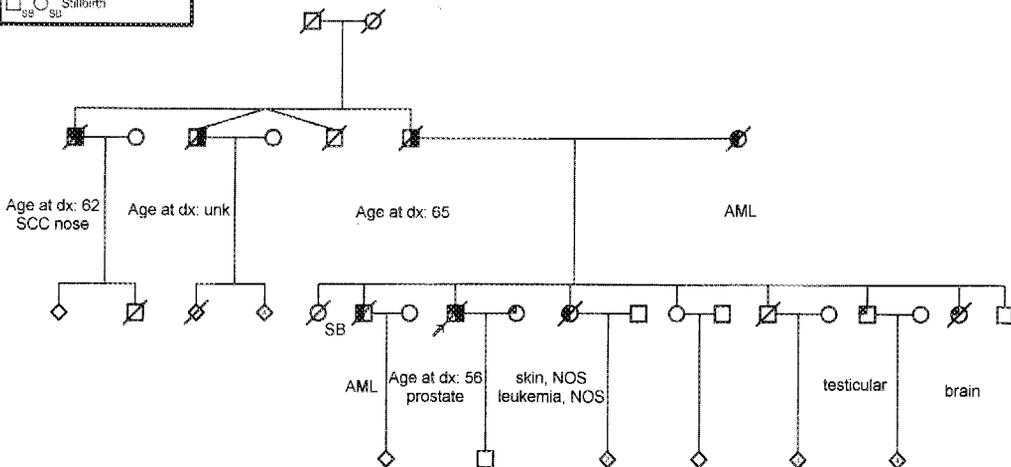
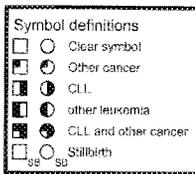
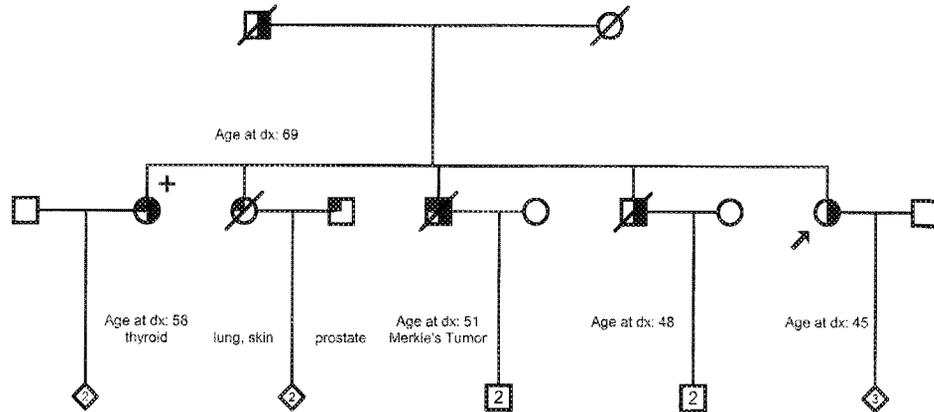
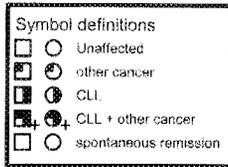


FIGURE 1 (a and b) – Representative Pedigrees from CLL Families. AML – Acute Myelogenous Leukemia SCC – Squamous Cell Carcinoma NOS – not otherwise specified (See Color Plate at the back of this issue)

and squamous cell carcinoma of the skin (n=3). There were 16 total solid tumors among the 14 cases with two individuals developing two different solid

tumors. Three solid tumors were diagnosed prior to the diagnosis of B-CLL, with the mean time interval between the two diagnoses being 7.7 ( $\pm 2.6$ ) years.

Twelve of the solid tumors were diagnosed after B-CLL. The mean time from diagnosis of B-CLL to diagnosis of the second solid tumor was 8.5 ( $\pm$  4.1) years ( $n=11$ ). The age distribution for the second tumors did not appear to differ from the expected age distribution for these tumor types. Interestingly, the 14 cases with second tumors were clustered among 9 families. There was no difference in age of onset of B-CLL between these 9 families and kindreds who only reported B-CLL. However, differences in gender distribution in B-CLL cases were observed between these two categories. The ratios observed were 0.85:1 (male to female) for the CLL only families and 2.43:1 for the families with second tumors. Two individuals with second solid tumors (one squamous cell carcinoma and a Merkle cell tumor) had received chemotherapy for B-CLL consisting of alkylating agents in the interval between the diagnosis of B-CLL and the second solid tumor. On 4 of the second tumors there was no available data on treatment, and the remaining cases (7) had no treatment in the interval between diagnoses.

### Malignancies in First-Degree Relatives of B-CLL Cases

Among the first-degree relatives of B-CLL cases, 6.2% (31/499) had reported solid tumors (Table III). The most commonly reported solid tumor (excluding skin cancer, not otherwise specified) was prostate cancer (6 cases), followed by gastric cancer (3 cases). The age distribution for tumor type did not appear different from expected and there were 17 males and 13 females with solid tumors. These 31 solid tumors (one female had two solid tumors) occurred among 30 individuals in 14 kindreds. Of note, seven of these kindreds were the same families that reported second solid tumors in B-CLL cases. There were 10 reported cases of hematopoietic malignancies in first-degree relatives in seven kindreds: 5 cases of leukemia and 5 cases of lymphoma, all of which were non-Hodgkins. One kindred had 3 cases of leukemia among first-degree relatives: myelogenous leukemia (NOS), acute myelogenous leukemia and lymphocytic leukemia, NOS (Table III and Figure 1-b). In these first-degree relatives, there was a slight predominance of men, 1.5:1 (male to female).

TABLE II Solid Tumors in Familial B-CLL Cases

<i>Solid Malignancies</i>	<i>Total Number Diagnosed Among Familial B-CLL Cases</i>	<i>Number of Cases Diagnosed after B-CLL</i>	<i>B-CLL Treatment<sup>a</sup></i>
Bladder	3	2	No
Colon	2	2	No / N/A
Lung	2	1	No
Prostate	2	2	No/N/A
Thyroid	1	1	N/A
Skin (excluding basal cell)			
Melanoma	2	1	N/A
Merkle Cell	1	1	Yes
Squamous Cell	3	3 <sup>b</sup>	Yes (1)
<i>Hematopoietic Malignancies</i>			
Acute Myelogenous Leukemia	1	1	Yes
Acute Lymphocytic Leukemia	1	1	N/A
Non-Hodgkins Lymphoma	4	4	Yes (2)

N/A – not available.

- a. If the individual received treatment for B-CLL at the time of second tumor diagnosis.  
 b. 1 case of Squamous Cell Carcinoma was diagnosed simultaneously with B-CLL.

TABLE III Malignancies in First-Degree Relatives of Familial B-CLL Cases

<i>Solid Malignancies</i>	<i>Number of Cases Among First Degree Relatives</i>	<i>Ages at Diagnosis</i>
Brain, NOS	2	24, N/A
Breast	2	51, N/A
Colon	2	69, 69
Eye, Melanoma	1	53
Gastric	3	62, 73, 73
Kidney, Renal Cell	1	60
Lung		
Adenocarcinoma	1	58
Squamous Cell Carcinoma	1	66
Mesothelioma	1	58
Ovarian	1	63
Pancreatic	2	60, NA
Prostate	6	64, 89 (4 N/A)
Testicular, NOS	1	
Skin (excluding basal cell)		
Melanoma	1	81
NOS	6	49, 62, 75, 88 (2 N/A)
<i>Hematopoietic Malignancies</i>		
Leukemia		
Acute Myelogenous	2	46, 59
Myelogenous, NOS	1	63
Lymphocytic, NOS	1	24
Hairy Cell	1	N/A
Lymphoma	5	
Non-Hodgkin's		
T-cell	1	N/A
B-cell	4	39 (3 N/A)

NOS – not otherwise specified. N/A – not available.

### Transformations

Four individuals (6%) had a disease transformation from B-CLL to non-Hodgkin's lymphoma (NHL) and two individuals with B-CLL transformed to acute leukemia. Two of these individuals were affected sisters. The pathological subtypes of NHL were diffuse histiocytic, large cell lymphosarcoma (two sisters), immunoblastic and reticulosarcoma. The mean interval from time of diagnosis of B-CLL to time of transformation was 31.5 ( $\pm 16.6$ ) months. Two of the four

patients had received treatment for their B-CLL prior to transformation: one patient received chlorambucil and cyclophosphamide and the other received 2-chlorodeoxyadenosine. Two individuals with B-CLL underwent transformation to acute leukemia – one to acute myeloid (AML) and one to acute lymphoid (ALL). The individual who developed AML had received treatment with cyclophosphamide, prednisone and total body irradiation for progression of B-CLL, 9 years after the initial diagnosis. One year

after treatment, this individual developed acute leukemia. Details on the individual with the transformation to ALL were not available.

### Spontaneous Remissions

One individual in our kindreds appears to have had a spontaneous remission. An affected female sibling with 3 affected siblings and an affected parent was diagnosed with B-CLL (Rai Stage II) in 1974. Evaluation at the NIH revealed axillary lymphadenopathy, splenomegaly (confirmed by abdominal radiograph) and a WBC of 32,200 with 63% lymphocytes and many smudge cells. Bone marrow biopsy demonstrated 60% small lymphocytes. She was followed without treatment, and in 1975, her peripheral WBC was 6500 with 28% lymphocytes. Data on the status of the lymphadenopathy or splenomegaly was not available at this time. In 1981, FACS (fluorescent activated cell sorting) analysis showed no B-cell clone. In 1999, a repeat evaluation revealed no lymphadenopathy or splenomegaly on clinical exam. Abdominal ultrasound revealed the spleen to be normal size. Total WBC was 4,400 with 29% lymphocytes. Flow cytometry was repeated with a large panel of markers, including CD19, CD20 and CD22 for T, B and NK cells, which were all normal. The kappa lambda analysis was carried out with several different reagents, and a predominance of lambda bearing cells that were estimated to be less than 1% of her total lymphocytes was observed. This was interpreted as minimal residual disease.

### DISCUSSION

The twenty-eight kindreds described here, along with families reported previously, clearly identify familial aggregation of CLL. We have not identified any common extrinsic factor in the families, and epidemiological studies investigating potential risk factors of B-CLL have been unable to identify any major exposures that may account for this disease. Only Caucasian ancestry, increasing age, and family history of

hematological disorders have consistently been observed to be associated with the development of B-CLL. In addition, no studies have assessed potential differences between sporadic and familial CLL. In this study of 28 B-CLL families with 73 affected individuals, differences between familial and sporadic B-CLL were observed.

Familial B-CLL may differ biologically from sporadic B-CLL. In this study, an earlier age of onset than that reported for sporadic B-CLL was observed. While earlier age at onset is often observed in conditions where heredity plays a role in susceptibility, this may also be attributable to a screening bias, where families with a history of B-CLL are screened at a younger age. The finding of an earlier age of onset may also be due to the phenomenon known as anticipation, which has been previously reported in our families [25] as well as other CLL families [26;27]. However, the mean age of onset in families with only affected siblings is 61 years old, which is still younger than the age of onset observed in sporadic cases.

A higher percentage of second primary tumors among familial CLL cases was observed compared to reports in sporadic CLL. A second tumor frequency of 19% was twice as high as the 8.8% rate observed in a study of sporadic B-CLL cases in the SEER Registry [28]. In SEER, nearly 75% of second cancers in sporadic B-CLL cases occurred within 4 years of B-CLL diagnosis, whereas only 25% of our familial cases developed a second primary tumor in the same time interval. This difference in the time to second tumor development may be due to an earlier stage at diagnosis observed in our familial patients.

The pattern of second tumors also appears to be different from that reported for sporadic cases. Travis [28] observed an increased number of cases of Hodgkin's disease (HD) following CLL, whereas we observed no cases of HD in our familial CLL cases. In addition, three cases of bladder cancer were observed in our kindreds; however, no significant increase in this tumor type has been seen in sporadic cases [28;29]. The transformation rate to NHL does not appear to be different from that reported for sporadic cases [30].

It is interesting that the second tumors in cases and malignancies in first-degree relatives unaffected with CLL appear to cluster in kindreds. Fourteen of the 28 families did not report second tumors in affected members or any other malignancies in first-degree relatives (i.e., "pure" CLL families). While this may suggest the possibility of a common genetic defect predisposing the kindred to the development of multiple cancers, the clustering observed may also be due to a reporting bias, a shared exposure or lifestyle risk factor or extended follow-up time in earlier identified kindreds. It may be that some families openly discuss health issues and are more aware of cancer diagnoses in extended family members, or undergo more frequent exams or screening. In addition, this difference in cancer pattern may be attributable to a participation or referral bias.

Spontaneous remissions in CLL have been reported, [31;32] but are thought to be infrequent. We report one such instance in our kindreds. In 1999, clinical reevaluation showed no evidence of CLL, however flow cytometry revealed a pattern felt to be consistent with residual disease or B-cell monoclonal lymphocytosis (BCML) [33]. While the significance of this finding in an individual with a previous history of CLL is unclear, the stable clinical status of this individual over 25 years seems to suggest a benign clinical picture.

There is no accepted specific precursor, such as a laboratory finding or a clinical syndrome, to identify familial CLL. BCML is a presumptive laboratory finding of monoclonality detected by flow cytometry in the presence of a normal white blood count. Guidelines for the definition of BCML have been proposed: 1) lymphocytes are 50 per cent or more B cells; or 2) the absolute number of B-cells is 1,000 cells per ml or greater; or 3) CD5 positive cells are 50% or greater, and 4) light chain restriction and 5) evidence of Ig gene rearrangement [34]. However, the precise definition, natural history, incidence and prevalence are under investigation. Currently, it is unknown whether BCML will resolve spontaneously, remain stable indefinitely or progress into B-CLL. A longitudinal, clinical study by Faguet [33] of individuals with apparent BCML, found that 32% (8/25) of these patients progressed to clinically recognizable B-CLL over a time period of 6–71 months. We are currently planning a more extensive study to better characterize

the pattern and prevalence of these markers in the NCI B-CLL families.

BCML may represent a pattern that confers a higher probability of progression to disease or it may simply be a characteristic of high-risk families. Further family and population-based studies will be required to better understand the prevalence of BCML and its relationship to B-CLL and other lymphoproliferative diseases.

There are many biases inherent to the study of families. A recruitment bias exists due to referral patterns (e.g., physician's knowledge of familial CLL study, access to the Web). Ascertainment of the families was not performed in a systematic manner, i.e. from a defined population using systematic sampling. Therefore, it is not possible to estimate the frequency of familial CLL from these data. The information collected on each study participant was also dependent on his or her willingness to participate; hence, the degree to which data is complete on each subject and kindred is different. In addition, the completeness of the data collected by patient report for a kindred is influenced by communication patterns within a family. These features limit our ability to infer a mode of inheritance, but the families comprise an invaluable resource for the discovery and characterization of genes that may contribute to familial CLL.

The strength of this study is the collection of biological specimens over a 25-year period and the continued cooperation of the existing families and ongoing recruitment of new families. The current data suggest potential differences between familial and sporadic B-CLL and emphasizes the importance of studying families. Our families and the collected biological specimens provide an ideal opportunity to conduct whole genome searches and to study candidate genes as well as other biomarkers of interest in investigating the etiology of CLL.

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