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Familial Chronic Lymphocytic Leukemia in Norway and Denmark. Comments on Pleiotropy and Birth Order

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Abstract. *Aim: To investigate the genetics of chronic lymphocytic leukemia (CLL). Materials and Methods: In 56 (7%) out of 800 CLL patients with concomitant malignant hematological disease, 51 families and 141 cases were ascertained. Result: 106 cases (75%) of CLL, 27 cases (19%) of nonCLL and 8 cases (6%) of myeloproliferative disorders. Paternal disease was transmitted primarily to the youngest sons in the sibship while maternal disease was transmitted equally to all sibs, demonstrated by means of matrix conjugation and confirmed with Cox regression on parity and birth order (maternal-offspring combination: relative risk (RR), 95% confidence interval (CI)=1.47 (0.89 – 2.43), p=0.12, compared with paternal-offspring combination: RR=3.25, 95% CI=(1.57–6.72), p<0.001). The B-cell expression in familial and sporadic CLL was indistinguishable. Conclusion: Parental genomic imprinting is pointed out as one possible mechanism behind this non-Mendelian genomic output.*

Chronic lymphocytic leukemia (CLL) and related lympho-proliferative disorders (LPD) such as the other lymphoid leukemias, lymphomas, myeloma and the subclinical conditions, monoclonal gammopathy of uncertain significance (MGUS) and monoclonal B-lymphocytosis (MBL) constitute an inheritable entity, reflected by the familial occurrence of these disorders (1-9). Ethnical variation in predisposition to CLL and other subtypes of LPD supports the concept of a genetic entity (10, 11). Six susceptibility loci for CLL have recently been identified: 2q13, 2q37.1, 6p 25.3, 11q 24.1, 15q 23, and 19q13.32 (12), but the mode of transmission of the susceptibility genes from generation to generation is as yet largely unknown, without evident signs of a traditional Mendelian segregation (*e.g.* dominant, recessive, X-linked inheritance) from genealogical interpretation of pedigrees.

In the present paper, we present data on the genetics of CLL relating pleiotropy (4-6, 9, 3, 14) (*viz.* the polymorphism in the inheritance of CLL/LPD) to a birth order effect (15-17) (viz. a non-random occurrence of affected sibs in a sibship) where a distinct maternal and paternal pattern of transmission to offspring is seen in affected families from Norway and Denmark.

Materials and Methods

Patients. Eight hundred and twenty-four CLL patients have been interviewed during the past five years in Oslo and Copenhagen. Twenty-four patients left the cohort without follow-up so that 800 patients constitute the final sample size. Each CLL patient underwent a face-to-face interview about other family members with CLL or any other malignant hematological disease and the family tree was drawn up. The interviews were individually adjusted and modulated to include old terminology if necessary, lymphogranulomatosis and lymphosarcoma, for example, have routinely been mentioned to ensure maximal ascertainment of familial cases. Each patient was asked about the number and positions in the family tree of healthy members, stillborns and extramarital individuals. All patients were allowed sufficient time for discussion with other family members. Each patient was informed about the purpose of the study, that data were confidential and unrecognisable outside the study and that the study was approved by the Scientific-Ethical Committees and the Data Protection Agencies in Norway and Denmark.

Contribution: VJ, GET, TBJ, BL and JHO collected and cross-checked data in Norway and Denmark. All authors contributed to the interpretation of data. VJ wrote the draft based on invaluable inputs from all authors. None of the authors have financial interest involved.

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Information provided by patients was validated by crosschecking information with the Cancer Registry in Denmark or Norway in all cases. Hospital records, and review of histopathological and laboratory reports including information from flow cytometry and cytogenetics were crosschecked when available. All diagnoses were based on standard criteria (18-22). CLL was confirmed by flow cytometry, fluorescence *in situ* hybridization (FISH) cytogenetic characterization and estimation of the immunoglobulin heavy chain V (IgHV) status in all patients alive at the time of investigation. All persons investigated were Caucasians and all families were of Scandinavian or European origin.

Inclusion. Sampling of families and pedigrees: Fifty-one families with malignant hematological disorders in two or more family members were identified. These families were detected from 56 index CLL patients, *viz*. patients among the 800 CLL who had one or more affected family members, where 3 out of the 56 patients belonged to the same family. In 3 instances, 2 patients happened to be members of the same family. To obtain consecutive data, 6 previously reported families were included: 5 families were part of a sole birth order estimation (17) and 1 family was described as a case history (23) but none of data regarding the 51 crosschecked families have been published before as part of a comprehensive genetic analysis. For the description of pleiotropy (Table I), all subtypes of hematological malignancies in the 51 families were recorded and grouped into CLL, other types of lymphoproliferative disease (LPD), designated nonCLL, or myeloproliferative disease (MPD). In this study the few cases of myelodysplasia (MDS) were grouped together with MPD.

Scoring of data for interpretation of segregation: The affiliation of each affected family member in all 51 pedigrees were grouped into the following categories: (A) parent-offspring pairs and (B) grandparent-parent-offspring combinations (vertical inheritance); (C) aunt/uncle-nephew/niece combinations (oblique inheritance); and (D) sib concordance, which denotes two or more affected siblings without other affected relatives (horizontal inheritance).

The number of pairs of affected family members between two or more generations in A, B and C, and the total number of affected sibs in D, all related to the pleiotypic diagnoses, were recorded (Tables II and III). Furthermore, the age at onset of disease, the sex of patients and healthy family members, and the range of affected family members in the sibship were recorded (Tables IV-V, Figures 1-4).

Where ascertained pairs do not comprise CLL, as for example the lymphoma-myeloma parent-offspring pair in skipped generation pedigree no 5 (Figure 2), or the CML-AML grandparent-offspring pair shown in the same pedigree, an index CLL patient will always be seen at another place in the pedigree, and no criteria other than index CLL have been used for entry.

Since each pair of affected family members (parent-offspring, grandparent-parent-offspring, aunt/uncle-nephew/niece, and sib-sib combinations) was counted each time it was independently ascertained, inevitable duplicates cause a difference between the number of patients in pairs and the real number of patients included (Tables II and III). This difference arises each time an affected family member is part of more than one pair, for example when an affected parent has two affected offspring giving two parent-offspring pairs, or in cases when two pairs, grandfather-parent and parent-offspring, are scored from a grandparent-parent-offspring combination. Furthermore, doublets of families occur in larger families contributing multiple combinations of affected family members, for example both a parent-offspring pair and an uncle-nephew pair.

Table I. *Lymphoproliferative and myeloproliferative diagnoses in 51 families with familial CLL.*

CLL n=106	NonCLL[a] n=27	Myeloproliferative disorders n=8
	5 Diffuse large B cell lymphoma	4 [b] Polycythemia vera
	4 [c]Follicular B cell lymphoma	2 Myelodysplasia
	4 B cell lymphoma, NOS	1 AML
	4 Multiple myeloma	1 CML
	3 Hodgkin's lymphoma	
	2 Pre B ALL	
	1 Lymphoplasmacytic lymphoma	
	1 Waldenström's disease	
	1 IgM MGUS	
	1 T-cell PLL	
	1 Diffuse small T-cell lymphoma, NOS	
Healthy family members in 51 families with familial CLL (n=523)		

[a]NonCLL denotes lymphoproliferative disorders other than CLL. [b]Two of the PV patients were JAK2 V617F negative, one was JAK2 V617F positive, and the fourth PV patient died before investigation. [c]Stage I, II and III together. ALL, Acute lymphoblastic leukemia; AML, acute myelogenous leukemia; CLL, chronic lymphocytic leukemia; CML, acute myelogenous leukemia; MGUS, monoclonal gammopathy of uncertain significance; NOS, not otherwise specified; PLL, prolymphocytic leukemia.

The doublets were carefully recorded (Tables II and III) and only used for the identification of pairs between generations, and not for the estimation of the total number of affected family members, frequencies or the number of families involved.

Cumulative data from the 51 pedigrees were sorted into the following groups.

Group 1. LPD, parent-offspring combination: One affected parent: Ninety patients, 66 CLL and 24 nonCLL from 38 families were parent-offspring related, making 51 pairs (Table II). 1.1. There were 31 one parent-one offspring pairs. 1.2. Furthermore, we found 20 one parent->1 offspring combinations (12 CLL-CLL and 8 nonCLL-CLL or CLL-nonCLL) from the following combinations: 1.2.1. One affected parent and two affected offspring (3 patients) making 2 parent-offspring pairs (4 patients) in 2 families with a total of 2 CLL doublets and 6 real LPD patients (5 CLL, 1 low-grade T-cell lymphoma). 1.2.2. A grandparent-parent-offspring combination with one patient in each of the three generations (3 patients), making 2 pairs (4 patients) in 3 families with 3 doublets (1 CLL, 1 follicular B-cell lymphoma, 1 lymphoplasmocytic lymphoma) and 9 real LPD patients (4 CLL, 2 follicular B-cell lymphoma, 1 Hodgkin's lymphoma, 1 lymphoplasmocytic lymphoma, 1 multiple myeloma). 1.2.3. A combination of (1.2.1) and (1.2.2) (4 patients) making 3 pairs (6 patients) in 2 families with a total of 4 doublets (3 CLL, 1 unclassified, low-grade B-cell lymphoma different from CLL) and 8 real LPD patients (6 CLL, 1 unclassified low-grade B-cell lymphoma different from CLL, 1 multiple myeloma). 1.2.4. In one family we found affected members in four generations, one patient in the 1st, 2nd, and in the

Table II. *Lymphoproliferative disorders in affected parent-offspring pairs (group 1).*

Group	Parent offspring	Pairs n	Patients n	CLL n	nonCLL n	Families n
1.1	CLL-CLL	18	36	36	0	18
	CLL-nonCLL nonCLL-CLL nonCLL-nonCLL	13	26	11	15	12
1.2	CLL-CLL	12	24	24	0	8
	CLL-nonCLL nonCLL-CLL nonCLL-nonCLL	8	16	4	12	8
Total group 1		51	102	75	27	38

Group 1.2 doublets, cf. text	n	n	d	n	d	n	d	n
1.2.1	4	6	2	5	2	1	0	2
1.2.2	6	9	3	4	1	5	2	3
1.2.3	6	8	4	6	3	2	1	2
1.2.4	4	5	3	4	3	1	0	1
	20	28	12	19	9	9	3	8
Total Group 1n+d	51	90	12	66	9	24	3	38

n=Real numbers of new patients included; d=duplicates, ascertained first time in another group.

Table III. *Familial lymfoproliferative and myeloproliferative disorders in 51 families (group 1-5).*

Group	Combination	Pairs		Patients		CLL		nonCLL		MPD		Families	
		n	d	n	d	n	d	n	d	n	d	n	d
1	Parent-offspring	51	0	90	12	66	9	24	3	0	0	38	0
2	Two affected parents	5	1	12	1	12	0	0	0	0	1	1	2
3	Skipped generation	6	0	11	1	9	1	0	0	2	0	1	4
	Carriers	4	0	8	0	5	0	2	0	1	0	2	2
4	MPD-LPD	3	2	5	5	2	2	0	0	3	3	2	3
1-4	Total	69		126		94		26		6		44	
5	Sib concordance		15	0	12	0	1	0	2	0	7	0	
1-5	Total		141		106		27		8		51		

n=Real number of new patients included; d=duplicates, ascertained first time in another group.

4th generations and 2 patients in the 3rd generation, making 4 pairs (8 patients with 3 CLL duplicates and 5 real LPD patients (4 CLL, 1 Hodgkin's lymphoma).

Group 2. LPD, parent-offspring combination: Two affected parents: This group comprised 3 families (Figure 1) and 13 affected family members with a marked predominance of CLL (12) and one case of PV in a grandfather. There were no healthy members at the time of investigation, the male in family 2 (Family 2, 3rd generation, no. 2) had died of astrocytoma at the age of 26, and a boy in family 3 (Family 3, 2nd generation, no.1) died in a traffic accident.

Pedigrees 1 and 2 (Figure 1) are part of a larger family also included in group 1 without overlap so that group 2 contributes only one new family and five pairs. The case of polycythemia vera in the PV-CLL parent offspring pair has been counted in group 4 (Table III). In pedigree 2, the grandfather with CLL is counted here as a new CLL patient to avoid his drop out from the data because this particular parent-offspring CLL-CLL combination is regarded as being different from the solitary CLL-CLL parent-offspring pairs in group 1.

Group 3. Skipped generations and carriers: This group comprised nine families, 5 skipped generations (vertical inheritance), and 4 carriers (oblique inheritance) (Figure 2).

Other parts of the skipped generation pedigrees 1, 2, 4 and 5 are encoded into group 1 so that skipped generations contribute 1 new family (Table III). There are six pairs of grandparent-offspring

Table IV. *Lymphoproliferative disorders, maternal and paternal parent-offspring pairs, all combinations.*

Diagnoses and pairs	Affected offspring					Unaffected offspring (healthy sibs)	
		Males		Females			
	n	n	%	n	%	n	alive
CLL – CLL	30	16	53	14	47	61	35 (57%)
Mater-offspring	17	7	41	10	59		
Pater-offspring	13	9	69	4	31		
Other combinations[a]	21	15	71	6	28	35	27 (77%)
Mater-offspring	9	5	56	4	44		
Pater-offspring	12	10	83	2	17		
Total	51	31	61	20	39	96	62 (65%)
Mater-offspring	26	12	46	14	54		
Pater-offspring	25	19	76	6	24		

[a]Other combinations comprise CLL-nonCLL, nonCLL-CLL and nonCLL-nonCLL pairs (described in Table V).

combinations: one in each of the first four pedigrees 1-4, and two pairs in the skipped generation pedigree 5. In skipped generation pedigree 4, the youngest brother with CLL to the CLL grandfather proband is counted as a new CLL patient here because otherwise he would not be included. In skipped generation pedigree 5, the two nonCLL LPD patients have already been included in group 1.

Two of the carrier families (1 and 4) are parts of a larger family encoded into other groups so that the carriers contribute two new families, while the patients in all four families are new to the data.

Group 4. Mixed LMP and MPD: All five MPD-LPD/LPD-MPD combinations between two or more generations are shown in Figure 2: Skipped generation no. 5, carrier pedigree no. 4 together with the three parent-offspring pedigrees 1-3, leaving the single-generation family (sib concordance, group 5 pedigree 1). Thus, group 4 (Table III) contributes a total of five families, of which three have been counted before (skipped generation no. 5 counted in group 1, carrier no. 4 in group 3 and the no. 3 parent-offspring MPD-LPD in group 2). The CML-AML grandfather-offspring pair in skipped generation pedigree no. 5 and the MDS-CLL uncle-nephew combination in carrier pedigree no. 4 have been counted with group 3, so that group 4 contribute three new pairs and two doublets. Furthermore, in parent-offspring MPD-LPD no. 3, the son with CLL has been counted with group 2 so that this pedigree contributes only one new PV patient.

Group 5. Sib concordance without other affected relatives: This group conprised 7 families (Figure 3, Table III) with 15 patients (12 CLL, 1 unclassified, low-grade B-cell lymphoma different from CLL, 1 PV and 1 MDS with multiple chromosomal abnormalities 1).

Group 6. Healthy persons and family size: A total of 523 healthy family members (Table I), of whom 34% were alive at the time of investigation, were confirmed to be members of the 51 families by crosschecking with the Civil Person Registry. In the few cases of extramatrimonial relationships, the identity of the offspring was crosschecked with The Midwife Book Registry. Furthermore, it was ascertained that no healthy family members had a hematological hospital record, nor were known to the cancer registries of Norway or Denmark. Registrations of healthy family members comprised close relatives of the affected index patients. Spouses, siblings and parents were always recorded and crosschecked. The 96 healthy siblings of the affected offspring in group 1 (Table IV) are included in the total number of healthy persons. The position of each healthy family member in the pedigree was carefully recorded with registration of sex and rank related to the patients. Data on gender and rank of the unaffected, healthy family members in each generation are reported in Figure 4.

From the number of healthy family members and the findings in Table III we found 2.8 (141/51) patients per family investigated (mean) and 13 (523/51 + 141/51) members per family (mean) who underwent systematic crosscheck on entry to this investigation.

Statistics. Traditional segregation analysis by comparing the proportion of affected family members with the proportion of those expected to become affected according to a given hypothesis (24) is not possible because a given hypothesis (dominant, recessive, sex-linked *etc.*) is uncertain. Instead, all affected individuals and the total number of individuals in the sample were unitized in matrices for a standard pedigree on pooled, conjugated, data to visualize a trend (Figure 4) (25-27). This technique is usually used in biology to construct a family tree for phylogenetic investigation based on multiple parameters, allowing uniform and nonparametric data processing no matter the size of the family and the number of healthy and affected persons per family (26, 27).

The matrix used arrange (i) each of the affected family members according to diagnoses, rank in the sibship, gender, and generation (1st, 2nd, 3rd or 4th) and (ii) the number of healthy (undiagnosed) members of the family according to rank in the sibship, gender, and generation (1st, 2nd, 3rd or 4th generation). None of the duplicates from parent-offspring combinations (group 1) were included. The set of the unitary orthogonal matrices involved is listed in the legend to Figure 4. Sets of conjugates for the matrix of the generalized data (Figure 4) were regarded as commutative when at least two generations were involved. Vector determinants (tendency) and metric space estimation (phenetic resemblance) were based on matrix conjugation with Bayesian approaches (25, 26), calculated by means of common matrix processing and confirmed from Perl programming, available from http://www.perl.com, project definition and code design as previously described (28, 29).

The parental birth order effect was confirmed by means of Cox regression analysis. The p-value of 0.05 was considered statistically significant in all analyses and significance values are two-sided.

The B-cell expression in familial and in sporadic CLL was compared by means of Cox regression analysis and Mann Whitney, Wilcoxon rank sum test where a two-sided p-value <0.05 was considered significant.

Results

Pleiotropy. Of the 800 CLL patients interviewed, 56 index CLL patients (7%) had other affected family members, 51 families and a total of 141 patients were identified (Table I) The concomitant disorders of the index CLL patients were 75% (106/141%) CLL, 19% (27/141%) nonCLL, and 6% (8/141%) MPD (Table I). The same figures for the CLL cohort were 13% (106/800%) CLL, 3% (27/800%) nonCLL, and 1% (8/800%) MPD, where the diffuse large B-cell lymphoma

Table V. *Lymphoproliferative disorders, maternal and paternal parent-offspring pairs, other combinations than CLL-CLL.*

Diagnoses and pairs	Affected offspring					Parent - offspring
		Males		Females		
	n	n	%	n	%	
CLL-non CLL	7	3	43	4	57	Mater-offspring
Mater-offspring	3	1		2		CLL - Waldenström's disease
Pater-offspring	4	2		2		CLL - pre B ALL
						CLL - FL
						Pater - offspring
						CLL - small T cell lymphoma NOS
						CLL - IgM MGUS
						CLL - DLBCL
						CLL - FL
nonCLL-CLL	8	8	100	0	0	Mater - offspring
Mater-offspring	2	2		0		T PLL - CLL
Pater-offspring	6	6		0		DLBCL - CLL
						Pater - offspring
						FL NOS - CLL
						FL - CLL
						FL NOS - CLL
						FL NOS - CLL
						FL - CLL
						DLBCL - CLL
nonCLL-non CLL	6	4	67	2	33	Mater - offspring
Mater-offspring	4	2		2		MM - MM
Pater-offspring	2	2		0		FL - HL
						MM - LP
						LP - FL
						Pater - offspring
						DLBCL - HL
						FL NOS – MM

ALL, Acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular B-cell lymphoma; HL, Hodgkin's lymphoma; LP, lymphoplasmacytic lymphoma; MGUS, monoclonal gammopathy of uncertain significance; MM, multiple myeloma; NOS=not otherwise specified; PLL=prolymphocytic leukemia.

(DLBCL) was predominant in nonCLL and PV was predominant in MPD. Mixed LPD and MPD were seen in 6 (12%) of the families, while 45 families (88%) had only LPD.

The number of pleiotropic diagnoses in the 800 interviewed CLL patients is higher than expected from sheer coincidence and when compared with the expected occurrence in the population. The observed numbers are seen in Table I. The expected numbers are calculated from the crude incidences in Norway and Denmark as a mean for the five years of observation, *viz*. the total number of patients with a certain diagnosis (all ages, women and men together from all parts of the two countries) per 100,000 people per year as a mean of data from the past 5 years (30-32). For the observation of PV, however, no such mean crude incidence

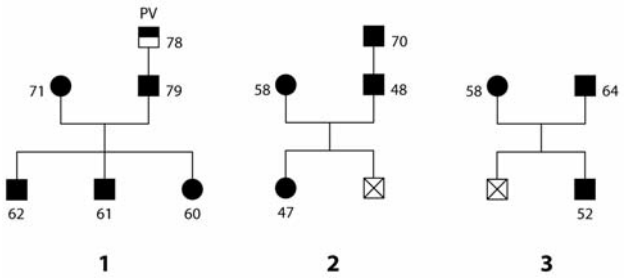

Figure 1. *Lymphoproliferative disease, three families with two affected parents. Circle=female; square=male; white=unaffected; black=CLL; crossed square=died at young age; black/white split =myeloproliferative disease. The numerical values show the age at onset of disease. PV, polycythemia vera.*

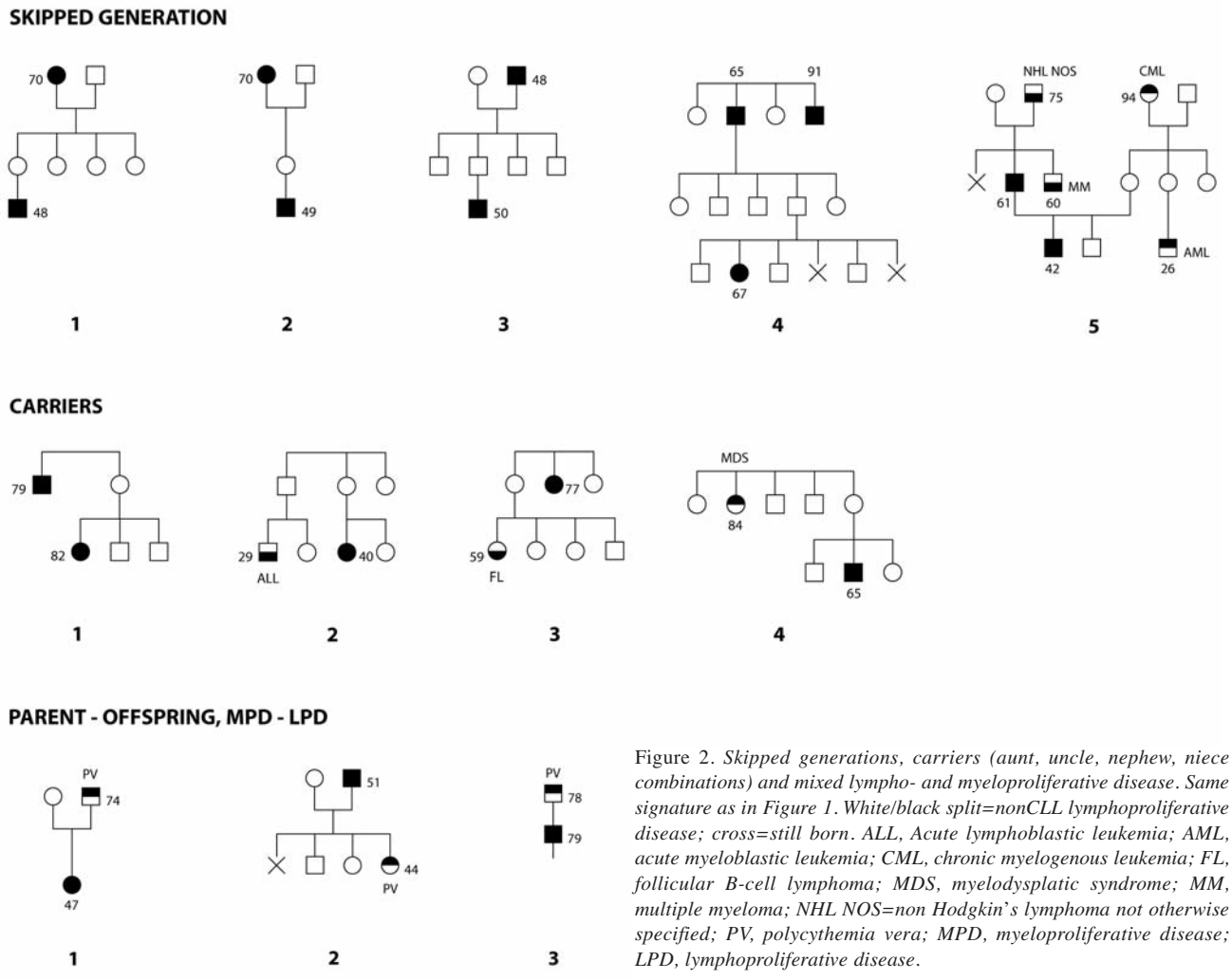

Figure 2. *Skipped generations, carriers (aunt, uncle, nephew, niece combinations) and mixed lympho- and myeloproliferative disease. Same signature as in Figure 1. White/black split=nonCLL lymphoproliferative disease; cross=still born. ALL, Acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CML, chronic myelogenous leukemia; FL, follicular B-cell lymphoma; MDS, myelodysplatic syndrome; MM, multiple myeloma; NHL NOS=non Hodgkin's lymphoma not otherwise specified; PV, polycythemia vera; MPD, myeloproliferative disease; LPD, lymphoproliferative disease.*

is available from Norway and Denmark and a closely related Swedish normal data has been used for comparison instead (33). Only diagnoses with an observed number higher than two (Table I) were accepted for comparison to avoid the risk of random influence from single observations. The comparison between the observed and the expected number of cases shows these remarkable differences in mean crude incidence: CLL=5.4 cases /100,000 people/year=4.3×10^{-2} expected cases of CLL in 800 people from the population *vs.* the 106 observed; diffuse large B cell lymphoma 8.2/100,000=6.6×10^{-2} expected cases in 800 people *vs.* the 5 observed; follicular B cell lymphoma stage I, II and III 3.3/100,000=2.6×10^{-2} expected cases in 800 people *vs.* the 4 observed; multiple myeloma 3.9/100,000=3.1×10^{-2} expected cases in 800 people *vs.* the 4 observed; PV 2.6/100,000=2.1×10^{-2} expected cases in 800 people *vs.* the 4 observed; Hodgkin's lymphoma 2.5/100,000=2.0×10^{-2} expected cases in 800 people *vs.* the 3 observed.

Segregation. Birth order effect, parent-offspring (group 1): Table IV shows that CLL-CLL parent-offspring combinations were predominant over other combinations (30 of 51 parent-offspring pairs) with a nearly equal number of maternal and paternal pairs (17 and 13 pairs, respectively). Transmission was seen to take place mainly to sons, especially in affected father-offspring pairs with CLL-CLL, $p<0.005$ (Table IV), and in pairs with parental nonCLL (Table V) where there was a 100% transmission of CLL to sons and a 67% transmission of nonCLL to sons (Table V), $p<0.005$.

In three out of 51 families (6%) where both parents had CLL we found an extremely high rate of CLL among the offspring (Figure 1).

Birth order effect, all familial combinations between generations (group 1-4): The parental birth order effect is seen from Figure 4. In matrilineal inheritance, the affected children are randomly distributed in the sibship while in

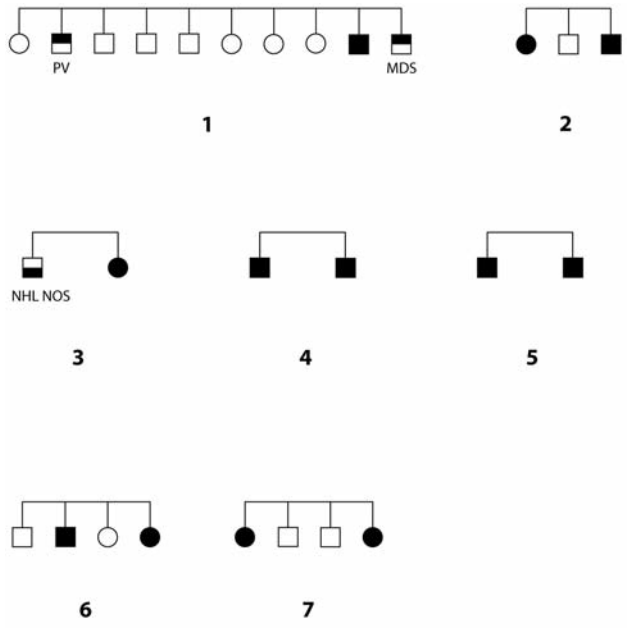

Figure 3. *Affected sibs without other affected relatives showing marked lymphoproliferative predominance. Same signature as in Figures 1-2.*

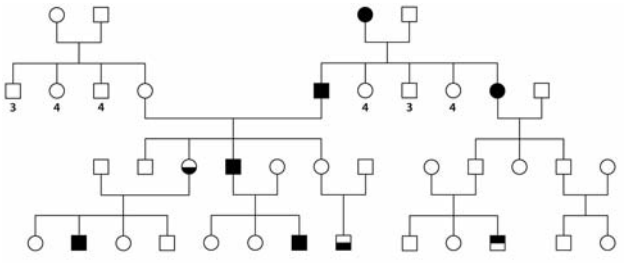

Figure 4. *Pedigree with conjugated data from all families with affected members in two or more generations. Same signature as in Figures 1-3. The numbers below the 2nd generation indicate the estimated numbers of healthy offspring to the unaffected parents from 2nd generation. Sets of the following unitary orthogonal matrices were included: (1) The proportions of patient-offspring pairs (one affected parent) between the generations 1-2, 2-3, and 3-4 (g1, g2, g3) with one of the six combinations CLL-CLL, CLL-nonCLL, nonCLL-CLL, nonCLL-nonCLL, LPD-MPD, MPD-MPD were designated (g1,g2g3/a1,1; a1,2; a1,3; a1,4, a1,6, respectively). Similarly, the proportions of affected parent-offspring pairs with two affected parents (g1,g2,g3/ a2,1; a2,2….a2,5), affected grandparent-offspring pairs (g1,g2,g3/a3,1; a3,2….a3.6), and affected uncle, aunt-nephew, cousin pairs (g1,g2,g3/a4,1; a4,2…..a4,6) were noted. (2) These proportions were grouped into the combinations: mother-son, mother-daughter, father-son, father-daughter designated (g1,g2,g3/a5,1; a5,2…..a5,4) and also allocated to subgroups depending upon the rank of the affected offspring in the sibship, for example 1/1 (only child), ½ (first of two children), 3/5 (the third child in a sibship of 5 children) etc., designated (g1,g2,g3/a6,1; a6,2;….. a6,n). Max (n) in 6a,n is 10 since the largest family in the present material has 10 offspring. Therefore, 6a,n provides a total of 55 subgroups=∑ (1/1, 1/2 - 2/2, 1/3 - 3/3, ….. 1/10 - 10/10)=∑ (1/1, 1/2, 2/2, 1/3, 2/3, 3/3, 1/4 .....8/10 9/10 10/10)=1 + 2 + 3 + 4 + 5 + 6 + 7 + 8 + 9 + 10=55 per generation g2-g4. Data on the first generation, g1, with family members born before 1890 cannot be safely crosschecked and is too uncertain to allow an estimation of the rank of affected family members. (3) Combinations of g1-g3 with a1,n - a6,n resulted in a predominant number of subgroups with no affected family members (value zero) Such empty subgroups show the position of unaffected (healthy) members in the pedigree and thus the affiliation of (i) healthy-healthy family members between two or more generations as the majority of empty combinations, (ii) affected parent-healthy offspring, and (iii) healthy parent-affected offspring. (4) For the empty groups of matrices (3) above, the number and sex of 511 healthy family members of groups 1-4 (total 523 minus 12 normal persons from group 5, Figure 3) and their (older/younger) range related to affected family members in each generation was compared with the mean family size per woman since 1890, covering the observation period of the present investigation in Scandinavia (cf. ref. 56-58). Data from families with sib concordance (Figure 3, Table III) is evidently non-commutative due to the lack of an affected predecessor in the family and are therefore not included.*

patrilineal inheritance, the affected offspring are mainly sons late in the sibship. Figure 4 shows eight pairs:

Six parent-offspring pairs: 1st-2nd generation, 2 pairs (0 patrilineal pair; 2 matrilineal pairs where the numbers of older/younger sibs to the affected offspring is 0/4 and 4/0, respectively). 2nd-3rd generation, 2 pairs (2 patrilineal pairs, 1/2 and 2/1; 0 matrilineal pair). 3rd-4th generation, 2 pairs (1 patrilineal pair 2/0; 1 matrilineal pair 1/2), *and two grandparent-offspring pairs:* 2nd-4th generation, 2 pairs (1 patrilineal 2/0; 1 matrilineal 0/0).

Such matrix models used for phylogenetic investigations have only described tendencies in terms of posterior probabilites (25, 26) avoiding the standard statistical approach of specifying a null hypothesis and asking whether or not data are strong enough to reject the null hypothesis. However, to address the question whether the hypothesis on birth order effect should be believed, and how strongly in terms of standard probability, the sum of the posterior probabilities, *viz*. the tendencies in terms of older/younger healthy sibs in the eight parent-offspring pairs extracted from Figure 4, can be converted to standard probability (26, 27):

∑ patrilineal (older/younger sibs)=(1 + 2 + 2 + 2/ 2 + 1 + 0 + 0)=7/3=2.3

∑ matrilineal (older/younger sibs)=(0 + 4 + 1 + 0/ 4 + 0 + 2 + 0)=5/6=0.8

The deviation of ∑ (older/younger sibs) to 1.0 (where 1.0 denotes no birth order effect) (27) expresses the degree of birth order where the patrilinieal sum is significantly deviated ($p<0.01$), while the matrilineal is not, with the reservation that only the number but not the rank of the affected female in the 1st generation is included in the calculation due to lack of proper crosscheck of her family data and uncertain data available on the real family size in the population before 1890. Since the patrilineal sum is upper deviation (2.3 larger than 1.0), this also shows that the patrilineal birth order is

characterized by a significant number of older sibs to the affected offspring, in other words that the affected offspring is among the youngest in the sibship.

For control, this is in accordance with the findings from a Cox regression analysis on parity and birth order with the same data as described in the matrices (1)-(3) from all 51 families (Legend to Figure 4) which showed: maternal-offspring combination: relative risk (RR), 95% confidence interval (CI)=1.47, (0.89-2.43), p=0.12 (score and likelihood ratio test) compared with paternal-offspring combination: RR=3.25, 95% CI=(1.57-6.72), p<0.001 (score and likelihood ratio test).

The power of the birth order parameter is furthermore seen from the fact that Cox regression analysis of patrilineal and matrilineal segregation (score and likelihood ratio test) shows no significant difference if data on the rank of sibs to the affected offspring are omitted.

The limit of impact is close to the square root of the value (older/younger sibs to the affected offspring), p=0.078. This means that a reduction of this birth order parameter, *viz*. the value (older/younger sibs to the affected offspring), by its square, and all other parameters constant, brings about a difference between patrilineal and matrilinieal segregation which is only a little above the traditional 5% limit (p=0.078 compared with p=0.050).

Sib concordance (group 5): The data of group 5 are fully included in the calculations of pleiotypic frequencies but not used for the estimation of a birth order effect since the data of group 5 is clearly non-commutative. In contrast to all other parts of the data used, that from group 5 covers only one generation. The Perl processing described above is unconditional with data from group 1-4 while the inclusion of group 5, requiring an elusive converting factor, would turn the data processing into a conditional stage with consequences for the overview and interpretation.

No evident sex concordance was seen between the affected sibs in any family of group 5 (3 male-male combinations; 1 female-female combinations, 3 mixed male-female combinations) in spite of a marked male predominance among the affected sibs: 10 out of 15 (67%) affected siblings were males.

B-cell expression in familial CLL. Sixty-eight out of the 106 CLL patients included were alive at the time of investigation. Our diagnostic test battery for CLL provided findings from flow cytometry, FISH cytogenetic testing, and the degree of VH homology of the IgH gene rearrangement measured by means of multiple PCR where less than 98% homolog was accepted as a VH mutated status.

CLL was only accepted as a combined CD5-, CD19-, CD20- and CD23-positive B-cell monoclone with weak light chain expression kappa or lambda. The tumor size scoring (viz. the number of CLL cells in the blood and/or in the bone marrow, the number of regions with enlarged lymph nodes, and the degree of lymph node enlargement and splenomegaly) in combination with the FISH profile and furthermore in combination with the VH mutation status (40 (59%) mutated and 28 (41%) unmutated) was not significantly different when comparing the 68 cases of familial CLL with their sex and age matches of sporadic CLL (p>0.05).

The findings from FISH testing in the 68 CLL patients alive at the time of investigation were: 29 (43%) 13q deletion, 23 13q deletion alone and 6 in combination with other abnormalities; 8 (12%) trisomy 12q; 11 (16%) 11q deletion; 4 (6%) 17p deletion; and 15 (22%) normal. The 6q deletion FISH test was carried out in only 19 of the 68 CLL patients (1 case positive).

Neither did we see any significant difference when comparing the expression of the CLL cells in affected sibs of patrilineal and matrilineal parent-offspring pairs (p>0.05). Thus, in our trial with samples from only 68 patients in a multifactorial testsystem, the B-cell monoclone in familial and in sporadic CLL is indistinguishable (p>0.05).

Discussion

The precise mechanism for the transfer of the CLL susceptibility genes from generation to generation is unknown and no simple Mendelian pattern has so far been described. Male predominance, the presence of pleiotropy, and a birth order effect are undoubtedly parts of this mechanism. In the present paper, a conjugated pedigree based on matrices with parameters from a large number of affected families reveals both a patrilineal and a matrilineal inheritance of CLL (Figure 4). Such a sex-specific, pleiotropic segregation gives association to an epigenetic (*viz*. outside the DNA) segregation of polygenes where genomic imprinting provides a plausible explanation when interpreting both the existence of pleiotropy and the fact that the CLL susceptibility genes must be available for the meiosis at the fertile age (34-39).

Genomic imprinting, a parental specific gene expression based on intrauterine regulation of the fetal genetic material, gives rise to monoallelic genes depending on the paternal or maternal origin of the allele (35-39). Genomic imprinting enables the female to transfer selected alleles to her offspring, making a birth order effect possible (35-39). Such a birth order effect has recently been discussed in relation to CLL and LPD (15, 17, 40, 41). When estimated by means of Haldane Smith's test for birth order calculation (17, 42, 43), or by means of matrice conjugation in the present work, we find that patrilineal CLL is mainly given to the youngest sibs while matrilineal CLL is equally distributed in the sibship, where CLL pleiotropy is seen in an excess of males (Tables IV-V). Sex-specific imprinting, where sons are more prone to be affected than daughters in patrilineal families but not in matrilineal families, seems more likely than a subset of

familial CLL associated with Y-linked inheritance, because an association between CLL and the Y-chromosome has so far not been established and because Y-linked inheritance alone can explain the sex-specific segregation but not a birth order effect. Genomic imprinting based on the maternal tolerance produced by microchimerism induced by the male haplo-load per pregnancy and per male partner (44-47) may well explain the birth order effect where paternal LPD genes in particular are transferred to sons late in the sibship, when an increased tolerance due to earlier pregnancies makes a transfer of foreign, paternal LPD susceptibility genes feasible. If so, male predominance in CLL (10, 11) finds an explanation with this mechanism and explains why no difference was seen in the monoclonal B-cell expression when comparing familial and sporadic CLL, neither in our small material nor in a larger investigation of VH expression (48). However, genomic imprinting in explanation of a birth order effect is not the only model available. Smouldering stimulation of inborn CLL susceptibility genes from autoimmunity prior to the development of CLL has been pointed out as one likely mechanism (49) where the impact of female-predominant autoimmunity is obvious. In this way, a relationship between chronic infection and birth order emerge from the well-known risk of autoimmunity after long-lasting infection (49), or from the influence of environmental life-style factors in childhood such as exposure to lymphotrop infection from older siblings (15, 16, 40, 41).

The CLL susceptibility genes are supposedly clustered polygenes (12). The impact of mutations in the susceptibility genome is renewal of the pleiotropic repertoire (Tables II-III) which also comprises a minor proportion of MPD at a rate higher than would be expected from coincidence. Thus, mutations in the CLL susceptibility genes seem to affect both lympho- and myeloid cell lines, with a wider spectrum of chromosomal alterations in the mutated cell lines than the traditional profiles used for identification of subtypes of CLL (*e.g.* 13q14, trisomy12, del6q21, 11q22-q23, and del17p13 subsets of CLL with either VH-positive or VH-negative prefix) (18, 21, 22). Familial MPD is well known (50, 51) but very little is known about the susceptibility gene or genes for comparison with LPD.

Bias may have an impact on data due to a systematic underestimation of sample size. Only dead family members have been sufficiently, *viz.* life-long, observed to rule out the development of disease, especially with regard to CLL and other disorders in elderly people. The concern about bias is obvious in the present study, where 34% of the normal family members were alive at the time of investigation, and this was even higher (65%) in the healthy persons related to the parent-offspring pairs (Table IV). Overlooked low-grade silent diagnoses such as MGUS, MBL, low-grade non-Hodgkin's lymphomas, smouldering myeloma and stage A CLL in otherwise healthy persons who were not tested for blood disease are very likely (3, 14, 52) and such overlooked cases are in part the explanation behind the presence of skipped generations, carriers and horizontal inheritance which cannot be ascribed to a genetic manifestation alone, even if all possible precautions related to ascertainment in sequential sampling of pedigrees have been taken (42, 43, 53, 54). The age at diagnosis has undoubtedly changed during the long observation period of the present study. The increased incidence of CLL in very old patients in Scandinavia in recent decades (30) partly reflects a more efficient diagnostic process, while we are uncertain to what extent medical advances have influenced the age at onset of disease in the youngest LPD patients, for example those with ALL and Hodgkin's disease. In the present study, we saw a younger age at onset of disease in offspring than in parents (Figures 1-3), but due to these reservations, we find them hard to be ascribed to anticipation (4, 55), in spite of the fact that from a basic genetic point of view, birth order and parent's age are usually closely related parameters (43). Furthermore, the sample size of 15% early spontaneous abortions in females in Scandinavia in the time window used (56-58) is an underestimation. Such spontaneous abortions, experienced only as a strong late menstruation, counts as pregnancies in an evaluation of parity and the number of male partners related to epigenetic parental imprinting. Furthermore, we are aware of the influence of a change in male fertility in the latest generations investigated, every fourth man in Scandinavia has no children of his own at present (59). Finally, the 105:100 ratio between newborn male and female babies, the slightly higher death rate in newborn males than in females, and the generally increased life especially in recent generations and especially in women, are theoretical factors to take into account when the numbers of affected sons and daughters are considered (56-58).

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