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Inherited susceptibility to chronic lymphocytic leukemia: evidence and prospects for the future

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Abstract: Chronic lymphocytic leukemia (CLL) is the most common leukemia in the United States and one of the most heritable cancers. A family history of the disease is perhaps the best defined risk factor, and approximately 15–20% of CLL patients have a family member with CLL or a related lymphoproliferative disorder. Much effort has been devoted to trying to elucidate the mechanisms underlying this genetic risk. Familial CLL appears to be clinically and biologically similar to sporadic CLL, and most if not all CLL appears to be preceded by monoclonal B-cell lymphocytosis (MBL), which does appear to occur at higher frequency in relatives in families with CLL. Neither linkage studies nor candidate gene association studies have proven particularly informative in CLL, but genomewide association studies have identified multiple low-risk variants that together explain about 16% of the familial risk of CLL. Studies of individual families have identified higher-risk single nucleotide polymorphisms or copy number variants associated with disease risk in those families. Current efforts to identify additional risk loci are focused on applying next-generation sequencing to the germline of informative CLL families as well as individuals with sporadic CLL.

Keywords: CLL, copy number variant, familial, genomewide association study, linkage, monoclonal B lymphocytosis

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Inherited predisposition to CLL

Chronic lymphocytic leukemia (CLL) remains the most common adult leukemia in the United States, with about 16,000 people expected to be diagnosed this year alone, and substantially more living with the disease. Although the cause of CLL remains unknown, a family history of CLL is perhaps the best characterized risk factor. Families with what appears to be Mendelian autosomal dominant inheritance of CLL are not uncommon in the literature [Brown *et al.* 2012a; Caporaso *et al.* 2004; Jonsson *et al.* 2005; Sellick *et al.* 2006]. In fact, approximately 12% of patients with CLL report a family history of a lymphoproliferative disorder (LPD) [Brown *et al.* 2008a], and 6–9% have a relative who also has CLL [Capalbo *et al.* 2000; Mauro *et al.* 2006; Yuille *et al.* 1998]. Not surprisingly, then, the risk of developing CLL is also increased in first-degree relatives of CLL patients. The most definitive study looking at this question used the Swedish Family Cancer Database to evaluate risk in the

relatives of CLL probands and matched controls and found a factor of 7.5 increased risk of CLL in first-degree relatives of CLL probands [Goldin *et al.* 2004]. This result is consistent with other population-based epidemiologic studies, most of which have found a factor of 3–8 relative risk of CLL in first-degree relatives [Tjonnfjord *et al.* 2012].

In the Swedish Family Cancer Database study, relatives of CLL probands also had increased risks of Hodgkin's lymphoma (HL; factor of 2.35) and non-Hodgkin's lymphomas (NHL; factor of 1.45), but not multiple myeloma (MM) [Goldin *et al.* 2004]. Multiple studies have reported similarly increased relative risks of NHLs and HLs [Chang *et al.* 2005; Pottern *et al.* 1991] and other LPDs are consistently seen in some families with CLL [Brown *et al.* 2008a]. Clustering of other malignancies is not as well recognized, although breast cancer has been reported frequently in a CLL family [Jonsson *et al.* 2005], and increased

risks of CLL have been associated with breast cancer in mothers or sisters [Pottern *et al.* 1991].

Additional evidence for the heritability of CLL comes from the variation in its incidence based on ethnicity [Ruchlemer and Polliack, 2013]. For example, Asian countries have a very low incidence of CLL, particularly Japan and China, and these low rates appear to be maintained even after migration to the United States, suggesting that genetic factors may weigh more heavily in this difference than environmental factors [Pang *et al.* 2002]. Worldwide the incidence is highest among whites in the US, Europe and Israel, with lower rates among African-Americans and Hispanics [Ruchlemer and Polliack, 2013]. The reasons for this remain poorly understood and more studies are required.

Biology of familial CLL

Familial CLL has generally been defined as a family with at least two first-degree relatives who are both affected with CLL. Although one might suspect that CLL would have a younger age of onset in familial cases, the literature is conflicting on this point. The Swedish Registry study, which should not be subject to selection bias, found no significant difference in age of onset between familial and sporadic CLL [Goldin *et al.* 2004]. Familial and sporadic CLL have also been reported to show similar stage at diagnosis and 10-year overall survival [Mauro *et al.* 2006]. Interestingly, women report a family history more commonly than men [Crowther *et al.* 2005; Mauro *et al.* 2006], and the 2:1 male:female sex ratio typical of CLL is reduced in familial CLL.

Although familial CLL could in principle differ biologically from sporadic CLL, most studies to date have not found substantial differences [Goldin *et al.* 2010b]. V gene usage at the immunoglobulin heavy chain gene has been reported to be similar between familial and sporadic CLL, with VH3 predominating, followed by VH1 and VH4 [Crowther *et al.* 2005; Pritsch *et al.* 1999; Rassenti *et al.* 2003; Sakai *et al.* 2000]. Chang and colleagues performed the most extensive analysis of V gene usage and somatic hypermutation at the immunoglobulin heavy chain variable region gene, comparing familial to sporadic CLL [Crowther *et al.* 2005]. Mutated CLL (<98% homologous to the closest germline V region) was observed in 68% of the familial cases, as compared with 47% of the sporadic cases. However,

mutation status was correlated within families, and 86 families in this study were represented by more than one affected individual, suggesting that the shift toward mutated CLL could be due to enrollment of multiple members of the same family on this study [Crowther *et al.* 2005]. An earlier study also found greater somatic hypermutation in familial than sporadic CLL [Pritsch *et al.* 1999] but this finding was not seen in a report from the CLL Research Consortium database on over 3000 individuals with CLL [Brown *et al.* 2008b].

Other biologic features have not been as well studied in familial CLL. No difference in ZAP-70, CD38 or CD23 expression or serum beta-2-microglobulin levels was seen between familial and sporadic CLL in two CLL Research Consortium studies [Brown *et al.* 2008b; Rassenti *et al.* 2003]. No systematic large-scale evaluation of cytogenetics has been performed in familial CLL [Goldin and Caporaso, 2007; Ng *et al.* 2007]. Both deletion 13q and trisomy 12 have been reported, with a suggestion of increased frequency of 13q deletion [Ng *et al.* 2007]. We compared cohorts of familial to sporadic CLL using a technique called array comparative genomic hybridization (CGH), which detects chromosomal imbalances using differentially labeled tumor and normal DNA that is hybridized to probes on an array. This analysis found a lower frequency of 11q deletion in the familial patients, but a higher frequency of 14q11 gain [Setlur *et al.* 2010]. Overall, the limited data suggest that the biologic range of familial CLL is similar to that of sporadic CLL, with a possible shift toward lower risk markers (mutated *IGHV*, absence of 11q deletion). That possible shift will need to be confirmed in larger studies, however.

Monoclonal B-cell lymphocytosis: precursor condition

The last 10–20 years have witnessed an explosion of research about the newly defined entity monoclonal B-cell lymphocytosis (MBL). Work in individuals living near hazardous waste sites as well as in families with CLL led to the identification of small populations of monoclonal B cells in peripheral blood in individuals who were otherwise hematologically normal [Marti *et al.* 2003; Vogt *et al.* 2007]. The most common flow cytometry phenotype was found to be CLL-like, positive for CD5, with dim CD20 [Ghia *et al.* 2004; Marti *et al.* 2005; Rawstron *et al.* 2002a]. However, both an atypical CLL phenotype, positive for CD5

with bright CD20, and a non-CLL-like phenotype, which is negative for CD5 with bright CD20, have also been described [Ghia *et al.* 2004; Marti *et al.* 2005].

In order to clearly distinguish it from CLL, MBL has been defined as a condition in which a monoclonal B-cell population of appropriate immunophenotype is present in less than 5000 lymphocytes per microliter in the peripheral blood of individuals who have normal complete blood counts otherwise and no evidence of a LPD. Studies of the general population have found that the frequency of monoclonal B-cell populations in hematologically normal outpatients increases with age and with the sensitivity of the flow cytometry assay employed [Nieto *et al.* 2009; Rawstron *et al.* 2008]. Such populations are very rare under age 40; 2.1% in adults 40–59; 5–5.5% in adults age 60–89; and detectable in as many as 50% of individuals over age 90 [Ghia *et al.* 2004; Rawstron *et al.* 2008, 2013]. Two categories of MBL are now recognized: low count MBL, typically less than 50 cells per microliter, found by applying very sensitive flow cytometry to the general population and carrying minimal risk of progression to CLL; and clinical or high count MBL, typically identified clinically based on relative or absolute lymphocytosis with a CLL phenotype but below the 5,000 per microliter cutoff for CLL. Low count MBL has a different V gene repertoire than CLL [Dagklis *et al.* 2009; Nieto *et al.* 2009] and is enriched for lower risk CLL markers [Lanasa *et al.* 2011], while the biology of high count MBL parallels the spectrum seen in CLL [Rawstron *et al.* 2008; Rossi *et al.* 2009].

MBL is more common in unaffected relatives in CLL families than in the general population. Four studies have now detected MBL cell populations in 13.5–18% of unaffected first-degree relatives in CLL families [Goldin *et al.* 2010a; Lanasa *et al.* 2011; Marti *et al.* 2003; Rawstron *et al.* 2002b]. The relative risk varies with age, highest in younger adults, due to the lower likelihood of MBL in the sporadic population at that age [de Tute *et al.* 2006]. However, one study found a 61% probability of developing MBL by age 90 among relatives in high-risk CLL families [Goldin *et al.* 2010a]. Among relatives of sporadic CLL patients, the overall rates of MBL are comparable to the general population, but older relatives over age 60 showed increased risks of MBL more similar to those seen in unaffected individuals in CLL families [Matos *et al.* 2009]. This finding is interesting

and suggests that some or all of the genetic predisposition underlying sporadic and familial CLL may be shared.

The current model suggests that MBL is likely a precursor condition for CLL. Evidence for this model comes from a study in which samples were available from a median of approximately 3 years prior to CLL diagnosis in a cohort of patients subsequently diagnosed with CLL, and MBL was detectable in those earlier samples in 44 of 45 cases [Landgren *et al.* 2009]. However, the converse, namely the likelihood of MBL progressing to CLL over time, is not precisely known. The overall prevalence of MBL, which is significantly greater than that of CLL, is consistent with the expectation that most MBL will not progress to CLL. Certainly for low count MBL, progression appears very unlikely [Rawstron *et al.* 2010]. For high count MBL in an unselected (sporadic) population, the risk of progression to CLL treatment is approximately 1% per year [Rawstron *et al.* 2008; Shanafelt *et al.* 2009]. Two studies have identified the B-cell count as a predictor of progression [Rawstron *et al.* 2008; Shanafelt *et al.* 2009], while one study also identified CD38 expression as a risk factor for progression [Shanafelt *et al.* 2009].

These data from an unselected MBL or CLL population may not apply in the familial MBL context where one might imagine the likelihood of progression could be higher, although this is unknown. In the familial CLL context, MBL is generally considered to be a marker of the at-risk genotype, which if true would certainly indicate that the individual has a somewhat higher risk of developing clinical CLL. The degree of this increased risk would obviously depend on the relative risk and the penetrance of the at-risk genotype. Long-term follow up of familial MBL cases is currently lacking, and defined genetic markers of the at-risk genotype are also lacking, so we do not have any data to address these hypotheses. It therefore seems reasonable at present to consider familial MBL cases as affected in biologic studies of familial CLL, but ultimately this assumption will need to be confirmed with more data.

Early studies looking for genes

Linkage

Early efforts to identify loci important in familial CLL focused on traditional genetic approaches

including linkage, which uses markers throughout the genome to assess the statistical likelihood that a particular phenotype is associated with a given marker. Even markers tightly linked to a particular phenotype are not necessarily causative, however, but may be near a causative genetic variant. Linkage studies in CLL have been hampered by small numbers of families and few affected individuals per family, many of whom may be deceased. The largest study was a collaborative effort of UK and American groups and included 206 families whose germline DNA was studied with Affymetrix 10K single nucleotide polymorphism (SNP) arrays. This study identified 2q21 as the most promising susceptibility locus, followed by 18q21 and 6p22 [Sellick *et al.* 2005, 2007]. At 2q21 the most likely candidate gene was CXCR4, which has been implicated in homing of CLL to microenvironmental niches [Davids and Burger, 2012]. A subsequent follow-up study attempted to validate the association of the relevant SNP rs2228014 with CLL, in 1058 CLLs and 1807 controls, and found no evidence of association with CLL [Crowther-Swanepoel *et al.* 2009]. Germline sequencing of the CXCR4 gene in 186 familial CLLs compared to 213 controls identified three previously undescribed variants with possible functional consequences, one truncating and two missense, all in the familial CLLs [Crowther-Swanepoel *et al.* 2009]. No further study of these mutations has been published, however, and their frequency is low. A possible candidate SNP at the 18q21 locus, rs12953717, had been previously implicated in colon cancer risk and was near the SMAD7 gene, which may be involved in regulation of B-cell apoptosis [Sellick *et al.* 2007]. A follow-up study therefore evaluated whether this SNP was associated with CLL in 984 cases and 4831 controls and again found no evidence of an association [Broderick *et al.* 2008]. Finally, this study identified linkage to 6p22, the site of the MHC locus, in familial CLL. Many early studies of specific HLA loci in relation to familial CLL have yielded conflicting results and as yet there is little clarity on the significance if any of this association [Brown, 2008]. Thus, the overall results of the largest linkage analysis performed across multiple families in CLL have unfortunately been largely inconclusive.

Despite this, a genomewide linkage analysis performed on a single family with six affected individuals was informative, identifying possible linkage to 9q22 [Raval *et al.* 2007]. The death-associated protein kinase 1 (DAPK1) gene in that

region was found to be silenced in most sporadic CLLs and also showed reduced expression in this family in the affected individuals, all of whom carried what was ultimately identified as a regulatory SNP (c.1-6531A>G) that increased binding of the HOXB7 repressor, leading to decreased DAPK1 expression [Raval *et al.* 2007]. Although segregating with disease and of demonstrated functional effect, this allele proved to be a private event in this family, not seen in any of 338 additional CLLs examined.

Association

Another early approach to gene discovery was to investigate the role of polymorphisms in individual candidate genes thought to be important in B-cell biology, such as the ATM gene, using an association analysis approach. Such studies investigate whether the population of interest, in this case CLL patients, has a statistically higher frequency of a given genetic variant than a control population, suggesting that the variant is associated with the disease. Although some of these studies have found significant associations with CLL, typically these findings have not been replicated in subsequent studies [Brown, 2008], likely due to patient selection, the small size of most studies and underlying genetic heterogeneity between the experimental and control populations [Deng, 2001; Deng *et al.* 2001]. Association studies have also been performed on a larger but subgenomewide scale, for example to assess many polymorphisms in a given pathway like the DNA damage response [Rudd *et al.* 2006]. One of the largest of these studies looked at 1467 coding nonsynonymous SNPs in 865 genes, and tested their association with CLL in 992 patients and 2707 controls. This study did find approximately twofold associations between CLL and several polymorphisms in ATM, CHEK2 and BRCA2 among others [Rudd *et al.* 2006]. However, in subsequent genomewide association analyses these SNPs and/or genes have not been identified.

Moving to genomewide analyses: association

The failure of linkage to identify mutations conferring high relative risks of CLL suggested that CLL risk might come from the combinatorial effects of common relatively low-risk variants. Such variants are identified through genomewide association studies (GWAS) that use high-density

SNP arrays to look throughout the genome to identify SNPs that are statistically associated with a phenotype of interest, in this case CLL, in thousands of cases and controls. The SNPs that are investigated are typically common in the population, with minor allele frequency at least 5%. These studies are large enough to identify alleles associated with small relative risks with high statistical confidence. For example, the first GWAS conducted for CLL analyzed 299,983 SNPs in a total of 1529 cases and 3115 controls from a European cohort [Di Bernardo *et al.* 2008]. Seven SNPs representing six CLL risk loci were identified in this study, located at 2q13, 2q37.1, 6p25 near the *IRF4* gene, 11q24, 15q23 and 19q13 [Di Bernardo *et al.* 2008]. The SNPs identified in this and all GWAS are generally located in noncoding regions, may only be markers for the actual risk locus, and lack known direct functional effects in most cases, but show strong statistical association with disease.

The same group went on to extend their findings by adding a UK and Spanish replication cohort, followed by an additional Swedish replication cohort [Crowther-Swanepoel *et al.* 2010a]. This second study confirmed five of the loci from the initial study and identified four additional loci, including 2q37.3, 8q24.21 within the region near *MYC* known to be associated with disease risk for multiple solid tumors, 15q21.3, and 16q24.1 near *IRF8* [Crowther-Swanepoel *et al.* 2010a]. A subsequent case-control study investigated the role of these 10 SNPs in MBL using 419 cases and found associations with 9 SNPs, 6 of which were statistically significant, suggesting a shared genetic predisposition to MBL and CLL [Crowther-Swanepoel *et al.* 2010c]. Ultimately two additional loci at 15q25.2 and 18q21.1, which had shown a trend toward significance in the initial study, were subsequently confirmed through the addition of new cohorts from Poland, Italy and the UK [Crowther-Swanepoel *et al.* 2011]. Thus, a total of 12 loci were identified in these GWAS. These loci should be associated with all CLL risk, not just familial CLL risk, although the initial cohorts were enriched in familial CLL cases.

Slager and colleagues performed a GWAS that particularly targeted familial CLL, with an initial discovery set of 407 American Caucasian CLL cases, including 102 familial cases, and 296 controls [Slager *et al.* 2011]. A second-stage validation included 252 familial CLL cases and 965 controls. They found four SNPs, all in introns of

the *IRF8* gene and all with genomewide significance, located at the previously identified 16q24.1 locus. All four risk alleles were associated with increased *IRF8* mRNA levels in lymphocytes, providing some evidence for *IRF8* as a candidate target gene. A recently published follow-up study performed sequencing of *IRF8* to identify additional polymorphisms, and ultimately found the strongest association signal with rs1044873, a SNP in the 3' untranslated region (UTR) of *IRF8*. However this variant did not associate with altered *IRF8* expression as determined from publicly available gene expression profiling data, nor did it have a predicted miRNA binding site, so its significance remains unknown [Slager *et al.* 2013]. The other region identified in the Slager and colleagues study and which was particularly associated with familial CLL was the 6p21.3 region, interestingly similar to the linkage results discussed earlier that identified the *MHC* region. In this study the signal was specifically focused on the *HLA-DQA1* and *HLA-DRB5* genes [Slager *et al.* 2011]. An earlier study had associated *HLA-DRB1* with familial CLL [Theodorou *et al.* 2002]. This region and the *IRF8* region discussed above were both also associated with MBL risk in their high-risk CLL families [Slager *et al.* 2011].

Understanding the risk alleles at 6p21 has proven complex. Given the recently described ability to predict classic HLA alleles from SNP data, the UK group performed a SNP and haplotype analysis of a 5 Mb region at the *MHC* locus at 6p21 [DiBernardo *et al.* 2013]. This analysis identified the *HLA-A* region as the primary source of signal, and found that the following alleles were significantly associated with CLL: *HLA-A*0201*; *HLA-A*3101*; *HLA-B*1401*; *HLA-C*0802*; and *HLA-DRB*1101* [DiBernardo *et al.* 2013]. However only *HLA-A*0201* remained significant after correction for multiple testing [DiBernardo *et al.* 2013]. Interestingly, despite the expectation that the key genes in this region would be in the *HLA* region, a recent meta-analysis of the above three GWAS found an additional independent locus at 6p21.33, rs210142, which lies in the first intron of the *BCL2* antagonist killer 1 gene, or *BAK1* [Slager *et al.* 2012]. *BAK1* antagonizes *BCL2*, and as one would therefore predict, the risk genotype was associated with decreased gene expression [Slager *et al.* 2012]. Thus, in CLL, multiple independent associations have been found in the 6p21 region and larger studies and/or functional analyses will be required to confirm the independent findings.

An additional largely unexplored area relates to defining ethnic differences in risk. A recent study looked at the frequency of the known GWAS alleles in an African-American population and found that most of these alleles were less common in African-American CLL patients compared with Caucasian patients [Coombs *et al.* 2012]. Furthermore the allele frequency was not different among African-Americans with CLL compared with African-American control populations, suggesting that these alleles are not major contributors to disease risk in African-Americans [Coombs *et al.* 2012]. Further work will be required to define genetic risk in African-Americans as well as other ethnic groups.

GWAS: getting to biology?

Although the statistical evidence implicating these GWAS alleles in CLL is compelling, in only a few cases is there a clear biologic hypothesis about how the alleles might promote risk. For example, in the initial GWAS, the strongest statistical evidence pointed to two SNPs that map to 6p25.3 near the *IRF4* gene, one in the 3' UTR and one about 10 kb downstream. Fine-scale mapping of the 6p25.3 locus narrowed the association signal to an 18-kb region containing the 3' UTR of *IRF4* [Crowther-Swanepoel *et al.* 2010b]. The rs872071 SNP in the 3' UTR correlated with lower expression of *IRF4* in lymphoblastoid cell lines. Lower expression of *IRF4* as the risk-inducing phenotype fits with the phenotype of the *IRF4* knockout mouse, which shows a block in lymphocyte maturation and the development of lymphadenopathy [Klein *et al.* 2006; Mittrucker *et al.* 1997]. Although the biology of *IRF4* in CLL remains obscure, several other lines of evidence also implicate *IRF4* in CLL. Rare case reports in the literature have identified *IRF4* as a translocation breakpoint partner in CLL, although the functional effects of these translocations are unclear. In addition, somatic mutations of *IRF4* have been identified in 1.5% of CLLs and reported to activate its transcriptional activity [Havelange *et al.* 2011]. We have also recently reported on a large CLL kindred with apparently autosomal dominant inheritance of CLL in which the disease is associated with a unique germline 720 kb copy number gain at 6p25 [Brown *et al.* 2012a]. Affected individuals have four copies of 6p25, show allele-specific gain of the GWAS risk allele rs872071, and show reduced expression of *IRF4*. Resequencing of this region in this family identified no somatic mutations or novel germline variants, suggesting that the copy gain is the

primary pathogenetic mechanism [Brown *et al.* 2012a]. However, how that copy gain promotes CLL remains obscure, just as the effect of the GWAS allele remains obscure despite the various lines of evidence implicating *IRF4* in CLL. This example is similar to the few other known examples of genes involved in familial CLL, however; specifically, a family with Mendelian inheritance of CLL carries a genetic change affecting a gene more broadly involved in sporadic CLL, but private to that family.

Another perhaps better understood example from GWAS although not yet implicated in familial CLL comes from the region of 8q24.1, a gene desert region approximately 400 kb from *MYC* which has been identified repeatedly in GWAS as carrying risk alleles for solid tumors. In a couple of cases, this region has been shown to function as an enhancer for *MYC*, with risk alleles that alter transcription factor binding [Ahmadiyeh *et al.*, 2010; Wright *et al.*, 2010; Yochum *et al.*, 2010]. Although in CLL this mechanism has not been formally demonstrated, it seems likely to apply, particularly since *MYC* has been implicated in CLL through a transgenic mouse model in which *MYC* and *BALF* collaborate to induce a CLL-like disease in transgenic mice [Zhang *et al.* 2010]. In addition, in our studies of copy number in CLL, we have identified two individuals who had focal somatic gains of the 8q24.1 region, without gains of *MYC*, but who nonetheless showed increased *MYC* expression [Brown *et al.* 2012b]. This mechanism of somatic copy number alteration of GWAS risk alleles has been described only relatively rarely to date but may come to be seen more widely with more detailed testing [Dworkin *et al.*, 2010; Wang *et al.*, 2011].

However, for the majority of SNPs found by GWAS, the actual location of the risk locus itself is not known, and how that risk locus may promote disease is therefore also unknown. Most risk SNPs identified by GWAS are located in noncoding or intergenic regions, prompting the hypothesis that their function lies in regulating gene expression. Efforts to identify genes whose expression is altered by GWAS SNPs, called expression quantitative trait loci (eQTLs), are ongoing. A recently published study in breast cancer found that 1.2% of the variation in tumor gene expression was due to eQTLs and identified three variants associated with transcript levels as well as several new risk loci [Li *et al.* 2013]. Efforts to identify eQTLs in CLL are just beginning. Sille

and colleagues performed eQTL analysis in CLL by integrating genomewide SNP data with publicly available gene expression data from lymphoblastoid cell lines [Sille *et al.* 2012]. They found 19 SNPs associated with differential gene expression in the lymphoblastoid cell lines: 16 SNPs associated with expression of *SP140*, a putative tumor suppressor gene, and 3 SNPs linked to expression of *DACT3*, a member of the WNT/ β -catenin pathway, and *GNG8*, involved in G protein-coupled receptor signaling. Of these, 14 were in predicted regulatory elements [Sille *et al.* 2012]. We have performed a similar analysis, using our own gene expression data from CLL tumor cells, and SNP profiling data from both CLLs and normal tissues [Tesar *et al.* 2012]. The strongest finding in our dataset is an association between the risk allele rs674313 on 6p21 and *HLA-DQA1* expression [Tesar *et al.* 2012], which is interesting given the variety of studies implicating *HLA* in CLL risk. Our data did not replicate the findings of Sille and colleagues which may relate to the differences in gene expression between lymphoblastoid cell lines and CLL. These discordant results illustrate the difficulties of these analyses and suggest that further biologic validation will be required to be certain of these associations.

Applying genomic technologies to familial and sporadic CLL

Our laboratory has taken a somewhat different approach to the problem of identifying genes involved in familial CLL. We have been interested in using high-resolution genomic technologies applied to both tumor and germline of familial as compared with sporadic cases in hopes of identifying events of interest in the familial cases. In a study we recently performed of 44 families with familial CLL in which we used high-resolution Affymetrix SNP6 arrays to scan through the tumor and normal genomes, we identified two families with germline copy number variants associated with familial CLL [Brown *et al.* 2012a]. The first family is the kindred described above, which shows a unique germline copy number gain at 6p25 likely targeting the *IRF4* gene. The region contains four protein-coding genes, none of which show somatic mutations, and the only gene with altered expression due to this CNV is *IRF4*. Furthermore, the copy number gain is allele-specific, affecting the known GWAS risk allele. The second family carries a single-copy germline deletion of 500 kb affecting the *DLEU7* and *RNASEH* genes [Brown *et al.* 2012a]. This

region is immediately adjacent to the known minimal region of somatic deletion at 13q14, which affects *miR-15a/16-1* [Cimmino *et al.* 2005]. Two germline mutations have also been reported in the *miR-15a/16-1* precursor, one of which occurred in a familial CLL case [Calin *et al.* 2005]. The great majority of somatic 13q deletions also extend through the *DLEU7* region [Lia *et al.* 2012], and knockout mice whose deletions extend through this region show a more severe phenotype than those with just deletion of *miR-15a/16-1*. Furthermore in the individuals affected with CLL in our family, this deletion CNV behaves as a classic tumor suppressor gene, showing loss of the second allele in the tumor [Brown *et al.* 2012a]. Our data in these 2 out of 44 families suggest that germline copy number variation affecting genes known to be important in CLL may be a not uncommon mechanism for altering the expression or function of these genes. These findings are consistent with a now extensive body of literature that genes that predispose to cancer or are involved in Mendelian disorders can be mutated not just by point mutations but often by copy number variation or rearrangement [Krepischi *et al.* 2012; Schlien *et al.* 2009].

Where are we now?

The data to date suggest that highly penetrant gene variants that confer high relative risks of developing CLL are rare. In a subset of families with apparently Mendelian inheritance of CLL, this type of causative allele has been found, but generally these events are private to that given family. Interestingly, the genes implicated in these cases are typically also involved more generally in CLL, for example the previously described point mutations in the *miR-15a/16-1* precursor, the copy number loss at *DLEU7*, the *DAPK* gene which is silenced in all CLL, and the copy number variant gain at 6p25 which likely affects *IRF4*.

Nonetheless, these types of families with highly penetrant disease and readily detectable mutational events do not explain the bulk of even familial CLL much less all CLL. The GWAS alleles identified to date, which represent common variants with small relative risks, are estimated to account for about 16% of the familial risk of CLL [Slager *et al.* 2012]. Thus, there is a wide gap in the genetic causation of CLL not explained by what has been found to date. One hypothesis is that this gap may be filled with

relatively rare variants, less common than what was studied in GWAS, but which confer moderate risks, higher than typical for a GWAS allele but lower than typical for genes conferring near Mendelian inheritance [Lupski *et al.* 2011]. Current efforts by our group and others are focused on this latter hypothesis, using next-generation sequencing to analyze the germline sequence of a variety of CLLs both familial and sporadic, to try to identify key recurrent variants which when taken together may account for a significant additional component of the heritability of CLL. Elucidation of the mechanisms underlying familial CLL and genetic predisposition to CLL has clearly proven to be a very difficult problem, but we hope that harnessing the increasing power of genomics will start to yield more answers in the coming years.

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Conflict of interest statement

The author has no conflicts of interest to declare.

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