**Genome-wide association analysis implicates dysregulation of immunity genes in chronic lymphocytic leukemia**

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**Several chronic lymphocytic leukemia (CLL) susceptibility loci have been reported, however much of the heritable risk remains unidentified. We performed a meta-analysis of six genome-wide association studies, imputed using a merged reference panel of 1000 Genomes and UK10K data, totaling 6,200 cases and 17,598 controls after replication. We identified nine novel risk loci at 1p36.11 (rs34676223, *P* = 5.04x10-13), 1q42.13 (rs41271473, *P* = 1.06x10-10), 4q24 (rs71597109, *P* = 1.37x10-10), 4q35.1 (rs57214277, *P* = 3.69x10-8), 6p21.31 (rs3800461, *P* = 1.97x10-8), 11q23.2 (rs61904987, *P* = 2.64x10-11), 18q21.1 (rs1036935, *P* = 3.27x10-8), 19p13.3 (rs7254272, *P* = 4.67x10-8) and 22q13.33 (rs140522, *P* = 2.70x10-9). New and established risk loci map to areas of active chromatin and show an over-representation of transcription factor binding for key determinants of B-cell development and immune response.**

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**INTRODUCTION**

Chronic lymphocytic leukemia (CLL) is an indolent B-cell malignancy that has a strong genetic component, as evidenced by the eight-fold increased risk seen in relatives of CLL patients[1](#_ENREF_1). Our understanding of CLL genetics has been transformed by genome-wide association studies (GWAS) that have identified risk alleles for CLL[2-9](#_ENREF_2). So far common genetic variation at 32 loci has been shown to influence CLL risk. Although projections indicate that additional risk variants for CLL can be discovered by GWAS, the statistical power of the individual existing studies is limited.

To gain a more comprehensive insight into CLL predisposition, we analyzed genome-wide association data from populations of European ancestry from Europe, North America, and Australia, identifying nine new risk loci. Our findings provide additional insights into the genetic and biological basis of CLL risk.

**RESULTS**

**Association analysis**

After quality control, the six GWAS provided single-nucleotide polymorphism (SNP) genotypes on 4,478 cases and 13,213 controls (**Supplementary Tables 1** and **2**). To increase genomic resolution, we imputed >10 million SNPs using the 1000 Genomes Project[10](#_ENREF_10) combined with UK10K[11](#_ENREF_11) as reference. Quantile-Quantile (Q-Q) plots for SNPs with minor allele frequency (MAF) >0.5% post imputation did not show evidence of substantive over-dispersion (*λ* between 1.00–1.10 across the studies; **Supplementary Fig. 1**). Meta-analyzing the association test results from the six series, we derived joint odds ratios (OR) per-allele and 95% confidence intervals (CI) under a fixed-effects model for each SNP and associated *P* values. In this analysis, associations for the established risk loci were consistent in direction and magnitude of effect with previously reported studies (**Fig. 1** and **Supplementary Table 3**).

We identified 16 loci where at least one SNP showed evidence of association with CLL (*P* < 1.0 × 10−7) and which were not previously implicated with CLL risk at genome-wide significance (*i.e.* *P* < 5.0 × 10−8) (**Table 1** and **Supplementary Table 4 and 5**). Where the signal was provided by an imputed SNP we confirmed the fidelity of imputation by genotyping (**Supplementary Table 6**). We substantiated the 16 SNPs using *de novo* genotyping in two studies and *in-silico* replication in two additional studies, totaling 1,722 cases and 4,385 controls. Meta-analysis of the discovery and replication studies revealed genome-wide significant associations for eight novel loci (**Table 1**) at 1p36.11 (rs34676223, *P* = 5.04x10-13), 1q42.13 (rs41271473, *P* = 1.06x10-10), 4q35.1 (rs57214277, *P* = 3.69x10-8), 6p21.31 (rs3800461, *P* = 1.97x10-8), 11q23.2 (rs61904987, *P* = 2.64x10-11), 18q21.1 (rs1036935, *P* = 3.27x10-8), 19p13.3 (rs7254272, *P* = 4.67x10-8) and 22q13.33 (rs140522, *P* = 2.70x10-9). We also confirmed 4q24 (rs71597109, *P* = 1.37x10-10), which has previously been identified as a suggestive risk locus[9](#_ENREF_9). Conditional analysis of GWAS data showed no evidence for additional independent signals at these nine loci. In the remaining seven loci which did not replicate with genome-wide significance, the 9q22.33 locus (rs7026022, *P* = 7.00x10-8) remains suggestive (**Supplementary Table 5**). By analyzing the germline exomes of 141 CLL cases from 66 families we excluded the possibility that the any of the association signals were a consequence of LD with a rare, disruptive coding variant.

Several of the newly identified risk SNPs map in or near to genes with established roles in B-cell biology, hence representing credible candidates for susceptibility to CLL. The 4q24 association marked by rs71597109 (**Fig. 2**) maps to intron 1 of the gene encoding BANK1 (B-cell scaffold protein with ankyrin repeats 1), a B-cell-specific scaffold protein. SNPs at this locus have been associated with systemic lupus erythematosus risk[12](#_ENREF_12). *BANK1* expression is only seen in functional B-cell antigen receptor (BCR)-expressing B-cells, mediating effects through LYN-mediated tyrosine phosphorylation of inositol triphosphate receptors. *BANK1*-deficient mice display higher levels of mature B-cells and spontaneous germinal center B-cells[13](#_ENREF_13), whilst studies in humans found lower *BANK1* transcript levels in CLL versus normal B-cells[14](#_ENREF_14). The 19p13.3 association marked by rs7254272 (**Fig. 2**) maps 2.5kb 5’ to *ZBTB7A* (zinc finger and BTB domain-containing protein 7a, alias *LRF*, leukemia/lymphoma-related factor, pokemon). ZBTB7A is a master regulator of B versus T lymphoid fate. Loss of *ZBTB7A* results in aberrant activation of the NOTCH pathway in lymphoid progenitors. NOTCH is constitutively activated in CLL and is a determinant of resistance to apoptosis in CLL cells. rs34676223 at 1p36.11 maps around 10kb upstream of *MDS2* (**Fig. 2**), which is the fusion partner of ETV6 in t(1;12)(p36;p13) myelodysplasia. Based on RNA-seq data from patients, *MDS2* is over-expressed in CLL versus normal cells and also differentially expressed between two experimentally determined CLL sub-groups[14](#_ENREF_14). The SNP rs57214277, maps to 4q35.1 and resides approximately 140kb centromeric to *IRF2* (interferon regulatory factor 2, **Fig. 2**). IFNαβ, a family of antiviral immune genes, induces IRF2 which inhibits the reactivation of murine gammaherpesvirus[15](#_ENREF_15" \o "Mandal, 2011 #196). Furthermore, SNPs in strong linkage disequilibrium (LD) with rs57214277 are associated with increased expression of *IRF2* as well as *trans*-regulation of a network of genes in lipopolysaccharide and IFNγ treated monocytes[16](#_ENREF_16). rs140522 maps to 22q13.33 (**Fig. 2**), which has previously been associated with multiple sclerosis risk[17](#_ENREF_17). This region of LD contains four genes, of which only *NCAPH2* (non-SMC condensin II complex subunit H2) shows differential expression between CLL and normal B-cells[14](#_ENREF_14) (~2.5-fold lower levels in CLL), and plays an essential role in mitotic chromosome assembly and segregation. rs41271473, rs3800461, rs61904987, and rs1036935 mark genes which have roles in WNT-signaling (*RHOU*), autophagy (*C6orf106*), transcriptional activation (*CXXC1*), kinetochore association (*SKA1*, *ZW10*), and protein degradation (*USP28*, *TMPRSS5*) (**Fig. 2**).

**Relationship between new CLL risk SNPs and clinical phenotype**

We tested for differences in the associations by sex or age at diagnosis for each of the nine risk SNPs using case-only analysis, and observed no relationships (**Supplementary Data 1**). Additionally, case-only analysis in a subset of studies provided no evidence for associations between risk SNP genotypes and IGHV mutation subtype (**Supplementary Data 1**) or overall patient survival (**Supplementary Table 7**). Collectively, these data suggest that these nine risk variants have generic effects on CLL development rather than tumor progression per se.

**Functional annotation of new risk loci**

To gain insight into the biological basis underlying the novel association signals, we first evaluated profiles for three histone marks (H3K4me1, H3K27ac marking active chromatin, and the repressive mark H3K27me3) at each locus, in GM12878 LCL[18](#_ENREF_18) as well as primary CLL samples[19](#_ENREF_19) (**Supplementary Fig. 2**). We also examined ATAC-seq profiles from CLL samples and primary B-cells as a marker of chromatin accessibility[19](#_ENREF_19),[20](#_ENREF_20). Since the strongest associated GWAS SNP may not represent the causal variant, we examined signals across an interval spanning all variants in LD r2>0.2 with the sentinel SNP (based on the 1000 Genomes EUR reference panel). These data revealed regions of active chromatin state at all nine risk loci, in at least one of the cell types. Furthermore, based on the analyses of Hnisz *et al*[21](#_ENREF_21), five of the loci fall within regions designated as ‘super-enhancers’ in either LCLs or CD19 B-cells (**Supplementary Fig. 2**). Overall, these findings suggest that the risk loci annotate regulatory regions and may therefore impact upon CLL risk through modulation of enhancer or promoter activity.

Given the possibility that SNPs might influence enhancer or promoter activity by causing changes in transcription factor (TF) binding, we next evaluated the SNPs at each GWAS locus based on their overlap with TF binding sites. In the absence of comprehensive TF ChIP-seq data from CLL samples, we used regions of chromatin accessibility defined by ATAC-seq data[19](#_ENREF_19) as a surrogate marker for TF binding, identifying 47 SNPs in LD r2>0.2 with the sentinel SNPs that also overlapped ATAC-seq peaks. Using motifbreakR[22](#_ENREF_22" \o "Coetzee, 2015 #198) to predict whether these SNPs might disrupt TF-binding motifs we found 478 potentially disrupted motifs, corresponding to 349 TF-binding sites (**Supplementary Table 7**). Moreover at 10 of the SNPs, the altered motif matched the TFs bound in ChIP-seq data from the ENCODE project (**Supplementary Table 8**, **Supplementary Fig. 3**). In particular, we noted that rs13149699 at 4q35 (r2 = 0.83 with lead SNP rs57214277) was predicted to disrupt SPI1 binding. In addition rs13149699 showed evidence of evolutionary constraint, and in LCL ChIP-seq data, the SNP was bound by SPI1 as well as other TFs with roles in B-cell function including IRF4, PAX5, POU2F2 (alias OCT2) and RELA (**Supplementary Table 8**).

We explored whether there was any association between the genotypes of the nine new risk SNPs and the transcript levels of genes within 1Mb of each respective variant by performing expression quantitative trait loci (eQTL) analysis using gene expression profiles of 468 CLL cases. Additionally, we interrogated publicly accessible expression data on whole blood and lymphoblastoid cell lines (LCLs) (**Supplementary Data 2**). There were significant (FDR < 0.05) and consistent eQTLs between rs3800461 and *C6orf106*, rs1036935 and *SKA1*, rs140522 and *ODF3B*, and rs140522 and *TYMP.*

**Biological inference of all CLL risk loci**

Given our observation that the nine novel risk loci annotate putative regulatory regions, we sought to examine the epigenetic landscape of CLL risk loci on a broader scale, evaluating the enrichment of both histone modifications (N=11) and TF binding (N=82) in GM12878 LCLs, across the new and previously published CLL GWAS risk SNPs. Using the variant set enrichment method of Cowper-Sal lari *et al*[*23*](#_ENREF_23), we identified regions of strong LD (defined as *r*2 > 0.8 and D’ > 0.8) and determined the overlap between these variants and ENCODE ChIP-seq data. Imposing a *P*-value threshold of 5.37x10-4 (*i.e.* 0.05/93), we identified a significant enrichment of histone marks associated with active enhancer and promoter elements (HK4Me1, H3K27ac, H3K9ac) as well as actively transcribed regions (H3K36me3). We also identified an over-representation of TF binding for POLR2A, IRF4, RUNX3, NFATC1, STAT5A, PML and WRNIP1(**Fig 3**). Additionally, although not statistically significant, POU2F2 showed evidence for enriched binding (*P*=7.78x10-4). Several of these TFs have established roles in B-cell function. OCT2, IRF4 and RUNX3, have been shown to be targeted for hypomethylation in B-cells[24](#_ENREF_24). MYC is a direct target of IRF4 in activated B-cells with IRF4 being itself a direct target of MYC transactivation. It is noteworthy that variation at IRF4 and 8q24-MYC are recognized risk factors for CLL[2](#_ENREF_2),[3](#_ENREF_3). Collectively these findings are consistent with CLL GWAS SNPs mapping within regions of active chromatin state that exert effects on B-cell *cis*-regulatory networks.

We investigated the genetic pathways between the gene products in proximity to the GWAS SNPs using the LENS pathway tool[25](#_ENREF_25). These gene products were primarily involved in immune response, BCR-mediated signaling, apoptosis and maintenance of chromosome integrity, as well as inter-connectivity between the gene products (**Fig. 4**). Pathways that were enriched included those related to interferon signaling and apoptosis (**Supplementary Data 3**).

**Impact of risk SNPs on heritability of CLL**

By fitting all SNPs from GWAS simultaneously using Genome-Wide Complex Trait Analysis, the estimated heritability of CLL attributable to all common variation is 34% (± 5%); thus having potential to explain 57% of the overall familial risk. This estimate represents the additive variance and therefore, does not include the potential impact of interactions or dominance effects or gene-environment interactions impacting on CLL risk. The currently identified risk SNPs (newly discovered and previously identified) only account for 25% of the additive heritable risk.

**DISCUSSION**

As well as providing additional evidence for genetic susceptibility to CLL, the new and established risk loci identified further insights into the biological basis of CLL development. These loci annotate genes that participate in interconnecting cellular pathways which are central to B-cell development. In particular, we note the involvement of BCR-mediated signaling with immune responses and apoptosis. Importantly, gene discovery initiatives can have an impact on the successful development of new therapeutic agents[26](#_ENREF_26). In this respect it is notable that Ibrutinib[27](#_ENREF_27" \o "Byrd , 2013 #192) (a BTK-inhibitor) and Idelalisib[28](#_ENREF_28" \o "Furman , 2014 #191) (a PI3KCD inhibitor) mediate their effects through interference of BCR-signaling, and Venetoclax[29](#_ENREF_29" \o "Roberts, 2016 #193) targets the anti-apoptotic behavior of BCL-2. The power of our GWAS to identify common alleles conferring relative risks of 1.2 or greater (such as the rs35923643 variant) is high (~80%). Hence, there are unlikely to be many additional SNPs with similar effects for alleles with frequencies greater than 0.2 in populations of European ancestry. In contrast, our analysis had limited power to detect alleles with smaller effects and/or MAF<0.1. Hence, further GWAS studies in concert with functional analyses should lead to additional insights into CLL biology and afford the prospect of development of novel therapies.

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**AUTHOR CONTRIBUTIONS**

R.S.H. and S.L.S. developed the project and provided overall project management; R.S.H., S.L.S., P.J.L., H.E.S., G.P.S. drafted the manuscript. At The ICR: P.J.L., G.P.S. and H.E.S. performed bioinformatic and statistical analyses; H.E.S. performed project management and supervised genotyping; G.P.S. and A.H. performed sequencing and genotyping. In Newcastle, J.M.A. and D.J.A. conceived of the NCLLC; J.M.A. obtained financial support, supervised laboratory management and oversaw genotyping of cases with NCLLC; N.J.S. and H.M. performed sample management of cases; A.G.H. developed the Newcastle Haematology Biobank, incorporating NCLLC; and T.M-F, G.H.J., G.S., R.J.H., A.R.P., D.J.A., J.R.B., G.P., C.P. and C.F. developed protocols for recruitment of individuals with CLL and sample acquisition and performed sample collection of cases. In Leicester M.J.S.D. performed overall management, collection and processing of samples; and S.J. and A.M. performed DNA extractions and IGHV mutation assays. In Spain, S.B., G.C., D.M-G., I.Q., A.C. and E.C. performed sample collection, genotyping, and expression analysis in CLL cells. In Sweden, L.M. and R.R. performed collection of cases, and H.H. and K.E.S. performed sample collection in the Scandinavian Lymphoma Etiology (SCALE) study. At NCI GWAS/GEC GWAS: S.L.S., S.I.B., N.R., S.J.C. conducted and supervised the genotyping of samples. S.I.B., N.J.C., C.F.S., J.V., A.N., A.M., L.R.G., L.R.T., B.M.B., S.J., W.C., K.E.S., Q.L., A.R.B.-W., M.P.P., C.M.V., P.C., Y.Z., T.Z., G.G.G., C.L., T.G.C., M.L., M.Melbye, B.G., M.G., K.C., W.R.D., B.K.L., L.C., P.M.B., E.A.H., R.D.J., L.F.T., Y.B., P.Boffetta, P.Brennan, M.Maynadie, J.M., D.A., S.W., Z.W., N.E.C., L.M.M., R.K.S., E.R., P.V., R.C.H.V., M.C.S., R.L.M., J.C., S.T., J.J.S., P.K., M.G.E., G.S., G.F., R.J.H., L.M., A.R.P., K.E.N., J.F.F., K.O., H.H., S.J.C., R.R., S.d.S., J.R.C., N.R., and S.L.S. conducted the epidemiological studies and contributed samples to the GWAS. Utah GWAS: N.J.C. designed and directed all aspects of the study; M.G. provided clinical oversight; K.C. provided statistical expertise. UCSF GWAS: C.S. supervised all aspects of the overall study; P.M.B. provided project management; L.C. performed bioinformatic and statistical analyses.

**COMPETING INTERESTS STATEMENT**

The remaining authors declare no competing financial interests.

**DATA ACCESSION**

Accession codes for supporting data: EGAS00001000090, EGAD00001000195, EGAS00001000108, EGAD00000000022, EGAD00000000024, EMBL-EBI E-TABM-1140, EMBL-EBI E-TABM-1036, E-MTAB-945, E-MTAB-1708, EMBL-EBI E-GEUV-1, NCBI dbGaP phs000424.v6.p1. The remaining data are contained within the paper and Supplementary files or available from the authors upon request.

**ONLINE METHODS**

**Ethics**

Collection of patient samples and associated clinico-pathological information was undertaken with written informed consent and relevant ethicalreview board approval at respective study centers in accordance with the tenets of the Declarationof Helsinki. The diagnosis of CLL (ICD-10-CM C91.10, ICD-O M9823/3 and 9670/3) was established in accordance with the International Workshop on Chronic Lymphocytic Leukemia guidelines[30](#_ENREF_30).

**Genome-wide association studies**

The meta-analysis was based on six GWAS[2](#_ENREF_2),[6](#_ENREF_6),[7](#_ENREF_7),[9](#_ENREF_9" \o "Berndt, 2016 #186) (**Supplementary Tables 1 and 2)**. Briefly, the six GWAS comprised – UK-CLL1: 517 CLL cases and 2,698 controls; UK-CLL2: 1,403 CLL cases, 2,501 controls; Genetic Epidemiology of CLL (GEC) Consortium: 396 CLL cases and 296 controls; NHL GWAS Consortium: 1,851 CLL cases and 6,649 controls; UCSF: 214 CLL cases, 751 controls; Utah: 331 CLL cases, 420 controls.

**Quality control of GWAS**

Standard quality control measures were applied to the GWAS[31](#_ENREF_31). Specifically, individuals with low call rate (<94%) as well as all individuals evaluated to be of non-European ancestry (using the HapMap version 2 CEU, JPT/CHB and YRI populations as a reference) were excluded. For apparent first-degree relative pairs, we removed the control from a case-control pair; otherwise, we excluded the individual with the lower call rate. SNPs with a call rate <95% were excluded as were those with a MAF < 0.01 or displaying significant deviation from Hardy-Weinberg equilibrium (*i.e.* *P*<10‑6). GWAS data were imputed to >10 million SNPs with IMPUTE2 v2.3 software[32](#_ENREF_32) using a merged reference panel consisting of data from 1000 Genomes Project (phase 1 integrated release 3, March 2012)[10](#_ENREF_10) and UK10K[11](#_ENREF_11). Genotypes were aligned to the positive strand in both imputation and genotyping. Imputation was conducted separately for each study, and in each, the data were pruned to a common set of SNPs between cases and controls before imputation. We set thresholds for imputation quality to retain potential risk variants with MAF > 0.005 for validation. Poorly imputed SNPs defined by an information measure <0.80 were excluded. Tests of association between imputed SNPs and CLL was performed using logistic regression under an additive genetic model in SNPTESTv2.5[33](#_ENREF_33). The adequacy of the case-control matching and possibility of differential genotyping of cases and controls were formally evaluated using Q-Q plots of test statistics (**Supplementary Fig. 1**). The inflation factor *λ* was based on the 90% least-significant SNPs[34](#_ENREF_34). Where appropriate, principal components, generated using common SNPs, were included in the analysis to limit the effects of cryptic population stratification that otherwise might cause inflation of test statistics. Eigenvectors for the GWAS datasets were inferred using smartpca (part of EIGENSOFT[35](#_ENREF_35)) by merging cases and controls with Phase II HapMap samples.

**Replication studies and technical validation**

The 16 SNPs in the most promising loci were taken forward for *de novo* replication (**Supplementary Table 2)**. The UK replication series comprised 645 cases collected through the NCLLC and Leicester Haematology Tissue Bank and 2,341 controls comprised 2,780 healthy individuals ascertained through the National Study of Colorectal Cancer (1999–2006)[36](#_ENREF_36). These controls were the spouses or unrelated friends of individuals with malignancies. None had a personal history of malignancy at the time of ascertainment. Both cases and controls were British residents and had self-reported European ancestry. The Mayo replication series comprised 407 newly diagnosed cases and 1,207 clinic-based controls from the Mayo Clinic CLL case-control study[37](#_ENREF_37). The eligibility criteria of the cases were age 20 years and older, consented within 9 months of their initial diagnosis at presentation to Mayo Clinic, and no history of HIV. The eligibility criteria for the controls were age 20 years and older, a resident of Minnesota, Iowa, or Wisconsin at the time of appointment at Mayo Clinic, no history of lymphoma or leukemia, and no history of HIV infection. Controls were frequency matched to the regional case distribution on 5-year age group, sex, and geographic area. *In silico* replication was performed in 444 cases and 609 controls from International Cancer Genome Consortium (ICGC), and 226 cases and 228 controls from the Women’s Health Initiative (WHI) study[38](#_ENREF_38),[39](#_ENREF_39).

The fidelity of imputation as assessed by the concordance between imputed and directly genotyped SNPs was examined in a subset of samples (**Supplementary Table 5**). Replication genotyping of UK samples was performed using competitive allele-specific PCR KASPar chemistry (LGC, Hertfordshire, UK); replication genotyping of Mayo samples was performed using Sequenom MassARRAY (Sequenom Inc. San Diego, USA). Primers, probes, and conditions are available on request. Call rates for SNP genotypes were >95% in each of the replication series. To ensure the quality of genotyping in all assays, at least two negative controls and duplicate samples (showing a concordance of >99%) were genotyped at each center. To exclude technical artefacts in genotyping, we performed cross-platform validation of 96 samples and sequenced a set of 96 randomly selected samples from each case and control series to confirm genotyping accuracy. Assays were found to be performing robustly; concordance was >99%.

**Meta-analysis**

Meta-analyses were performed using the fixed-effects inverse-variance method based on the β estimates and standard errors from each study using META v1.6[40](#_ENREF_40). Cochran's Q-statistic to test for heterogeneity, and the *I*2 statistic to quantify the proportion of the total variation due to heterogeneity were calculated[41](#_ENREF_41). Using the meta-analysis summary statistics and LD correlations from a reference panel of 1000 Genomes Project combined with UK10K we used GCTA to perform conditional association analysis[42](#_ENREF_42). Association statistics were calculated for all SNPs conditioning on the top SNP in each loci showing genome-wide significance. This is carried out in a step-wise fashion.

**Exome sequencing data**

Exome sequencing of 141 cases from 66 CLL families was performed using the Nextera Rapid Capture Exome Enrichment kit and a HiSeq2000 machine (both Illumina). Sequence alignment, variant calling and variant filtering were performed as previously described[43](#_ENREF_43).

**Mutational status**

IGHV gene mutation status was determined according to BIOMED-2 protocols as described previously[44](#_ENREF_44). Sequence analysis was conducted using Chromas software version 2.23 (Applied Biosystems) and the international immunogenetics information system database. In accordance with published criteria, we classified sequences with a germline identity of ≥98% as unmutated and those with identity of <98% as mutated.

**Association between genotype and patient outcome**

To examine the relationship between SNP genotype and patient outcome we analysed two patient series: (1) 356 patients from the UK Leukaemia Research Fund (LRF) CLL-4 trial[45](#_ENREF_45) which compared the efficacy of fludarabine, chlorambucil, and the combination of fludarabine plus cyclophosphamide; (2) 377 newly diagnosed patients from Mayo Clinic who were prospectively followed. Cox-regression analysis was used to estimate genotype-specific hazard ratios (HR) and 95% confidence intervals (CIs) with overall survival (OS). Statistical analyses were undertaken using R version 2.5.0.

**Expression quantitative trait locus analysis**

Expression quantitative trait locus (eQTL) analyses were performed by examining the gene expression profiles of 452 CLL cases (Affymetrix Human Genome U219 Array)[46](#_ENREF_46). Additional data was obtained by querying publicly available eQTL mRNA expression data using MuTHER[47](#_ENREF_47" \o "Grundberg, 2012 #112), the Blood eQTL browser[48](#_ENREF_48), and data from the GTEx consortium[49](#_ENREF_49). MuTHER contains expression data on LCLs, skin and adipose tissue from 856 healthy twins. The Blood eQTL browser contains expression data from 5,311 non-transformed peripheral blood samples. We used the whole blood RNA-seq data from GTEx which consisted of data from 338 individuals.

**Functional annotation**

Novel risk SNPs and their proxies (*i.e.* *r*2 > 0.2 in the 1000 Genomes EUR reference panel) were annotated for putative functional effect based upon histone mark ChIP-seq/ChIPmentation data for H3K27ac, H3K4Me1 and H3K27Me3 from GM12878 (LCL)[18](#_ENREF_18) and primary CLL cells[19](#_ENREF_19). We searched for overlap with “super-enhancer” regions as defined by Hnisz *et al*[21](#_ENREF_21), restricting the analysis to the GM12878 cell line and CD19+ B-cells. We also interrogated ATAC-seq data from CLL cells[19](#_ENREF_19) and primary B-cells[20](#_ENREF_20). The novel risk SNPs and their proxies (*r*2 > 0.2 as above) were intersected with regions of accessible chromatin in CLL cells, as defined by Rendeiro *et al*[19](#_ENREF_19),which were used as a surrogate for likely sites of TF binding. SNPs falling within accessible sites (n=47) were taken forward to TF binding motif analysis and were also annotated for genomic evolutionary rate profiling (GERP) score[50](#_ENREF_50) as well as bound TFs based on ENCODE project[18](#_ENREF_18) ChIP-seq data.

**Transcription factor binding disruption analysis**

To determine if the risk variants or their proxies were disrupting motif binding sites, we used the motifbreakR package[22](#_ENREF_22). This tool predicts the effects of variants on TF binding motifs, using position probability matrices to determine the likelihood of observing a particular nucleotide at a specific position within a TF binding site. We tested the SNPs by estimating their effects on over 2,800 binding motifs as characterized by ENCODE[51](#_ENREF_51), FactorBook[52](#_ENREF_52" \o "Wang, 2012 #200), HOCOMOCO[53](#_ENREF_53) and HOMER[54](#_ENREF_54). Scores were calculated using the relative entropy algorithm.

**Enrichment analysis for transcription factor binding and histone marks**

To examine enrichment in specific transcription factor (TF) binding across risk loci we adapted the variant set enrichment method of Cowper-Sal lari *et al*[*23*](#_ENREF_23). Briefly, for each risk locus, a region of strong LD (defined as *r*2 > 0.8 and D’ > 0.8) was determined, and these SNPs were termed the associated variant set (AVS). Transcription factor ChIP-seq uniform peak data was obtained from ENCODE for the GM12878 cell line, which included data for 82 TF and 11 histone marks. For each of these marks, the overlap of the SNPs in the AVS and the binding sites was determined to produce a mapping tally. A null distribution was produced by randomly selecting SNPs with the same characteristics as the risk associated SNPs, and the null mapping tally calculated. This process was repeated 10,000 times, and approximate *P*-values were calculated as the proportion of permutations where null mapping tally was greater or equal to the AVS mapping tally. An enrichment score was calculated by normalizing the tallies to the median of the null distribution. Thus the enrichment score is the number of standard deviations of the AVS mapping tally from the mean of the null distribution tallies.

**Heritability analysis**

We used Genome-wide Complex Trait Analysis (GCTA)[42](#_ENREF_42) to estimate the polygenic variance (*i.e.* heritability) ascribable to all genotyped and imputed GWAS SNPs. SNPs were excluded based on low MAF (MAF < 0.01), poor imputation (info score < 0.4) and evidence of departure from HWE (P < 0.05). Individuals were excluded for poor imputation and where two individuals were closely related. A genetic relationship matrix (GRM) of pairs of samples was used as input for the restricted maximum likelihood analysis to estimate the heritability explained by the selected set of SNPs. To transform the estimated heritability to the liability scale, we used the lifetime risk[55](#_ENREF_55),[56](#_ENREF_56) for CLL, which is estimated to be 0.006 by SEER (http://seer.cancer.gov/statfacts/html/clyl.html). The variance of the risk distribution due to the identified risk loci was calculated as described by Pharoah *et al*[57](#_ENREF_57), assuming that the relative risk when a first degree relative has CLL is 8.5[1](#_ENREF_1).

**Pathway analysis**

To investigate the interaction between the gene products of the GWAS hits, we performed a pathway analysis. We selected the closest coding genes for the lead associated SNPs and then performed pathway analysis using the LENS tool[25](#_ENREF_25), which identifies gene product and protein-protein interactions from HPRD[58](#_ENREF_58) and BioGRID[59](#_ENREF_59" \o "Chatr-Aryamontri, 2013 #190). Enrichment of pathways was assessed using Fisher’s exact test, comparing the overlap of the genes in the network with the genes in the pathway. Pathway data was obtained from REACTOME[60](#_ENREF_60). Cytoscape was used to perform network analyses[61](#_ENREF_61), and the Hive Plot was drawn using HiveR (academic.depauw.edu/~hanson/HiveR/HiveR.html).**FIGURE AND TABLE LEGENDS**

**Figure 1: Manhattan plot of association *P* values.** Shown are the genome-wide *P* values (two sided) of >10 million successfully imputed autosomal SNPs in 4,478 cases and 13,213 controls from the discovery-phase. Text labeled in red are previously identified risk loci, and text labeled in blue are newly identified risk loci. The red horizontal line represents the genome-wide significance threshold of *P* = 5.0 x 10−8.

**Figure 2: Regional plots of association results and recombination rates for new risk loci for chronic lymphocytic leukemia.** Results shown for 1p36.11, 1q42.13, 4q24, 4q35.1, 6p21.31, 11q23.2, 18q21.1, 19p13.3, 22q13.33 (a-i).Plots (drawn using visPig[62](#_ENREF_62)) show association results of both genotyped (triangles) and imputed (circles) SNPs in the GWAS samples and recombination rates. −log10 *P* values (*y* axes) of the SNPs are shown according to their chromosomal positions (*x* axes). The sentinel SNP in each combined analysis is shown as a large circle or triangle and is labeled by its rsID. The color intensity of each symbol reflects the extent of LD with the top genotyped SNP, white (*r*2 = 0) through to dark red (*r*2 = 1.0). Genetic recombination rates, estimated using 1000 Genomes Project samples, are shown with a light blue line. Physical positions are based on NCBI build 37 of the human genome. Also shown are the chromatin-state segmentation track (ChromHMM) for lymphoblastoid cells using data from the HapMap ENCODE Project, andthe positions of genes and transcripts mapping to the region of association.

**Figure 3: Enrichment of transcription factors and histone marks.** The enrichment and over-representation of (a) histone marks and (b) transcription factors using the new risk SNPs and known CLL risk SNPs. The red line represents the Bonferroni corrected *P*-value threshold.

**Figure 4: Hive Plot of common protein-protein interactions in CLL.** Each arm represents a functional annotation term, each arc represents an interaction between two proteins, and the distance from the center of the plot corresponds to a greater number of protein-protein interactions (higher degree of the node). The left arm represents proteins annotated as being involved in B-cell receptor (BCR)-signaling; the top arm represents proteins annotated as immune response; the right arm represents proteins involved in apoptosis; and the bottom arm represents proteins involved in DNA damage and chromosomal integrity. Selected proteins known to be involved in CLL risk are shown.

**Table 1: Summary results for SNPs associated with CLL risk.** RAF is risk allele frequency across all of the discovery and replication datasets, respectively. Odds ratios are derived with respect to the risk allele.

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