Acute lymphoblastic leukemia

**Genetic predisposition to B-cell acute lymphoblastic leukemia at 14q11.2 is mediated by a CEBPE promoter polymorphism**

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**Abstract**

Acute lymphoblastic leukaemia (ALL) is the most common paediatric malignancy. Genome-wide association studies have shown variation at 14q11.2 influences ALL risk. We sought to decipher causal variant(s) at 14q11.2 and the mechanism of tumorigenesis. We show rs2239630 G>A resides in the promoter of the CCAT enhancer-binding protein epsilon (CEBPE) gene. The rs2239630-A risk allele is associated with increased promoter activity and CEBPE expression. Depletion of CEBPE in ALL cells reduces cell growth, correspondingly CEBPE binds to the promoters of electron transport and energy generation genes. RNA-seq in CEBPE depleted cells demonstrates CEBPE regulates the expression of genes involved in B-cell development (IL7R), apoptosis (BCL2), and methotrexate resistance (RASS4L). CEBPE regulated genes significantly overlapped in CEBPE depleted cells, ALL blasts and IGH-CEBPE translocated ALL. This suggests CEBPE regulates a similar set of genes in each, consistent with a common biological mechanism of leukemogenesis for rs2239630 associated and CEBPE translocated ALL. Finally, we map IGH-CEBPE translocation breakpoints in two cases, implicating RAG recombinase activity in their formation.

**Introduction**

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer in western countries. While the aetiology of ALL is poorly understood evidence implicates initiating transforming events occurring in utero [1–3], with secondary events required for transition to malignancy [4].

Our understanding of ALL susceptibility has been transformed by genome-wide association studies (GWAS) [5–8]. GWAS have identified 11 loci associated with ALL, including the 14q11.2 risk locus, which has been replicated in multiple independent series [9, 10]. ALL risk loci often map in the vicinity of B-cell development genes, including IKZF1, GATA3, ARID5B and at 14q11.2 mapping to CCAT enhancer-binding protein epsilon (CEBPE) [6], suggesting a central role for dysregulated B-cell development in leukemogenesis. Correspondingly, crucial B-cell development transcription factors IKZF1, PAX5, and EBF1 are also the targets of frequent somatic mutation [11, 12], highlighting that GWAS signals and somatic mutations may impact upon the same genes.

Around 60% of BCP-ALL is characterised by translocations, most common of which, t(12;21) produces the chimeric, ETV6-RUNX1, transcription factor. Three percent of BCP-ALL features a translocation at the immunoglobulin heavy chain (IGH) locus that often involves a CEBP family member. These juxtapose IGH with the coding region of CEBP, increasing CEBP expression [13], raising the possibility that the 14q11.2
GWAS association is also mediated through increased $CEBPE$ expression.

We sought to identify the causal polymorphism(s) for the 14q11.2 association. Our data are compatible with rs2239630 in the promoter of $CEBPE$ driving the association and increasing $CEBPE$ expression.

**Material and methods**

**Ethics**

Samples and clinicopathological information were collected with ethical board approval. ALL blast RNA sequencing and genotyping sample collection was approved by the Lund University, nos 2011/289 and 2017/796. Informed consent was granted from all participants.

**GWAS**

UK GWAS and German GWAS have been previously reported [6, 7]. Briefly, the UK GWAS comprised 824 cases, genotyped using Illumina Human 317K arrays; WTCC2 controls were of 2699 of 1958 British Birth Cohort and 2501 from the UK Blood Service. German GWAS comprised 834 cases, genotyped using Illumina Human OmniExpress-12v1.0 arrays. Controls comprised 2024 individuals from the Heinz Nixdorf Recall study [14]. GWAS QC has been described previously [7]. Untyped genotypes were imputed using IMPUTE2 v2.3 [15] with combined UK10K (ALSPAC and TwinsUK) [16] and 1000 Genomes Project (phase III) [17] references. Poorly imputed SNPs (INFO score <0.8) were excluded. Association between SNP and ALL was performed using SNPTESTv2.5 [18]. Meta-analysis was undertaken using META v1 [18].

**Hi-C data analysis**

Hi-C data in Supplementary Fig.1 derived from GM12878 (MboI plus replicate) was obtained from (https://www.aidenlab.org/juicebox/) [19]. Data were viewed using balanced Knight-Ruiz normalisation at 5 kb resolution. Hi-C data in Supplementary Fig.7 (GM12878 and human embryonic stem cells) were from (http://yunliweb.its.unc.edu/HUGInu/; GSE87112).

**Cell lines and lentiviral transduction**

REH, NALM6, and SEM B-ALL, Jurkat T-ALL cell lines were obtained from DMSZ (Braunschweig Germany). GM12815 and GM12760 were obtained from the Coriell Institute (Camden NJ, US). Cell lines were maintained at 37 °C, with 5% CO2 in RPMI with 10% FBS and GlutaMAX (Thermo Fisher Scientific). Doxycyclin inducible $CEBPE$ shRNA knockdown, $CEBPE$ and ZNF148 over-expressing cell lines were generated by lentiviral transduction using $CEBPE$ (V3THS_150517(A13), V3THS_404312 (G3)), Empty, Non-targeting pTRIPZ or pCW57.1 vectors (GE Life Sciences or from David Root Addgene plasmid # 41393) into REH. Lentiviral particles were produced in HEK239T cells as described [20, 21]. Transduced REH cells were selected with 750 ng/ml puromycin for 1 week. Polyclonal populations were used for all assays. Cells were tested for mycoplasma using Promokine PCR Mycoplasma Test Kit I/C, no positive results were obtained. Cell identity was confirmed using Promega PowerPlex 16 microsatellite testing kit.

**Plasmid construction and luciferase assays**

The $CEBPE$ promoter containing rs2239630, rs2239632 and rs2239633 and ZNF148 coding sequence cloned in pGL3 Promoter Vector (Promega) or pCW57.1 doxycycline inducible vectors, respectively. For luciferase assays, cells were electroporated and promoter activity was assayed using a Dual-Luciferase assay (Promega). Full methods are described in Supplementary material.

**Genotyping and sequencing**

B-cell epigenetic profiles were obtained from Roadmap [22] and Blueprint [23] projects and ENCODE. Chromatin state dynamics (ChromHMM) projects for E031 is from (http://egg2.wustl.edu/roadmap/data/byFileType/chromhmm Segmentations/ChmmModels/coreMarks/jointModel/final/) and for GM12878 from (http://hgdownload.cse.ucsc.edu/goldenPath/hg19/encodeDCC/wgEncodeBroadHmmp/ wgEncodeBroadHmmpGm12878HMM.bed.gz). DNase hypersensitivity data from the ALL blast p294 (European Genome-phenome Archive EGAD00001002499) is from (ftp://ftp.ebi.ac.uk/pub/databases/blueprint/data/homo_sapiens/GRC h38/bone_marrow/pz_294/Acute_Lymphocytic_Leukemia_ CTR/DNase-Hypersensitivity/NCMLS/) and for GM12 878 from (http://hgdownload.cse.ucsc.edu/goldenPath/ hg19/encodeDCC/wgEncodeUwDnase/ wgEncodeUwDna
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Fig. 1 Regional association plot for 14q11.2. a and b show SNPs (red circles) plotted by GWAS P-values ($-\log_{10}$, y-axis) and location (x-axis, GRCh37/hg19). Recombination rate (cM/Mb) light blue line (y-axis). SNP colour denotes linkage disequilibrium with the lead SNP, ($r^2 = 0$, white, $r^2 = 1.0$, dark red). a Association plot annotated with genes from Gencode v27. Multicolour bar shows B-cell chromatin states from ChomHMM. b Association plot of SNPs within 1 kb of the lead SNP. Blue lines denote transcription factor ChIP-Seq peaks. Black bar, B-cell DNAse hypersensitivity peak.
addition of 400 µl of buffer, all analyses were on a LSRII cytometer (Becton Dickinson).

**Cell line RNA-sequencing**

shRNA expression was induced in REH cells with 1 µg/ml doxycycline (Sigma). Cells were lysed after 144 h and RNA prepared using Qiagen RNAeasy. CEBPE knockdown was verified by qRT-PCR. RNA integrity was assessed using a TapeStation 2200 (Agilent). Three biological replicates were processed each with two controls, (empty, non-targeting shRNA) and two CEBPE shRNAs (A13 and G3). Libraries were prepared using NEBNext Ultra II Directional RNA Library Prep Kit and sequenced on an Illumina HiSeq 2500 using 2 × 101 version 4 paired end chemistry. RNA QC metrics are provided in Supplementary Table 4. FASTQs were filtered to remove adapter contamination using Trimomatic v0.32 [27] and aligned to the human reference genome (human_g1k_v37) and transcript reference (GRCh37.87) using STAR v2.5.1 [28]. Gene and transcript abundance were quantified using RSEMv1.3.0 [29]. Non-coding genes and those with <10 mapped reads in all samples were excluded. Raw gene counts were analysed using EdgeR [30] and DeSeq2 [31].

**ALL blast RNA-sequencing**

IGH-CEBPE translocation BCP-ALL expression data originated from two cohorts. A total of 195 diagnostic cases [32] from Lund University analysed using TCGA RNA sequencing version 2 pipeline quantified by RSEM value and 231 diagnostic [33] cases from the National University of Singapore analysed as per ‘Alignment and Bioinformatic Processing of RNA-Sequencing.’

Gene expression in 2 IGH-CEBPE cases was quantified by Z-score calculated from RSEM TPM (transcripts per 10⁶) based on the mean per gene expression of all non-IGH-CEBPE translocated cases in each cohort.

RNA-Seq from 117 diagnostic BCP-ALL cases from TARGET was downloaded from ftp://caftpd.nci.nih.gov/pub/OCG-DCC/TARGET/ALL/mRNA-Seq/Phase2/L3/expression/BCCA/). Gene expression data were converted to TPM before Spearman correlation analysis in R3.4.2 Blasts were unselected based on molecular subtype.

**SNP arrays**

A total of 150 BCP-ALL cases from the Lund cohort with expression data were genotyped using either Illumina Human 1M-duo Infinium BeadChip, HumanOmni1-Quad BeadChip or IlluminaOmni5M BeadChips as previously described [34, 35], and untyped SNPs imputed from the UK10K and 1000 Genomes project reference panels using the Sanger Imputation Server (https://imputation.sanger.ac.uk/).

**Data availability**

Raw fastq data of RNA-seq from CEBPE-depleted REH cells and CEBPE ChIP-seq from REH has been deposited in the European Genome Phenome archive (https://www.ebi.ac.uk/ega/home EGAS00001002877).
Results

Epigenomic profiling of the 14q11.2 locus

We reviewed a meta-analysis of UK and German GWASs [8], fine-mapping the 14q11.2 risk locus by imputation. The strongest association was the imputed SNP rs2239630 ($P = 1.66 \times 10^{-19}$, odds ratio = 1.45, $P_{\text{het}} = 0.38$, Fig. 1a and Supplementary Table 5). We verified imputation of rs2230630 by sequencing 133 cases. Conditional analysis provided no evidence for an additional independent association. Referencing SNPdb.v150 confirmed imputation captured 100% of variants (minor allele frequency [MAF] > 0.01) within the linkage disequilibrium (LD) block containing rs2239630 (pairwise $r^2 \geq 0.4$).

We examined the regulatory potential of SNPs in LD with rs2239630 using chromatin state modelling [36] in primary B-cells, showing lead SNPs reside in an active promoter. Additionally, a region of B-cell DNase hypersensitivity overlaps rs2239630 (Fig. 1a, b). Encode [37] and Chip Atlas (http://chip-atlas.org/) ChIP-Seq data also revealed binding sites for MAX, YY1, ELF1, SPI1, and TCF3 overlapping rs2239630 (Fig.1b).

rs2239630 influences CEBPE expression and promoter activity

We examined whether 14q11.2 risk SNPs influence CEBPE expression by performing expression quantitative trait loci analysis (eQTL) in ALL blast cells. Restricting our analysis to chr14 disomic blasts ($n = 44$), the rs2239630-A risk allele was associated with 1.8-fold increased CEBPE expression ($P = 0.046$, Fig. 2a). A similar association was seen in MuTHER ($P = 0.017$) [38] and Blood ($P = 4 \times 10^{-4}$) [39] lymphoblastoid cell line datasets. To exclude the potential of a looping cis-regulatory interaction we examined GM12878 Hi-C data [19]. This revealed an interaction between the 14q11.2 locus and the promoter of SLC7A8 (Supplementary Fig. 1A), however no significant association between rs2239630 genotype and SLC7A8 expression was seen in either ALL blasts ($P = 0.14$, Supplementary Fig.1B), MuTHER ($P = 0.31$) or blood ($P > 0.05$) eQTLs datasets.
We assessed whether risk SNP genotype influences CEBPE promoter activity, performing luciferase reporter assays. REH cells transfected with constructs containing the rs2239630-A risk allele displayed 1.9-fold higher luminescence (T-test $P = 0.0014$, Fig. 2b). The same effect was seen in additional ALL cell lines (Supplementary Fig.2). rs2239633 and rs2239632 have been reported to affect CEBPE promoter activity in non B-cell lines [40], however no relationship was shown in ALL cell lines (Supplementary Fig.2).

rs2239630 alleles differentially bind SPI1 and MAX

To examine SPI1, MAX, ELF1, YY1, and TCF3 binding at rs2239630 we performed ChIP in REH and NALM6. Only SP1 and MAX bound rs2239633 and rs2239632 have been reported to affect CEBPE promoter activity in non B-cell lines [40], however no relationship was shown in ALL cell lines (Supplementary Fig.2).

SPI1 but not MAX regulate CEBPE expression

We investigated whether differential SPI1 or MAX binding at rs2239630 accounts for eQTL and luciferase assays by performing siRNA knockdowns. Reduced MAX expression had no impact on CEBPE. Conversely, SPI1 depletion resulted in decreased CEBPE (Fig. 4a, b), a finding inconsistent with rs2239630-A increasing CEBPE expression.

ZNF148 binds the risk allele of rs2239630 and inhibits expression of CEBPE

To search for additional allele-specific protein binding at rs2239630 we performed electrophoretic mobility shift assays (EMSA). Greater protein or complex binding was shown for the rs2239630-A allele in REH, SEM, and JURKAT (Supplementary Fig.5). We used HaploReg v4.1 [41], RegulomeDB [42], and MotifBreakR [43] to identify motifs...
disrupted by rs2239630 (Fig. 5b). Using the criteria of preferential affinity for rs2239630-A allele, a predicted strong effect and expression in REH, identified ZNF148 and ZNF589 (Supplementary Table 6). Two mismatches within the motif of ZNF589 overlapping rs2239630 (Fig. 5b) suggested ZNF148 as the most credible candidate. Performing EMSA antibody supershift assays with REH nuclear extract overexpressing ZNF148 identified ZNF148 as the protein preferentially bound to rs2239630-A (Fig. 5c). Allele-specific binding of ZNF148 was confirmed by ChIP-qPCR (Fig. 6a, b). To investigate allele-specific binding of ZNF148 on CEBPE we generated inducible ZNF148-overexpressing REH

**Fig. 5** ZNF148 binds the rs2239630 risk allele. **a** EMSA allele-specific probes for rs2239630 incubated with REH nuclear protein. **b** Position weighted matrices for ZNF589 and ZNF148 with corresponding genomic sequence below. Yellow boxes highlight base mismatches. **c** EMSA in REH showing differential allelic binding is caused by ZNF148. Supershift assay performed by addition of anti-ZNF148 antibody or IgG isotype control.
cells. Overexpression of ZNF148 reduced CEBPε expression (T-test \( P = 0.046 \), Fig. 6c).

rs2239630-A is associated with marks of active transcription

Since effects of SPI1 and ZNF148 interaction at rs2239630 were inconsistent with higher risk allele CEBPε expression we sought evidence of allele-specific expression (ASE). As it is not possible to directly measure ASE of CEBPε, in the absence of a proxy coding SNP for rs2239630, we assayed CEBPε mRNA expression in ZNF148-overexpressing REH. Target gene expression normalised to geometric mean of PPIA and TUBB, shown relative to empty vector control. Data points, mean of biological replicates \( \pm \) SEM. (d) H3K27ac ChIP-seq in REH. Reads mapping to each allele lead SNPs in the 14q11.2 risk loci are enumerated on the y-axis, hashed bars show the enrichment for reads mapping to risk alleles. * binomial \( P \)-values <0.05

Functional and transcriptional profiling of CEBPε

To investigate CEBPε function in ALL we generated doxycycline inducible CEBPε shRNA cell lines, in REH. CEBPε knockdown reduced cell growth (Fig. 7a), but did not affect cell cycle or apoptosis (Supplementary Fig. 6A). CEBPε-depleted cells did not show differential apoptosis either alone or in combination with cisplatin or TNFα (Supplementary Fig. 6B-D).

To identify genes and pathways regulated by CEBPε, we performed ChIP-Seq and RNA-Seq in CEBPε-depleted cells. Peak calling of ChIP-Seq data identified 313 enriched loci, 83 mapping within 1kb of a transcription start site (TSS) (Supplementary Table 7). We identified enriched motifs using HOMER 4.9.1, revealing the CEBPε consensus motif as TTGCGCAA (binomial \( P = 1 \times 10^{-150} \)) (Supplementary Table 8). Protein-coding genes showing the highest promoter enrichment were GAS7, SPRY4, ANKRD13D, TFEB, and ADGB. Pathways enriched for putative CEBPε-regulated genes were interrogated using REACTOME [44] and PANTHER [45]. Analyses were consistent with CEBPε binding sites being enriched in the promoters of genes involved in precursor metabolites and energy biogenesis (\( P_{\text{REACTOME}} = 5.8 \times 10^{-3}, P_{\text{Panther}} = 4.2 \times 10^{-3} \)) and respiration/electron transport (\( P_{\text{REACTOME}} = 0.01, P_{\text{Panther}} = 0.067 \)), which may contribute to reduced metabolic activity and growth of CEBPε-depleted cells.
We identified genes regulated by CEBPE by performing RNA-Seq in CEBPE-depleted cells (Supplementary Table 9). Consistent with a role in granulocyte development, CEBPE positively regulated MPO and PLD1, genes of B-cell relevance included IL7R, PRAME, BCL2, and RASSF4, each positively regulated. To identify differentially expressed genes directly regulated by CEBPE we mapped ChIP-Seq peaks and gene TSS using Hi-C data in lymphoblastoid and embryonic stem cells [46]. Findings were consistent with direct regulation for 13 of 73 top differentially expressed genes. Hi-C contacts between ChIP-Seq peaks and TSS were seen for 10 of 73 genes, including BCL2, MPO, and FAM69C (Supplementary Table 9). The resolution of Hi-C and proximity of UGT3A2 and CDH12 TSS with ChIP-Seq peaks precluded demonstration of an interaction. Finally, the promoter of C4orf32 bound CEBPE directly.

To evaluate the similarity of CEBPE-regulated genes in CEBPE-depleted and BCP-ALL blast cells we examined RNA-Seq from 117 diagnostic cases from TARGET [47].

Fig. 7 CEBPE depletion reduces cell growth. Genomic breakpoints in two IGH-CEBPE ALL cases. a Cell viability/growth assay in CEBPE-depleted cells. Absorbance (562 nm) normalised to 0 h matched shRNA control shown on y-axis. * T-test P-value < 0.05. Data points, mean of four biological replicates ± SEM. b Schematic of IGH-CEBPE translocation breakpoints. Upper panel, RNA and Sanger sequencing reads spanning ~83 Mb between breakpoints, denoted by dashed lines. Case-specific breakpoints are denoted by coloured vertical lines. Lower panel, genes mapping to the region and GM12878 H3K27ac ChIP-seq.
First, we identified CEBPE-correlated genes, restricting our analysis to those correlated at \( P < 5 \times 10^{-4} \), a significant enrichment in differentially expressed genes from CEBPE-depleted cells was seen (binomial \( P = 5.4 \times 10^{-7} \)). We next examined the similarity of CEBPE-correlated gene profiles from two IGH-CEBPE translocated BCP-ALL cases and 117 ALL blasts. CEBPE was highly over-expressed in IGH-CEBPE translocated cases (mean Z-score = 4.6) and the top 150 differentially expressed genes were highly enriched for CEBPE-correlated genes in the 117 BCP-ALL cases (Binomial \( P = 2.9 \times 10^{-77} \)).

**Mapping IGH-CEBPE breakpoints**

To explore the integrity of the CEBPE transcript associated with t(14;14) IGH-CEBPE ALL, we mapped chr14 breakpoints by RNA and Sanger sequencing of genomic DNA. In contrast to the breakpoints in one previously reported IGH-CEBPE case [13] we observed breakpoints in the 3′ of CEBPE and the 5′ of IGHJ transcripts in both cases (Fig. 7b and Supplementary Figure 7 and 8). Although in one case breakpoints truncated the terminal 13 amino acids of CEBPE this affected a region of no known function suggesting no functional impact, and in a second case the coding sequence was unaffected.

The locus upstream of IGHJ breakpoints contains extensive H3K27ac, associated with enhancer activity, consistent with this region driving increased CEBPE expression (Fig. 7). The position breakpoints at IGHJ implicate aberrant RAG1 and/or RAG2 somatic VDJ recombination in their formation [48]. However, examination downstream of CEBPE breakpoints revealed only consensus RAG hexamer sequence, and not the nonamer sequence required for canonical RAG activity.

**Discussion**

Our data provide a plausible mechanism of increased ALL risk at 14q11.2, driven by increased CEBPE expression mediated via the rs2239630-A risk allele. Epigenetic, eQTL and luciferase data suggest differential promoter activity conferred by rs2239630 results in allele-specific expression of CEBPE.

We identified allele-specific interaction of SPI1 and ZNF148 at rs2239630. The regulatory impact of which is to attenuate increased CEBPE expression associated with rs2239630-A. This suggests that modulation of expression due to allele-specific binding of transcription factors is complex and the aggregate of multiple interacting proteins.

Previously, Wiemels et al. [49] proposed rs2239655 as the 14q11 functional variant for ALL. rs2239635, is weakly correlated with rs2239630 (\( r^2 = 0.52 \)) mapping to the 5′-UTR of CEBPE (Supplementary Figure 9). In contrast to rs2239630, neither GTEx v7, Blood [39] nor MuTHER [38] datasets showed a relationship between rs2239635 genotype and CEBPE expression and RNA-Seq data in chr14 disomic ALL blasts heterozygous for rs2239635 showed no allele bias (\( P = 0.92, n = 20 \)). The authors proposed IKZF1 binding at rs2239635 based on low ChIP enrichment (<2-fold) in bone marrow, which contains a low proportion of B-cell lineages. An analysis of eight B-cell ChIP-Seq datasets in five B-cell types, including precursors, showed no evidence of IKZF1 binding rs2239635 [50–53]. Additionally in silico data, cited by the authors, predicts only a minimal impact of rs2239635 on IKZF1 binding despite apparent abolition of risk allele occupancy (Supplementary Table 10). Furthermore, data from ENCODE suggest rs2239635 is unbound by transcription factors and possesses reduced marks of active transcription relative to rs2239630.

CEBPE is a member of the CBP transcription factor family primarily expressed during granulocye differentiation. Homozygous mutations in CEBPE cause congenital specific granule deficiency [54] and mice with CEBPE disruption have myelopietic defects [55]. However BCP-ALL featuring IGH translocations involving CEBP genes establishes their role in B-cell oncogenesis [13].

We have shown CEBPE depletion reduces growth in ALL cells. Transcriptional profiling of CEBPE-depleted cells demonstrates that it regulates the expression of genes with a role in B-cell development (IL7R [56]), apoptosis inhibition (BCL2 [57]), methotrexate resistance (RASSF4 [58]) and cell survival (PRAME [59]), identifying potential mediators of disease development.

IL7 signalling is required for adult bone marrow B-cell production, where it increases B-cell differentiation, proliferation, and survival [60]. Correspondingly, IL7R−/− mice do not respond to IL7 and have severely reduced B- and T-cell counts [61]; whereas, transgenic IL7-overexpressing mice develop lymphoproliferative disorders of B- and T-cell compartments [62].

BCL2 (B-cell lymphoma 2) expression is elevated in many tumours, including ALL [63, 64], and has been associated with an adverse prognosis. Correspondingly, BCL2 inhibition induces apoptosis in a number of ALL cell lines [57], but not REH, this response has been associated with serine 70 phosphorylation of BCL2 [65], a mark lacking in these cells [66]. BCL2 is currently under investigation as a target for therapy [67].

PRAME (preferentially expressed antigen in melanoma) expression is elevated in ALL [68] and other malignancies [59]. PRAME antagonises retinoic acid signalling promoting cell survival [69]. It encodes an antigen recognised by autologous cytolytic T lymphocytes and has been suggested as a target for immunotherapy.
Inherited variation and expression of RASSF4 are associated with accumulation of methotrexate polyglutamates [58]. Methotrexate is one of the main components of ALL treatment and conversion of the pro-drug to a polyglutamated form is required for efficacy.

Taken together this suggests CEBPE influences the expression of genes potentially contributing to various stages of disease development and progression.

Comparing the expression profiles of CEBPE-depleted cells to BCP-ALL blasts we find CEBPE expression affects similar transcriptional programs in both. We also compare CEBPE-regulated genes in two cases with IGH-CEBPE translocation with BCP-ALL blasts, again demonstrating significant similarities suggesting that the mechanism by which CEBPE drives leukemogenesis is the same for 14q11.2 associated and translocated ALL.

In conclusion, we have shown increased CEBPE expression in ALL patients carrying the rs2239630-A risk allele, and identified genes, involved in B-cell development and apoptosis, via which CEBPE may influence the risk of disease, thus providing a mechanistic basis for the 14q11.2 risk association for ALL. Further functional studies, however, are required to fully decipher the biological basis of differential CEBPE expression on ALL oncogenesis.

**Author contributions** J.B.S. and R.S.H. designed the study and drafted the manuscript; J.B.S. and Y.L. performed laboratory analyses; J.B.S., M.Y., Z.L., and K.P. performed bioinformatic and statistical analyses.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

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