Molecular allelokaryotyping of T-cell prolymphocytic leukemia cells with high density single nucleotide polymorphism arrays identifies novel common genomic lesions and acquired uniparental disomy

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ABSTRACT

Background
T-cell prolymphocytic leukemia is a rare aggressive lymphoproliferative disease with a mature T-cell phenotype and characteristic genomic lesions such as inv(14)(q11q34), t(14;14)(q11;q32) or t(X;14)(q28;q11), mutation of the *ATM* gene on chromosome 11 and secondary alterations such as deletions of chromosome 8p and duplications of 8q.

Design and Methods
We analyzed malignant cells from 18 patients with T-cell prolymphocytic leukemia using high density 250K single nucleotide polymorphism arrays and molecular allelokaryotyping to refine understanding of known alterations and identify new target genes.

Results
Our analyses revealed that characteristic disruptions of chromosome 14 are frequently unbalanced. In the commonly deleted region on chromosome 11, we found recurrent microdeletions targeting the microRNA 34b/c and the transcription factors *ETS1* and *FLI1*. On chromosome 8, we identified genes such as *PLEKHA2*, *NBS1*, *NOV* and *MYST3* to be involved in breakpoints. New recurrent alterations were identified on chromosomes 5p, 12p, 13q, 17 and 22 with a common region of acquired uniparental disomy in four samples on chromosome 17q. Single nucleotide polymorphism array results were confirmed by direct sequencing and quantitative real-time polymerase chain reaction.

Conclusions
The first high density single nucleotide polymorphism array allelokaryotyping of T-cell prolymphocytic leukemia genomes added substantial new details about established alterations in this disease and moreover identified numerous new potential target genes in common breakpoints, deletions and regions of acquired uniparental disomy.

Key words: T-cell prolymphocytic leukemia, SNP array, uniparental disomy, copy number change.

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The online version of this article contains a supplementary appendix.

Introduction

T-cell prolymphocytic leukemia (T-PLL) is a rare lymphoproliferative disease with a mature T-cell phenotype. The median age at presentation is 63 years.[1] Its clinical course is generally aggressive with a poor response to chemotherapy and median survival times ranging from 5 months to 2 years in patients receiving therapies containing alemtuzumab.[2,3]

T-PLL has several characteristic and recurring molecular lesions. These include an inversion or translocation of chromosome 14: inv(14)(q11q34) or t(14;14)(q11;q32), which lead to juxtaposition of the *T-cell receptor (TCR) α/δ* enhancer regions to the *T-cell leukemia 1 (TCL1)* locus causing deregulated expression of oncogenes located in this region.[4] An alternative translocation associated with T-PLL is the t(X;14)(q28;q11) juxtaposing the *TCR α/δ* to the *MTCP1* gene.[5] Other common molecular abnormalities in T-PLL are deletions on chromosome 11 involving the *ataxia-telangiectasia mutated (ATM)* gene, which has been shown to be mutated in patients with T-PLL, and common chromosomal gains of 8q and losses of 8p.[6-11]

The *TCL1* family of oncogenes enhances proliferation and survival in several lymphocytic malignancies by binding and augmenting activation of *AKT*[12] and inhibiting activation induced cell death via impairment of the *PKCθ* and *ERK* pathways.[13] This is also reflected by the clinical observation of hyperproliferative subsets of T-PLL with high levels of expression *TCL1*.[14] Mutations in the *ATM* gene are known to be the cause of the rare autosomal recessive disorder ataxia-telangiectasia, which is characterized by cerebellar degeneration, immunodeficiency and increased risk of cancer.[15] *ATM* plays a prominent role in the recognition and repair of DNA double strand breaks[16,17] and the frequent disruption of this gene in T-PLL may be an explanation for the genomic instability observed in this disease. The common genomic abnormalities observed on chromosome 8 have not yet yielded any specific target genes, but the breakpoints occurring on chromosome 8 in T-PLL cluster to two regions which contain the *fibroblast growth factor receptor-1 gene* (*FGFR1*) and the *MOZ* gene, suggesting them as possible candidate genes.[9]

In searches for new T-PLL specific target genes, recent studies have employed techniques such as comparative genomic hybridization (CGH) and 50K single nucleotide polymorphism (SNP) arrays combined with gene expression analysis. These studies have described several differentially regulated genes possibly due to gene dosage effects[18] and *CDKN1B* haploinsufficiency as a new pathogenic mechanism in T-PLL.[19]

Recently, SNP arrays with a higher resolution (250,000 SNPs interrogated per array) have been developed for whole genome mapping.[20] The analysis of genomic DNA with SNP arrays provides two different types of information. One is a data set comprising the intensity data of all SNPs. Since the human genome is diploid, the intensity values are raised to two after normalization, which represents the normal expression of SNPs on somatic chromosomes. A homozygous deletion results in an expression value of zero and a heterozygous deletion in an expression value of one. Amplifications result in expression values of three or higher integer copy numbers. Apart from copy number data, the method also yields a genotype data set which contains the SNP calls of either AA, AB or BB standing for the alleles of the SNPs. This, combined with the copy number data, which allows the detection of acquired uniparental disomy (UPD), which represents allelic imbalance when one allele is deleted and the other one is duplicated or amplified leading to regions with homozygous SNP calls but a copy number of two or higher. These regions typically contain a mutant tumor suppressor gene or oncogene with loss of their normal allele. Use of these high density SNP arrays in combination with a new computational calculation algorithm termed *molecular allelokaryotyping*[21] allows robust and detailed detection of the described alterations without a need for paired normal DNA samples. In the current study, we used this new interrogational power to assess the genomes of 18 T-PLL samples and thereby identify more precisely common submicroscopic genomic lesions and breakpoints and detect novel common genomic lesions and acquired UPD as potential new pathogenic factors in T-PLL.

Design and Methods

Patients and samples

We studied 18 cases of T-PLL (TP followed by the case number). Samples TP-04, -21, -22, -25, -28, -34, -35, -37, -41, -43, -56 and -57 were obtained from the Institut Curie, Centre de Recherche (Paris, France) and are identical to the samples used in the study by Le Toriellec *et al*.[19] Samples TP-651 and -799 came from the Department of Haematology, University Hospitals Leicester (United Kingdom) and samples TP-166, -168, -170 and -172 were from the Department of Hematology and Oncology, School of Medicine, University of Tokyo (Japan). The acquisition and analysis of patients' DNA samples was conducted with the approval of the local ethical committees of the respective institutions.

The diagnosis of T-PLL was established according to the World Health Organization (WHO) classification of hematopoietic and lymphoid tumors. T-PLL genomic DNA was isolated from residual frozen mononuclear cells from leukemic peripheral blood taken at the time of the initial diagnosis. DNA was extracted using a NucleosSpin Tissue kit (Macherey-Nagel, Hoerdt, France). Paired normal DNA was isolated from Epstein-Barr-virus-transformed lymphoblastoid cell lines, which were generated from frozen blood samples of the corresponding patients. All patients had major lymphocytosis. One case (TP56) arose in an individual with ataxia telangiectasia.

High density single nucleotide polymorphism-array analysis

High quality genomic DNA from the 18 T-PLL cases was processed according to the genomic mapping 250K NspI protocol and hybridized to 250K NspI SNP arrays

using the GeneChip Fluidics station 400 and GeneChip scanner 3000 (Affymetrix, Santa Clara, CA, USA) as described previously.[21,22] Data analysis of deletions, amplifications and UPD was carried out using the CNAG software with non-matched references, as previously described.[21,22] Size, position and location of genes were identified with the UCSC Genome Browser *http://genome.ucsc.edu/* and the Ensemble Genome Browser *http://www.ensembl.org/*.

Validation of acquired uniparental disomy and genomic copy number change

For confirmation of genomic copy number changes, quantitative real-time polymerase chain reaction (PCR) was performed on the genomic DNA from the hybridized T-PLL samples and from matched normal DNA from the same patients according to the calculation method described by Weksberg *et al.*[23] Thereby, we confirmed the deletion of the *FOXP1* gene on chromosome 3 in two samples and used a random region on chromosome 2p21 as a reference. Detection of acquired UPD was validated by PCR of genomic DNA and subsequent direct sequencing of SNPs in a region of acquired UPD versus a heterozygous region in sample TP28 on chromosome 17 and compared to direct sequencing of SNPs in the corresponding matched normal sample. All primer sequences are available on request.

Results

Copy number analysis

As expected from previous studies summarizing genomic lesions in T-PLL,[18,24] we found a great abundance of copy number alterations present in the 18 T-PLL samples. In an initial step, we evaluated all losses and gains of genomic material detected by the allelokaryotyping software, which are depicted for each chromosome in Figure 1. We excluded alterations, which were determined to be due to background noise of SNP signals or copy number polymorphisms as recorded in data bases of the UCSC genome browser. The results of this analysis are documented in *Online Supplementary Table S1*. The data re-confirmed characteristic genomic lesions described in T-PLL. Moreover, a variety of other alterations containing interesting putative target genes were detected. In the first systematic measure, we sought to review the above mentioned characteristic lesions on chromosomes 14, 11 and 8.

Chromosome 14

The characteristic inversion inv(14)(q11q34) or translocation t(14;14)(q11;q32) in T-PLL should not be detected by SNP array as no copy number changes occur by these balanced alterations. Interestingly, however, several copy number alterations involving the *TCL* oncogenes were detectable. Sample TP22 displayed a heterozygous deletion in region chr14:95130522-95211348 which partly covers *T-cell leukemia/lymphoma 6 isoform TCL6a3*. Furthermore, five samples had duplications of the telomeric end of chromosome 14 (example, Figure 2A) and a putative breakpoint within the *TCL* oncogenes or in their direct vicinity, distal to them, suggesting that the alterations affecting this site are unbalanced in these samples. The breakpoints leading to duplication or amplification in the respective samples are schematically displayed in Figure 2B, which shows that they lie in typical regions also described for the known translocations.[4] An analysis of the alternate translocation t(X;14)(q28;q11) on chromosome X revealed no unbalanced lesions.

Chromosome 8

The high density genomic mapping carried out in our study revealed broad heterozygous deletions of chromosome 8p in nine samples (50%) and duplications or amplification of chromosome 8q in 13 samples (72%). The detailed mapping of the breakpoints leading to these imbalances revealed that they were scattered over a broad region on chromosome 8p and often displayed highly complex copy number alterations with numerous breakpoints (Figure 2C). In search of genes in the commonly affected regions, we analyzed all chromosomal breaks in all samples on chromosome 8 also including alterations on chromosome 8q. Although an unambiguous common breakpoint could not be identified and most breakpoints were located in regions, that did not

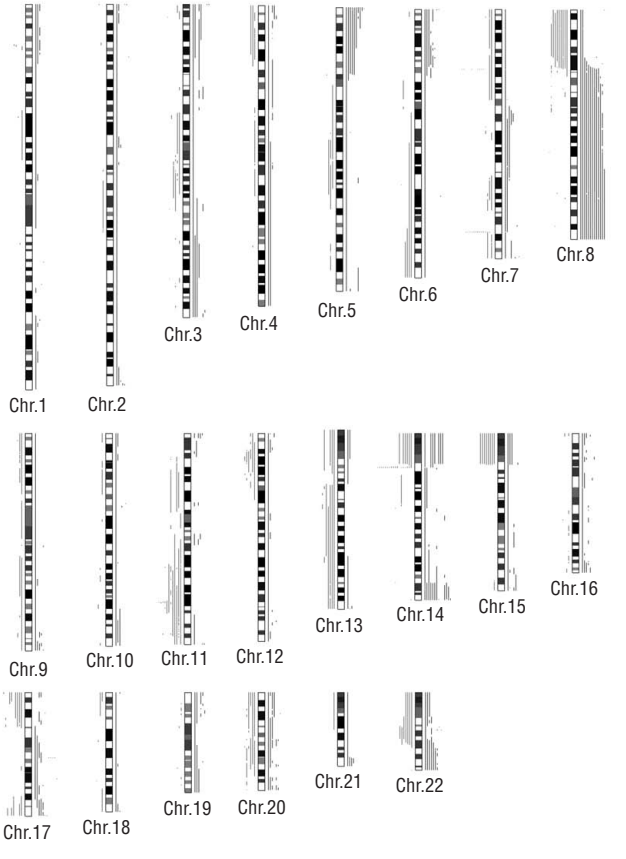

Figure 1. Overview of gains and losses detected by the CNAG software. Lines to the right of the cytobands document gains. Lines to the left of the cytobands represent losses. Each line represents one sample.

contain any genes, some breaks were notable because they were positioned directly within genes and recurred in at least two samples. Breakpoints, that were intragenic are summarized in Table 1. Genes commonly affected by breaks on chromosome 8 were *general transcription factor IIE polypeptide 2* (*GTF2E2*) (two samples), *peroxidasin homolog-like* (*PXDNL*) (two samples), *pleckstrin homology domain containing family A* (*PLEKHA2*) (two samples), *nibrin isoform 2* (NBN) (two samples), *CUB and Sushi multiple domains 3 isoform 2* (CSMD3) (two samples) and *minichromosome maintenance complex component 4* (*MCM4*). Furthermore, in single cases, interesting target genes such as *nephroblastoma overexpressed precursor* (*NOV*) or *MYST histone acetyltransferase monocytic* (*MYST3*) were involved directly in chromosomal breakpoints detected by SNP array.

Chromosome 11

In our study, 12 samples (67%) displayed heterozygous deletions on chromosome 11q, all including the *ATM* gene. Deletions either affected broad regions of chromosome 11q (seven samples) or very discrete deletions specifically targeting the *ATM* locus (five samples). Interestingly, several small defined regions other than the *ATM* deletions were also identified on chromosome 11q.

Sample TP4 displayed a 700 kb heterozygous microdeletion on chromosome 11q33.1 (110891599-111596604), which has its proximal breakpoint in the direct vicinity of two micro RNA, *hsa-mir-34b and hsa-mir 34c*. This micro RNA locus was affected either by chromosomal breakage in the direct vicinity or heterozygous deletion in eight samples (44%). Another region affected by small, confined lesions in several samples contains two members of the *ETS* family of transcription factors, *v-ets erythroblastosis virus E26 oncogene* (*ETS1*) and *Friend leukemia virus integration 1* (*FLI1*). These two genes were contained in heterozygous deletions in seven samples (39%).

Homozygous deletions

Homozygous deletions were abundantly detected on chromosomes 14 and 7 in the T-cell receptor loci. These deletions have to be understood as physiological as part of the T-cell receptor rearrangements and were, there-

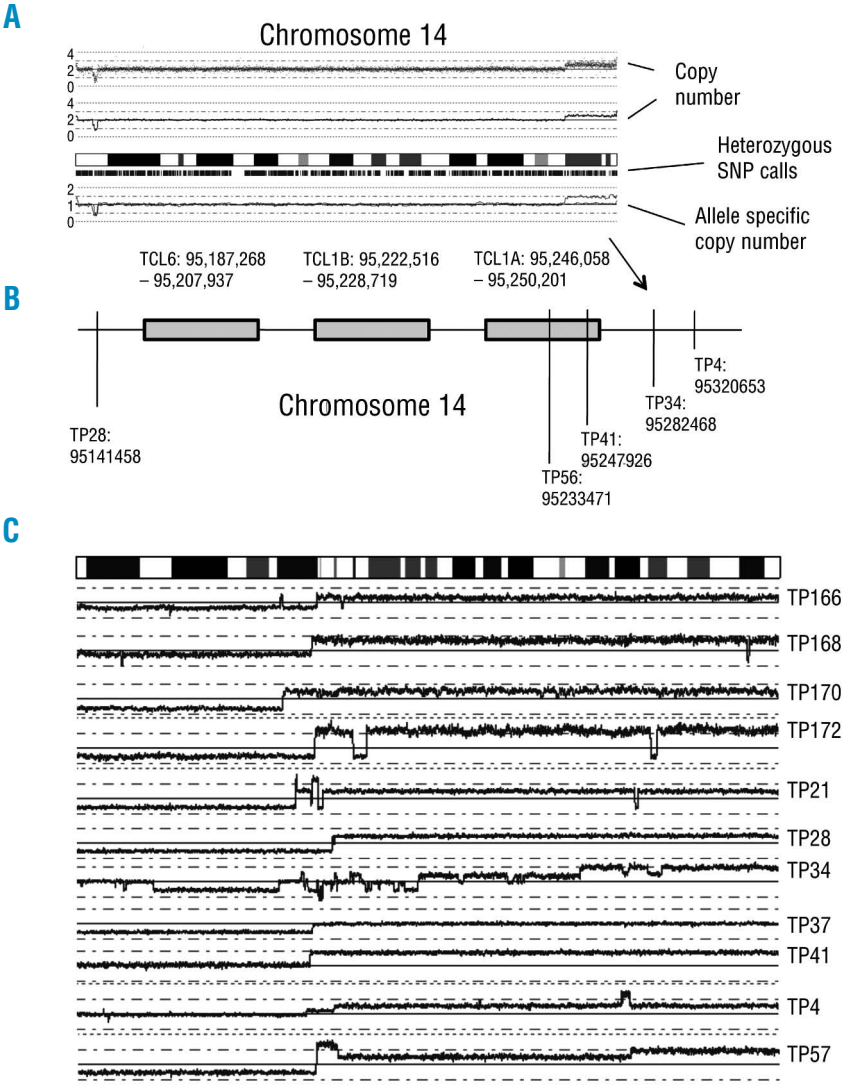

Figure 2. Breakpoints of unbalanced translocations involving the *TCL1* locus and breakpoints on chromosome 8. (A) A view of chromosome 14 in sample TP34 showing a duplication of the chromosomal region 14q32.13 – 14q32.33 harboring a breakpoint in the *TCL1* locus. Similar unbalanced translocations were detected in four other samples, and their breakpoints in relation to the three putative oncogenes *TCL6*, *TCL1B* and *TCL1A* are displayed in the lower panel (B). (C) T-PLL commonly displays a loss of chromosome 8p and gain of 8q. The regions of chromosomal breakpoints leading to this imbalance, as analyzed by high density SNP arrays are displayed. The images show that the breakages are frequently of highly complex nature, containing multiple changes of copy number and breakpoints in individual samples.

fore, excluded from the analysis. Apart from these, scattered homozygous deletions were detected in single samples and are summarized in Table 2. Most of these deletions were single events, not recurring in other samples except for the circumscribed deletion of the transcription factor *forkhead box P1 isoform 1* (*FOXP1*), which was additionally heterozygously deleted in two other samples. Due to this targeted deletion of *FOXP1*, we performed a mutation analysis of this gene by directly sequencing all exons in the samples affected by a deletion. However, this yielded no mutations.

Novel common lesions and acquired uniparental disomy

Besides the common lesions already described, our data showed a cumulation of noteworthy genomic alterations in other loci, which have not yet been described in T-PLL. As shown in Figure 1 and documented in Table 1, the allelokaryotyping software detected ten duplications and amplifications on chromosome 5p. Here, one lesion stood out to be common in three samples (TP172, TP28 and TP56). These three samples displayed a common breakpoint clustering on chromosome 5p within the direct vicinity of the gene *dynein axonemal heavy chain 5* (*DNAH5*). Potentially, this region may represent a common fusion partner to cancer-relevant genes in these samples.

Another hot-spot of alterations was on chromosome 12p, a location long known to be a region for frequent chromosomal rearrangements in hematologic malignancies.[25] Six samples featured heterozygous deletions and four samples had stretches of duplication in this region.

Table 1. Breakpoints involving genes on chromosome 8.

Sample	Breakpoints	Gene symbol / Ref seq (NCBI)	Gene name
TP166	30525448-30556394	*RBPMS (NM_001008711)*	RNA-binding protein with multiple splicing
		GTF2E2 (NM_002095)	**General transcription factor IIE, polypeptide 2**
		ZNF703 (NM_025069.1)	Zinc finger protein 703
TP170	30556573-30567360	***GTF2E2 (NM_002095)***	**General transcription factor IIE, polypeptide 2**
TP172	52424024-52449065	***PXDNL (NM_144651.4)***	**Peroxidasin homolog-like**
	55475848-55486603	*No gene*	
	119646159- 119658006	*SAMD12 (NM_207506.2)*	Sterile α motif domain containing 12 isoform
	120496451- 120498925	*NOV (NM_002514.3)*	Nephroblastoma overexpressed precursor
TP21	32725125-32748740	*NRG1 (NM_013956.2)*	Neuregulin 1 isoform HRG-β1
	38923786-38940822	***PLEKHA2 (NM_021623.1)***	**Pleckstrin homology domain containing family A**
	116672278-116694882	*TRPS1 (NM_014112.2)*	Zinc finger transcription factor TRPS1
TP25	18187886-18309434	*NAT2 (NM_000015.2)*	Arylamide acetylase 2
	90873782-91022515	***NBN (NM_001024688.1)***	**Nibrin isoform 2**
TP34	35377596-35400272	*AY358147 (AY358147)*	Netrin receptor UNC5D precursor
	38784934-38800233	*TACC1 (NM_006283.2)*	Transforming acidic coiled-coil containing
	38901784-38923786	***PLEKHA2 (NM_021623.1)***	**Pleckstrin homology domain containing family A**
	38959748-39003333	*HTRA4 (NM_153692.2)*	HtrA serine peptidase 4
		ADAM9 (NM_003816.2)	ADAM metallopeptidase domain 9 isoform 1
	39992705-40000344	*INDOL1 (NM_194294.2)*	Indoleamine-pyrrole 2 3 dioxygenase-like 1
	41930678-42004277	*MYST3 (NM_006766.3)*	MYST histone acetyltransferase
	48723259-48854459	*KIAA0146 (NM_001080394.1)*	Hypothetical protein LOC23514
		PRKDC (NM_006904.6)	Protein kinase DNA-activated catalytic
	49037364-49184635	***MCM4 (NM_005914.2)***	**Minichromosome maintenance complex component *4***
	51712398-51723458	*SNTG1 (NM_018967.2)*	Syntrophin γ 1
	52578754-52584231	*PXDNL (NM_144651.4)*	Peroxidasin homolog-like
	56339119-56371801	*XKR4 (NM_052898.1)*	XK Kell blood group complex subunit-related
	62477485-62513049	*RLBP1L1 (NM_173519.1)*	Retinaldehyde binding protein 1-like 1
	90991278-91018273	***NBN (NM_001024688.1)***	**Nibrin isoform 2**
	114118249-114142324	***CSMD3 (NM_198124.1)***	**CUB and Sushi multiple domains 3 isoform 2**
	121449563-121485532	*COL14A1 (NM_021110.1)*	Collagen type XIV α 1
		MRPL13 (NM_014078.4)	Mitochondrial ribosomal protein L13
TP4	113993350-114031134	***CSMD3 (NM_198124.1)***	**CUB and Sushi multiple domains 3 isoform 2**
TP57	49037364-49184635	***MCM4 (NM_005914.2)***	**Minichromosome maintenance complex component 4**
		UBE2V2 (NM_003350.2)	Ubiquitin-conjugating enzyme E2 variant 2

A list of all genes that were directly involved in a breakpoint (i.e. a change of copy number) on chromosome 8 in the respective TPLL samples as detected by molecular allelokaryotyping with 250K SNP arrays. Breakpoint positions are defined by the border SNPs of the detected copy number changes. Sometimes several genes were affected in one sample, as the breakpoints were often complex (see also Figure 3). Genes, that were commonly affected by a breakpoint in more than one sample are shown in bold.

The smallest commonly deleted region encompassed 1.8 Mbp (chr12:14746099-16521376) and contained 16 genes. This suggests that besides haploinsufficiency of *CDKN1B*, another tumor suppressor gene may be involved.[19] Of note, two samples (TP166, TP34) harbored breakpoints directly upstream of the promiscuous fusion partner *ETV6*, which is also heterozygously deleted in these samples. This could be an indicator that

Table 2. Homozygous deletions: a list of all homozygous deletions detected in our data set and the genes affected.

Sample	Region	Start-end	Gene symbol / Ref seq (NCBI)	Gene name
TP168	8p23.2	4917841-4975677	No gene	
TP25	20p12.1	14985065-15034862	*MACROD2 (NM_080676.5)*	MACRO domain containing 2 isoform 1
TP34	8p11.23	38923786-38959748	*PLEKHA2 (NM_021623.1)*	Pleckstrin homology domain containing family A
			HTRA4 (NM_153692.2)	HtrA serine peptidase 4
	8p11.21	40000344-40068118	No gene	
	9p12	42937610-43863873	No gene	
	9q31.1	102041415-102711737	*BC038565*	
	22q11.1	15962234-16119107	*IL17R (NM_014339)*	Interleukin 17 receptor precursor
		CECR6 (NM_031890.2)		Cat eye syndrome chromosome region candidate 6
		CECR5 (NM_033070.2)		Cat eye syndrome chromosome region candidate 5
		CECR1 (NM_177405.1)		Cat eye syndrome critical region protein 1
TP35	6q12	67068322-67105350	No gene, *CNP*	
TP4	3p13	71276619-71682261	*FOXP1 NM_032682.4*	Forkhead box P1 isoform 1

Table 3. Regions of acquired uniparental disomy or allelic imbalance.

Sample	Chromosome	Copy number	Start	End	Length (Mbp)
TP166	17q11.1-q25.3	2	22691152	78598059	55.91
TP168	12q21.33-q24.33	2	91163204	132387995	41.22
TP21	5q23.1-q23.2	2	115257555	123818130	8.56
	17q11.2-q25.3	2	27219405	78598059	51.38
TP28	4q13.3-q24	2	70683042	102463716	31.78
	17q21.2-q25.3	2	36884548	78598059	41.71
TP34	8p11.22-22.3	variable	39639901	105716979	66.08
TP37	2p25.1-22.3	2	7952368	32254818	24.30
	3p25.3-21.31	2	10996002	45379802	34.38
	3q23-25.1	2	141171610	151356745	10.19
	3q28-q29	2	191479491	197803570	6.32
	13q31.3-33.1	2	90823104	102071466	11.25
	20p13-12.3	2	169537	7213069	7.04
	20p11.1-q13.33	2	26170699	60036888	33.87
	22q12.3-13.31	2	35263007	43891222	8.63
TP41	8p23.3-p12	2*	180568	36965267	36.78
	11p15.4-q25	2*	3568345	134437775	130.87
	13q11-q34	2*	18380972	114092980	95.71
	17p13.3-p11.2	2*	18901	21459693	21.44
	17q24.3-25.3	2*	68346414	78598059	10.25
	22q11.22-12.3	2*	19767837	30681028	10.91
TP43	5q11.2-q13.2	2	57251185	71082801	13.83
TP57	5q13.1-q14.2	2	67311044	82317258	15.01
	16p13.2-p12.3	2	8822406	16884402	8.06
	18q22.3-q23	2	70382731	75795156	5.41
TP651	2p21	2	42208606	46496503	4.29
	20q11.21-q11.23	3	30400408	35663935	5.26
	20q13.12	3 or 4	43326890	45079291	1.75
	20q13.31-q13.32	3 or 4	54307144	56564113	2.26
	20q13.33	3 or 4	59395068	62111653	2.72

*A list of all regions of acquired UPD (i.e. loss of heterozygosity despite a genomic copy number of two) detected by molecular allelokaryotyping with 250K SNP arrays in our T-PLL samples. *occurred in a sample with tetraploidy.*

ETV6 may be the target of these two deletions. As reported for other hematologic malignancies, the presence of fusions involving the *ETV6* gene is often associated with a deletion or lack of expression of the other *ETV6* allele.[26,27]

A common heterozygous deletion on chromosome 13 occurred in six samples (33%) measuring 1.5 Mbp (chr13: 48879065-50392988). This region is commonly deleted in chronic lymphocytic leukemia and contains two microRNA (*miR-15a and miR-16-1*),[28] which regulate a significant number of cancer-related genes.[29]

Chromosome 17 often has genomic alterations in can-

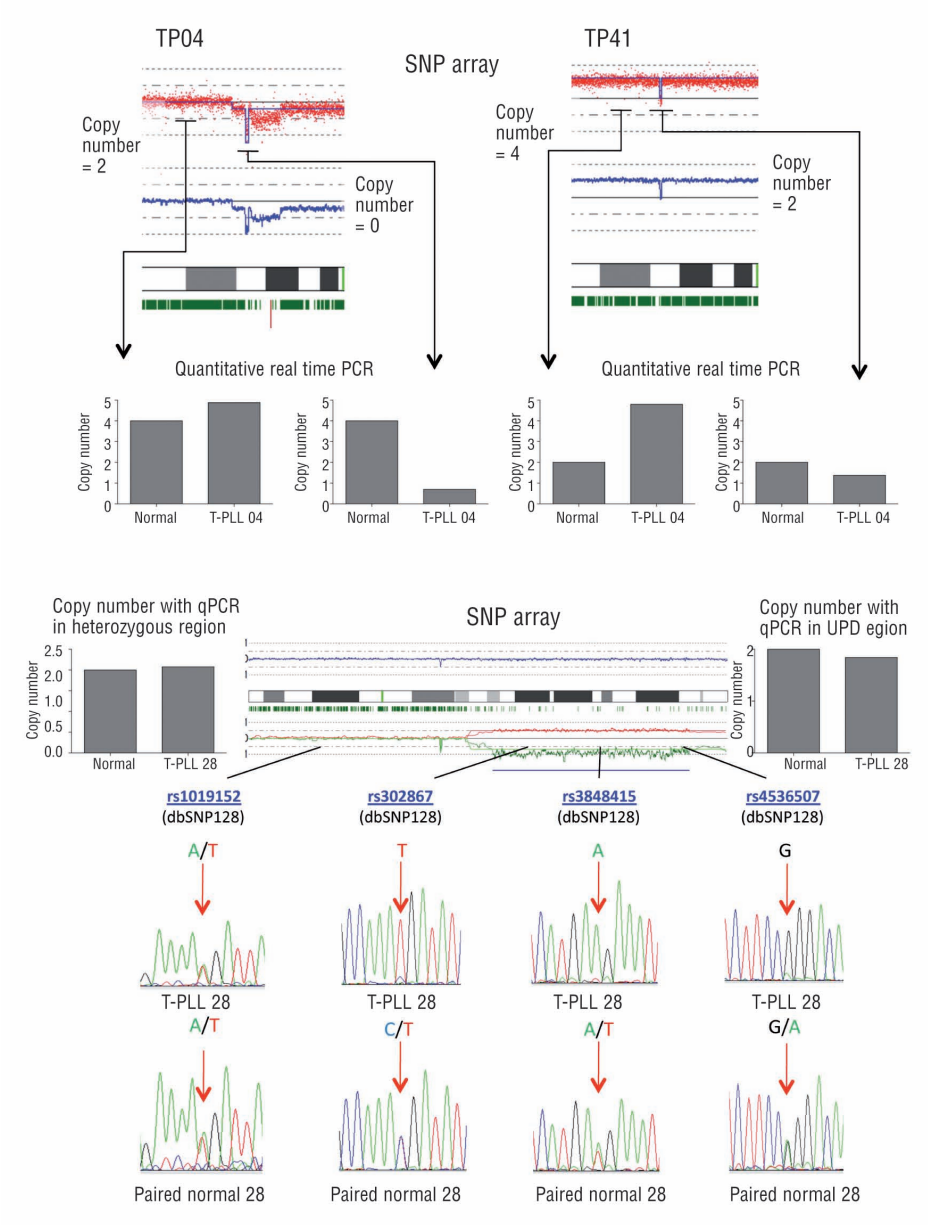

Figure 3. Validation of copy number analysis and acquired UPD. (A) Copy number results obtained by SNP arrays were validated by performing quantitative real-time PCR on genomic DNA of samples containing putative deletions of the *FOXP1* gene. Chromosome views of chromosome 3 are displayed. In sample TP04, *FOXP1* was putatively homozygously deleted (left image, *FOXP1* locus = copy number 0, adjacent regions = copy number: two), while in sample TP41, *FOXP1* was deleted heterozygously in a tetraploid setting (right image, *FOXP1* locus = copy number: two, adjacent regions = copy number: four). Quantitative real-time PCR in the corresponding T-PLL samples and their matched normal DNA confirmed the copy number states estimated by the allelokaryotyping software. (B) Acquired UPD detected by SNP arrays was validated using sample TP28 on chromosome 17. The chromosome view of chromosome 17 in sample TP28 is depicted. Acquired UPD is present on chromosome 17q, as visualized by the divergence of the estimated allele specific copy number (red and green lines in the lower panel of the image), indicating the duplication of one allele and concomitant loss of the other allele and loss of heterozygosity, as evidenced by the abrupt absence of heterozygous SNP calls (vertical green bars directly below the cytoband image). Chromatographs of sequenced SNPs within the acquired UPD region and the adjacent heterozygous region in T-PLL DNA (TP28) and matched normal DNA (paired normal DNA) show that SNPs were homozygous in the T-PLL sample in the acquired UPD region and heterozygous in the matched normal DNA. In the adjacent region, which did not display acquired UPD, the sequenced SNPs were heterozygous in both T-PLL and matched normal samples. Quantitative real-time PCR confirmed a copy number state of two in all regions sequenced in each of the samples.

cer. In total, chromosome 17 was affected by deletion, amplification or acquired UPD in 12 samples. Regions of chromosome 17p were heterozygously deleted in seven cases and affected by an acquired UPD in sample TP41, which featured tetrasomy of most of its chromosomes but showed a copy number of two with deletion of one allele on chromosome 17p. Therefore, eight samples (44%) showed loss of heterozygosity of various lengths on chromosome 17p and in five of these samples, the tumor suppressor *p53* was contained in the affected regions.

Chromosome 17q was affected by heterozygous deletions in five samples (TP22, TP34, TP35, TP4 and TP799) and acquired UPD in four samples (TP21, TP28, TP166 and TP41) and therefore exhibited loss of heterozygosity in nine cases (50%). The smallest commonly deleted region spanned 4.87 Mbp (chr17: 73729578-78599918) and contained potential target genes such as *baculoviral IAP repeat containing protein 5* (*BIRC5*) and *suppressor of cytokine signaling 3* (*SOCS3*). Of special interest is the recurrent acquired UPD or allelic imbalance in this region in four samples. In total, 30 regions of acquired UPD were detected in all samples (Table 3); among these, the acquired UPD observed on chromosome 17q was the only recurring acquired UPD lesion.

Of note, the starting point of acquired UPD of one sample (TP28) lies in the 17q21.2 region which contains the *signal transducer and activator of transcription genes STAT5 A/B and STAT3*, which are key factors in malignant transformation.[30]

Like chromosome 17, chromosome 22 was also commonly affected by loss of heterozygosity either by heterozygous deletion or acquired UPD. Seven T-PLL samples had heterozygous deletions of chromosome 22, with the smallest common lesion measuring 7.4 Mbp (chr.22: 22180211-29584212); two further samples (TP37 and TP41) displayed acquired UPD in the region, so that nine samples (50%) displayed loss of heterozygosity on chromosome 22, making this another interesting site for screening for mutated tumor suppressor genes.

Validation of copy number changes and acquired uniparental disomy

Copy number changes, loss of heterozygosity and acquired UPD detected by high density SNP arrays and molecular allelokaryotyping[21] have been validated extensively by alternative methods in previous studies.[27,31] In the current study, we confirmed copy number results by quantitative real-time PCR on the genomic DNA of the putatively deleted region and the adjacent region to the lesion in patients and matched normal samples in accordance to a method specifically designed to analyze genomic DNA by real-time PCR.[23] We confirmed a homozygous deletion of the *FOXP1* gene on chromosome 3p in sample TP4 and a heterozygous deletion of this gene in sample TP41 (Figure 3A). Acquired UPD was confirmed on chromosome 17 in sample TP28 by sequencing SNPs in the region displaying acquired UPD as compared to the adjacent heterozygous region on the same chromosome (Figure 3B).

Discussion

In this study, we aimed to utilize the increased interrogational power of high density SNP arrays and molecular allelokaryotyping[21] to refine the understanding of known genomic lesions and discover new ones present in the malignant cells of patients with T-PLL. With high quality genomic DNA from 18 T-PLL patients analyzed by 250K SNP arrays, this study currently represents the most detailed genomic examination of this hematologic malignancy.

In a first step of analysis, we sought to re-evaluate established molecular hallmarks of T-PLL. By doing this, we demonstrated that the well-known disruptions of chromosome 14, inv(14)(q11q34) or t(14;14)(q11;q32),[4,12,13] are often unbalanced, indicating excess copies of the involved chromosome 14q fragments.

We refined knowledge of the breakpoints leading to characteristic abnormalities on chromosome 8[18,24,9] at a submicroscopic level and showed that they were often of a highly complex nature. Although a common breakpoint was not identified for all these cases, certain genes were recurrently and directly involved in breakpoints in several samples. *PLEKHA2* is an adaptor protein with a pleckstrin homology domain involved in signaling after activation of lymphocytes.[32] *Nibrin isoform 2* (*NBN*), the *Nijmegen Breakage Syndrome protein* is an important member of the DNA breakage recognition and repair complex consisting of *Mre11*, *Rad50*, *Nbs1* and *ATM*[16] and was disrupted in one of the few samples that did not exhibit an *ATM* deletion (TP25). *Nephroblastoma overexpressed precursor* (*NOV*) is a critical regulator of human hematopoiesis[33] and *MYST histone acetyltransferase monocytic* (*MYST3*) is a histone acetyltransferase commonly involved in translocations in acute myeloid leukemia.[34] All these genes are, therefore, interesting targets for further analysis in the respective samples.

Deletion and mutation of the *ATM* gene is so far the only target gene identified in the commonly deleted region on chromosome 11 in T-PLL and other hematologic malignancies.[6,7,18] The presence of a second tumor suppressor has been hypothesized in this region.[31] Indeed, several other small circumscribed lesions similar to the focal *ATM* deletions were found on chromosome 11. The micro RNA *hsa-mir-34b and hsa-mir 34c* were involved in heterozygous deletions in a total of eight samples. *Hsa-mir-34b* and *hsa-mir 34c* are induced by *p53* and are important regulators in *p53*-dependent pathways[35] and may represent interesting targets, as reduced expression of *miR-34s* has been found in several tumors.[36-38] Furthermore, the oncogenes *ETS1* and *FLI1* were encompassed in small confined deletions in two samples. Disruption of both of these genes has been determined to be an initiating event in malignant transformation of hematologic diseases and solid tumors.[39,40]

In the subsequent search for new common genomic lesions in T-PLL, the *FOXP1* gene was detected in a small homozygous deletion in one sample and was heterozygously deleted in two other samples. *FOXP1* is a member of the FOX family of transcription factors and is involved in the development of the heart, lungs and lym-

phocytes.[41] Deletion and loss of expression in breast cancer confers a worse prognosis[42] and this gene is targeted by recurrent chromosome translocations in mucosal-associated lymphoid tissue (MALT) lymphoma.[43] We confirmed the deletion of this gene by quantitative real-time PCR. Although screening for mutations of this gene in samples containing heterozygous deletions showed no alterations in this respect, the *FOXP1* gene could also be involved in a fusion gene, as recently detected in acute lymphoblastic leukemia.[44] The underlying mechanism for accumulated gene fusions in T-PLL could be increased aberrant V(D)J recombination due to mutation of the *ATM* gene.[45] The concept of perturbed V(D)J recombination has recently also been shown to be responsible for common deletions of the *Ikaros* gene in Philadelphia chromosome-positive acute lymphoblastic leukemia.[46]

Newly detected common lesions with recurrence in six or more samples were found on chromosomes 5p, 12p, 13q, 17 and 22. Loss of heterozygosity, either by heterozygous deletion or acquired UPD, in nine of 18 samples on both chromosome 17 and chromosome 22 suggests common lesions specific to T-PLL. Loss of chromosome 17p is a common phenomenon in chronic lymphocytic leukemia; it is known to confer a worse prognosis and bad response to chemotherapy, possibly through disruption of the *p53* pathway.[47] However, loss of heterozygosity of chromosome 17q at such high frequency has not been reported for leukemic diseases. This finding was further corroborated by the observation of a common region of acquired UPD in four of our samples on chromosome 17q. UPD can either arise through several mechanisms at the level of the gametes such as trisomy rescue, compensatory UPD or gametic complementation or can develop due to a somatic recombinational event.[48] The UPD regions detected in our experiments are most probably acquired isodisomy that evolved due to somatic recombination events shown in our validation of acquired UPD, which demonstrated that loss of heterozygosity was only detectable in the tumor sample but not in the matched control. While acquired UPD has previously been described as a new genomic lesion in T-PLL,[18] this is the first study to report a recurring (n=4) acquired UPD lesion on chromosome 17q.

In conclusion, the use of high density SNP arrays to genotype T-PLL has refined our knowledge of established genomic alterations and revealed numerous new candidate lesions by directly pinpointing affected genes for ongoing functional studies to elucidate the pathogenesis of T-PLL.

Authorship and Disclosures

DN analyzed the data, carried out validation experiments and wrote the paper. ELT and MHS designed the study, acquired samples and wrote the paper. NK designed this study, analyzed the data, acquired samples and wrote the paper. TA analyzed the data, MJD designed the study and acquired samples, WKH wrote the paper, SO designed the study, performed SNP array experiments, molecular allelokaryotyping and acquired samples, HPK designed the study, analyzed the data and wrote the paper.

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