

# Mutations of *PHF6* are associated with mutations of *NOTCH1*, *JAK1* and rearrangement of *SET-NUP214* in T-cell acute lymphoblastic leukemia

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## ABSTRACT

### Background

Mutations in the *PHF6* gene were recently described in patients with T-cell acute lymphoblastic leukemia and in those with acute myeloid leukemia. The present study was designed to determine the prevalence of *PHF6* gene alterations in T-cell acute lymphoblastic leukemia.

### Design and Methods

We analyzed the incidence and prognostic value of *PHF6* mutations in 96 Chinese patients with T-cell acute lymphoblastic leukemia. *PHF6* deletions were screened by real-time quantitative polymerase chain reaction and array-based comparative genomic hybridization. Patients were also investigated for *NOTCH1*, *FBXW7*, *WT1*, and *JAK1* mutations together with *CALM-AF10*, *SET-NUP214*, and *SIL-TAL1* gene rearrangements.

### Results

*PHF6* mutations were identified in 11/59 (18.6%) adult and 2/37 (5.4%) pediatric cases of T-cell acute lymphoblastic leukemia, these incidences being significantly lower than those recently reported. Although *PHF6* is X-linked and mutations have been reported to occur almost exclusively in male patients, we found no sex difference in the incidences of *PHF6* mutations in Chinese patients with T-cell acute lymphoblastic leukemia. *PHF6* deletions were detected in 2/79 (2.5%) patients analyzed. *NOTCH1* mutations, *FBXW7* mutations, *WT1* mutations, *JAK1* mutations, *SIL-TAL1* fusions, *SET-NUP214* fusions and *CALM-AF10* fusions were present in 44/96 (45.8%), 9/96 (9.4%), 4/96 (4.1%), 3/49 (6.1%), 9/48 (18.8%), 3/48 (6.3%) and 0/48 (0%) of patients, respectively. The molecular genetic markers most frequently associated with *PHF6* mutations were *NOTCH1* mutations ( $P=0.003$ ), *SET-NUP214* rearrangements ( $P=0.002$ ), and *JAK1* mutations ( $P=0.005$ ). No differences in disease-free survival and overall survival between T-cell acute lymphoblastic leukemia patients with and without *PHF6* mutations were observed in a short-term follow-up.

### Conclusions

Overall, these results indicate that, in T-cell acute lymphoblastic leukemia, *PHF6* mutations are a recurrent genetic abnormality associated with mutations of *NOTCH1*, *JAK1* and rearrangement of *SET-NUP214*.

Key words: T-cell acute lymphoblastic leukemia, mutation, *PHF6*, *NOTCH1*, *SET-NUP214*.

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The online version of this article has a Supplementary Appendix.

## Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymocytes with multiple genetic abnormalities, accounting for 15% and 25% of newly diagnosed cases of ALL in children and adults, respectively. T-ALL often presents with more unfavorable clinical features than precursor B-ALL, such as a high white blood cell count, bulky adenopathy, and central nervous system infiltration.<sup>1</sup> Although the outcome has improved with current intensive therapy and hematopoietic stem cell transplantation, about 10% of children with T-ALL<sup>2-5</sup> and 37% of adult T-ALL patients relapse and ultimately die.<sup>6</sup> Further improvements in survival rates will require new therapies and targets. In the wake of the Human Genome Project, genes deregulated in specific disease entities may now be readily identified by a fast evolving variety of molecular genetic methods, relevant to disease stratification.

In recent years, genome-wide profiling of DNA copy-number variations and candidate gene sequencing have revealed abundant recurrent gene abnormalities affecting *RAS*,<sup>7</sup> *FLT3*,<sup>8</sup> *LMO2*,<sup>9</sup> *NOTCH1*,<sup>10</sup> *MYB*,<sup>11</sup> *FBXW7*,<sup>12</sup> *JAK1*,<sup>13</sup> *WT1*,<sup>14</sup> *PTPN2*,<sup>15</sup> and *LEF1*<sup>16</sup>.

Inactivating mutations of the plant homeodomain (PHD)-like finger 6 (*PHF6*) gene were originally linked to a form of syndromic X-linked mental retardation, Börjeson-Forssman-Lehmann syndrome (BFLS), originally observed in males.<sup>17</sup> Recently, *PHF6* was identified as a new key tumor suppressor gene in T-ALL. *PHF6* mutations were detected in 38% (16/42) of adult and 16% (14/89) of pediatric T-ALL patients and rarely also in adults with acute myeloid leukemia.<sup>18,19</sup> *PHF6* is X-linked, and tumorigenic mutations have been almost exclusively detected in male patients with either T-ALL or acute myeloid leukemia. This gender-bias has been proposed as contributing to the 3-fold higher incidence of T-ALL in males over females.<sup>18</sup>

In this study, we investigated the incidence and prognostic value of *PHF6* mutations by polymerase chain reaction (PCR) and direct sequencing in a cohort of 96 T-ALL patients. Patients were also assessed for the presence of *NOTCH1*, *FBXW7*, *WT1*, and *JAK1* mutations and *CALM-AF10*, *SET-NUP214* and *SIL-TAL1* rearrangements.

## Design and Methods

### Patients' samples and cell lines

Between June 1998 and August 2010, 297 T-ALL patients were identified following admission to the Jiangsu Institute of Hematology. Overall, genomic DNA from 96 patients was available for use in the present study. The median age of the case series was 23 years (range, 7-56 years), the majority were male (80.2%) and all were from the same ethnic group: the Han Chinese. The main characteristics of the patients in this study and the entire group are summarized in Table 1. The clinical characteristics of these 96 T-ALL patients were representative of the entire group. T-cell phenotype was defined by positivity for CD3, either on the cell surface or in the cytoplasm, with at least two of the B-cell antigens (CD19, CD20, CD22, CD79a) and three of the myeloid markers (CD11b, CD13, CD33, myeloperoxidase) testing negative.

Sample preservation and genetic analysis required informed consent and approval by the Ethics Committee of the First Affiliated Hospital of Soochow University in accordance with the

**Table 1. Clinical characteristics of 96 patients with T-ALL.**

Characteristics	The entire group	The present study
Number of patients	297	96
<b>Clinical features</b>		
Median age, y(range)	23.0(4-76)	23.0(7-56)
Male/female	218/79	77/19
Splenomegaly, %	60.0	63.6
Hepatomegaly, %	29.5	36.5
Lymphadenopathy, %	71.8	60.9
<b>Laboratory data</b>		
WBC, ×10 <sup>9</sup> /L, median(range)	59.5(1.0-899.4)	98.0(1.5-899.4)
Hb, g/L, median(range)	97(19-164)	104(38-164)
PLT, ×10 <sup>9</sup> /L, median(range)	58(4-505)	60(10-286)
LDH, U/L, median(range)	549.6(118-11993)	1150(124-9300)
BM blasts (%), median(range)	80(20-97.5)	85.5(20-97.5)
<b>French-American-British classification</b>		
L1	112	37
L2	94	43
NA	91	16
<b>Cytogenetics, n.(%)</b>		
Normal karyotype	166(55.9)	49(51.0)
Abnormal karyotype	122(41.1)	45(46.9)
Unsuccessful karyotype	9(3.0)	2(2.1)

WBC: white blood cell; Hb: hemoglobin; PLT: platelet; LDH: lactate dehydrogenase; BM: bone marrow; NA: not available.

Declaration of Helsinki. Genomic DNA was extracted from frozen bone marrow mononuclear cells of 68 T-ALL cases after Ficoll gradient centrifugation using standard procedures. In another 28 patients, genomic DNA was extracted from methanol/acetic acid-fixed cells that had been routinely prepared for cytogenetic analysis.

The CCRF-CEM, MOLT-4, and Jurkat T-ALL cell lines (detailed in *www.dsmz.de*) were cultured in RPMI-1640 medium (Gibco BRL, Grand Island, NY, USA) supplemented with 20% heat-inactivated fetal calf serum (Gibco BRL) at 37°C in a humidified incubator with 5% CO<sub>2</sub>.

### Cytogenetic analysis

Cytogenetic studies were carried out on bone marrow cells at diagnosis. Samples were processed using standard 24-h unstimulated cultures. Conventional R-banding assay was used for karyotypic analysis. When possible, at least 20 metaphases per sample were analyzed. Clonal karyotypic abnormalities are described using the International System for Human Cytogenetic Nomenclature (ISCN, 2005).

### Molecular characterization of T-cell acute lymphoblastic leukemia samples

*PHF6* mutations were analyzed by PCR amplification of the entire coding region of *PHF6* exons 2-10 followed by direct bidirectional DNA sequencing as previously described.<sup>18</sup>

*NOTCH1* mutations were detected by PCR amplification and direct DNA sequencing of exons 26, 27, and 34 of the gene.<sup>10</sup> *WT1* mutations were screened by PCR amplification and direct DNA sequencing of exons 7 and 9.<sup>14</sup> *JAK1* mutations were screened for by PCR amplification and direct DNA sequencing of the entire *JAK1* coding region.<sup>13</sup> *FBXW7* mutation analysis was performed by PCR amplification and direct DNA sequencing of exons 9 and 10.<sup>20</sup>

Expression of *SIL-TAL1*, *SET-NUP214*, and *CALM-AF10* was assessed by reverse transcription-PCR (RT-PCR) using previously

**Table 2.** Characteristics of 13 T-ALL patients with a *PHF6* mutation.

Case n.	Sex	Age	Mutation(s)	Exon	Type of mutation	Protein level	Status
1	M	20	c.820T>C	8	nonsense	p.Arg274X	R
2	F	16	c.76-95del20+insTTGG	2	frame-shift	p.Pro26fs	N
3	M	23	c.808C>T	8	nonsense	p.Gln270X	N
4	M	17	c.933_934insT	9	nonsense	p.Ala311X	N
5	M	39	c.525_526delGT	6	frame-shift	p.Ser176fs	N
6	M	34	c.1024C>T	10	nonsense	p.Arg342X	R
7	M	45	c.986A>G	10	missense	p.His329Arg	R
8	M	23	c.835delA	9	frame-shift	p.Lys279fs	N
9	M	24	c.903C>A	9	nonsense	p.Tyr301X	R
10	M	23	c.823G>A	8	missense	p.Gly275Arg	N
11	M	26	c.973T>C	10	missense	p.Tyr325His	N
12	F	46	c.289A>T	4	nonsense	p. Lys97X	N
13	M	16	c.779insCGGGAGGATCC	8	frameshift	p. Asp262fs	N

M: male; F: female; R: reported; N: novel.

published primers and PCR conditions.<sup>17-19</sup> Briefly, total RNA was extracted from bone marrow mononuclear cells of 48 T-ALL cases prepared with Ficoll gradient centrifugation. RNA concentrations were measured by fluorometry. Afterwards cDNA was synthesized using the SuperScript II reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA) with random hexamers. A single PCR was performed in a volume of 25  $\mu$ L using 1  $\mu$ L of cDNA. PCR products were separated on 2% agarose and visualized with GelRed. The quality of RNA was checked by amplification of *GAPDH* gene transcripts. Leukemic cells from two T-ALL patients known to contain the *SIL-TAL1* or *SET-NUP214* fusion transcripts and the U937 cell line with *CALM-AF10* fusion transcript were utilized as positive controls. Primer sequences and PCR conditions are shown in *Online Supplementary Table S1*.

#### Real-time quantification of DNA copy number

Real-time quantitative DNA PCR analysis was performed to screen for genomic *PHF6* deletions in 79 T-ALL patients for whom genomic DNA was available, using TaqMan Universal PCR Mastermix (Applied Biosystems) and the 7500 real-time PCR system (Applied Biosystems) as previously reported.<sup>18</sup> Data were analyzed using the comparative  $\Delta$ Ct method (Applied Biosystems).

#### Array-based comparative genomic hybridization analysis

Array-based comparative genomic hybridization (array-CGH) analysis was performed in 19 T-ALL patients for whom at least 1.5  $\mu$ g genomic DNA was available. Agilent human CGH microarrays (Agilent Technologies, Santa Clara, CA, USA) containing 244 000 probes (244k) with an average spatial resolution of 6.4 Kb were used for the array-CGH experiments according to protocols provided by the manufacturer. Image analysis, normalization and annotation were based on Feature Extraction 9.1 (Agilent), while data were visualized with Agilent Genomic Workbench Lite Edition 6.5 software.

#### Statistical analysis

The patients' characteristics were analyzed by the chi-squared ( $\chi^2$ ) or Fisher's exact tests for univariate analysis. Overall survival was calculated from the date of diagnosis until the date of death or last follow-up through October 31, 2010. Relapse-free survival

was measured from the date of complete remission to the date of first relapse. Kaplan-Meier analysis was used to evaluate patients' survival. The log-rank test was used to compare differences in survival. *P* values less than 0.05 were deemed statistically significant. All calculations were performed using the SPSS software package (version 13.0).

## Results

### *PHF6* mutations and deletions in T-cell acute lymphoblastic leukemia

*PHF6* mutations were identified in 11/59 (18.6%) adult and 2/37 (5.4%) pediatric T-ALL patients analyzed. While just falling short of statistical significance ( $\chi^2$  *P*=0.065), our data indicate an adult preference for *PHF6* mutations in T-ALL. However, we were unable to confirm the 13-fold male excess reported recently in T-ALL.<sup>18</sup> Although 11/13 (84.6%) cases with *PHF6* mutations were male and only 2 of 13 (15.4%) female, this skewing reflected that of our population of patients, which comprised 77 males and 19 females (*P*>0.5), in turn attributable to the approximately 3-fold excess of T-ALL commonly seen in males.

Overall, 13 *PHF6* mutations were documented in this study (Table 2), involving exons 2, 4 and 6 (one case each), and exons 8, 9 and 10 (4, 3 and 3 cases, respectively). *PHF6* mutations were not detected in CCRF-CEM, MOLT-4, or Jurkat cell lines.

The *PHF6* mutations detected in the present study are listed in Table 2 and graphically depicted in Figure 1. Among these, nonsense, frameshift, and missense mutations accounted for, respectively, 46.2% (6/13), 30.8% (4/13) and 23.0% (3/13) of all *PHF6* mutations. The mutation R342X in exon 10 was previously described in the germline of a child with BFLS who developed T-ALL.<sup>24</sup> Two nonsense mutations and one missense mutation have already been described.<sup>18</sup> The remaining nine mutations have not been reported previously. The patients' characteristics are listed in *Online Supplementary Table S2*. In all four of 13 T-ALL cases with *PHF6* mutations tested, analysis of matched newly-diagnosed and remission genomic

DNA confirmed the somatic origin of *PHF6* mutations (nonsense mutations: Arg274X, Tyr301X, Ala311X; missense mutations: Gly275Arg).

In addition, *PHF6* gene deletions were identified in 2/79 (2.5%) T-ALL patients (one male and one female) by quantitative PCR analysis and a 1.4 Mb deletion at Xq26 including *PHF6* confirmed in the female patient by array-CGH (Figure 2).

### NOTCH1, FBXW7, WT1, and JAK1 mutations in T-cell acute lymphoblastic leukemia

*NOTCH1* mutations were detected in 44 (45.8%) of the 96 T-ALL patients in the following domains and with the following frequencies: HD, 59.1%; PEST, 25.0%; HD+PEST 11.4%; and TAD, 4.5%. *FBXW7* mutations were identified in 9/96 (9.4%) T-ALL cases; alone in three, and associated with *NOTCH1* HD mutations in the other six cases. *WT1* mutations were found in 4/96 (4.2%) cases; one patient harbored a missense mutation at codon 462 (R462W) located in exon 9, while the others all had frameshift mutations located in exon 7. *JAK1* mutations were found in 3/49 (6.1%) cases; the mutation was missense in two cases, and a frameshift in another. In all eight available T-ALL cases with *NOTCH1* or *FBXW7* mutations, analysis of matched newly-diagnosed and remission genomic DNA confirmed the somatic origin of mutations (*NOTCH1* mutations: R1597P, L1599P, K1606fs, I1615V, delF@1605+insLGEN@1605/1606, del9@1605-1613+insLGK, delF@1605+insLGVTH@1605/1606; *FBXW7* mutations: R465Y).

### SET-NUP214, SIL-TAL1, and CALM-AF10 fusions in T-cell acute lymphoblastic leukemia

The expression of *SIL-TAL1*, *SET-NUP214*, and *CALM-AF10* fusion transcripts was determined by RT-PCR in 48 patients. *SIL-TAL1* was positive in 9/48 (18.8%) patients. The nine cases with *SIL-TAL1* fusion were younger than the 39 cases without *SIL-TAL1* fusion ( $P=0.018$ ). *SET-NUP214* fusion transcript was detected in 3/48 (6.3%) patients. All three patients with a *SET-NUP214* fusion harbored *PHF6* mutations. No *CALM-AF10* fusion was detected in 48 T-ALL patients tested.

### Correlation of PHF6 mutations with clinical features and prognosis

The clinical features of *PHF6* mutant and *PHF6* wild-type patients are compared in Table 3. *PHF6* mutations were significantly associated with lower lactate dehydrogenase serum levels ( $P=0.01$ ). Cases with *PHF6* mutations had a higher platelet count ( $P=0.001$ ) and higher bone marrow blast count ( $P=0.004$ ), than cases without *PHF6* mutations. No significant differences between the cases with and without *PHF6* mutations were found for median white blood cell count ( $P=0.078$ ) or for median hemoglobin concentration ( $P=0.314$ ).

Of the 96 T-ALL patients undergoing conventional induction chemotherapy, 46/66 patients (69.7%) achieved complete remission. The complete remission rates of cases with and without *PHF6* mutations were not significantly different (80% versus 67.9%, respectively;  $P=0.692$ ). Similarly, no significant difference in 1-year overall survival was found between patients with and without *PHF6* mutations (30.0% versus 51.1%, respectively;  $P=0.059$ ) with a short-term follow-up (median follow-up of 12.5 months; range, 1.0-62 months).

**Table 3. Patients' characteristics and *PHF6* mutations.**

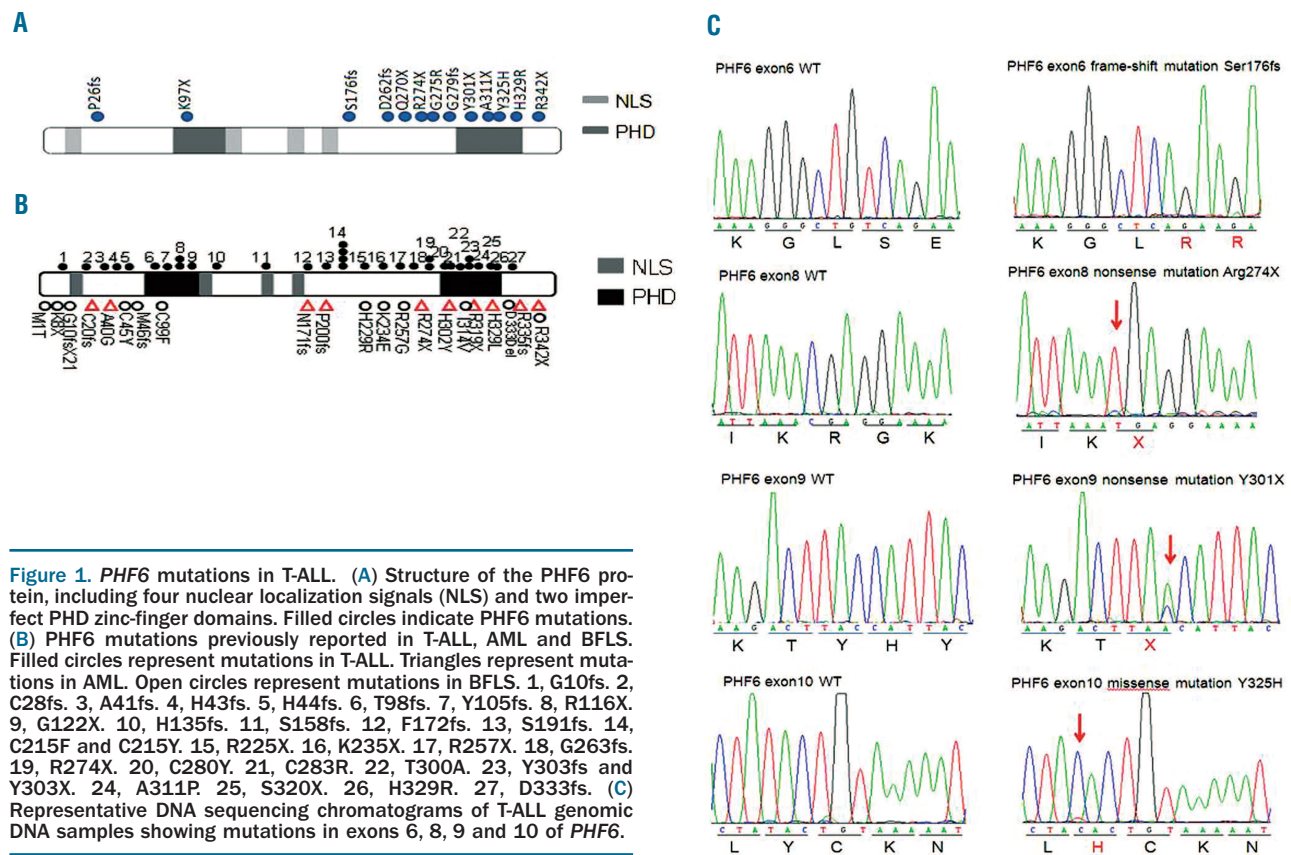
Patients' characteristics	<i>PHF6</i> mutated	<i>PHF6</i> wild type	P
<b>Statistically significant data</b>			
Median PLT, $\times 10^9/L$ (range)	106 (34-184)	51 (10-286)	0.001*
Median LDH, U/L (range)	433 (124-1171)	1359 (189-9300)	0.010*
Median BM blasts, % (range)	90.5 (85.5-96.5)	83 (20-97.5)	0.004*
<i>NOTCH1</i> mutation (n=96)			0.003 <sup>§</sup>
<i>NOTCH1</i> (+)	11	33	
<i>NOTCH1</i> (-)	2	50	
<i>JAK1</i> mutation (n=49)			0.005 <sup>§</sup>
<i>JAK1</i> (+)	3	0	
<i>JAK1</i> (-)	6	40	
<i>SET-NUP214</i> expression (n=48)			0.002 <sup>§</sup>
<i>SET-NUP214</i> (+)	3	0	
<i>SET-NUP214</i> (-)	4	41	
<b>Not statistically significant data</b>			
Gender			0.956 <sup>§</sup>
Male	11	66	
Female	2	17	
Age (years)			0.065 <sup>§</sup>
$\leq 18$	2	35	
$> 18$	11	48	
Median WBC count, $\times 10^9/L$ (range)	33.7 (2.2-530)	106.3 (1.5-899.4)	0.078*
Median Hb, g/L (range)	93 (38-141)	102 (40-164)	0.314*
Karyotype (n=94)			0.054 <sup>§</sup>
Normal karyotype, n (%)	10	39	
Abnormal karyotype, n (%)	3	42	
<i>FBXW7</i> mutation (n=95)			1.000 <sup>§</sup>
<i>FBXW7</i> (+)	1	8	
<i>FBXW7</i> (-)	12	74	
<i>WT1</i> mutation (n=96)			0.447 <sup>§</sup>
<i>WT1</i> (+)	1	3	
<i>WT1</i> (-)	12	80	
<i>SIL-TAL1</i> expression (n=48)			0.395 <sup>§</sup>
<i>SIL-TAL1</i> (+)	0	9	
<i>SIL-TAL1</i> (-)	7	32	
<i>LMO1/LMO2</i> (n=67)			0.526 <sup>§</sup>
<i>LMO1/LMO2</i> (+)	0	9	
<i>LMO1/LMO2</i> (-)	8	50	

WBC: white blood cell; Hb: hemoglobin; PLT: platelets; LDH: lactate dehydrogenase; BM: bone marrow. P value: \*rank sum test; <sup>§</sup>Fisher's test.

### Association of *PHF6* mutation with other cytogenetic and molecular abnormalities

Among 96 T-ALL patients undergoing cytogenetic analysis, karyotypic data were available for 94 cases; karyotyping failed in two cases because of lack of metaphases. Among the 94 patients with cytogenetic data, abnormal karyotypes were detected in 45 cases (47.9%). These included numerical changes (6 cases, 13.3%), structural changes (30 cases, 66.7%) and both types of changes (9 cases, 20%). Although *PHF6* mutations were more frequent in T-ALL patients with normal karyotypes (10/49, 20.4%) than in those with abnormal ones (3/45, 6.7%), the difference just failed to reach statistical significance ( $P=0.054$ ).

Patients with *PHF6* mutations were more likely than *PHF6* wild-type patients to harbor the following:



*NOTCH1* mutations (84.6% versus 39.8%,  $P=0.003$ ), *JAK1* mutations (33.3% versus 0%,  $P=0.005$ ) and *SET-NUP214* fusions (42.9% versus 0%,  $P=0.002$ ). (Table 3) There were no differences in the incidence of *FBXW7* mutated cases ( $P=1.000$ ), *WT1* mutated cases ( $P=0.447$ ), and *SIL-TAL1* fusion ( $P=0.395$ ), between patients with and without *PHF6* mutations. Primer sequences and PCR conditions are shown in *Online Supplementary Table S1*.

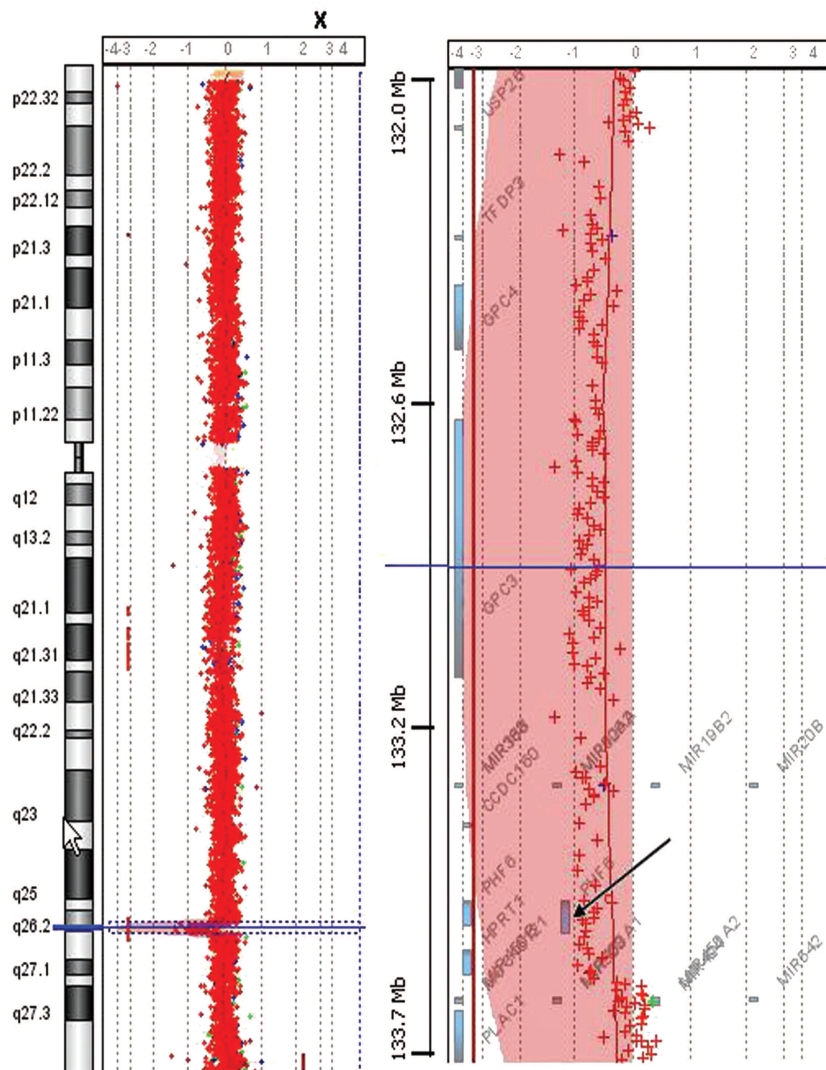
## Discussion

The *PHF6* gene is located on Xq26 - a chromosome region undergoing random X-inactivation in female embryos. It has been confirmed that *PHF6* does not (like a few other X-linked genes) escape inactivation.<sup>18</sup> Germline *PHF6* mutations cause BFLS, an X-linked recessively inherited mental retardation syndrome.<sup>17</sup> Though X-linked and recessive, BFLS may also occur in women. Remarkably, a 13-fold male excess of *PHF6* mutated cases in T-ALL and a 7-fold male excess in acute myeloid leukemia have been reported recently,<sup>18,19</sup> implying skewed inactivation of *PHF6* mutations, X-homologs in females, or some other unknown mechanism for maintaining *PHF6* expression. However, unlike Van Vlierberghe,<sup>18</sup> we observed no male excess of *PHF6* mutations in T-ALL patients, and although we were able to confirm the reported excess of adult (18.6%) over pediatric (5.4%) cases of *PHF6* mutations in T-ALL, both our estimates were significantly lower than the corresponding estimates of 38.1% and 15.7% in previ-

ous reports. Further series are needed to clarify the issue, and in particular whether ethnic factors might contribute to the discrepancies in gender risks between the respective series.

*PHF6* contains four nuclear localization sequences and two imperfect plant homeodomain (PHD) zinc finger domains suggesting a role for *PHF6* in transcriptional regulation and/or chromatin remodeling.<sup>17,25</sup> The *PHF6* mutations identified in our series were clustered in exons 8, 9, and 10 (Figures 1A, B) while those reported recently in T-ALL were more evenly distributed. Overall, 77% of the *PHF6* mutations were loss of function alleles comprising four frameshift and six nonsense mutations. Missense mutations accounted for the remaining 23% of *PHF6* mutations. Most *PHF6* mutations (9/13) are described here for the first time: notably, two nonsense mutations (Ala311X and Tyr301X), and two missense mutations (His329Arg and Tyr325His) involving the second (C-terminal) PHD-like domain of *PHF6*. Two nonsense mutations (Arg274X and Gln270X) and three frameshift mutations (Ser176fs, Lys279fs, and Asp262fs) occurred upstream of the second PHD-like domain causing its loss from the predicted protein (Figure 1A). Clustering of *PHF6* mutations in the second PHD-like domain or immediately upstream highlights a possible role for this region in tumor suppression by *PHF6*.

T-ALL is an aggressive malignancy requiring multiple genetic abnormalities, which are now being identified in increasing numbers thanks to new sequencing technologies. The associations displayed by these pathological



**Figure 2.** Whole-genome array CGH. Image shows array CGH (244K) analysis in one female T-ALL patient which revealed a 1.4 Mb deletion at Xq26 including the *PHF6* gene (black arrow).

alterations already aid disease stratification and should improve intelligent therapeutic targeting. Of note, our study demonstrated that *PHF6* mutations were particularly prevalent in T-ALL patients harboring mutations of *NOTCH1* and *JAK1* and those with a *SET-NUP214* rearrangement. *NOTCH1* mutation is the most common genetic alteration in T-ALL leading to constitutive activation of NOTCH signaling. *JAK1* is mutated in about 20% of adult patients with T-ALL and connotes an unfavorable prognosis. The *SET-NUP214* fusion is associated with elevated expression of *HOXA* genes in T-ALL. We also found that *PHF6* mutations were significantly associated with lower serum lactate dehydrogenase levels, higher platelet counts, and higher bone marrow blast counts. Our studies suggest that *PHF6* mutations may cooperate with these genetic abnormalities during T-cell leukemogenesis.

Collectively, this study demonstrated *PHF6* mutations in a significant proportion of adult T-ALL patients and in a small percentage of pediatric T-ALL patients, occurring less frequently than hitherto reported. *PHF6* mutations were clustered inside or immediately before DNA sequences encoding the second (C-terminal) PHD domain

highlighting the importance of this region in tumor suppression. We found no difference in the rates of *PHF6* mutated cases between male and female patients from China, in contrast to a previous study<sup>18</sup> which examined an ethnically undefined variety of patients. Again in contrast to previous findings, we found positive associations between both *PHF6* and *NOTCH1* mutation, and also *JAK1* mutation and rearrangement of *SET-NUP214* implying leukemic cooperation. Taken together with previous findings, our data call for closer examination of *PHF6* mutations in T-ALL patients of both sexes in ethnically defined populations.

### Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at [www.haematologica.org](http://www.haematologica.org).

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