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CASE REPORT

Poor responses to tyrosine kinase inhibitors in a child with precursor B-cell acute lymphoblastic leukemia with *SNX2-ABL1* chimeric transcript

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Abstract

In addition to *BCR*, various rare fusion partners for the *ABL1* gene have been reported in leukemia. We have identified the fusion gene *SNX2-ABL1* in a pediatric case of acute lymphoblastic leukemia (ALL), which has only once previously been reported in an adult patient. Cytogenetic analysis detected this fusion gene arising from a t(5;9)(q22;q34) translocation. ALL cells carrying a *SNX2-ABL1* fusion exhibited a *BCR-ABL1*+ ALL-like gene expression profile. The patient poorly responded to dasatinib but partially responded to imatinib. Treatment using tyrosine kinase inhibitors requires further investigation to optimize the genotype-based treatment stratification for patients with *SNX2-ABL1* fusion.

Key words *SNX2-ABL1*; precursor B-cell acute lymphoblastic leukemia; tyrosine kinase inhibitors; imatinib; dasatinib

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Constitutively activated tyrosine kinase ABL1 plays an important role in several leukemias (1). The most frequent *ABL1* fusion gene is *BCR-ABL1*, which results from t (9;22)(q34;q11) in patients with chronic myeloid leukemia (CML) and Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (ALL). Targeting BCR-ABL1 fusion proteins with tyrosine kinase inhibitors (TKIs) has been a major breakthrough in the management of patients with CML and Ph+ALL. Imatinib, a first-generation specific inhibitor of ABL1, binds ABL1 kinase in its inactive conformation and specifically inhibits activated kinases. Dasatinib, a second-generation inhibitor of ABL1, binds ABL1 both in its active and inactive conformation, resulting in a higher potency than imatinib and is active against most imatinib-resistant ABL1 kinase domain mutants (2). A number of *ABL1*-related fusion genes that have various fusion partners other than *BCR*, including *NUP214* (3–5), *RANBP2* (5), *RCSD1* (6), *ZMIZ1* (7), and *ETV6* (8), have been described. They are uncommon but important therapeutically because they also have sensitivities to TKIs (6, 9). Ernst T *et al.* reported the first case of the *SNX2-ABL1* fusion gene from t(5;9)(q23;q34) in an adult patient with B-cell precursor (BCP-)ALL who transiently responded to imatinib after early relapse (10). Here, we describe the second reported case of *SNX2-ABL1* fusion gene-positive BCP-ALL. The patient exhibited a *BCR-ABL1*+ ALL-like gene expression profile whereas poorly responded to dasatinib but partially responded to imatinib.

Materials and methods

Whole transcriptome sequencing

Total RNA was extracted from bone marrow-derived leukemic cells of the patient using the miRNeasy Mini Kit (Qiagen, Valencia, CA, USA). After qualification using Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA, USA), 1 μg of total RNA was prepared for sequencing with the TruSeq RNA sample preparation kit v2, Set B (Illumina, Inc., San Diego, CA, USA) according to the manufacture's protocol. The resulting cDNA libraries were quantified and qualified using KAPA Library Quantification Kit (KAPA Biosystems, Inc., Woburn, MA, USA) and Agilent High Sensitivity DNA Kit (Agilent), respectively. The cDNA libraries with average length of 380 bp were loaded on to the cBot (Illumina) for clustering on a flow cell, then sequenced using a HiSeq1000 (Illumina). A paired-end (2 × 101) run was performed using the SBS Kit (Illumina). Real-time analysis and basecalling was performed using the HiSeq Control Software Version 1.5 (Illumina). The sequence data have been deposited in DNA Data Bank of Japan Sequence Read Archive (DDBJ, http://trace.ddbj.nig.ac.jp/dta/index_e.html) under the accession number DRA001161. The chimeric transcripts were investigated in a way similar as described previously by employing deFuse, an algorithm for gene fusion discovery (11, 12).

Polymerase chain reaction (PCR) and sanger sequencing

One microgram of total RNA was transcribed to cDNA using the ReverTra Ace qPCR RT Master Mix (TOYOBO Co., LTD., Osaka-shi, Osaka, Japan). For detection of the *SNX2-ABL1* fusion transcript, PCR was carried out using the primers described previously (10). The PCR products were analyzed by electrophoresis on a 2.0% agarose gel. As an internal control for the intactness of the RNA, cDNA of the GAPDH gene was also amplified. PCR products were cloned into pGEM-T Easy vector system (Promega, Madison, WI, USA). Sequencing reactions of the cloned PCR products were carried out using Big Dye Terminator Cycle Sequencing for an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

Gene expression profiling

We analyzed the gene expression profile of the patient by employing SurPrint G3 Human GE 8x60K v2 Microarray (Agilent) according to the manufacture's protocol. To assess similarity of the gene expression profile between our case and the signature of *BCR-ABL1*+ ALL, gene set enrichment analysis (GSEA) (5) was performed. Ranked gene list of the top up- and down-regulated genes in the signatures of *BCR-ABL1*+ ALL in comparison with chimera-negative BCP-ALL were created with public repositories of databases of *BCR-ABL1*+ ALL (16 cases) and BCP-ALL without recurrent chromosomal changes (19 cases) obtained from a gene expression data set from the St. Jude Children's Research Hospital web site (http://www.stjuderesearch.org/ALL1) (13). We then determined the fraction of the top 300 differentially expressed probe sets in our case and performed GSEA.

Case report

A 7-yr-old boy was diagnosed with BCP-ALL based on an initial peripheral white blood cell (WBC) count of 115.4 × 10^9/L with 93% of lymphoblast and immunophenotyping (CD19+, CD10+, cCD79a+, TdT+, CD34+, HLA-DR+). The cytogenetic analysis revealed t (5;9) (q22;q34), described in more detail below. Treatment was initiated according to the standard chemotherapy for childhood ALL based on the ALL-BFM95 regimen. The patient achieved a morphological remission after the induction course. Despite continuing the postinduction chemotherapy, the patient experienced a bone marrow relapse 15 months after the initial diagnosis. After the relapse, he could not achieve a second remission, because response to the chemotherapy became limited. The patient received an umbilical cord blood transplantation with refractory disease. He achieved a second morphological remission after the transplantation. However, at 6 months from transplantation, the patient developed bone marrow relapse again. Since that time he has never achieved a remission despite salvage chemotherapies.

At the time of diagnosis, a clonal karyotype, 46XY, t (5;9) (q22;q34) was detected. Since the involvement of the 9q34 breakpoint had suggested the presence of an *ABL1*-related fusion gene, we performed whole-transcriptome sequencing on the bone marrow specimens at the initial diagnosis and first relapse and identified in-frame fusion transcripts of *SNX2* and *ABL1*. The presence of fusion transcripts of *SNX2-ABL1* was further confirmed by RT-PCR (Fig. 1A) and a Sanger sequencing (Fig. 1B). The sequencing of PCR products showed that *ABL1* exon 4 was fused in frame to *SNX2* exon 3. Upon a search using Basic Local Alignment Search Tool (BLAST), we found that the *SNX2-ABL1* sequence of our patient was completely identical with that reported by Ernst T *et al.* (6) in an adult case with ALL. We analyzed the gene expression profile of the patient and GSEA revealed that our patient had a gene expression profile that was enriched in that of *BCR-ABL1*+ ALL (Fig. 1C).

During the course, the patient received TKIs without other anti-leukemia drugs (Table 1). After the first relapse, imatinib was introduced at a dose of 240 mg/m^2/d once daily. Although the patient had never achieved a remission, the disease stabilized for 2 months after starting imatinib. The first course of imatinib was discontinued because of the

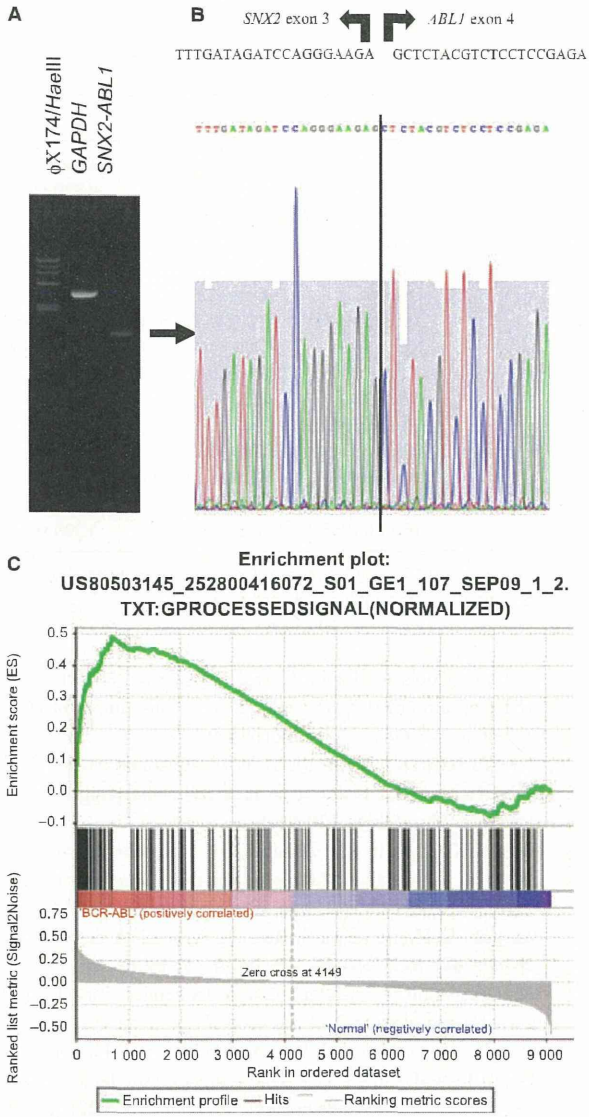

Figure 1 *SNX2-ABL1* fusion gene resulting from t(5;9)(q22;q34). (A) PCR product (B) amplified *SNX2-ABL1* mRNA fusion transcript. (C) Gene set enrichment analysis for *BCR-ABL1*-like signature.

umbilical cord blood transplantation (Table 1, column 1, 2). The patient received TKIs again after the second relapse. Because response to imatinib worsened (Table 1, column 3, 4), dasatinib was introduced at a dose of 80 mg/m^2/d once daily. However, the number of blast cells in peripheral blood rapidly increased (Table 1, column 5). Dasatinib was attempted again at the same dose after reduction of blast cells using vincristine and prednisolone. Again, the number of blast cells in peripheral blood rapidly increased (Table 1, column 6). At 3 months after discontinuing dasatinib, starting imatinib at a dose of 600 mg/m^2/d once daily rapidly decreased the number of blast cells in peripheral blood (Table 1, column 6).

Discussion

We have reported herein an *SNX2-ABL1* fusion resulting from the rearrangement of chromosome 9q34 and 5q22 in a pediatric BCP-ALL case. As we demonstrated, our patient with *SNX2-ABL1* exhibited a gene expression profile of *BCR-ABL1*+ ALL-like signature. In the literature, *SNX2-ABL1* has been reported only once in an adult patient with BCP-ALL (10), and thus, this is the second reported case to the best of our knowledge. SNXs are a family of oligomeric proteins that contain a variety of domains for protein-protein and protein-lipid interactions and are found distributed between membranes and the cytosol (14). While the precise function of SNX protein is not known, it is understood that SNXs are involved in various aspects of protein trafficking. SNX2 exhibits interactions with platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and insulin classes of receptor tyrosine kinases (15). Therefore, it is expected that fusion with SNX2 induces the constitutive activation of ABL1 kinase, but the details are still unclear.

Most *ABL1* fusions reported to date, including *BCR-ABL1*, result in the partner gene fusing to *ABL1* exons 2 (3, 16, 17). However, as in the report by Ernst (10), *SNX2-ABL1* fusion in this patient revealed that *SNX2* exon 3 was fused in frame to *ABL1* exon 4. Such a fusion may lack the amino acid W127-K183 to form an intact Src homology 2 (SH2) domain (18, 19). Since SH2 domain is postulated to be important for the regulation of ABL1 kinase acivity, *SNX2-ABL1* lacking SH2 domain may activate pathways different from those activated by BCR-ABL1. From a clinical point of view, it is therefore possible that patients with *SNX2-ABL1* show a clinical course different from those with *BCR-ABL1* or *BCR-ABL1*-like alterations. Indeed, the patient showed a transient and partial response to imatinib at 600 mg/m^2/d with a reduction in blast cells and the WBC count, while the effect of dasatinib seemed to be rather limited. The patient described by Ernst (106) also experienced early relapse after the initial chemotherapy, transiently responded to treatment with imatinib, and died despite salvage chemotherapies. While prognostic relevance of *SNX2-ABL1* is unknown, the clinical course of this case is very similar to that of the patient described by Ernst. We further confirmed the poorer sensitivity of *SNX2-ABL1* kinase toward TKIs in compared to BCR-ABL1 kinase by introducing *SNX2-ABL1* into murine Ba/F3 cells (unpublished data).

Although more studies are needed to assess the outcomes associated with treatment using tyrosine kinase inhibitors in patients with *SNX2-ABL1* fusion, therapeutic molecules designed to disrupt the SH2 domain of *ABL1* would be ineffective for patients with BCP-ALL possessing an *SNX2-ABL1* aberration. Therefore, possibilities of genotype-based treatment stratification should be explored, especially for chromosomal aberrations like *SNX2-ABL1*. Further investigation should be directed toward the identification of novel

Table 1 Responses to TKI treatment in a child with *SNX2-ABL1* positive precursor B-cell acute lymphoblastic leukemia

	TKI Dose, Duration	Previous anti-leukemia treatment other than TKI (within 2 wk before starting TKI)	Day after starting TKI WBC count/mm^3 (Blast%)			
			At the time of starting TKI	~7 d	~14 d	14 d~
1	Imatinib	Day −16~ to −12	Day 0	Day +6	Day +12	Day +24
	200 mg/d 1×, 24 d	DEX, 6MP, VDS, MTX, Dauno, IFO, IT	830 (39%)	1700 (0.5%)	3420 (0%)	1270 (1%)
2	Imatinib	Day −2~ to −1	Day 0	Day +5	Day +14	Day +34
	200 mg/d 1×, 34 d	VP16	860 (0%)	1390 (0%)	660 (0%)	1320 (0%)
3	Imatinib	–	Day0	Day +7	Day +13	Day +32
	200 mg/d 1×, 32 d		11 180 (26%)	10 990 (22%)	10 120 (24.5%)	21 960 (86%)
4	Imatinib	–	Day 0	NE	Day +10	NE
	300 mg/d 1×, 14 d		18 170 (60%)		21 960 (77%)	
5	Dasatinib	–	Day 0	Day +7	NE	NE
	70 mg/d 1×, 7 d		21 960 (86%)	55 190 (94%)		
6	Dasatinib	Day −12~ to −1	Day-1	Day +5	Day +13	NE
	70 mg/d 1×, 13 d	PSL, VCR	4040 (42%)	9440 (64%)	66 360 (93%)	
7	Imatinib	Day −25~ to −12	Day 0	Day +6	Day +14	Day +24
	500 mg/d 1×, 24+ days	DEX, CA	66 250 (93%)	16 410 (95%)	3430 (53%)	1390 (42%)

TKI, tyrosine kinase inhibitor, DEX, dexamethasone, 6-MP, mercaptopurine, VDS, vindesine, MTX, methotrexate, Dauno, daunorubicin, IFO, ifosfamide, IT, intrathecal chemotherapy, VP16, etoposide, PSL, prednisolone, VCR, vincristine, CA, cytarabine, NE, not evaluated.

genetic abnormalities, which would aid in developing risk-stratified treatment regimens for patients with BCP-ALL.

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Conflict of interest

The authors declare no conflict of interest.

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bjh short report

ATF7IP as a novel *PDGFRB* fusion partner in acute lymphoblastic leukaemia in children

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Summary

We identified *ATF7IP* as a novel *PDGFRB* fusion partner in B-progenitor acute lymphoblastic leukaemia (B-ALL) and showed that B-ALL with *ATF7IP/PDGFRB* translocation is included within the genomic lesions of a Philadelphia chromosome (Ph)-like ALL subgroup. Comprehensive analyses of previous repositories of gene expression data sets disclosed that B-ALL cases with high *PDGFRB* expression level in the context of the Ph-like ALL gene are likely to have a *PDGFRB* translocation. Thus, it is possible that measurement of the *PDGFRB* expression level can be utilized as a screening test for the detection of the cryptic *PDGFRB* translocation, especially within the Ph-like ALL subgroup.

Keywords: *PDGFRB*, *ATF7IP*, acute lymphoblastic leukaemia, Ph-like, screening.

The *PDGFRB* gene (located in 5q33) is a frequent target of chromosomal translocation in a broad spectrum of haematological malignancies, and myeloid neoplasm patients with *PDGFR* translocation are now grouped into a distinct clinical entity of the World Health Organization classification: myeloid neoplasm associated with eosinophilia and *PDGFRA* or *PDGFRB* rearrangement (Tefferi & Vardiman, 2008). Despite increasing evidence of *PDGFR* translocations in haematological malignancy, little is known about *PDGFRB* involvement in B-progenitor acute lymphoblastic leukaemia (B-ALL), presumably due to the limitation of the detection of cryptic *PDGFRB* translocation with conventional diagnostic procedures. Current advances in RNA sequence analysis of B-ALL identified a novel fusion chimera involving *PDGFRB* in high-risk B-ALL, namely Philadelphia Chromosome (Ph)-like ALL (Roberts *et al*, 2012). Herein, we report an 8-year-old boy

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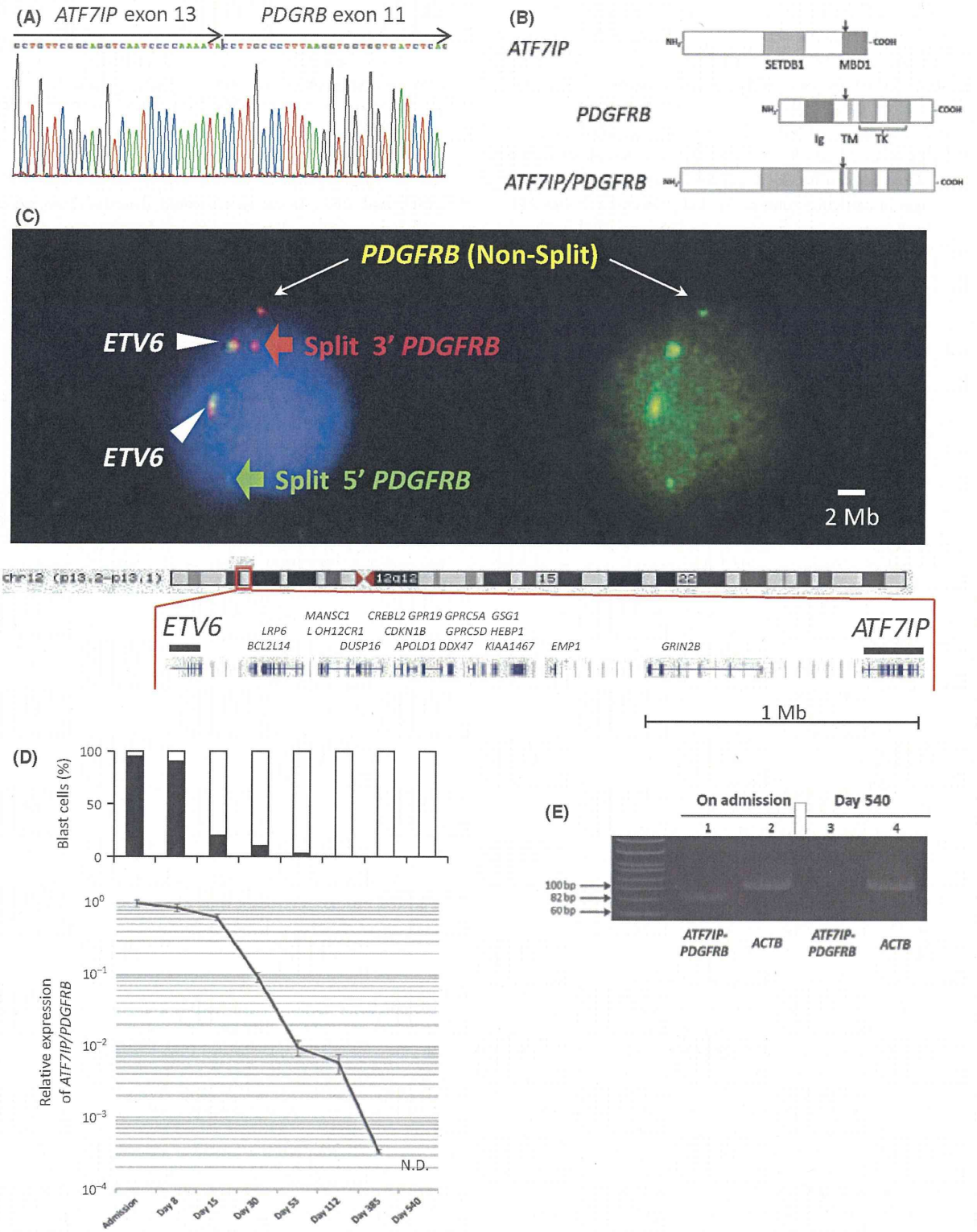
with B-ALL in whom *ATF7IP* was identified as a novel fusion partner in the *PDGFRB* translocation. We examined the molecular characteristics of B-ALL with *PDGFRB* translocation in comparison with previous repositories of gene expression data regarding paediatric B-ALL (Yeoh *et al*, 2002; Roberts *et al*, 2012), and also ascertained that the measurement of *PDGFRB* mRNA expression should be included as a screening test for the detection of such cryptic cytogenetic changes in particular subtypes of ALL, especially Ph-like ALL.

An 8-year-old boy presented in February 2011 with general fatigue of 2 months duration. Physical examination was normal except for pallor and mild hepatomegaly. Laboratory examination showed haemoglobin 47 g/l, platelet count $13{\cdot}1 \times 10^{10}$/l, and leucocyte count $4{\cdot}9 \times 10^{9}$/l (band forms: 30%, segmented neutrophils: 27%, monocytes: 4%, lymphocytes: 23%, and blasts: 16%). A bone marrow (BM) aspirate showed 95% blast cells that stained positive for CD10, CD19, HLA-DR and TdT, but not for myeloperoxidase. Based on these findings, the type of leukaemia of the patient was classified as standard risk B-ALL and he was treated with conventional chemotherapy according to the standard arm of the Tokyo Children's Cancer Study Group Study L99-15 protocol (Manabe *et al*, 2008). He showed good clinical and haematological response with 7 d of prednisolone monotherapy (60 mg/m^2), and blast cells had disappeared from the peripheral blood film on day 8. At the initial diagnosis, a karyotype analysis of blast cells disclosed 45, XY, -7, add (12)(p13). The chromosomal changes involving 12p13 suggested the possibility of t (12;21)(p13;q22); *ETV6/RUNX1* (previously termed *TEL/AML1*), which is known to be the most common translocation in paediatric B-lALL (Jamil *et al*, 2000). The translocation, however, was not detected by either reverse transcription polymerase chain reaction (RT-PCR) or fluorescence *in situ* hybridization (FISH) analysis. Given that the gene locus at 12p13 is frequently involved in chromosomal translocations other than *ETV6*, we performed mRNA sequence analysis (McPherson *et al*, 2011) and identified an in-frame transcript fusing exon 13 of *ATF7IP* with exon 11 of *PDGFRB* (Fig 1A, B). FISH analysis showed cryptic t(5;12)(q33;p13), in which a *ETV6* Dual colour probe (12p13) was fused with a split 3′*PDGFRB* probe (5q33) with a 2-Mb gap (Fig 1C). We also evaluated DNA copy number changes by multiplex ligation-dependent probe amplification analysis (SALSA MLPA KIT P335-A3, MRC-Holland, Amsterdam, the Netherlands), focusing on the common genetic alterations in B-ALL within the *SOX* region, *CRLF2, IKZF1, IL3RA, EBF1, CDKN2A/B, ETV6, BTG1,* and *RB1*. As far as we could determine, no significant copy number alterations except heterozygous deletions within chromosome 7 were present (data not shown). The fusion transcript was readily detected in a BM specimen by quantitative RT-PCR analysis at the time of admission, but its expression decreased with the continuation of chemotherapy. The patient has remained free of disease for 24 months, with the disappearance of the *ATF7IP/PDGFRB* transcripts at the time of this report (Fig 1D, E). To the best of our knowledge, this is the first report showing *ATF7IP* as a fusion partner of *PDGFRB* translocation in haematological malignancy.

ATF7IP has been identified to mediate methylated DNA-binding domain protein 1 (MBD1)-dependent transcriptional repression, recruiting complexes containing SET domain bifurcated 1 (SETDB1) (Fujita *et al*, 2003). Interestingly, a recent analysis suggested that *ATF7IP* facilitates *TERT* and *TERC* expression and is frequently overexpressed in cancer cells (Liu *et al*, 2009). As the N-terminal domain of *ATF7IP* lacked the MBD1 domain, it is likely that the fusion protein does not induce deregulation of *ATF7IP*-mediated transcriptional regulation, but rather is involved in the activation of tyrosine kinase signalling. Indeed, the fusion protein coding ATF7IP locus is predicted to contain a coiled coil structure that is known to contribute to the constitutive activation of kinase and cytokine receptor signalling in *PDGFRB* translocated leukaemia (Lupas *et al*, 1991; Ross & Gilliland, 1999). It is particularly noted that *ATF7IP* fused with *PRGFRB* exon 11, which is the same break point as that of previously reported Ph-like ALL cases with *EBF1/PDGFRB* translocation (Roberts *et al*, 2012).

Fig 1. Detection of *ATF7IP/PDGFRB* fusion gene. (A) Electropherogram of the transcript fusion sequence disclosed in-frame transcript fusing exon 13 of *ATF7IP* with exon 11 of *PDGFRB*. (B) A schematic diagram of the structure of *ATF7IP, PDGFRB,* and novel *ATF7IP-PDGFRB* fusion genes. The arrows indicate the breakpoints. Abbreviations: SETB1, SET domain bifurcated 1; MBD1, methylated DNA-binding domain protein 1; Ig, Immunoglobulin-like domain; TM, transmembrane region; TK, tyrosine kinase domain. (C) Fluorescence *in situ* hybridization (FISH) analysis showing chromosomal change of t (5;12)(q33;p13). Signal detection was carried out on metaphases according to the manufacturer's protocols with the following probes: *PDGFRB* Break (KI-10004 KREATECH) and *ETV6* Dual colour Break Apart Rearranged Probe (Vysis Abbott Molecular, Abbot Park, IL). Left upper panel: *PDGFRB* translocation was represented with split signals of 5′*PDGFRB* (broad green arrow) and 3′*PDGFRB* (broad red arrow), respectively. We used *ETV6* Dual colour probes to depict an *ATF7IP* locus, which is located at approximately 2 Mb centromeric from the *ETV6* loci. The resultant translocation of *ATF7IP/PDGFRB* developed fused signals with *ETV6* Dual colour signals (large arrowhead) and 3′*PDGFRB* (broad red arrow) with a 2-Mb gap. Right upper panel: The FITC filter discriminates the presence of split 5′*PDGFRB* (broad green arrow) and the Non-split *PDGFRB* (arrow), respectively. Scale bar, 2 Mb. Bottom: Genomic view of chromosome 12p13 from the UCSC genome Bioinformatics showing the loci of *ETV6* and *ATF7IP*, respectively. (D) Clinical course of the patient with a longitudinal monitoring of the fusion transcript. Top: Percentage of blast cells in a bone marrow specimen. Bottom: The *ATF7IP/PDGFRB* mRNA level was determined by quantitative reverse transcription polymerase chain reaction (RT-PCR). The expression levels were normalized with *ACTB* before calculating the expression ratios. N.D.: the chimeric mRNA was no longer detectable on day 540. (E) RT-PCR analysis of the expression of the *ATF7IP/PDGFRB* fusion transcript (81 bp). *ACTB* confirms the integrity of cDNA (100 bp). Lane 1–2 at diagnosis; lane 3–4 at day 540.



It is strongly suggested that *PDGFRB* translocation in B-ALL comprises a distinct clinical entity, as was shown in acute myeloid leukaemia (Tefferi & Vardiman, 2008). Therefore, we performed microarray analysis of a BM specimen obtained at the time of admission, and compared its expression profiles with public repositories of expression data sets of B-ALL with *BCR-ABL1* translocation (16 cases) and B-ALL without recurrent chromosomal changes (19 cases) (Yeoh *et al*, 2002). By employing gene set enrichment analysis (GSEA) (Subramanian *et al*, 2007), significant enrichment of the *BCR-ABL1* gene expression signature was demonstrated in our case, indicating that B-ALL with *ATF7IP/PDGFRB* translocation can be classified as Ph-like ALL (Fig 2A, B). Interestingly, we found that our case of *ATF7IP/PDGFRB* translocation showed high expression of *PDGFRB* at the mRNA level (Fig 2C). This observation prompted us

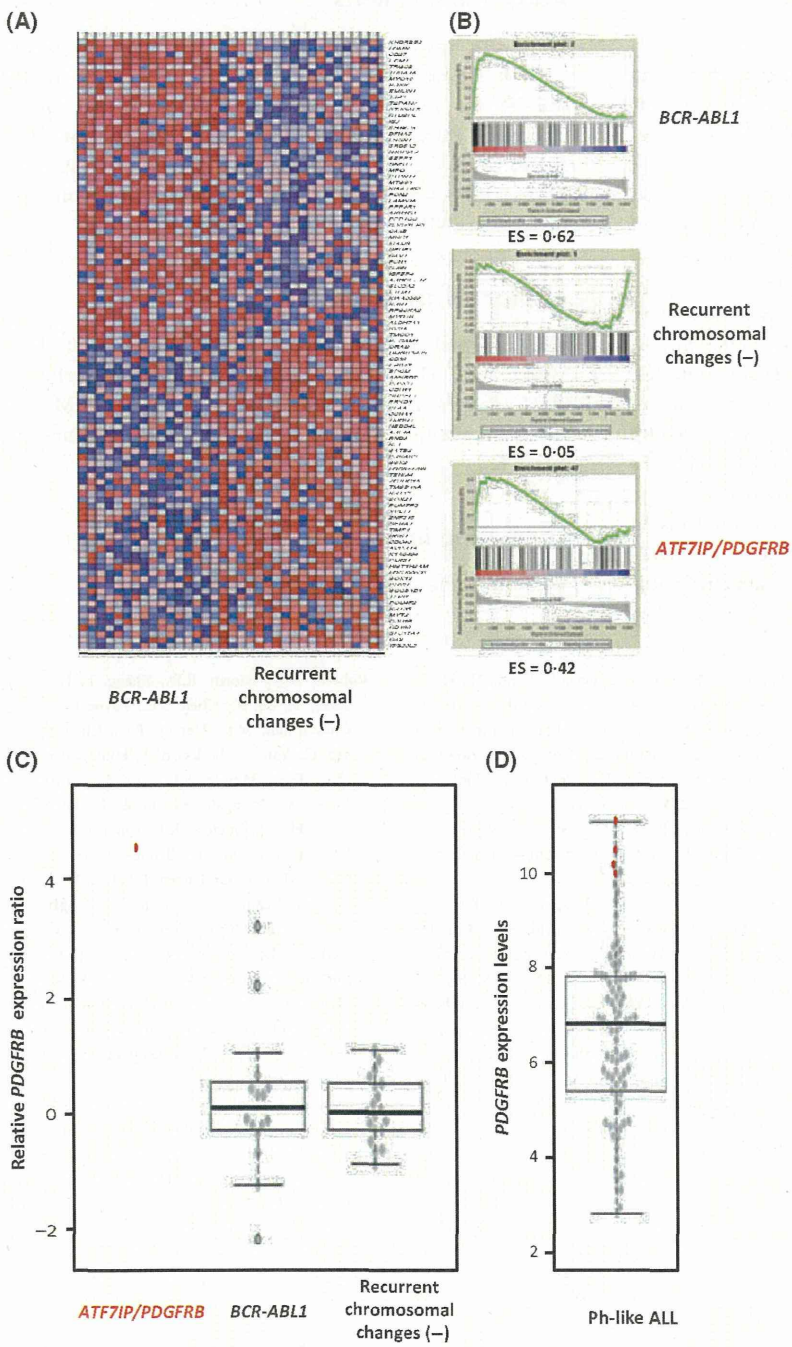


Fig 2. Characterization of gene expression profiles of B-ALL cases with *PDGFRB* translocation. (A–C) Microarray analyses of B-ALL cases with translocation of *ATF7IP/PDGFRB*, *BCR-ABL1* and B-ALL cases without recurrent chromosomal changes. Microarray data set of *ATF7IP/PDGFRB* was obtained from a bone marrow specimen at the time of admission. Expression data sets of both *BCR-ABL1* translocated B-ALL and B-ALL without recurrent chromosomal changes were obtained from those reported by Yeoh *et al* (2002). (A) The top 50 probe sets differentially expressed between *BCR-ABL1* translocated B-ALL and B-ALL without recurrent chromosomal changes are presented as a heat map. (B) Representative GSEA plots of the each subtype of B-ALL i.e. B-ALL with translocation of *BCR-ABL1*, B-ALL without recurrent chromosomal changes and *ATF7IP/PDGFRB* are shown. The enrichment score (ES) is shown at the bottom of the graph. (C) Distribution of the *PDGFRB* expression in B-ALL patients. The expression levels were normalized with *GAPDH* and calculated as the relative *PDGFRB* expression ratio. The ratio in each patient was represented as bee-swarm plots with boxplots. Horizontal bars, median; box, 25–75th percentile; error bars, 10–90th percentile. (D) Distribution of the *PDGFRB* expression within Ph-like ALL patients. Expression data were obtained from the high-risk ALL cohort data set from Children's oncology group (Roberts *et al*, 2012). Four patients with *EBF1/PDGFRB* translocation are indicated as red plots. Data presentation and analyses were performed as in Fig 2C.