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SHORT REPORT

Haematological Malignancy – Biology

Genocopy of *EVI1*-AML with paraneoplastic diabetes insipidus: *PRDM16* overexpression by t(1;2)(p36;p21) and enhancer hijacking

Julian List[1] | **Etienne Sollier**[2] | **Fiona Brown-Burke**[2] | **Katherine Kelly**[2] | **Dietmar Pfeifer**[1] | **Valeria Shlyakhto**[3] | **Kristina Maas-Bauer**[1] | **Milena Pantic**[1] | **Christoph Plass**[2] | **Michael Lübbert**[1]

[1]Department of Hematology, Oncology and Stem Cell Transplantation, University of Freiburg Medical Center, Faculty of Medicine, Freiburg, Germany

[2]Division of Cancer Epigenomics, German Cancer Research Center (DKFZ), Heidelberg, Germany

[3]Oncology Department, St. Claraspital, Basel, Switzerland

Correspondence
Julian List and Michael Lübbert, Department of Internal Medicine I, University Hospital of Freiburg, Hugstetter Str. 55, D-79106 Freiburg, Germany.
Email: julian.list@uniklinik-freiburg.de and michael.luebbert@uniklinik-freiburg.de

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Summary
Diabetes insipidus (DI) in patients with acute myeloid leukaemia (AML) and chromosome 3q alterations (*EVI1/PRDM3/MECOM* overexpression) constitutes a poorly understood paraneoplasia. A 44-year-old patient presented with clinical and morphological features of this syndrome but, surprisingly, disclosed the rare translocation t(1;2)(p36;p21), with massive *PRDM16* overexpression. WGS and RNA sequencing suggest enhancer hijacking of the *ZFP36L2* enhancer region as underlying mechanism. Methylome alterations were similar to those in *EVI1/PRDM3/MECOM* AML, indicating converging pathways. The patient was successfully allografted, she is in complete remission 14 months later. We conclude that t(1;2)(p36;p21), with massive *PRDM16* overexpression, can result in a faithful genocopy of *EVI1/PRDM3/MECOM* AML, including DI.

KEYWORDS
AML, genes rearrangement, malignant haematology

INTRODUCTION

Acute myeloid leukaemia (AML) presenting with diabetes insipidus (DI) is an infrequent but well-documented phenomenon.[1] Infection, infarction and hypophysial leukaemic infiltration are regarded as likely pathomechanisms.[2] Pathological homing of AML blasts to the pituitary gland may be the result of genetic abnormalities. Remarkably, about half of these cases display genetic alterations of the *PRDM3/EVI1* region (e.g. inv(3) or t(1;3)) and approximately 75% of the cases harbour a monosomy 7.[3] Since *PRDM3/EV1* alteration in AML is a rather rare phenomenon, this observation raises speculation about a possible functional link between *PRDM3/EVI1* and paraneoplastic DI in AML, although there is no direct proof. The role of monosomy 7 can also be discussed, albeit it occurs generally more frequently in AML. *PRDM3/EVI1* repositioning is stated as an AML-defining genetic abnormality associated with a specific cytomorphology (dysmegakaryopoesis), distinct clinical features (e.g. thrombocytosis) and an unfavourable prognosis.[4]

In this report, we present the first case of an AML with t(1;2)(p36;p21) associated with DI which provides inspiring insights into genetic pathomechanisms. The translocation t(1;2)(p36;p21) is a rare genetic alteration occurring in different haematological neoplasms and is to our knowledge only described in seven AML cases (Table 1).

METHODS

DNA and RNA were extracted from flash-frozen samples using the Qiagen Qiasymphony system with manufacturer's

TABLE 1 Overview of all detected cases of AML harbouring t(1;2)(p36;p21).

Age	Sex	Cytogenetics	Survival/ last follow-up (in months)	Additional mutations	Remarks	Reference
n.a.	F	50,XX,t(1;2)(p36.1;p21),+8,+14,+18,+20[20]	18,9	*FLT3 ITD, SF3B1, NRAS, RUNX1*		(5)
n.a.	M	46,XY,t(1;2)(p36;p21),t(14; 21)(q22;q22) [4]/45,idem, Y, del(5)(q23q35or q22q34) [2]/46,idem,del(5), t(2;12) (p21;p11) [1]/46,XY [11]	n.a.	*n.a.*	t-AML	(6)
62	M	46,XY,t(1;2)(p36.1;p21) [15]/46,XY [5]	n.a.	*n.a.*	t-AML	(7)
38	M	46,XY,t(1;2)(p36;p21),t(14;21)(q22;q22) [4]/45,idem,-Y,del(5)(q23q35 or q22q34) [2]/46,idem,del(5),t(2;12) (p21;p11) [1]/46,XY [11]	n.a.	*n.a.*	t-AML	(6)
47	F	46,XX, t(1;2)(p36;p21) [2]/46,XX [18]	103	*n.a.*	[a]	(8)
35	F	46,XX,t(1;2)(p36.1;p21) [20]	n.a.	*n.a.*		(9)
44	F	45,XX,t(1;2)(p36;p21),-7 [19/22]	14+	*NRAS*		Our case

[a]t(1;2) was detected in relapse, initial karyotype was normal.

protocols (Qiagen). WGS was performed with TruSeq Nano library prep (Illumina), and NovaSeq 6000 (Illumina) Paired End 150 base pair sequencing. RNAseq was performed with TruSeq Stranded library prep (Illumina) and NovaSeq 6000 (Illumina) Paired End 100 base pair sequencing. For ChIP-seq data, we called peaks with MACS2 for H3K27ac and P300 data from the myeloid cell lines MOLM-1, K562 and Kasumi-1, ran ROSE separately on each of them and then averaged the results.[10]

RNA-seq data quantified as Reads Per Kilobase per Million mapped reads (RPKM) were downloaded from The Cancer Genome Atlas (TCGA) to identify *EVI1*-overexpressing AML samples (Figure 1I). Differential methylation between *EVI1*-overexpressing and other TCGA AML sample groups was computed at the level of 5'-C-phosphate-G-3' (CpG) sites using RnBeads. CpG sites with absolute mean beta-value difference >0.2 and False Discovery Rate (FDR)-adjusted *p*-value <0.05 were considered differentially methylated. All differentially methylated CpG sites with overlapping probes on the 450K and EPIC array were used for clustering of TCGA samples together with the *PRDM16*-overexpressing sample using Ward's hierarchical clustering method.

The search for AML cases with t(1;2) was performed using the Mitelman database. One additional case was identified during the literature search.

RESULTS

Case presentation

In March 2023, a 44-year-old female was admitted to our hospital with fever and respiratory infection. The blood count showed leucocytosis (31 810 Tsd/μL), anaemia (Hb 10.7 g/dL), and thrombocytosis (555 Tsd/μL). The blood film revealed 27% POX-negative blasts, poikilocytosis and platelet anisocytosis. We performed a bone marrow study, confirming the diagnosis of AML with an infiltration of 40%, massively expanded dysmegakaryopoiesis (Figure 1A) and dysplastic erythropoiesis, highly reminiscent of a previous AML case with inv(3) and DI.[2] FISH showed monosomy 7 [90/100], (Figure 1B) and chromosome analysis revealed 45,XX,t(1;2)(p36;p21),-7 [19/22] and additionally, an *NRAS* Q61R mutation (VAF 37%) was detected. We confirmed these results with WGS, which showed no further genetic changes (Figure 1E). Remarkably, the patient also reported a newly diagnosed central DI 6 months before the AML diagnosis; at the time of diagnosis of the DI the blood count had shown thrombocytosis. An MRI of the brain (7 days after the beginning of chemotherapy) showed a partially empty sella without leukaemic infiltration. Under therapy with desmopressin, the patient was symptom-free regarding the DI.

After 3 days of cytoreductive therapy with hydroxyurea, we administered induction with CPX-351. After prolonged cytopenia, the blood film on day 30 showed 38% blasts. The bone marrow study on day 31 confirmed AML persistence. Therefore we conducted a salvage therapy with 10-day decitabine/venetoclax, with subsequent blast reduction from 60% to 20% in the bone marrow. Eight weeks after AML diagnosis (May 2023) an allogeneic stem cell transplantation (HLA-matched sibling donor) was performed after conditioning with thiotepa, fludarabine and busulfan. Fourteen months after transplantation the patient is in complete haematological and molecular remission with a donor chimerism of 100%. The patient is still under treatment for the DI but in reduced desmopressin dosage and the platelet count is normalized.

Enhancer hijacking resulting in overexpression of *PRDM16*

To understand the effects of the not yet well-characterized translocation t(1;2)(p36;p21) observed in this case, we retrospectively compared the *PRDM16* expression of our patient to all other AML bone marrow samples which were

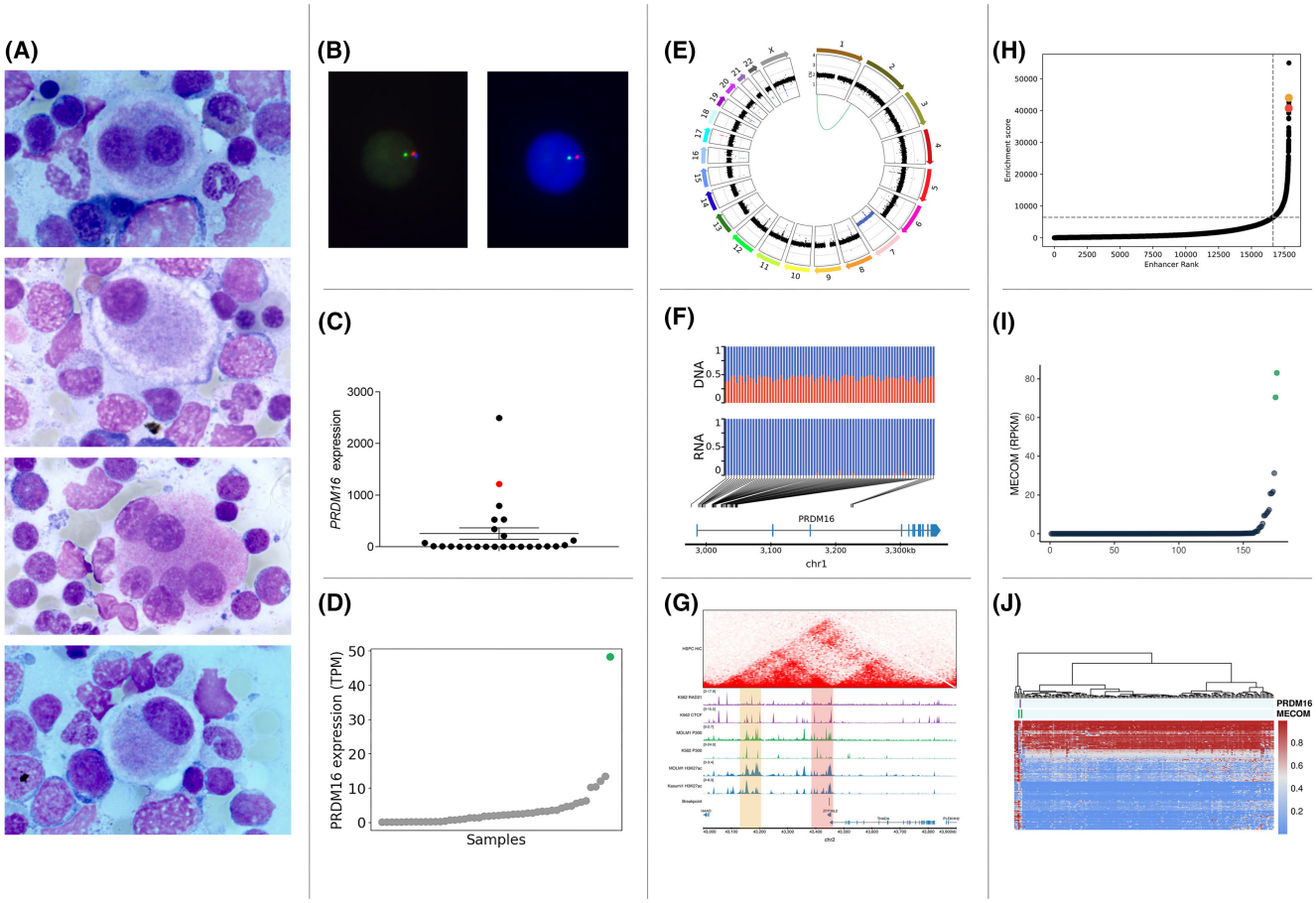

FIGURE 1 Enhancer hijacking causing massive *PRDM16* overexpression in AML with t(1;2)(p36;p21) and DNA Methylation patterns of AML with t(1;2)(p36;p21) and *PRDM16* overexpression resemble those of *EVI1* overexpressed AMLs. Enhancer hijacking was detected with the unpublished pyjacker tool (https://github.com/CompEpigen/pyjacker). (A) Representative bone marrow smears at time of diagnosis showing a striking expansion of micromegakaryocytes, mononuclear and polynuclear megakaryocytes. (B) FISH Analysis with DNA Probe: XL del(7)(q22q31) (MetaSystems, # Lot #26243) discovered Monosomy 7 (one red (KMT2A), one green (MET) and one blue Signal (D7Z1) in 90/100 interphase-nuclei. (C) Dot plot showing *PRDM16* mRNA expression in bone marrow of all AML patients ($n = 25$) analysed by cancer fusion panel at diagnosis in the years 2019–2023 at the Department of Hematology, Oncology and Stem Cell Transplantation, University Hospital Freiburg. The red dot indicates the index patient. The patient with the highest expression harboured a *NUP98-NSD1* fusion which is known to cause high *PRDM16* levels.[11] (D) Plot showing the *PRDM16* expression from the index patient (green), as well as other AML samples from the Heidelberg cohort without breakpoints close to *PRDM16* (grey). (E) Circos plot generated with figeno[12] showing the CNAs (losses in blue, gains in red and diploid regions in black) and SVs (green arcs) observed in WGS data. (F) Variant allele frequencies of heterozygous SNPs in *PRDM16* from DNAseq and RNAseq data. Only SNPs found to be heterozygous in the DNAseq and with coverage >6x in the RNAseq are shown. (G) Contact map generated with figeno[12] showing the TADs around the breakpoint. Tracks showing public H3K27ac and P300 ChIP-seq data in the region around the breakpoint, from the myeloid cell lines MOLM-1, K562 and Kasumi-1.[10] Putative enhancers are highlighted in yellow and red. (H) ROSE enrichment score for haematopoietic enhancers, based on ChIP-seq data of H3K27ac and P300. The enhancers are sorted according to their enrichment score, and the two superenhancers on chromosome 2 which are hypothesized to activate *PRDM16* are highlighted in yellow and red. (I) Gene expression (RPKM) of *EVI1* in TCGA AML samples. Highlighted samples (green dots) were considered as *EVI1*-overexpressing samples for downstream differential methylation analysis. (J) Heatmap depicts the hierarchical clustering of TCGA AML samples together with the *PRDM16* case using Ward's method. All CpG sites (rows) differentially methylated in two *EVI1*-overexpressing AML patients were used for clustering. Methylation beta-values are coloured red for methylated (1) and blue for unmethylated (0). The green bars represent the EV1-overexpresing samples and the purple bar our index patient.

analysed for *PRDM16* expression at the University Hospital Freiburg in the years 2019–2023 (Figure 1C). *PRDM16* located near the t(1;2)(p36;p21) breakpoint is a zinc finger transcription factor with histone methyltransferase activity, known to be involved in the pathogenesis of AML/MDS.[13] Compared to our index patient (red dot), only one case showed a higher expression harbouring a *NUP98-NSD1* fusion which is known for causing high *PRDM16* levels.[11] This result was confirmed with a second cohort which included our index patient and other AML cases from the ASTRAL-1 cohort (Figure 1D) in which the index patient showed the highest expression. *PRDM16* expression was monoallelic (Figure 1F), indicating that the expression was likely due to the translocation, rather than other mechanisms of upregulation such as histone modification or transcription factor regulation. On the other side of the breakpoint, on chromosome 2, we found two putative enhancers with H3K27ac and P300 enrichment in myeloid cells (Figure 1G). We ranked haematopoietic enhancers by their superenhancer potential using the ROSE algorithm

(ranking of superenhancers) and determined that these two putative enhancers are among the strongest haematopoietic superenhancers (Figure 1H). We therefore speculate that the translocation t(1;2)(p36;p21) may result in a hijacking of these superenhancers by *PRDM16*, leading to its aberrant expression.

Previously, this t(1;2)(p36;p21) translocation had been hypothesized to cause *PRDM16* overexpression because of a juxtaposition to the *THADA* promoter,[9] but the *THADA* promoter is not part of the region translocated to *PRDM16* in this sample (Figure 1G). This makes it unlikely that the *THADA* promoter is involved. The role of the superenhancers on chromosome 2 in the wild-type state remains unknown, but they are located in the same Topologically associating domain (TAD) as *ZFP36L2*, a gene that has a key biological role in haematopoietic cells and is known to serve as an oncogene in several gastrointestinal cancers.[14,15] To assess the epigenetic similarity of *PRDM16* and *EVI1*-overexpressing AML, we performed DNA methylation EPIC analysis on the *PRDM16*-overexpressing sample, which we integrated with publicly available methylation data from TCGA AML. Clustering of samples revealed that CpG sites differentially methylated in *EVI1*-overexpressing AML carried a similar methylation pattern in the *PRDM16*-overexpressing case (Figure 1J).

DISCUSSION

Chromosomal translocations resulting in AML with overexpression of *PRDM16*, typically t(1;3) involving *RPN1* (3q21), are very infrequent[16]; hence this entity cannot be delineated as detailed as *EVI1*-activated AML. Nonetheless, it is notable that both entities share abnormal megakaryocytes (mono- and binuclear forms) and can present with thrombocytosis[2,17] and, as this case illustrates, paraneoplastic DI. In that regard, the initial presentation of our patient strikingly phenocopies the syndrome of AML with *EVI1* overexpression. The parallels between *EVI1*- and *PRDM16*-activated AML are exemplified by the remarkable similarities in DNA methylation patterns as demonstrated. *EVI1* overexpression in AML is known to cause a specific methylation pattern.[18] Since *PRDM16* and *EVI1/PRDM3* are structurally and functionally very closely related (both displaying histone methyltransferase activity at H3K9me1) we speculate that *PRDM16* is shaping a specific *EVI1*-like DNA methylation pattern in this case. Another functional parallel can be surmised in the context of the observed thrombocytosis. *EVI1* overexpression induces megakaryocyte differentiation which might also be due to a similar mechanism in *PRDM16* overexpressed AML.[19] This hypothesis is supported by the fact that AML with *NUP98-NSD1* fusion, which is associated with high PRDM16 levels, also shows a thrombocytosis.[20]

Based on the above assumptions we sought to identify a report of AML with t(1;3) and paraneoplastic DI, but after an extensive literature search, we could not identify a single case. This might be explained by the fact that AML with *PRDM16* overexpression is a rare phenomenon in itself, and DI is an underdiagnosed as well as underreported disorder in the context of AML.

We could demonstrate that the rare translocation t(1;2)(p36;p21) causes a massive *PRDM16* overexpression most probably due to enhancer hijacking. In contrast to the previously assumed hijacking of the *THADA* promoter, we hypothesize an involvement of superenhancers possibly related to *ZFP36L2* as a new genetic pathomechanism in AML. This case also provides a new perspective on the discussion of a functional relation between cytogenetics and paraneoplastic DI in AML. There is certainly no direct evidence of a causal link, but since *PRDM3/EVI1* is associated with paraneoplastic DI and closely related to *PRDM16*, we can speculate that *PRDM16* overexpression might lead to paraneoplastic DI in this case.

Importantly, this case demonstrates that AML with DI and the associated cytogenetic aberrations are curable by allogeneic stem cell transplantation. The patient (with inv(3) and DI) reported by us in 2002[2] is still in remission 22 years after allografting without GvHD or other complications. Therefore vigorous efforts should be made to provide patients with DI and AML the curative approach of allografting.

AUTHOR CONTRIBUTIONS

J.L., M.L. and C.P. wrote the paper and developed the concept. E.S., D.P., F.B.-B., M.P. and K.K. carried out the analysis. V.S. and K.M.-B carried out critical revision of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

For original data, please contact julian.list@uniklinik-freiburg.de.

ETHICS APPROVAL STATEMENT

The patient consented to the use of biomaterial and publication of the results in scientific papers.

ORCID

Julian List https://orcid.org/0009-0005-6944-3752
Michael Lübbert https://orcid.org/0000-0003-1186-1650

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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