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CASE REPORT

Characterization of a t(1;2)(p36;p21) involving the *PRDM16* gene region by mate-pair sequencing (MPseq) in a patient with newly diagnosed acute myeloid leukemia with myelodysplasia-related changes

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Abstract
Per the 2017 WHO, several translocations have been described that are sufficient for the diagnosis of acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) (assuming no prior therapy and ≥ 20% myeloblasts present in blood or bone marrow), including the t(1;3)(p36;q21). This translocation juxtaposes the *RPN1* gene (3q21.2) promoter upstream of the *PRDM16* gene (1p36) resulting in *PRDM16* overexpression. While uncommon, *PRDM16* overexpression is considered an unfavorable prognostic finding in myeloid neoplasms. A variant *PRDM16* rearrangement t(1;2)(p36;p21) has been rarely described in various hematologic neoplasms, including two cases of myelodysplastic syndrome and one case each of myelofibrosis and T-lymphoblastic leukemia. We describe the first case to our knowledge of t(1;2)(p36;p21) observed in AML-MRC. In addition, a next-generation sequencing strategy, mate-pair sequencing (MPseq) was performed and revealed the promoter 2 region of *THADA* (2p21) was juxtaposed upstream from *PRDM16* which may be responsible for *PRDM16* overexpression that has been reported in hematologic neoplasms harboring the t(1;2)(p36;p21).

Keywords *PRDM16* · *THADA* · Acute myeloid leukemia with myelodysplasia-related changes (AML-MRC) · Mate-pair sequencing (MPseq)

Introduction

Recurrent balanced chromosomal rearrangements in acute myeloid leukemia (AML) have diagnostic-, prognostic-, and/or treatment-related significance and the majority result in gene fusions that encode chimeric proteins [1]. While less commonly observed in AML with myelodysplasia-related changes (AML-MRC), several reciprocal translocations have been described that are sufficient for the diagnosis of AML-MRC (assuming no prior therapy and ≥ 20% myeloblasts present in blood or bone marrow), including the t(1;3)(p36;q21) [2]. Instead of creating a gene fusion, the 1;3 translocation juxtaposes the *RPN1* gene (3q21) promoter upstream of the *PRDM16* gene (1p36), resulting in aberrant transcriptional upregulation of *PRDM16* [3, 4]. Highly homologous to the *MECOM* gene region (3q26), *PRDM16* has been shown to play a critical role in hematopoietic stem cell maintenance and renewal and is overexpressed in leukemic cells [3–5]. Furthermore, overexpression of the *PRDM16* gene has been associated with an unfavorable prognosis in myeloid neoplasms [4, 6, 7].

A rarely described *PRDM16* translocation variant t(1;2)(p36;p21) is hypothesized to result in *PRDM16*

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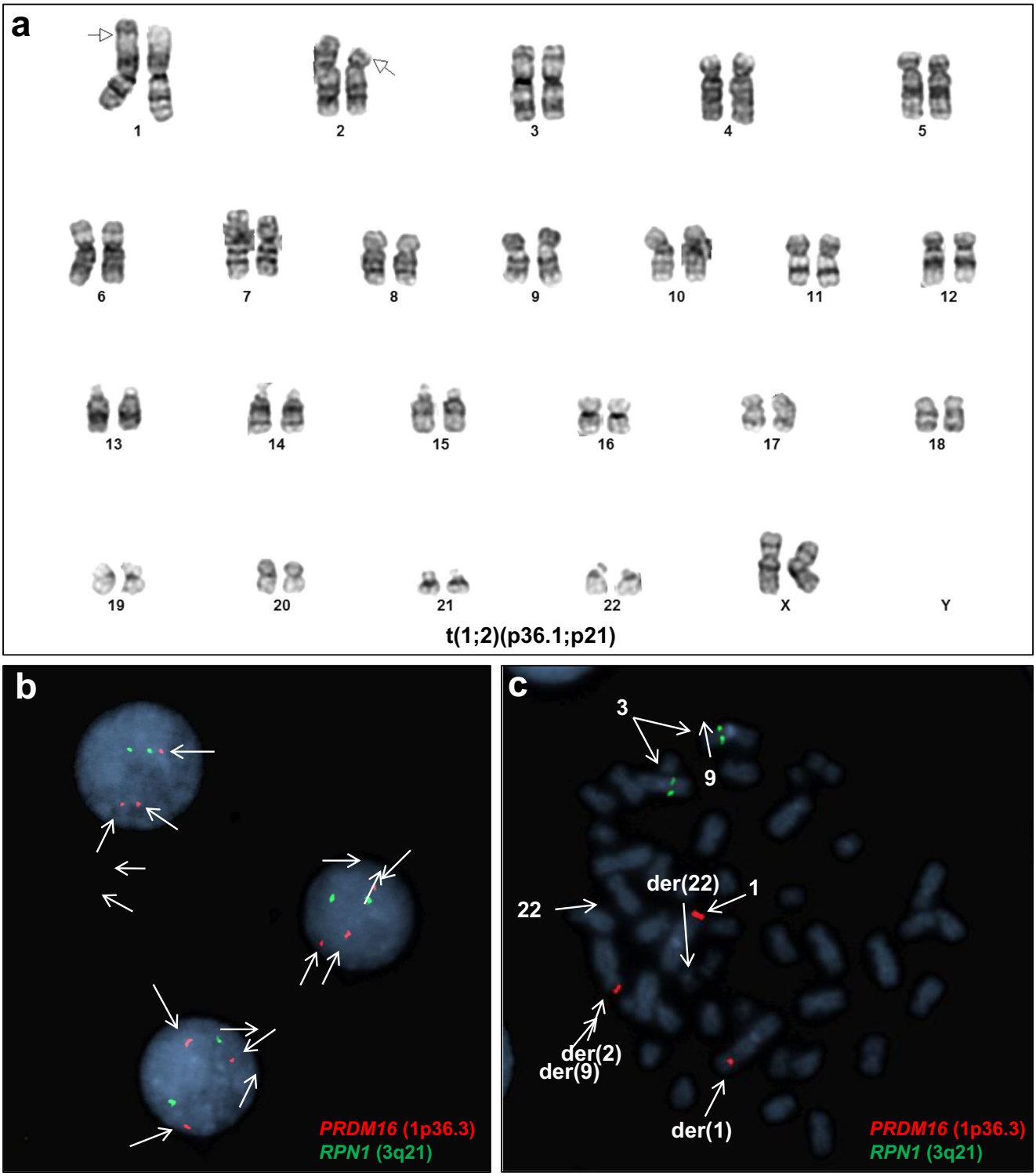

Fig. 1 Representative conventional chromosome and FISH images. **a** Karyogram demonstrating a t(1;2)(p36.1;p21). This translocation was observed in all 20 metaphases analyzed. **b** Representative interphase nuclei demonstrating three *PRDM16* signals (red) indicated by arrows. This signal pattern was observed in 413 (82.6%) of 500 interphase nuclei. **c** Sequential FISH studies using the *PRDM16*/*RPN1* D-FISH probe set illustrating the locations of the three *PRDM16* signals (red). The *PRDM16* FISH probe hybridized to the normal chromosome 1p and at the translocation breakpoints on the derivative chromosomes 1p and 2p (arrows)

overexpression due to the juxtaposition of regulatory elements from 2p21, specifically *THADA* [4, 8]. To date, only four cases have been described that harbor t(1;2)(p36;p21), including two cases of myelodysplastic syndrome (MDS) and one

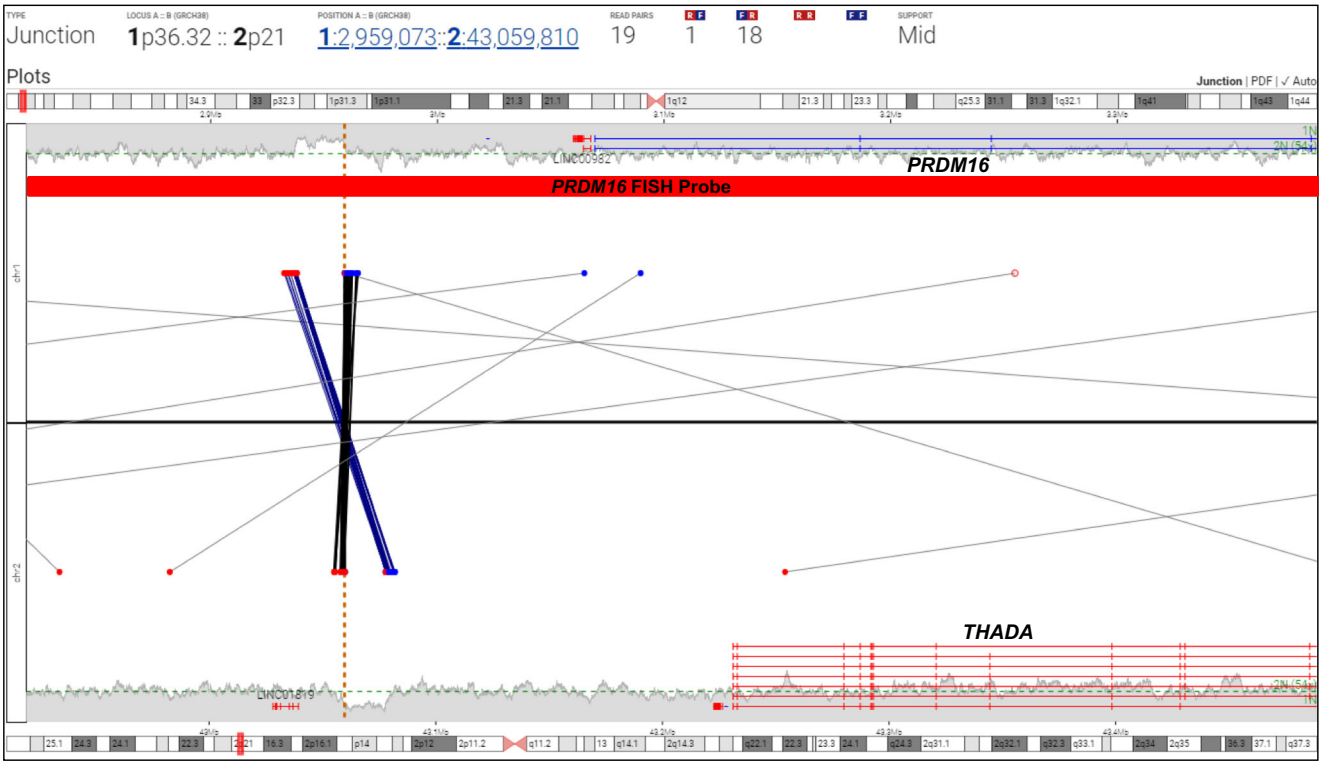

Fig. 2 Mate-pair sequencing (MPseq) results visualized in SVAtools. Junction plot demonstrating a translocation between 1p36.32 and 2p21. Note that while both breakpoints involved intergenic regions, the *PRDM16* FISH probe was disrupted (horizontal red bar)

case each of myelofibrosis and T-lymphoblastic leukemia (T-ALL) [4, 8, 9]. We describe the first case to our knowledge of a t(1;2)(p36;p21) observed in AML-MRC. To further characterize this rearrangement, we utilized a next-generation sequencing strategy, mate-pair sequencing (MPseq), to define the translocation breakpoints with greater resolution and precision compared to traditional cytogenetic methodologies.

Clinical history

A 35-year-old female underwent a bone marrow biopsy for pancytopenia evaluation. Hematologic data obtained from a peripheral blood specimen included a hemoglobin of 5 g/dL (12.0–15.5 g/dL), hematocrit of 14.7% (34.9–44.5%), red blood cell count of 1.2×10^{12}/L ($3.90–5.03 \times 10^{12}$/L), white blood cell count of 1.86×10^{9}/L ($3.5–10.5 \times 10^{9}$/L), and platelet count of 72×10^{9}/L ($150–450 \times 10^{9}$/L). The peripheral blood smear revealed macrocytic normochromic red cells with marked absolute neutropenia, a rare circulating blast, and moderately decreased platelets. The bone marrow aspirate smears showed decreased erythroid precursors with nuclear irregularity and asynchronous maturation, and decreased granulopoiesis with hypogranular and abnormal segmentation of the neutrophils. The bone marrow biopsy was mildly hypocellular (30–40%) with 16% myeloid blasts, 6% promonocytes, 17% monocytes, and increased numbers of dysplastic appearing megakaryocytes, several of which were small and hypolobated. Immunohistochemistry with CD34 demonstrated overall positivity in approximately 20% of nuclei with occasional small clusters. Flow cytometry of the bone marrow revealed 10% myeloid blasts [positive for CD117, CD34, HLA-DR (variable), CD33 (dim partial), CD13 (dim partial), and CD64 (dim partial)] and 17% monocytes with an aberrant immunophenotype [positive for CD14 (variable), CD64, HLA-DR, CD33, and CD13 (partial and variable)]. Based on these findings, a diagnosis of AML-MRC was rendered.

Materials and methods

Conventional chromosome and fluorescence in situ hybridization studies

All genomic studies were performed on the diagnostic bone marrow aspirate specimen. For conventional chromosome studies, G-banding by trypsin using Leishman stain was performed on bone marrow cells that were cultured and harvested as per protocol. A total of 20 metaphases were fully analyzed. The bone marrow aspirate specimen was processed for fluorescence in situ hybridization (FISH) according to specimen-

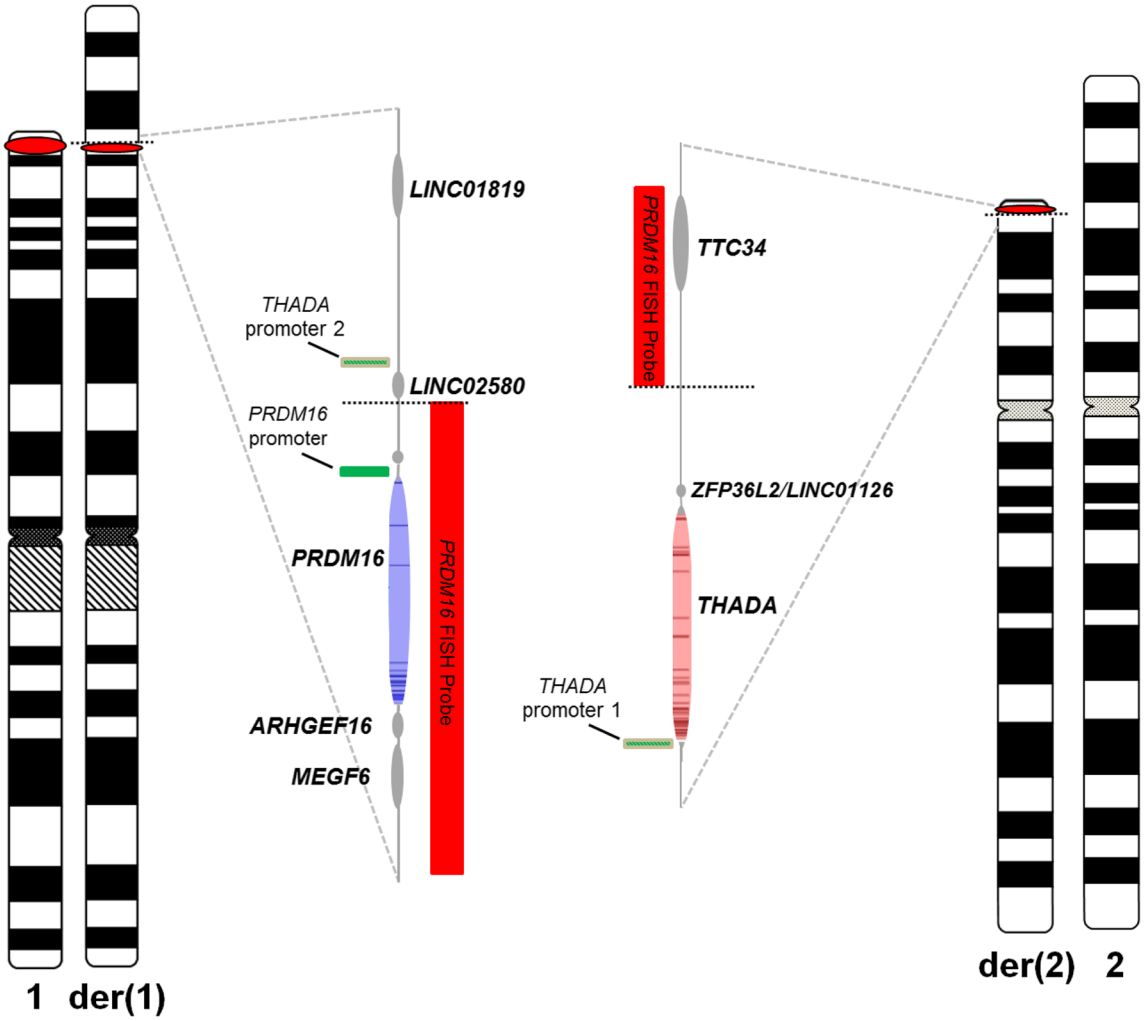

Fig. 3 A focused view of the t(1;2)(p36.32;p21). Dashed horizontal black lines indicate breakpoints on the derivative chromosomes 1 and 2. This translocation juxtaposes the promoter 2 region of *THADA* upstream from *PRDM16*. The translocation breakpoint on 1p is also consistent with a disruption of the *PRDM16* FISH probe and a translocation of a portion of the *PRDM16* FISH probe to the derivative 2p, as observed by *PRDM16*/*RPN1* D-FISH studies

specific laboratory protocols and subjected to standard pretreatment, hybridization, and fluorescence microscopy. A "laboratory developed" *PRDM16/RPN1* dual-color dual-fusion (D-FISH) probe set was utilized. Conventional chromosome and FISH results were interpreted by a board-certified clinical cytogeneticist (ABMGG).

Mate-pair sequencing

DNA was processed using Illumina Nextera Mate Pair library kit (Illumina, San Diego, CA), multiplexed at two samples per lane, and sequenced on the Illumina HiSeq 2500 using 101-basepair reads and paired end sequencing in Rapid Run mode. Data were aligned to the reference genome (GRCh38) using BIMAv3, and abnormalities were identified and visualized using SVAtools and Ingenium, both in-house developed bioinformatics tools [10, 11].

Results

Conventional chromosome studies identified an apparently balanced t(1;2)(p36.1;p21) in all 20 metaphases analyzed (Fig. 1a). The *PRDM16/RPN1* D-FISH probe set did not demonstrate fusion of these two probes, but revealed a third (additional) *PRDM16* signal in 413 (82.6%) of 500 interphase nuclei (Fig. 1b). Sequential FISH studies with the *PRDM16/RPN1* D-FISH probe set indicated the three *PRDM16* signals were located on the normal chromosome 1p, on the derivative chromosome 1p, and on the derivative chromosome 2p (Fig. 1c).

To further interrogate the 1;2 translocation, MPseq was performed and characterized the translocation between 1p36.32 and 2p21. This translocation did not result in a chimeric fusion gene (Fig. 2); however, this rearrangement juxtaposed the promoter 2 region of the *THADA* gene approximately 267 kb upstream from the *PRDM16* gene region

Table 1 Mate-pair (MPseq) and Sanger sequencing results for t(1;2)(p36;p21)

Event description	MPseq event/position	Breakpoint by Sanger [hg38]	Breakpoint by MPseq [hg38]	Gene/ locus	Primer sequence
t(1;2)(p36;p21)	FR/position A	chr1:2,958,736	chr1:2,959,073	No gene	CCCATCATCAGAGATGCATCCATTTAT
	FR/position B	chr2:43,060,174	chr2:43,059,810	No gene	GAGACAGGAAGTAGAAGATGACAGTTTC
	RF/position A	chr1:2,938,039	chr2:2,937,968	No gene	GTCCATCACTAAGTGGATAAAGAGGAA
	RF/position B	chr1:43,077,798	chr2:43,077,895	No gene	CTCTTCCACTATTAGCTCCACTCTATTC

(Fig. 3). Sanger sequencing subsequently confirmed the translocation breakpoints identified by MPseq (Table 1). While this translocation did not disrupt the *PRDM16* gene, the 1p36.32 breakpoint "disrupted" the *PRDM16* FISH probe footprint resulting in a *PRDM16* FISH signal located at the translocation breakpoints on each of the derivative chromosomes 1p and 2p (Fig. 3).

Discussion

Upregulation of the *PRDM16* gene by promoter swap is a distinct entity in myeloid neoplasms and is associated with an unfavorable prognosis [4]. While the t(1;3)(p36;q21) is the most common rearrangement associated with *PRDM16* upregulation, four cases of hematologic neoplasms have been described in the literature that harbor the rare variant, t(1;2)(p36;p21). Duhoux et al described a 63-year-old male with myelofibrosis and a 79-year-old female with T-ALL, each harboring a t(1;2) that was postulated to involve the *THADA* gene region (as defined by FISH studies) [4]. Moreover, overexpression of the *PRDM16* gene was confirmed in the case of T-ALL by gene expression studies. Similarly, a t(1;2) involving the *THADA* gene region by FISH was also observed in a 70-year-old female with MDS that resulted in upregulation of *PRDM16* by gene expression studies [8]. Lastly, Masuya et al described a similar t(1;2) in a 55-year-old female with therapy-related MDS, although neither FISH nor *PRDM16* gene expression studies were performed [9]. In addition, while only evaluated thus far in a single patient, gene expression of *THADA* may not be compromised due to the t(1;2) as demonstrated by gene expression studies performed on the 70-year-old female with MDS [8].

Adding to the literature, we are describing the youngest patient harboring a t(1;2)(p36;p21), a 35-year-old female with a diagnosis of AML-MRC. We verified a disruption of the *PRDM16* gene region by FISH analysis (Fig. 1), performed MPseq, and revealed a translocation between intergenic chromosomal regions 1p36.32 and 2p21 (Fig. 2). While a fusion gene was not created, promoter 2 of *THADA* was juxtaposed 267 kb upstream from *PRDM16* (Fig. 3). Although *PRDM16* gene expression studies were not performed on our case, MPseq results indicate the promoter 2 region of *THADA* likely represents the previously uncharacterized regulatory element within the 2p21 chromosome region that is responsible for *PRDM16* overexpression in these rare cases with a t(1;2)(p36;p21). While MPseq further characterized this translocation, conventional chromosome analysis would be sufficient to detect and monitor a hematologic neoplasm harboring the t(1;2)(p36;p21).

In summary, we further characterized t(1;2)(p36;p21) involving the *PRDM16* and *THADA* gene regions by MPseq in a newly diagnosed case of AML-MRC. Although *PRDM16* gene expression studies were not performed, juxtaposition of the promoter 2 region of *THADA* upstream of the *PRDM16* gene is likely responsible for *PRDM16* overexpression that has been reported in the various hematologic neoplasms harboring a t(1;2)(p36;p21) [4, 8].

Compliance with ethical standards

Conflict of interest PM, DLVD, MKB, BAP, SAS, JBS, WRS, PTG, RPK, LBB, and JFP declare that they have no conflict of interest. GV: Algorithms described in this manuscript for mate-pair sequencing are licensed to WholeGenome LLC owned by GV.

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References

1. Arber DA, Brunning RD, Le Beau MM et al (2017) Acute myeloid leukaemia with recurrent genetic abnormalities. In: Swerdlow SH, Campo E, Harris NL et al (eds) WHO classification of tumours of haematopoietic and lymphoid tissues, revised 4th edn. IARC, Lyon, pp 130–149
2. Arber DA, Brunning RD, Orazi A et al (2017) Acute myeloid leukaemia with myelodysplasia-related changes. In: Swerdlow SH, Campo E, Harris NL et al (eds) WHO classification of tumours of haematopoietic and lymphoid tissues, revised 4th edn. IARC, Lyon, pp 150–152
3. Mochizuki N, Shimizu S, Nagasawa T, Tanaka H, Taniwaki M, Yokota J, Morishita K (2000) A novel gene, MEL1, mapped to 1p36.3 is highly homologous to the MDS1/EVI1 gene and is transcriptionally activated in t(1;3)(p36;q21)-positive leukemia cells. Blood 96:3209–3214

4. Duhoux FP, Ameye G, Montano-Almendras CP et al (2011) PRDM16 (1p36) translocations define a distinct entity of myeloid malignancies with poor prognosis but my also occur in lymphoid malignancies. Br J Haematol 156:76–88
5. Aguilo F, Avagyan S, Labar A, Sevilla A, Lee DF, Kumar P, Lemischka IR, Zhou BY, Snoeck HW (2011) Prdm16 is a physiologic regulator of hematopoietic stem cells. Blood 117:5057–5066
6. Eveillard M, Delaunay J, Richebourg S, Lode L, Garand R, Wuilleme S, Duhoux F, Antoine-Poirel H, Godon C, Bene MC (2015) The closely related rare and severe acute myeloid leukemias carrying EVI1 or PRDM16 rearrangements share singular biological features. Haematologica 100:e114–e115
7. Yamato G, Yamaguchi H, Handa H, Shiba N, Kawamura M, Wakita S, Inokuchi K, Hara Y, Ohki K, Okubo J, Park MJ, Sotomatsu M, Arakawa H, Hayashi Y (2017) Clinical features and prognostic impact of PRDM16 expression in adult acute myeloid leukemia. Genes Chromosom Cancer 56:800–809
8. Storlazzi CT, Albano F, Guastadisegni MC, Impera L, Mühlematter D, Meyer-Monard S, Wuillemin W, Rocchi M, Jotterand M (2008) Upregulation of MEL1 and FLJ42875 genes by position effect resulting from a t(1;2)(p36;p21) occurring during evolution of chronic myelomonocytic leukemia. Blood Cells Mol Dis 40:452–455
9. Masuya M, Katayama N, Inagaki K et al (2001) Two independent clones in myelodysplastic syndrome following treatment of acute myeloid leukemia. Int J Hematol 75:182–186
10. Drucker TM, Johnson SH, Murphy SJ, Cradic KW, Therneau TM, Vasmatzis G (2014) BIMA V3: an aligner customized for mate pair library sequencing. Bioinformatics 30:1627–1629
11. Johnson SH, Smadbeck JB, Smoley SA, Gaitatzes A, Murphy SJ, Harris FR, Drucker TM, Zenka RM, Pitel BA, Rowsey RA, Hoppman NL, Aypar U, Sukov WR, Jenkins RB, Feldman AL, Kearney HM, Vasmatzis G (2018) SVAtools for junction detection of genome-wide chromosomal rearrangements by mate-pair sequencing (MPseq). Cancer Genet 221:1–18