INTRODUCTION

Advances in cytogenetic and molecular genetics have widened the recognition of hematologic malignancy. Chromosome abnormalities often influence related gene expression. Translocation and inversion of chromosomes can result in a split gene and form a fusion gene, which may be translated into a fusion protein and disrupt normal function. Today, many genes involved in abnormal karyotypes have been located, cloned, and sequenced, and the functions of relevant fusion proteins have been identified (Erickson et al., 1992; Gao et al., 1991). The considerable

Genetic analysis and clinical significance of a rare t(1;12)(q21;p13) in a patient with high-risk myelodysplastic syndrome

Fang Fang | Ru Jia | Congyan Liu | Hong Zhao | Wanling Sun

Department of Hematology, Xuanwu Hospital, Capital Medical University, Beijing, China

Correspondence
Wanling Sun, Department of Hematology, Xuanwu Hospital, Capital Medical University, No.45 Changchun Street, Beijing, 100053, P. R. China. Email: wanlingsun@xwhosp.org

Funding information
Natural Science Foundation of Beijing Municipality, Grant/Award Number: Z200022

Abstract
To explore the genetic and clinical features of a rare t(1;12)(q21;p13) in a patient with myelodysplastic syndrome (MDS). A 53-year-old male was diagnosed as high-risk MDS, and died in a short period. A complete cytogenetic analysis of bone marrow by conventional G-banding karyotyping was performed at the time of initial evaluation. On the basis of chromosome karyotype, interphase and metaphase fluorescence in-situ hybridization (FISH) were carried out to further confirm the abnormal karyotypes. Reverse-transcription polymerase chain reaction (RT-PCR) was performed to determine ETV6/ARNT fusion gene status. G-banding revealed karyotype 47, XY, +8, der(12) t(1;12)(q21;p13). FISH with the centromere 8 probe verified the trisomy 8, and the ETV6 break-apart probe suggested heterozygous loss of ETV6 allele located in short arm of chromosome 12. Subsequently, the painting probe of whole chromosome 12 further confirmed the part break of short arm of chromosome 12, and the 1q21/1p36 probe yielded three signals of 1q21 and two signals of 1p36. The results of FISH were in accordance with the karyotype completely. No ETV6/ARNT fusion gene was detected by PCR. T(1;12)(q21;p13) is a rare abnormal karyotype, and the limited reports cannot supply definite clinical significance. Rapid deterioration of our case suggests this translocation of chromosome might have a poor effect on the survival of MDS.

KEYWORDS
chromosome translocation, fluorescence in-situ hybridization, genetic analysis, karyotype
progress in genetics has provided an important basis for elucidating the molecular mechanisms in the pathogenesis of several hematological neoplasms (Al-Harbi et al., 2020; Peterson & Zhang, 2004). Some recurrent cytogenetic abnormalities have already applied critical evidence for the diagnosis and targeted therapy in certain disorders, for example, tyrosine kinase inhibitor therapy in chronic myeloid leukemia (CML) with t(9,22)(q34;q11) (Jabbour & Kantarjian, 2018) and all-trans retinoic acid therapy in acute promyelocytic leukemia with t(15,17) (q22;q21) (Wang & Chen, 2008). However, the clinical significance of other chromosome abnormalities still requires clarification.

This paper reports a patient carrying a rare karyotypic abnormality including the reciprocal translocation of chromosomes 1 and 12, t(1;12) (q21;p13). This translocation is rarely observed and has only been reported in hematologic diseases. Therefore, the cytogenetic and molecular genetics of the patient were further analyzed.

2 | PATIENT AND METHOD

2.1 | Patient information

A 53-year-old male was admitted to the hospital with a history of fatigue, poor appetite accompanied by weight loss for 6 months, and hoarseness with dyspnea for 2 months. Physical examination showed obvious pallor. The complete blood count was leukocytes 3.28 × 10⁹/L (including 5% blasts), hemoglobin 45 g/L, and platelets 57 × 10⁹/L. Bone marrow aspiration smear showed hypercellularity and dysplasia in granulocytic cells and megakaryocytes, 6% blast and 19% ring sideroblasts. Immunophenotyping by flow cytometry revealed that blast cells were positive for CD 13, CD34, CD38, CD117, and HLA-DR. No RUNX1-RUNX1T1, CBFB-MYH11, or PML-RARA fusion genes and no mutations of FLT3-ITD, CEBPA, or NPM1 were detected. Conventional cytogenetic analysis of bone marrow using a G-banding technique revealed a karyotype of 47, XY, +8, der(12)t(1;12)(q21; p13) in available 10 metaphases (Figure 1).

The arterial blood gas analysis indicated pH 7.411, PaO₂ 58.4 mmHg, SaO₂ 90.3%, and PaCO₂ 41.7 mmHg. Laryngoscopy showed incomplete paralysis of the vocal cords, with normal appearance. PET-CT scan, performed in another hospital, showed an abnormal radioactivity concentration between the right thyroid gland and cricoid cartilage. Biopsy of the vocal cords was recommended, but the patient refused the high-risk operation. Therefore, a diagnosis of myelodysplastic syndrome with excess blast-2, with ring sideroblasts (MDS-EB-2-RS), vocal cord incomplete paralysis, and type I respiratory failure was established. Decitabine 50 mg/d for 3 days were administrated, supplemented with erythropoietin and other supportive treatment. Another course of decitabine was given 1 month later and the patient was discharged home. One month later, the patient visited an emergency department and died of unknown cause in a short time, and pulmonary infection was highly suspected.

2.2 | Cytogenetic analysis

Cytogenetic analysis using standard G-banding techniques on heparinized BM samples was performed as described...
previously (Bates, 2011). Chromosome identification and karyotype description used the International System for Human Cytogenetic Nomenclature (ISCN) (McGowan-Jordan et al., 2016).

2.3 | Fluorescence in situ hybridization (FISH)

On the basis of chromosome karyotype, interphase and metaphase FISH were carried out to further confirm the abnormal karyotypes following the standard protocols (Kearney et al., 2002). Briefly, the common MDS FISH panel including CEPX/CEPY, CEP8, 5q33-34/5p15.2, 5q31/5p15, 7q31(D7S486)/CEP7, 7q31(D7S522)/CEP7, 7q31 (D7S522)/CEP7, 20q12, and ETV6 break-apart probe (Abbott) were performed first, then the whole chromosome 12 painting probe (Kreatech Diagnostics) and 1q21/1p36 probe (GPMEDICAL) were hybridized subsequently.

2.4 | Reverse-transcription polymerase chain reaction (RT-PCR)

RNA was extracted (Thermo Fisher Scientific) and reverse transcription (Promega) were operated according to the manufacturer’s instructions, and the product was used for the polymerase chain reaction (PCR) analyses of ETV6/ARNT fusion gene. The synthesized PCR products were electrophoresed onto a 2% agarose gel and stained with ethidium bromide. The primer designed for GAPDH (OMIM18400), ETV6 (OMIM126110), and ARNT (OMIM 600618) as follow.

GAPDH (NM 001289746.1). Forward primer 5’-ATCGCTCAGACACCATGGGGAAG-3’. Reverse primer 5’-CAAAGTTGTCATGGATGACC-3’.

ETV6-6 (NM 001987.4). Forward primer (primer 1) 5’-CTTGCAGCCAATTTACTGG-3’. Reverse primer (primer2) 5’-AGAGGGTAGGACTCCTGGTG-3’.

ARNT (NM 001286036.1). Forward primer (primer3) 5’-CAGAGCTCTGCTCATGGATGACC-3’. Reverse primer (primer4) 5’-CATGGCGGCGACTGCAACTGCAAC-3’.

3 | RESULTS

3.1 | FISH confirmation of the karyotype

The first line FISH revealed the trisomy 8 with three red signals of the centromere 8 probe (Figure 2a), and the heterozygous loss of ETV6 allele located in the short arm of chromosome 12 with one fusion signal of the ETV6 break-apart probe on the normal chromosome 12 (Figure 2b). At the same time, there were no -X/-Y, del(5q)/−5, del(7q)/−7, or 20q- found by FISH. These results verified +8 and the translocation of 12p13 with a breakpoint centromeric to the ETV6 allele, while the derivative chromosome carrying the broken 12p with ETV6 allele had been lost.

Furthermore, to verify the karyotype of t(1;12) (q21;p13), the metaphase FISH using a painting probe of whole chromosome 12 labeled by red fluorescence was further carried out. In metaphases, one chromosome 12 was painted completely red and another was partly labeled red, including the centromere. This further confirmed the break of short arm of one chromosome 12 (Figure 2c). The banding of the unlabeled part of the derivative chromosome 12 was consistent with the telomeric part of 1q in DAPI staining, supporting the partner chromosome of this translocation is chromosome 1.

On the basis of G-banding, there were two normal chromosome 1 and chromosome telomeric to 1q21 was translocated to the derivative chromosome 12. Therefore, 1q21/1p36 probe was chose to evaluate chromosome 1. The hybridization yielded three signals of the 1q21 (green signals) and two signals of 1p36 (red signals), both in metaphases and interphases (Figure 2d). Especially, the metaphases clearly showed two normal chromosome 1 and the derivative chromosome 12 carrying 1q21. Thus, the FISH results were in accordance with the karyotype 47, XY, +8, der(12) t(1;12)(q21;p13) completely.

3.2 | RT-PCR detecting ETV6/ARNT fusion gene

According to the previous FISH results, the breakpoint of t(1;12)(q21;p13) in chromosome 12 centromeric to ETV6 allele. As a result, ETV6 was not involved in the translocation. To confirm this inference, RT-PCR was conducted to detect ETV6/ARNT fusion gene, which had been reported the result of t(1;12)(q21;p13) (Otsubo et al., 2010; Salomon-Nguyen et al., 2000). In our case, the RT-PCR was negative and there was no ETV6/ARNT fusion gene detected. This result was in accordance with our expectation.

4 | DISCUSSION

Generally, patients with MDS exhibit genetic abnormalities, and some of such abnormalities represent independent prognostic variables in MDS. The patient we reported was diagnosed as MDS-EB2, with a 47, XY, +8, der(12) t(1;12)(q21;p13). Trisomy 8 is a relative common abnormality in various hematological diseases, which is neutral...
for the prognosis in MDS. While the t(1;12)(q21;p13) is rarely reported.

A retrospective review of literature revealed totally six cases carrying t(1;12)(q21;p13), all of which were associated with hematologic disease (Table 1). The first case with t(1;12)(q21;p13) was reported in 1984 (Lewis & MacKenzie, 1984), and the diagnosis was multiple myeloma. Then, another five cases were reported

FIGURE 2  FISH image of the bone marrow (a) chromosome 8 centromere probe: three red signals. (b) ETV6 break-apart probe: One fusion signal. (c) Whole chromosome 12 painting probes: red signals on one complete chromatid and part of the chromosome12 covering the centromere. (d) 1q21 (green)/1p36 (red) probe: two red signals and three green signals. (e) Schematic map of t(1;12)(q21;p31)
in succession, including one acute myeloid leukemia (AML) (Salomon-Nguyen et al., 2000), one high-risk myelodysplastic syndrome (Sánchez et al., 2000), one CML (Palandri et al., 2009), and two acute lymphoblastic leukemia (ALL) (Heerema et al., 2004; Otsubo et al., 2010). Among the six cases, three were three male, and three female. The ages ranged from 2 to 66 years. The MDS case exhibited only t(1;12) (q21;p13); the other five cases had one to three additional abnormalities. Among the six reported cases, molecular biological study of the bone marrow was performed in three cases (Otsubo et al., 2010; Salomon-Nguyen et al., 2000; Sánchez et al., 2000). The fusion gene ETV-6/ARNT was detected in two cases, and even the related fusion protein was verified in the AML patient (Salomon-Nguyen et al., 2000). We here reported the seventh patient with t(1;12)(q21;p13) and confirmed this rare finding by FISH.

Translocation resulting from the break apart of 12p13 usually involves ETV6 gene in hematologic malignancies, such as t(5;12) (q31-33; p13) in chronic myelomonocytic leukemia (Apperley et al., 2002; Di Giacomo et al., 2021) and t(12;21) (p13;q22) in ALL (Montaño et al., 2020; Shurtleff et al., 1995). The aryl hydrocarbon receptor nuclear translocator (ARNT) gene, also named HIF-1β, locates in 1q21, which play a critical role in driving tumor growth and metastasis (Lee et al., 2021). It has been reported as a partner gene of fusion gene in two cases with t(1,12)(q21;p13)(Otsubo et al., 2010; Salomon-Nguyen et al., 2000). Therefore, we also tried to detect this fusion gene in our case. The reported breakpoint of the ETV6 gene was located between exons 4 and 5 or between exons 3 and exon 4, and the ARNT gene was located between exons 1 and 2, suggesting these sites were fragile on the chromosomes. Therefore, primers for ETV6 were designed to target exon 3 (primer 1) and exon 5 (primer 2), and primers for ARNT were designed to target exon 1 (primer 3) and exon 2 (primer 4). Under this design scheme, RTPCRs were developed and had no positive results, supporting the preceding judgment that the breakpoint of chromosome 12 centromeric to ETV6 allele and ETV6 was not involved in this translocation.

Survival information was mentioned for only two previously reported cases (Table 1), so even a preliminary judgment about survival with t(1;12)(q21;p13) is difficult. However, all seven cases, including ours, were malignant hematologic disease, suggesting that t(1;12)(q21;p13) translocation might be a poor prognostic factor. The vocal cord paralysis and dyspnea in our patient, is a rare symptom in patients with MDS. PET-CT displayed an abnormal radioactivity concentration between thyroid gland and cricoid cartilage, which might be responsible for vocal cord paralysis. It is hardly to exclude the possibility of abnormal myeloid blasts infiltration, without biopsy. Anyway, the patient died in a short period less than 3 months, suggesting the poor prognosis of t(1;12)(q21;p13) in MDS.

**5 | CONCLUSION**

To date, t(1;12) (q21;p13) is an uncommon karyotype and may produce ETV-6/ARNT fusion genes and related proteins, but this was not observed in the case reported here. Moreover, t(1;12) (q21;p13) may be associated with a poor prognosis, but its rarity and the limited clinical data make it hard to clarify the mechanism and clinical significance. More cases need to be accumulated.

**ETHICAL COMPLIANCE**

All procedures were in accordance with the ethical standards of Xuanwu Hospital Capital Medical University
Ethics committee. Informed consent form was obtained from the patient's family.

ACKNOWLEDGMENT
Financial support was provided by the Beijing Natural Science Foundation (grant no. Z200022).

CONFLICT OF INTEREST
The authors declare no competing interests.

AUTHOR CONTRIBUTIONS
W.S. designed the study. F.F., R.J., and H.Z. collected the data. C.L. and R.J. performed the experiment. F.F. R.J., and W.S. interpreted the data. F.F. and W.S. wrote and revised the manuscript.

DATA AVAILABILITY STATEMENT
Data are available upon reasonable request.

ORCID
Wanling Sun https://orcid.org/0000-0001-9429-3024

REFERENCES
Al-Harbí, S., Aljurf, M., Mohty, M., Almohareb, F., & Ahmed, S. O. A. (2020). An update on the molecular pathogenesis and potential therapeutic targeting of AML with t(8;21)(q22;q22.1):RUNX1-RUNX1T1. Blood Advances, 4(1), 229–238. https://doi.org/10.1182/bloodadvances.2019001168
Apperley, J. F., Gardembas, M., Melo, J. V., Russell-Jones, R., Bain, B. J., Baxter, E. J., Chase, A., Chessells, J. M., Colombat, M., Dearden, C. E., Dimitrijevic, S., Mahon, F. X., Marin, D., Nikolova, Z., Olavarria, E., Silberman, S., Schultheis, B., Cross, N. C., & Goldman, J. M. (2002). Response to imatinib mesylate in patients with chronic myeloproliferative disorders with rearrangements of the platelet-derived growth factor receptor beta. The New England Journal of Medicine, 347(7), 481–487. https://doi.org/10.1056/NEJMoa020150
Bates, S. E. (2011). Classical cytogenetics: Karyotyping techniques. In P. H. Schwartz & R. I. Wesselschmidt (Eds.), Human pluripotent stem cells: Methods and protocols (pp. 177–190). Humana Press.
di Giacomo, D., Quintini, M., Pierini, V., Pellanera, F., la Starza, R., Garello, P., Matteucci, C., Crescenzi, B., Fiumara, P. F., Veltroni, M., Borlenghi, E., Albano, F., Forghieri, F., Maccaferri, M., Betteli, F., Luppi, M., Cuneo, A., Rossi, G., & Meucci, C. (2022). Genomic and clinical findings in myeloid neoplasms with PDGFRB rearrangement. Annals of Hematology, 101, 297–307. https://doi.org/10.1007/s00277-021-04712-8
Erickson, P., Gao, J., Chang, K. S., Look, T., Whisenant, E., Raimondi, S., Lasher, R., Trujillo, J., Rowley, J., & Drabkin, H. (1992). Identification of breakpoints in t(8;21) acute myelogenous leukemia and isolation of a fusion transcript, AML1/ETO, with similarity to drosophila segmentation gene, runt. Blood, 80(7), 1825–1831.
Gao, J., Erickson, P., Gardiner, K., le Beau, M. M., Diaz, M. O., Patterson, D., Rowley, J. D., & Drabkin, H. A. (1991). Isolation of a yeast artificial chromosome spanning the 8;21 translocation breakpoint t(8;21)(q22;q22.3) in acute myelogenous leukemia. Proceedings of the National Academy of Sciences of the United States of America, 88(11), 4882–4886. https://doi.org/10.1073/pnas.88.11.4882
Heerema, N. A., Nachman, J. B., Sather, H. N., la, M. K., Hutchinson, R., Lange, B. J., Bostrom, B., Steinherz, P. G., Gaynon, P. S., & Uckun, F. M. (2004). Deletion of 7p or monosomy 7 in pediatric acute lymphoblastic leukemia is an adverse prognostic factor: A report from the Children's cancer group. Leukemia, 18(5), 939–947. https://doi.org/10.1038/sj.leu.2403327
Jabbour, E., & Kantarjian, H. (2018). Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. American Journal of Hematology, 93(3), 442–459. https://doi.org/10.1002/ajh.25011
Kearney, L., Tosi, S., & Jaju, R. J. (2002). Detection of chromosome abnormalities in leukemia using fluorescence in situ hybridization. Methods in Molecular Medicine, 68, 7–27. https://doi.org/10.1385/1-59295-135-3:0007
Lee, S. H., Golinska, M., & Griffiths, J. R. (2021). HIF-1-independent mechanisms regulating metabolic adaptation in hypoxic cancer cells. Cells, 10(9), 2371. https://doi.org/10.3390/cells10092371
Lewis, J. P., & MacKenzie, M. R. (1984). Non-random chromosomal aberrations associated with multiple myeloma. Hematological Oncology, 2(4), 307–317. https://doi.org/10.1002/hon.2900020402
McGowan-Jordan J, Simons A, Schmid M. (2016). Cancer Genetics and Cytogenetics, 202(1), 22–26. https://doi.org/10.1016/j.cancergenet.2010.07.121
Palandri, F., Castagnetti, F., Allmima, G., Testoni, N., Breccia, M., Luatti, S., Rege-Cambrin, G., Stagno, F., Specchia, G., Martino, B., Levato, L., Merante, S., Liberati, A. M., Pane, F., Saglio, G., Alberti, D., Martinelli, G., Baccarani, M., & Rosti, G. (2009). The long-term durability of cytogenetic responses in patients with accelerated phase chronic myeloid leukemia treated with imatinib 600 mg: The GIMEMA CML working party experience after a 7-year follow-up. Haematologica, 94(2), 205–212. https://doi.org/10.3324/haematol.13529
Peterson, L. F., & Zhang, D. E. (2004). The 8:21 translocation in leukemogenesis. Oncogene, 23(24), 4255–4262. https://doi.org/10.1038/sj.onc.1207727
Sánchez, J., Serrano, J., Román, J., García, J. M., Nomdedéu, J., & Torres, A. (2000). A case of atypical myelodysplastic syndrome with a novel reciprocal translocation t(1;12)(q21;p13). Haematologica, 85(4), 434–435.
Salomon-Nguyen, F., Della-Valle, V., Mauchaffe, M., Bussur-Le Coniat, M., Ghysdael, J., Berger, R., & Bernard, O. A. (2000). The t(1;12)(q21;p13) translocation of human acute myeloblastic leukemia results in a TEL-ARNT fusion. Proceedings of the National Academy of Sciences of the United States of America, 97(12), 6757–6762. https://doi.org/10.1073/pnas.120162297
Shurtleff, S. A., Buijs, A., Behm, F. G., Rubnitz, J. E., Raimondi, S. C., Hancock, M. L., Chan, G. C., Pui, C. H., Grosveld, G., & Downing, J. R. (1995). TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. *Leukemia, 9*(12), 1985–1989.

Wang, Z. Y., & Chen, Z. (2008). Acute promyelocytic leukemia: From highly fatal to highly curable. *Blood, 111*(5), 2505–2515. https://doi.org/10.1182/blood-2007-07-102798

**How to cite this article:** Fang, F., Jia, R., Liu, C., Zhao, H., & Sun, W. (2022). Genetic analysis and clinical significance of a rare t(1;12)(q21;p13) in a patient with high-risk myelodysplastic syndrome. *Molecular Genetics & Genomic Medicine, 10*, e1893. https://doi.org/10.1002/mgg3.1893