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ARTICLE

Ectopia associated *MN1* fusions and aberrant activation in myeloid neoplasms with t(12;22)(p13;q12)

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Abstract

Chromosome translocation t(12;22)(p13;q12)/*MN1-ETV6* and *MN1* overexpression confer a subset of adverse prognostic AML but so far lack in-depth research. We focused on the clinical course and comprehensive genetic analysis of eight cases with t(12;22)(p13;q12) and one with t(12;17;22) (p13;q21;q13) to elucidate their molecular etiology and outcomes of allogeneic hemopoietic stem cell transplantation (allo-HSCT). The total incidence of t(12;22)(p13;q12) and related translocations was 0.32% in myeloid neoplasms. These patients were confirmed to have dismal prognosis when treated only with chemotherapy, and we firstly provided evidence that they can significantly benefit from timely allo-HSCT. Five cases were *MN1-ETV6* positive, and a novel *MN1-STAT3* fusion was identified in the patient with triadic translocation. Significant *MN1* overexpression was observed in all three *MN1*-fusion-negative cases. Genetic analysis highlighted the evidence of an ectopic super-enhancer associated orchestrated mechanism of *MN1* overexpression and *ETV6* haploinsufficiency in t(12;22)(p13;q12) myeloid neoplasms, rather than the conventional thought of *MN1-ETV6* fusion formation. We also disclosed the high concomitance of trisomy 8 and 531 Kbps focal 8q duplication in t(12;22)(p13;q12) cases. The new perspective about this entity of disease will enlighten further research to define the mechanism of tumorigenesis and discover effective treatments for *MN1*-driven malignancies.

Introduction

Translocation of t(12;22)(p13;q12) is a rare but recurrent chromosomal abnormality in hematologic malignancies involving meningioma 1 (*MN1*) and ETS variant 6 (*ETV6*) genes [1]. Although more than 40 cases with t(12;22)(p13;q12) have been reported so far, *MN1-ETV6* and the reciprocal *ETV6-MN1* fusion transcripts were confirmed in only a dozen of them [1]. The translocation has been reported only in myeloid neoplasms, most of which are acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) with poor responses to chemotherapy [1]. Also, *MN1* overexpression has been reported in several subsets of AML and has been accepted as adverse prognosticators in AML with normal karyotype [1–3].

The pathogenic mechanism of t(12;22)(p13;q12) and the fact that a considerable proportion of cases with this translocation lack fusion transcripts remain mysterious. Whether the central pathogenesis lies in the fusion of *MN1-ETV6* or *ETV6-MN1* also remains elusive [4]. We focused here on AML cases with t(12;22)(p13;q12) to elucidate their molecular etiology and outcomes of allogeneic hemopoietic stem cell transplantation (allo-HSCT) treatment.

Subjects and methods

Patients

Inclusion criteria for cases in this study were the diagnosis of myeloid neoplasms from April 2012 to October 2018, and karyotyping analyses showing t(12;22)(p13;q12) or

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related translocations. Data included in the analysis were gender, age, diagnosis, clinical course, morphology test, immunophenotypic test, cytogenetics, fluorescence in situ hybridization (FISH), and molecular genetics analysis. The diagnosis was made according to the 2008 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia [5]. The follow-up cutoff date was May 22, 2019. This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Hebei Yanda Lu Daopei Hospital. All patients or their legal guardians signed informed consent for sample collections and research.

Cytogenetic and molecular cytogenetic analysis

Cytogenetic analysis was performed on bone marrow (BM) mononuclear cells after a 24-h culture. Cultures were exposed overnight to 0.1 μg/mL colcemid (ThermoFisher Scientific Inc., Waltham, USA) and then harvested following standard procedures to obtain metaphases. The karyotype was interpreted according to the International System for Human Cytogenetic Nomenclature 2016 (ISCN 2016) after the analysis of at least 20 G-banded metaphases using the IKAROS software (MetaSystems Inc., Altlhusseim, Germany). FISH with an *MN1* break-apart probe was performed with the help of professor Jinlan Pan according to the protocols previously reported [1].

High-throughput sequencing (HTS) mutation screening

Mutational hotspots or whole coding regions of 58 genes that are known to mutate frequently in hematologic malignancies were sequenced using a targeted, multiplexed, amplicon-based high-throughput sequencing protocol as previously reported [6].

Whole-genome sequencing (WGS) analysis

Standard 30 × WGS was performed on diagnostic BM samples from cases that were negative for *MN1-ETV6*. The libraries were constructed with NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs Inc., Ipswich, USA) according to the manufacturer's instructions, followed by sequencing on Illumina HiSeq X Ten platform (Illumina, Inc., San Diego, USA) using HiSeq X Ten Reagent Kit v2.5 (Illumina, Inc., San Diego, USA) running on paired-end 150 bp mode. Reads were aligned to the Genome Reference Consortium Human Genome Build 37 (GRCh37/hg19) assembly of the human genome with bwa mem.

Structure variations, including translocations and copy number variations (CNVs), were analyzed according to the protocols we previously reported [7, 8]. The two super-enhancers (SEs) that have been annotated in the SEdb, with potential blood-cell specific functions within the genomic region of *ETV6*, were SE_00_00600344 (chr12:11898032-11928354), which spans *ETV6* exon 2, and SE_02_25500737 (chr12:11986329-12016687), which spans *ETV6* exon 3 [9].

Reverse-transcriptase polymerase chain reaction (RT-PCR) and PCR validation

MN1-ETV6, *ETV6-MN1*, and *MN1* expression were analyzed by RT-PCR according to the protocols previously reported [1]. The putative breakpoints and transcripts were validated by PCR and Sanger sequencing.

Results

Patient characteristics

Eight cases with t(12;22)(p13;q12) and one case with t(12;17;22)(p13;q21;q13) were detected by G-banding karyotyping in 2782 newly-diagnosed AML and MDS patients (Table 1), with a relatively low incidence of 0.32%. There were six males, three females, and the median age of onset was 45 years (range 4–60 years).

The cases included three AML-M5, one AML-M2, one AML progressed from MDS (MDS-AML), one MDS with excess blasts 2 (MDS-EB-2), one chronic myelomonocytic leukemia (CMML), and two myeloid/T mixed-phenotype acute leukemia (MPAL) (Table 1).

Clinical outcome

All cases underwent standard or intensive chemotherapy for myeloid malignancies, mainly according to the National Comprehensive Cancer Network guidelines [10, 11]. The three cases treated only with chemotherapy died 3, 7, and 11 months after diagnosis, respectively. Six patients underwent haploidentical allo-HSCT [12], five of them were alive until the last follow-up, and the overall survival time was 11, 49, 47, 29, and 18 months, respectively. The other case (P1), who underwent salvage allo-HSCT after 2 years of chemotherapy and multiple relapses, relapsed 13 months after transplantation and died from a lung infection (Fig. 1).

Karyotyping and FISH results

Three cases had t(12;22)(p13;q12) as the only karyotype abnormality. Six patients harbored additional chromosomal abnormalities, five of whom had trisomy 8. Four cases had complex karyotypes (Table 1 and Fig. 2a). FISH was

Table 1 Clinical and genetic characteristics of the nine cases.

	Sex	Age	Diagnosis	Karyotype	Gene mutation	Gene fusion	*MN1%*
P1	Female	49	AML-M5	46,XX,t(12;22)(p13;q12)[22]	*RUNX1* R166Sfs*46 *ASXL2* L629Pfs*142	–	387.63
P2	Male	46	AML-M5	49,XY, + 8,t(12;22)(p13;q12), + 21, + der(22), t(12;22)(p13;q13)[20]/50,idem, + 18 [1]	NA	*MN1-ETV6*; *ETV6-MN1* type I	15.76
P3	Male	59	MDS-EB-2	46,XY,t(12;22)(p13;q12)[7]/46,XY[20]	*IDH1* R132H *IDH2* R140Q *SRSF2* P95R	–	337.75
P4	Female	45	AML-M2	46,XX,t(12;22)(p13;q12)[21]	*DNMT3A* R882H *FLT3-ITD*	–	1101.50
P5	Male	11	MPAL myeloid/T	47,X,add(Y)(p11.2),t(4;7)(q31.3;q36), + 8, t(12;22)(p13;q12)[17]/47,idem,del(11)(q23)[2]/47,idem,t(10;13)(p11.2;q14)[1]	–	*MN1-ETV6*; *ETV6-MN1* type I	55.54
P6	Male	4	AML-M5	47,XY, + 8,t(12;22)(p13;q12)[1]/47,idem,der(1)t(1;13)(p32;q12),add(2)(p21),add(9)(q13),add(13)(q12)[1]/46,idem,der(1)t(1;13)(p32;q12),add(2)(p21),−8,add(9)(q13),add(13)(q12) [18]/46,XY[1]	–	*MN1-ETV6*; *ETV6-MN1* type I	59.87
P7	Male	19	MPAL myeloid/T	47,XY, + 8,t(12;22)(p13;q12)[14]/46,XY[6]	*NRAS* G13V *WT1* R462W	*MN1-ETV6*; *ETV6-MN1* type I	33.50
P8	Male	60	CMML	47,XY, + 8,t(12;22)(p13;q12),del(20)(q11.2)[20]	*U2AF1* S34Y	*MN1-ETV6*; *ETV6-MN1* type I	7.61
P9	Female	41	MDS-AML	46,XX,add(1)(p36.3),t(12;17;22)(p13;q21;q12)[10]/46,XX[12]	NA	*MN1-STAT3* exon 1-exon 22	13.28

AML acute myeloid leukemia, *CMML* chronic myelomonocytic leukemia, *MDS* myelodysplastic syndrome, *MDS-EB-2* MDS with excess blasts type 2, *MPAL* mixed-phenotype acute leukemia, *MN1% MN1* gene mRNA expression, *NA* not available

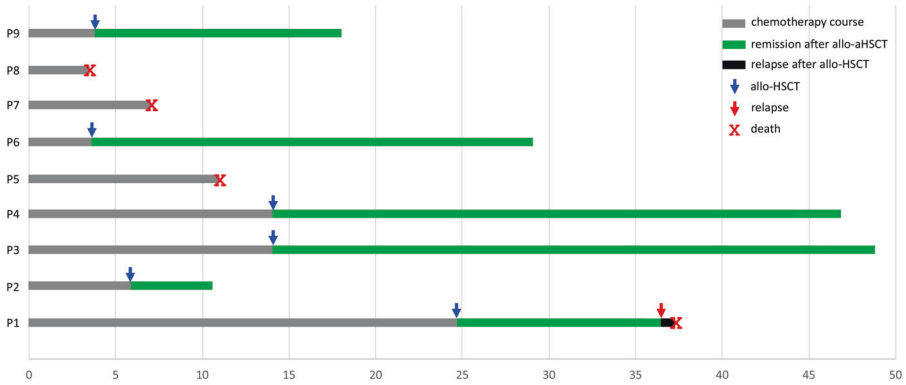

Fig. 1 Clinical course of the nine cases. *allo-HSCT* allogeneic hematopoietic stem cell transplantation.

performed using the *MN1* dual-color break-apart probe [1] on cases P4 and P9, who were *MN1-ETV6* negative, and showed split signals in both cases, indicating a fracture within or adjacent to *MN1* (Fig. 2b).

Fusion transcripts and *MN1* expression

Five cases were positive for both *MN1-ETV6* and *ETV6-MN1* fusions (all type I, Table 1, Fig. 2c, d). The expression of *MN1* was significantly upregulated in all three cases without *MN1* fusion (P1, P3, and P4), but not in the six cases that carried *MN1-ETV6* or *MN1-STAT3* fusion (Table 1).

HTS mutation screening

The HTS mutation screening results of 58 genes were available in seven cases, but no significant feature was found related to the chromosome subtypes or *MN1-ETV6* fusion (Table 1).

Genomic translocation analysis

The WGS analysis was performed on cases P1, P3, P4, and P9, who were negative for *MN1-ETV6*. In case P1 (Fig. 3a), WGS analysis showed the chr22:28117054 breakpoint located at 25 Kbps downstream of *MN1*, and the chr12:11942309 breakpoint located at *ETV6* intron 2. Thus, the translocation resulted in the intact *MN1* ectopia adjacent to the SE_02_25500737.

In case P3 (Fig. 3a), WGS analysis revealed tandem splicing, which sequentially involved telomeric chr22q with the breakpoint at chr22:28124526, a 5.3 Mbps chr16:51322289-56600706 fragment, a 5.3 Mbps chr5:14044903-19353114 fragment, and the chopped chr12 with the breakpoint at chr12:11574655. The breakpoint on chr22q was 18 Kbps downstream of *MN1* and adhered to the chr16 gene-deserted fragment. The chr12:11574655 breakpoint was located at 226 Kbps upstream of *ETV6*. Hence, the intact *MN1* translocated to a gene barren fragment in chr16, and there was no evidence of *ETV6* disruption in this case.

In case P4 (Fig. 3a), the breakpoints of chr22:28047879 and chr12:11845460 were located at 94 Kbps downstream of *MN1* and within *ETV6* intron 1, respectively. Thus, it would result in the translocation of intact *MN1* to *ETV6* intron 1, adjacent to SE_00_00600344. So, the *MN1* remained intact, but the *ETV6* was disrupted in this case. The *MN1* break-apart FISH probe used in this study was designed to label a ~710 Kbps region upstream and a ~620 Kbps region downstream of the 57 Kbps *MN1* separately. Therefore, it makes sense that the FISH test showed split signals.

In case P9 (Fig. 3a), the triadic t(12;17;22)(p13;q21;q13) resulted in a chr22:28168851-chr17:40473489 splicing and a predicted *MN1* exon 1-*STAT3* exon 22 in-frame fusion transcript, which had never been reported (Fig. 3b). The RT-PCR, followed by Sanger sequencing, further confirmed the novel *MN1-STAT3* transcript (Fig. 3c). Besides, a breakpoint in *ETV6* intron 4 and a chr12:12013281-chr1:2980818 translocation was found, indicating the breakage of *ETV6* and cryptic chr1 fragments. The assumed reciprocal *STAT3* exon 21-*MN1* exon 2 fusion transcript was not captured by RT-PCR, which was consistent with the triadic translocation trait.

Genomic CNV analysis

Notably, all five cases with *MN1-ETV6* in this study were accompanied by trisomy 8 (Table 1). Then duplications >10 Kbps of chr8 were analyzed with WGS data in the other four fusion-negative cases, and three of them shared a 531 Kbps focal duplication (Fig. 3d). As a comparison, only one of the five randomly selected acute lymphoblastic leukemia cases (C1–C5 in Fig. 3d) had duplication involving this region. Therefore, we disclosed a high concomitance of trisomy 8 or this 531 Kbps focal chr8 amplification in this disease entity.

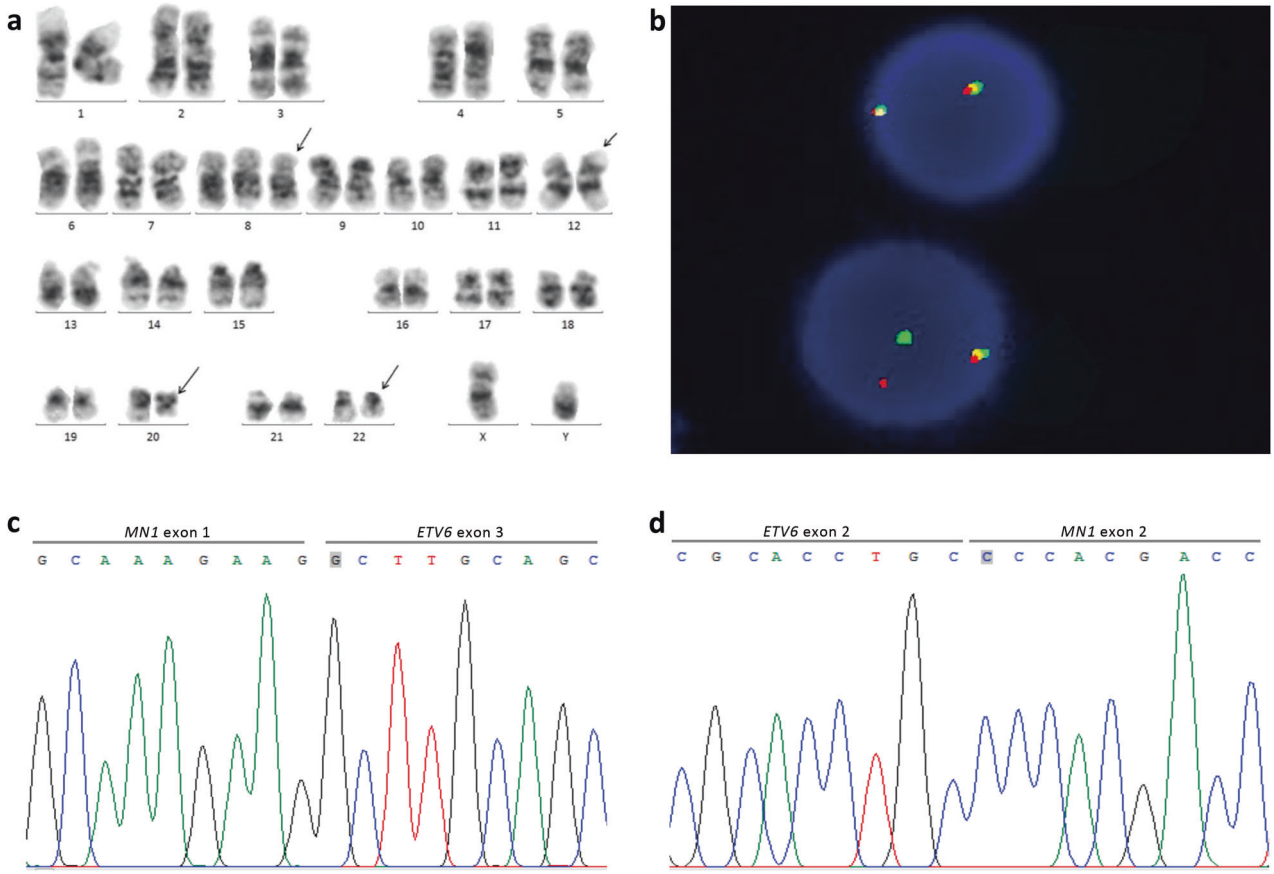

Fig. 2 Karyotype, FISH analysis, and Sanger sequencing of t (12;22)(p13;q12) and *MN1-ETV6/ETV6-MN1*. FISH fluorescence in situ hybridization. **a** One karyotype of case P8, the arrows indicate t (12;22(p13;q13), trisomy 8, and del(20) (q11.2). **b** *MN1* gene break-apart probe FISH test result of case P1. The separation of the red and green signals in one of the cells indicate a fracture of the *MN1* gene or its vicinity. **c, d** Sanger sequencing of *MN1-ETV6* (**c**) and *ETV6-MN1* (**d**) transcripts.

Discussion

In this study, we provided the analysis of t(12;22)(p13;q12) and related translocations in myeloid malignancies with the largest number of cases in a single center. We further performed *MN1* expression and WGS analysis in cases with this translocation but negative for *MN1-ETV6* fusion transcript. Our data showed a relatively low incidence of 0.32% of the translocation in myeloid malignancies. Clinical data also indicated that the translocation could occur at any age and with no predominance in AML morphological classifications, which were similar to the literature reports [1]. Nevertheless, the bi-phenotypic of P5 and P7 in this study further indicated that some cases might have mixed phenotypes of myeloid and T-cell lineages.

The clinical outcomes of our cases confirmed the dismal prognosis when treated only with chemotherapy, which is consistent with the literature reports [1]. Six cases underwent allo-HSCT in this study; five displayed notable efficacies, and the one who died from relapse suffered multiple relapses before the salvage allo-HSCT. Thus, we first provide evidence that these patients can benefit significantly from timely allo-HSCT.

It was reported that nearly all cases carrying t(12;22)(p13;q12) showed *MN1* split signals in FISH testing, but only half of them were *MN1-ETV6*-positive [1]. Three out of eight t(12;22)(p13;q12) cases were negative for *MN1-ETV6* and the reciprocal *ETV6-MN1* fusion in this study. Moreover, both cases that went through FISH analysis showed *MN1* split signals, which further confirmed that the form of fusion is unnecessary. We also identified a novel *MN1-STAT3* fusion in one case with triadic t(12;17;22)(p13;q21;q13) translocation. This novel *MN1* fusion also provides collateral evidence that it is the *MN1-ETV6* but not *ETV6-MN1* fusion that plays the essential pathological role in t(12;22)(p13;q12) leukemia.

We observed significantly upregulated *MN1* expression in *MN1*-fusion-negative cases but not in fusion-positive cases. *MN1* overexpression has been studied and accepted as an independent adverse prognostic marker in karyotype normal AML [13–15], and studies also indicated that the specific phenotype of *MN1*-leukemia depends on the

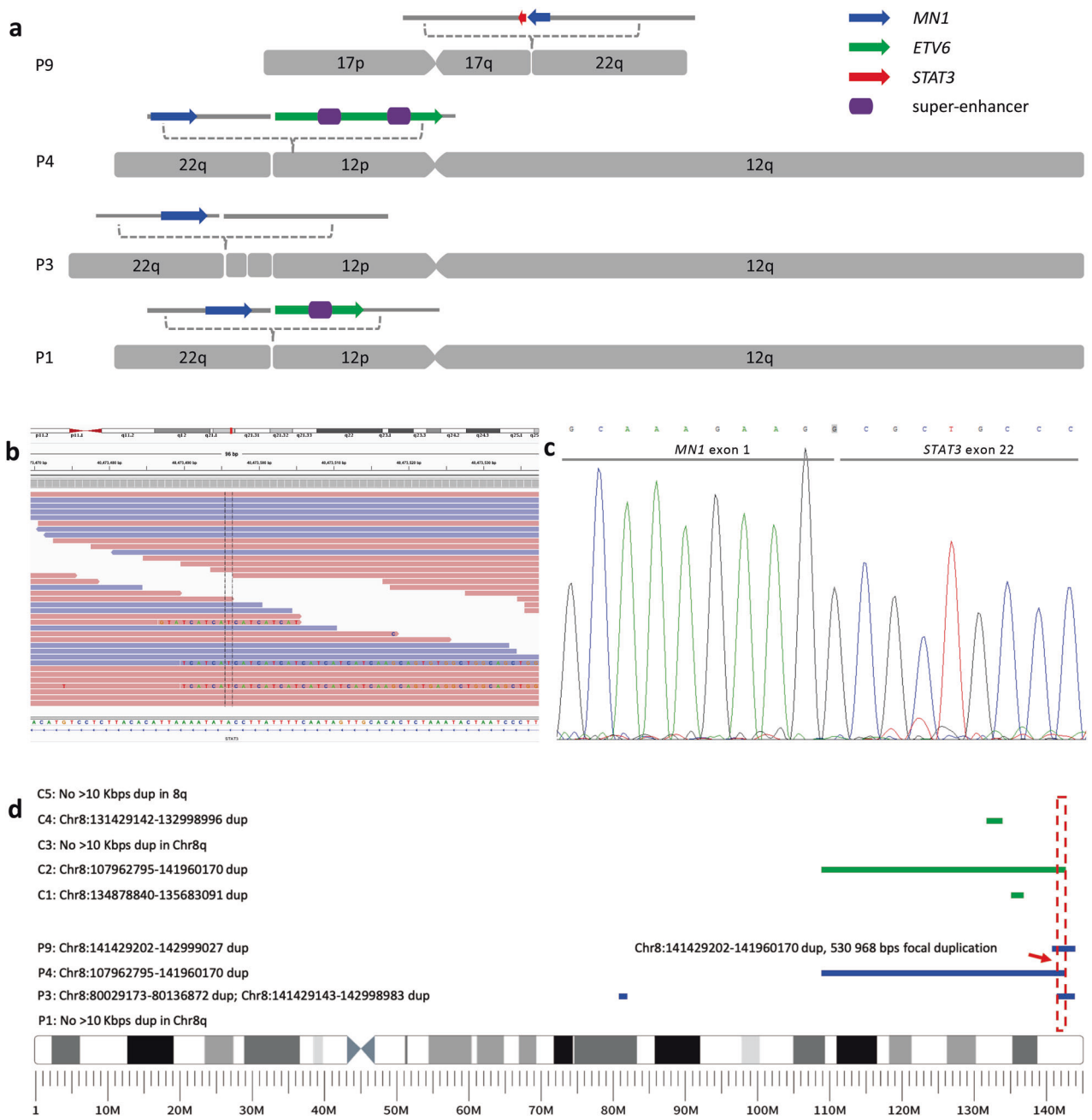

Fig. 3 Genomic analysis of *MN1-ETV6* negative cases. a Structural diagram of *MN1* ecotopia in *MN1-ETV6* negative cases. **b**, **c** Genomic sequence of *MN1* intron 1- *STAT3* intron 21 splicing (**b**) and Sanger sequencing of *MN1-STAT3* transcripts (**c**). **d** Chromosome (Chr) 8 duplication analysis.

cooperating mutations [16]. The *MN1* transcript consists of a 3781 bp exon 1, which encodes the main functional domains of MN1 protein, and a 182 bp exon 2, which encodes a short C-terminal sequence without an explicitly annotated domain (Fig. 4). The breakpoints of *MN1*, in reported cases, were all located in intron 1, so *MN1* retains its exon 1 in both the *MN1-ETV6* and *MN1-STAT3* transcripts. Thus, the RT-PCR primers designed to quantitatively detect *MN1* expression, which spanned *MN1* exon1 and exon 2 [1], could not amplify and reflect the abundance of the *MN1* fusion transcripts.

Overexpression of *MN1* but not the *MN1-ETV6* fusion has also been reported in the t(12;22)(p13;q12) positive AMU-AML1 cell line [17], and has been identified playing an essential synergistic role with *CBFB-MYH11* in the leukemogenesis of inv(16) AML [18]. Kandilci A. et al. reported that *CEBPA* downregulation contributes to *MN1*-modulated leukemogenesis, and reintroduction of *CEBPA*

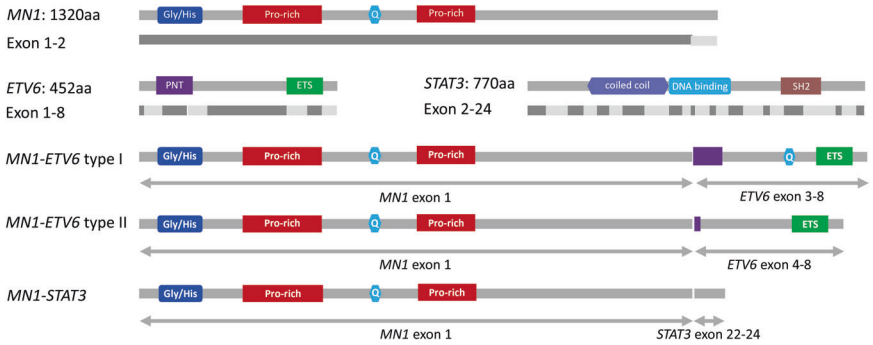

Fig. 4 Structural diagram of gene transcripts. Structural diagram of *MN1*, *ETV6*, *MN1-ETV6*, and *MN1-STAT3* transcripts.

in *MN1*-overexpressing hematopoietic cells prevents their hyperproliferation and restores myeloid differentiation [19]. Besides, *MN1* has been reported significantly upregulated in *ETV6-AML1*-positive B-cell acute lymphoblastic patients [20]. *MN1* has also been observed to fuse with another ETS family of transcription factors gene *FLI1* in acute megakaryoblastic leukemia [21]. These reports together well-founded indicate that *MN1* overexpression might closely cooperate with abnormalities of transcription-factor genes such as *ETV6*, *CBFB*, *CEBPA*, and *FLI1* in leukemogenesis.

Over 30 *ETV6* fusion partners have been characterized, the genomic breakpoint within *ETV6* is scattered, and the contribution of the fused *ETV6* fragment to the leukemogenesis remains elusive [22]. In most scenarios, the main pathogenic mechanism of *ETV6* fusion is ectopic activation of its partner gene, together with the *ETV6* insufficiency due to impaired integrity [22]. The newly reported *ETV6-IGH* translocation in primary central nervous system lymphoma, which lead to *IGH* overexpression together with *ETV6* haploinsufficiency but without *ETV6-IGH* fusion transcripts, also further support this interpretation [23].

The MN1-STAT3 fusion protein contains only 69 amino acids of the STAT3 residual without annotated functional domains, and *STAT3-MN1* was absent due to the triadic translocation. Then, the fusion protein reserves the primary functional domains of *MN1* but not *STAT3* in case P9 in this study. So, both type I/II *MN1-ETV6* and the novel *MN1-STAT3* in this study reserve the functional domains of *MN1* (Fig. 4). Taken together, we suggest it is reasonable to speculate that the upregulated function of MN1 protein plays the major pathological role in *MN1* overexpression, *MN1-ETV6*, and *MN1-STAT3* cases.

Impaired integrity of *ETV6* betides in all cases except one MDS-EB-2 case in this study, regardless of whether there is *MN1-ETV6* fusion. *ETV6* functions as a transcription repressor and plays a critical role in hematopoiesis [24]. Deletions and fusion-driven haploinsufficiency of *ETV6* have been reported widely in various hematological malignancies, and it also acts as a tumor suppressor, with haploinsufficiency enough to manifest partial effects [25, 26]. Thus the highly concomitant *ETV6* haploinsufficiency caused by gene truncation might play a synergistic or phenotypic determination role in t(12;22)(p13;q12) leukemia.

The mechanism of *MN1* overexpression in leukemia is far from disclosed. SEs are a kind of DNA elements that function as distal regulators of gene expression and sometimes hijacked in cancers to drive the oncogene activities [9, 27]. One paradigm in hematology is the *GATA2-MECOM* aberration in inv(3)(q21q26) AML. The formerly identified *RPN1-MECOM* fusion was once considered as the primary pathogenic factor of inv(3)(q21q26) AML and had ever been accepted as a genetic classification marker in WHO 2008 criteria [5]. However, further researches have confirmed that the central pathogenesis of this translocation was the ectopia of a *GATA2* SE that activated *MECOM* expression and conferred *GATA2* haploinsufficiency simultaneously [27–29]. These findings have led to the rewriting of the corresponding content in the WHO 2016 classification [3].

There are two annotated *ETV6* SEs with potential blood-cell specific functions spanning its exon 2 and exon 3, respectively [9]. The scenario of t(12;22)(p13;q12) portrays another analog of inv(3)(q21q26) in AML. The detectable or absent fusion transcript, oncogene activation, SE ectopia, and even haploinsufficiency caused by gene breakage are all similar. Nearly all *MN1-ETV6* fusion transcripts reported are type I, which reserves *ETV6* exon 3 together with the SE that spanning this exon. As to cases P1 and P4 who without *MN1-ETV6* fusion in this study, the unabridged *MN1* is adjacent to the truncated *ETV6* and its SEs due to ectopia. Therefore, it is more likely that the ectopia hijacks the SEs within *ETV6* and then activates the chimeric *MN1-ETV6* or *MN1* itself.

Trisomy 8 is often seen in the AML subgroup with transcription-factor-associated fusions *CBFB-MYH11*, *RUNX1-RUNX1T1*, and *PML-RARA* [30]. Notably, all five cases with *MN1-ETV6* in this study were accompanied by trisomy 8, and genomic CNV analysis further revealed a shared 531 Kbps focal chr8 amplification in three of the other four cases. Since *ETV6* is also a transcription regulator in hematogenesis, synergistic factors might lie in this genomic focal region in this disease entity.

Taken together, we provide the incidence, clinical course, and effectiveness of allo-HSCT of myeloid neoplasms with t(12;22)(p13;q12) in this study. We identify incomplete *MN1*-fusion penetrance, *MN1* overexpression in fusion-negative cases, decapitated *ETV6*, a novel *MN1-STAT3* fusion, and high concomitance of trisomy 8 or 8q focal duplication in t (12;22)(p13;q12) myeloid neoplasms by integrative genetic analysis. Our investigation highlights the evidence of an ectopia-associated orchestrated mechanism of *MN1* aberrant activation and *ETV6* haploinsufficiency in t(12;22)(p13;q12) myeloid neoplasms, rather than the conventional thought of *MN1-ETV6* fusion formation. The incidence of *MN1* overexpression is much higher than t(12;22)(p13;q12), and the mechanism remains to be explored [2]. Studies have reported that Mediator kinases can negatively regulate SE-associated gene expression and can be pharmacologically targeted as a therapeutic approach [29, 31]. The new perspective about this entity of disease will enlighten further research to define the mechanism of tumorigenesis and discover effective treatments for *MN1*-driven malignancies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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