Insertion (21;8)(q22;q22q22): A masked type t(8;21) in a patient with acute myeloid leukemia.

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Abbreviated title: A masked t(8;21) in AML-M2

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ABSTRACT

A 43-year-old man was diagnosed with AML with cellular maturation (AML-M2) according to the French-American-British classification criteria. A cytogenetic study with a G-banding method was initially reported as 45,X,-Y. However, dual-color, dual-fusion fluorescence in situ hybridization (FISH) with probes for the *AML1* and the *ETO* genes showed an unusual pattern of signals, presenting one fusion signal on chromosome 21. Molecular study by reverse transcriptase polymerase chain reaction revealed the presence of a typical *AML1/ETO* chimeric gene. FISH with whole chromosome painting probes targetting either chromosomes 8 and 21 revealed insertion of part of 8 chromosome into the long arm of chromosome 21. We concluded that complicated translocations involving chromosomes 8 and 21 in this patient resulted in development of the chimeric gene, *AML1/ETO*, on the long arm of chromosome 21. This aberrant location of *AML1/ETO* gene and a karyotype of 45, X, -Y, ins(21;8)(q22;q22q22) could not be determined without molecular analysis. This abnormality is considered a masked type of translocation 8;21.

INTRODUCTION

The translocation (8;21)(q22;q22) is frequently associated with the M2 subtype of acute myelogenous leukemia (AML) designated according to the French-American-British (FAB) classification [1, 2]. As large scale studies suggested that patients with AML carrying t(8;21) have a good prognosis, it is thought to be important for clinicians to differentiate this translocation from other chromosomal abnormalities. This translocation involves the *AML1* gene on chromosome 21 and the *ETO* gene on chromosome 8, leading to formation of a chimeric *AML1/ETO* fusion gene on the derived chromosome 8. However, the *AML1/ETO* fusion transcripts without cytogenetic evidence of the classical t(8;21)(q22;q22) was identified in some cases, which was designated as a masked type of t(8;21). Some patterns of complex chromosomal translocation were reported related to masked t(8;21). We describe a case of a masked t(8;21) in which insertion of 8q22 into 21q22 resulted in formation of the *AML1/ETO* fusion gene on chromosome 21.

CASE REPORT

In May 2001, a 43-year-old man visited a hospital because of malaise and left sided headache. Because of leukocytosis, thrombocytopenia and anemia, he was transferred to our hospital on June 7. Physical examination on admission showed purpura in the right lower extremity. Peripheral blood counts were: hemoglobin 6.5g/dl, platelets $0.6 \times 10^4/\mu l$, and leukocytes 15,100/μl with 2% promyelocytes, 5% myelocytes, 19% neutrophils, 18% lymphocytes, 0% monocytes, 0% eosinophils, 53% blasts. Bone marrow was hypercellular; 71.2% of the marrow consisted of blast cells that were strongly positive for peroxidase and naphthol ASD chroloacetate reaction,

some of which contained Auer rods. The blast cells expressed CD 13, 33, 34, 56 and HLA-DR. The patient was diagnosed as having the M2 subtype of AML according to the FAB classification. Induction chemotherapy with daunomycin, citarabine, 6-melcaptopurine and predonisolone was performed to achieve complete remission (CR). However, he relapsed after four courses of consolidating chemotherapy. Re-induction chemotherapy with high dose cytarabine was performed and achieved the second CR.

MATERIALS AND METHODS

Cytogenetics

We used standard cytogenetic techniques to determine the karyotype. Bone marrow cells were cultured for 24 hours in RPMI1640 (SIGMA, MO, USA) supplemented with 10% bovine serum (GIBCO BRL, NY, USA), and antibiotics. Chromosomal analysis was carried out on G-banded preparations.

RNA isolation and Reverse transcription-polymerase chain reaction (RT-PCR)

RNA isolation and RT-PCR were carried out according to a method designed and confirmed by the BML Laboratory (Tokyo, Japan). RNA was extracted from the patient's bone marrow samples using Trizol (GIBCO BRL, NY, USA) and precipitated with isopropyl alcohol. Reverse transcription (RT) of the RNA was performed to synthesize cDNA of interest. One μg of RNA was diluted in 10 μl of diethylpyrocarbonate distilled water, then incubated with 1 μl of the appropriate primer (5'-ATTGCTGAAGCCATTGGGTG-3') at 70℃ for 5 minutes. The mixture was incubated with 4 μl of RT buffer with 2 μl of 0.1M DTT, 1 μl of 10 mM dNTPs and 1 μl of RNasin at 42℃ for 2 minutes. The mixture was incubated with 1 μl of reverse

transcpriptase (Super Script RT, GIBCO BRL, NY, USA) at 37℃ for 2 hours for cDNA synthesis. One μl of the synthesized cDNA was amplified in a 50 μl reaction mixture with Taq polymerase and a pair of primers which span the juxtaportion of the chimeric *AML1/ETO* gene using a DNA thermal cycler (The Perkin-Elmer Corporation, CT, USA) for 40 cycles; the sense primer was 5'-CTTCACTCTGACCATCACTG-3' and the anti-sense primer 5'-GTGCTTCTCAGTACGATTTC-3'. The following temperature and time settings were applied: at 94℃ for 2 minutes for denaturing, at 60℃ for 1 minute for annealing and 72℃ for 1 minute and 30 seconds for extension of the product. PCR products were electrophoresed in a 3% agarose gel containing ethidium bromide and visualized on a UV transilluminator. The cDNA derived from the patient's sample and a control was simultaneously subjected to PCR for assessment. The *ETO* gene was amplified using an optimal set of primers as an internal control.

Fluorescence in situ hybridization (FISH)

Double color FISH was perfomed using probes for *AML1* and the *ETO* gene (LSI *AML1/ETO* dual color dual fusion translocation probe, Vysis, IL, USA). Whole chromosome painting probes of chromosomes 8 and 21 (Vysis) were used for whole chromosome painting FISH.

RESULTS

Cytogenetics

The karyotype of this patient at diagnosis was interpreted as 45, X, -Y [20/20] (Figure 1). The final interpretation of the karyotype was 45, X, -Y, ins(21;8)(q22;q22q22) after correction by the information obtained from FISH and RT-PCR analyses.

RT-PCR Analysis

RT-PCR showed a positive amplification for the *AML1/ETO* fusion transcript (Figure2).

FISH Analysis

Results of dual-color FISH with *AML1* and *ETO* probes are shown in Figure 3(A). In the control t(8;21) sample, fusion signals were located both on chromosomes 8 and 21. On the other hand, a single fusion signal was observed on chromosome 21 in the patient's sample. Whole chromosome painting FISH revealed insertion of a segment of chromosome 8 into a part of the long arm of chromosome 21(Figure 3(B)).

DISCUSSION

The translocation t(8;21)(q22;q22) is one of common chromosomal changes in AML. It is found in 40% of the M2 subtype in the FAB classification, including 3-4% of variant translocations [3, 4]. This subtype shows typical morphologic features, including prominent Auer rods, marrow eosinophilia, and large cytoplasmic globules and vacuoles. Loss of a sex chromosome, especially Y, is frequently associated with t(8;21). In our case, despite failure of t(8;21) detection by standard cytogenetics, characteristic morphology of the leukemic cells and Y deletion karyotype prompted us to look for a t(8;21) using molecular methodology. We added FISH and PCR analysis. RT-PCR revealed fusion transcripts, which indicate a hidden form of *AML1/ETO* gene differently processed from the usual t(8;21). Then, FISH analysis gave us precise information about the location and origin of the masked fusion gene. The whole painting probe in FISH analysis showed that paint of the long arm of chromosome 8, including

the *ETO* gene, was inserted right into the 3' portion of the *AML1* gene on the q22 of chromosome 21. From these results, it appears that the actual karyotype should be 45,X,-Y,ins(21;8)(q22;q22q22). As for the mechanism involved in this insertion, it remains unclear whether direct insertion of a part of chromosome 8 including *ETO* gene to chromosome 21 occurred or the chimeric *AML1/ETO* gene developed on chromosome 8 with t(8;21) was subsidiary inserted to chromosome 21.

The masked t(8;21) is defined as confirmation of the presence of a fusion gene by molecular study without cytogenetic findings of the usual t(8;21). Previously, 12 reports were published about masked t(8;21) [5-16] (Table.1). AML-M2 is the most prevalent subtype of AML in cases with masked t(8;21) similar to usual t(8;21). However, the M1, M4, M5 and RAEB-t subtypes have been also reported to have this aberrant disorder. In most of the cases, the fusion transcript was detected by RT-PCR or Southern blot analysis. In some cases, complex translocations involving chromosome 8 and 21 were revealed by standard cytogenetics. Thus, it is reasonable that these cases should be classified as variant types of t(8;21) rather than the masked t(8;21). On the other hand, Maseki et al. and Gamerdinger et al. reported cases of masked t(8;21) with apparently normal karyotypes and those with sex chromosome deletion as the sole chromosomal abnormality, similar to our case [5,16]. Only molecular analysis revealed the presence of *AML1/ETO* fusion gene in these cases that could be thought to have otherwise normal karyotype.

Submicroscopic translocations can also be detected by FISH analysis combined with other molecular studies. In 6 reports of masked t(8;21), the precise translocations were determined by FISH analysis [9,11,13,14,15,16]. Table 2 shows a list of patients with masked t(8;21)

whose precise karyotype was decided by FISH analysis. In each case, the precise karyotype could not be determined by standard cytogenetic analysis unless combined with molecular study. While RT-PCR provides evidence of a fusion transcript, FISH gives us detailed information about possible mechanisms of such complex chromosomal events. Various types of FISH probes are now available, such as dual color *AML1/ETO* probes, whole chromosome painting probes, and centromeric probes. Miyagi et al. recently reported a case of a masked t(8;21) which was confirmed by spectral karyotyping (SKY) analysis [15]. The SKY analysis involves the hybridization of 24 differentially labeled chromosome-specific painting probes to metaphase spreads, and permits simultaneous visualization of each of the chromosomes in a different color. This method is a powerful tool to detect the masked type of translocation, especially when complex chromosomal rearrangements are involved.

The prognostic significance of a masked t(8;21) is controversial. Some reports mentioned the importance of surveying *AML1/ETO*, as presence of this fusion gene is an indicator of good prognosis. Langabeer et al. reported that the usual t(8;21) and masked t(8;21) have a similar CR rate and prognosis [10]. On the other hand, Sarriera et al. reported that 8 patients with a masked t(8;21) had a lower CR rate and poorer prognosis than those with standard t(8;21) [14]. Because the involvement of complex gene rearrangement in cases of masked t(8;21) is suggested, it is conceivable that chromosomal instability in these cases may be much greater than in the usual t(8;21) cases. Therefore, it is possible that patients with masked t(8;21) and patients with usual t(8;21) may not be clinically equivalent.

With advanced technology such as FISH and RT-PCR, more cases either of complex or subtle chromosome changes such as masked t(8;21) will be identified, which will make their clinical

significance clear.

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Figure Legends

Figure 1. Karyotype

The G-banded karyotype of the patient's bone marrow cells, 45, X, -Y [20/20].

Figure 2. RT-PCR for *AML1/ETO*.

RT-PCR showed a positive amplification for the *AML1/ETO* fusion transcript from the patient's sample (lane 6). Lanes 1 and 7 are molecular size markers. The negative control (lanes 2 and 4), the internal control (lane 3), and the positive control (lane 5) are shown.

Figure 3

(A) The signal pattern of FISH with *AML1* (red) and *ETO* (green) probes. In the typical case of t(8;21), two fusion signals (yellow) should be observed both on chromosome 8 and 21. The *AML1/ETO* fusion gene is known to be developed on the long arm of chromosome 8. Only one fusion signal (yellow) was observed on chromosome 21 in this case. It is speculated that the *AML1/ETO* fusion gene was located on the long arm of chromosome 21. (B) FISH with the whole chromosome painting probes (chromosome 8 was stained green and chromosome 21 red) showed insertion of a fragment of chromosome 8 into the long arm of chromosome 21.

Table 1. List of patients with masked t(8;21)

Table 2. Patterns of revised karyotype in cases of masked t(8;21)

Fig. 1

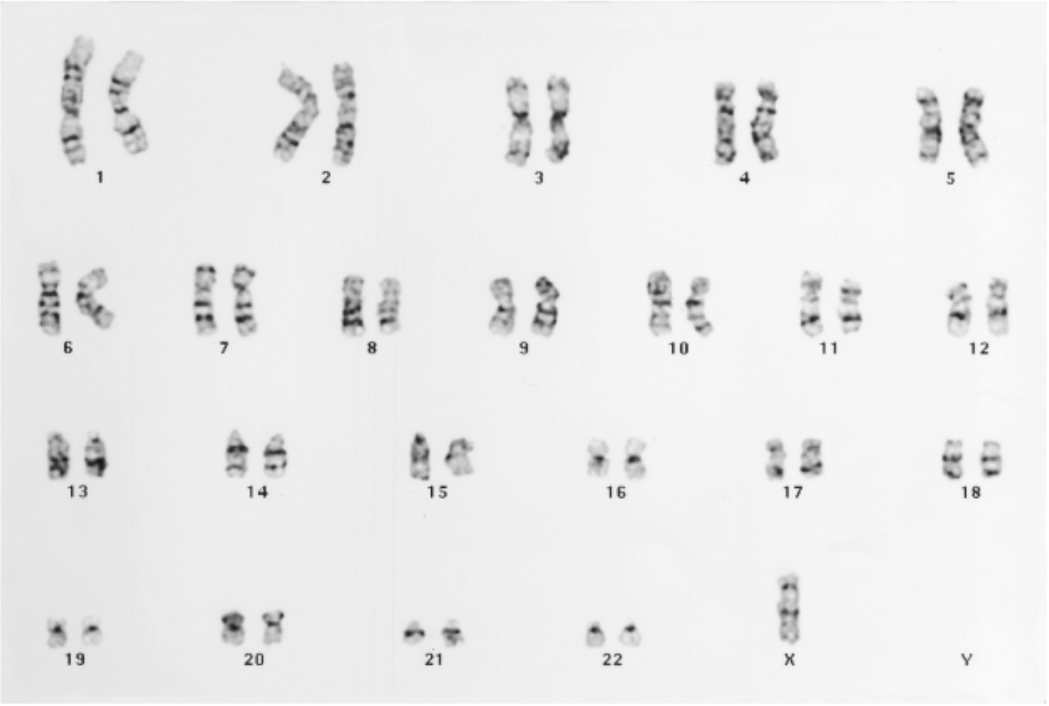

Fig. 2

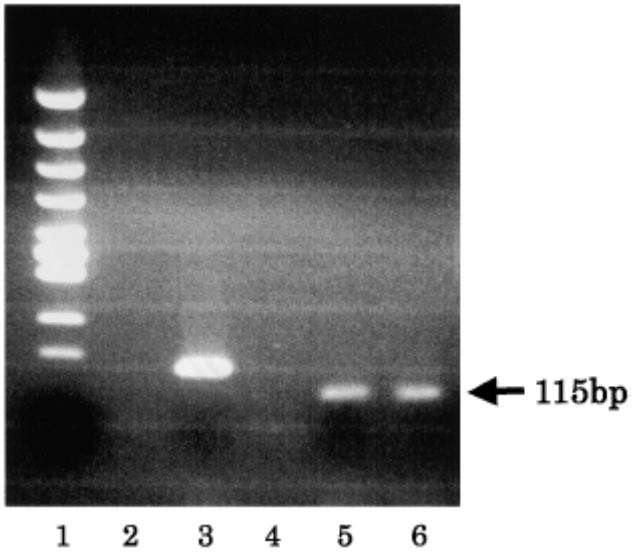

Fig. 3

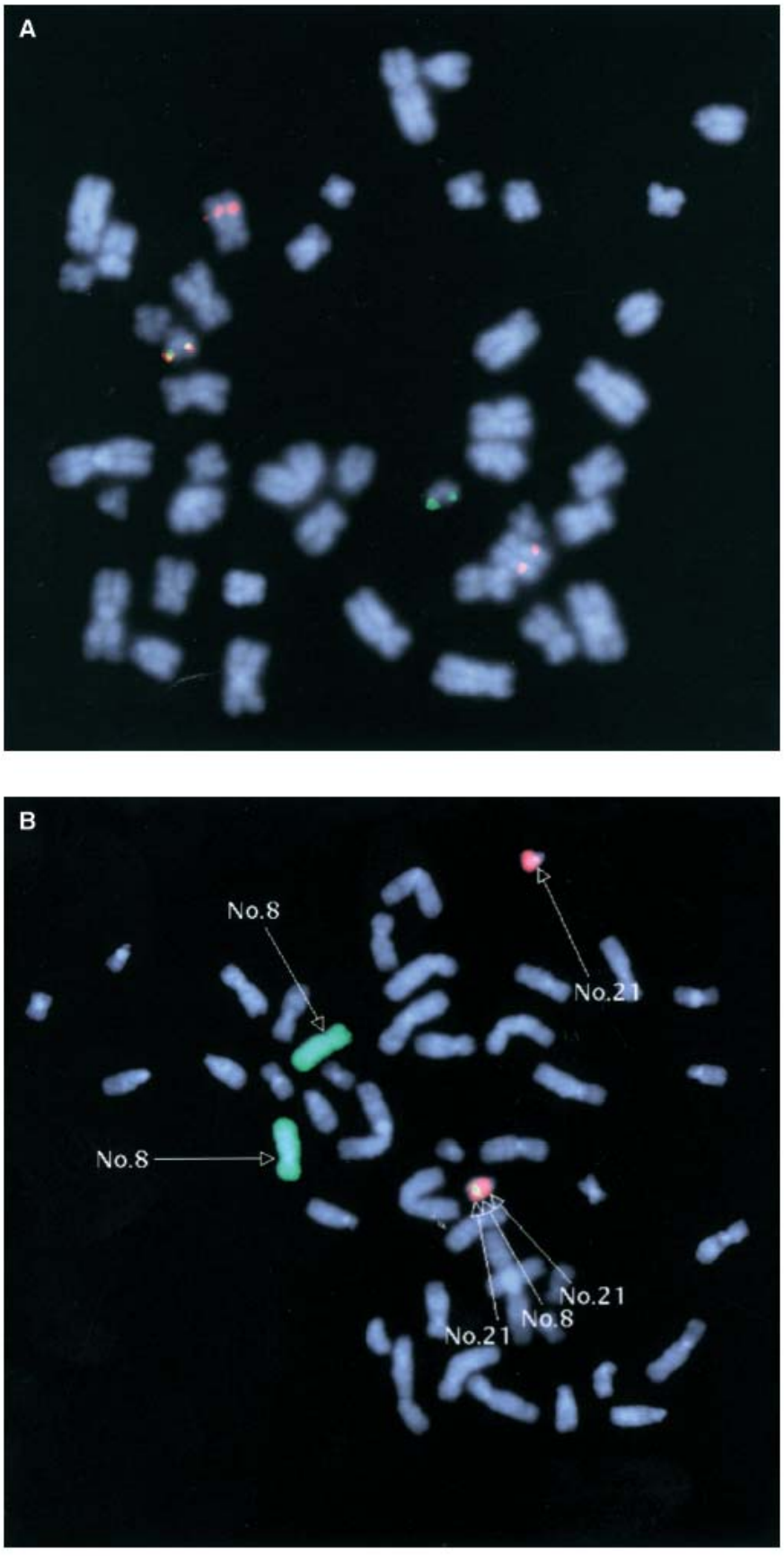

Table1.List of patients with masked t(8;21)

Author	Year	cases	FAB type	karyotype*	FISH
Maseki *et al* [5]	1993	3	M2	46,XY	
				46,XX	
				46,X,-X	
Maruyama *et al* [6	1993	2	M2		
Maruyama *et al* [7	1994	1	M2		
Oka *et al* [8]	1994	1	M2	45,X,-X,inv(9)(p12q13)	
Andrieu *et al* [9]	1996	3	M1,2	46,XY,del(8)(q?),del(9)(p?)	Painting probes 8
				46,XY,I(8)(q10)[4]/46,idem,del(7)(q31)[4]/46,XY[10]	
				46,XX,del(1)(q21q25),del(8)(q22),add(19)(?q13),del(21)(q22)[11]/45,idem,-/X[9]	
Langabeer *et al* [1(	1997	19	M1,2,4,5		
Saito *et al* [11]	1997	1	M2	45,X,-Y,t(8;12;21)(q22.1;q24.1;q22.1)	Painting probes 8,12,21 AML1
Wong *et al* [12]	1998	1	M2	45,X,-Y,t(8;20)(q22;q13)	
Harrison *et al* [13]	1999	4	M2	46,XY,t(8;8;21)(q22;p23;q22)	inting probes, centromere of 8, AML1
				46,XY,del(1)(q21q25),t(8;19;21)(q22;q?13;q22)	
				45,X,-Y,der(8),inv(8)(q22q24),t(8;21)(q22;q22)	
				46,XY,ins(21;8)(q22;q21q22)	
Sarriera *et al* [14]	2001	8	M0,1,2,4,RAEB-T		Centromeric probes
Miyagi *et al* [15]	2002	1	M2		Spectral karyotyping
Gamerdinger *et al*	2003	6	M1,2	45,X,-X,der(7)t(7;8)(q3?4;q?24),ins(21;8)(q22;q22q22)	Multicolor-FISH
				45,X,-X,der(8)ins(8;21)(q22;q22q22)inv(8)(p1?1q22)	
				46,XY,ins(21;8)(q22;q22q22)/47,XY,+8,ins(21;8)(q22;q22q22)	
				47,XX,der(3)t(3;8)(q2?9;q13)del(8)(q21q22),+8,der(8)t(3;8)(q2?9;q13),ins(21;8)(q22;q22q22)	
				46,XY,ins(8;21)(q22;q22q22)	
				45,X,-Y,t(8;12;21)(q22;p13;q22)	
our case		1	M2	46,X,-Y,ins(21;8)(q22;q22q22)	Painting probes 8,21 AML1/ET0

*final (reviesed) karyotype which mentioned

Table 2. Patterns of revised karyotype in cases of masked t(8;21)

Auther	Cytogenetic results	Revised cytogenetic results following FISH
Saito *et al* [11]	45,X,-Y,t(8;21)(q22.1;q24.1)	→45,X,-Y,t(8;12;21)(q22.1;q24.1;q22.1)
Harrison *et al* [13]	46,XY,i(8)(q10)[4]/46,idem,del(7)(q31)[4]/46,XY[10]	→46,XY,t(8;8;21)(q22;q23;q22)
	46,XX,del(1)(q21a25),del(8)(q22),add(19)(?q13),del(21)(q22)[11]/45,idem,-X[9]	→45,XX,del(1)(q21q25),t(8;19;21)(q22;?q13;q22)
	45,X,-Y,t(8;21)(q24;q22)	→45,X,-Y,der(8),inv(8)(q22q24)t(8;21)(q22;q22)
	46,XY,ins(21;8)(q22;q21q22)	→46,XY,ins(21;8)(q22;q21q22)
Gamerdinger *et al*[16]		
	45,X,-X[10]	→45,X,-X,der(7)t(7;8)(q3?4;q?24),ins(21;8)(q22;q22q22)
	45,XX[7]	→45,X,-X,der(8)ins(8;21)(q22;q22q22)inv(8)(p1?1q22)
	47,XY,+8[10]/46,XY[2]	→46,XY,ins(21;8)(q22;q22q22)/47,XY,+8,ins(21;8)(q22;q22q22)
	47,XX,der(3)t(3;?8)(q27;?q13),der(21)t(8;21)(q22;q22),+?der(21)t(8;21)(q22;q22)[14]	→47,XX,der(3)t(3;8)(q2?9;q13)del(8)(q21q22),+8,der(8)t(3;8)(q2?9;q13),ins(21;8)(q22;q22q22)
	46,XY[11]	→46,XY,ins(8;21)(q22;q22q22)
	45,X,-Y,t(8;12;?21)(q22;p13;q22)[13]	→45,X,-Y,t(8;12;21)(q22;p13;q22)
our case	45,X,-Y	→45,X,-Y,ins(21;8)(q22;q22q22)