Original Article

Chromosomal Abnormalities Associated with Local Recurrence or Pulmonary Metastasis of Giant Cell Tumor of Bone in Thai Adults: A Prospective Cohort Study with 6 Years of Follow-up

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Objectives: Giant cell tumor (GCT) of bone shows chromosome abnormalities. We aimed to assess the prognostic role of chromosome abnormalities of primary GCT of bone for local recurrence or pulmonary metastasis.

Method: A cohort study with 6 years of follow-up was performed in consecutive patients with primary GCT of bone surgically treated between 2011 and 2013. All patients underwent surgical resection with extended intra-lesional curettage and phenol local adjuvant therapy. Systematic cytogenetic analysis compared cytogenetic abnormalities between patients with local recurrence or pulmonary metastasis and those without. Fifteen patients were eligible, enrolled, and had successful cytogenetic analysis.

Results: The median follow-up time was 46 months (IQR 32, 58). Five patients experienced local recurrence or pulmonary metastasis [median time-to-recurrence 6 months (IQR 3.25, 10.5)]. The mean number of abnormal cells in the primary culture comparing those with local recurrence or pulmonary metastasis to those without was 24.4 vs. 9.6 ($p = 0.04$), respectively. Similar patterns were found in the cultures of the following four passages ($p < 0.05$). Forty-five patterns of clonal telomeric association (tas) were observed in passaged cultures. Six tas patterns were associated with local recurrence or pulmonary metastasis including tas(11;19) (p15;q13.4), tas(15;19) (q26.3;q13.4), tas(15;22) (p13;p13), tas(16;19) (p13.3;q13.4), tas(17;19) (p13;q13.4), and tas(19;22) (q13.4;q13).

Conclusion: The mean number of abnormal cells and the six TAS patterns identified could be candidate prognostic factors for local recurrence or pulmonary metastasis of GCT tumor of bone.

Keywords: Cytogenic study, Giant cell tumor, tas, predicting factor, local recurrence

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INTRODUCTION

Giant cell tumor (GCT) of bone is the most common benign aggressive tumor of bone. The incidence of this tumor in the Asian population is around 15% [1-4]. Post-operative recurrence rates ranging from 18 to 65% have been reported [5-7]. The recurrence rate is approximately 17.4% within the first five years of follow-up [8]. Intra-lesional curettage with applications of many adjuvant substances to control local recurrence of GCT of bone has been studied, including warm Ringer's lactate solution, phenol, bone cement, zolendronic load-bone cement, and liquid nitrogen [9-12]. Campanacci, et al. suggested that the presence of tumor at the surgical margin is the most important prognostic factor of recurrent GCT [13]. Other potential predictors have been studied extensively. However, radiographic findings, histological appearance, or immune-histochemical stain such as Ki-67 were not able to predict local aggressiveness and/or metastatic potential of the tumor after surgery [14-19]. Conversely, abnormalities found in genetic studies of DNA or chromosomal study of the tumor were strongly associated with local recurrence and lung metastasis [20-23]. Bridge, et al. found the presence of chromosomal abnormalities in GCT of bone, particularly telomeric fusion. Therefore, cytogenetic analysis has been suggested as a potentially useful tool to predict the aggressiveness of the tumor [24]. However, few cytogenetic studies have been reported to date.

Therefore, we aimed to identify chromosome abnormalities in GCT of bone associated with local recurrence or pulmonary metastasis by comparing tissue taken from primary lesions associated with the outcome and those without cytogenetic analysis.

MATERIALS AND METHODS

We conducted a prospective longitudinal cohort study. Our institutional review board approved this study. All patients presenting with primary GCT of bone were eligible for enrollment between October 2011 to October 2013. Complete history, physical examination, and investigation, including plain radiograph at the lesion, plain radiograph of the chest (postero-anterior and lateral views), computed tomograph (CT) chest, bone scan, and magnetic resonance imaging/CT scan at the lesion were performed in all patients. Open biopsy was performed for definite diagnosis in all patients. If the pathological diagnosis was conventional GCT of bone, the patient was enrolled in the study. Biographic data, age, gender, location of the tumor (axial/nonaxial skeleton), and preoperative tumor Campanacci's classification were recorded in all patients. All patients then underwent definitive treatment using extended intra-lesional curettage, and 1 cu mm of the solid parts of the tumor was sent for cytogenetic study. The primary tumor tissues derived intraoperatively were kept in sterile tubes containing fetal bovine serum cell culture media and were transferred to the cytogenetic laboratory immediately. Local treatment with high-speed burr, electrocautery, and phenol was done, and bone cement was used to fill the bony lesions. After surgery, the patients were followed-up every month during the first year and every three months, subsequently for six years as our routine practice. The outcome was a composite of local recurrence or pulmonary metastasis. Clinical presentations of the primary tumors before the operation and of the locally recurrent tumors or pulmonary metastasis after the operation were recorded in all patients.

Cytogenetic procedure

The cytogenetic study was conducted at the Cytogenetic Laboratory for Leukemia Diagnosis, Research Department. This laboratory is ISO 15189 and ISO 15190 accredited. Cytogenetic investigators were blinded to the clinical presentations. The primary tumor tissues were obtained directly from the surgery and placed into Dulbecco's Modified Eagle Medium (DMEM, Gibco, SIGMA, St. Louis, MO) with antibiotics (Biochrome) immediately. The specimens were subsequently disaggregated mechanically by mincing with a scalpel blade and enzymatically by incubating in collagenase (Biochrome). The short-term culture was performed following the Mandahl protocol. After removal of the collagenase solution, the cells were resuspended in DMEM with 20% fetal calf serum. The cultures were placed in a humidified 37 ° C, 5% CO2 incubator. After 10-14 days of cultivation, the cells were in the log phase. Three hours before harvesting, the cells were exposed to colchicine (0.25μg/mL). The cell suspension was then resuspended in the 0.075M KCl twice and fixed three times with a 3:1 mixture of methanol and acetic acid. The cell suspension was then dropped on slides, and Q-banding was performed.

Serial passaging was then performed on the cultures of all patients to study the dynamics of chromosome changes in GCT of bone. The culture conditions were as previously described, and the cell cultures were split 1:2 at subconfluency. The samples were studied from primary culture to five passages [25]. The 50 metaphases in every passage were analyzed according to the International System for Human Cytogenetic Nomenclature 2013. The number of abnormal cells and the total number of abnormalities and types of abnormalities, including numerical, structural, and telomeric associations were recorded. The association of the short arm of the acrocentric chromosome was also recorded as a telomeric association. Both the nonclonal and clonal chromosome changes along with the frequencies of the telomerase associations were calculated.

Study size and statistical analysis

No a priori sample size was calculated. All eligible patients were included. Categorical variables were presented as frequencies and percentages along with continuous variables as mean (SD) or median (IQR) as appropriate. The unpaired t-test was used to compare the means of the group with local recurrence or pulmonary metastasis and the group without. A p-value of less than 0.05 was considered statistically significant. Data analyses were performed using SPSS statistics version 18 (SPSS, Inc., Chicago, IL).

RESULTS

A total of 15 patients were screened for eligibility and enrolled. There was no loss of follow-up cases or death during the study period. All participants completed the study protocol, including successful cytogenetic analysis. Patient demographic and clinical characteristics are shown in Table 1. The mean age of the included patients was 35.4 years. Of the total of 15 patients, 10 (66.7%) were female. Most of our GCT cohort had non-axial skeleton involvement (86.6%) and Campanacci stage 2 (66.7%). There was only one case (6.7%), which confirmed pulmonary metastasis prior to operation. Five of the patients (33.3%) had either local recurrence or pulmonary metastasis during the 6-year post-operative follow-up period.

The mean number of abnormal cells in the primary culture of patients with the outcome was significantly higher than in those without the outcome (24.4 vs. 9.6, respectively; $p = 0.04$). Similar patterns were found in the following cultures for four passages ($p < 0.05$). Similar patterns were found in the following cultures for four passages ($p < 0.05$) (Table 2). Forty-five patterns of clonal telomeric association (tas) were observed in passaged cultures. However, only six pattern including tas(11;19) (p15;q13.4), tas(15;19) (q26.3;q13.4), tas(15;22) (p13;p13), tas(16;19) (p13.3;q13.4), tas(17;19) (p13;q13.4), and tas(19;22) (q13.4;q13) were consistently found in the cultured samples from primary GCT of bone from patients with recurrence (Fig. 1-6). These abnormalities were found repeatedly in passaged cultures.

Table 1. Patient demographic and clinical characteristics.

Clinical variables	Total
Age (years±SD)	35.4±14.8
Sex	
Female (N, %)	10 (66.7)
Male (N, %)	5(33.3)
Recurrent	
Non recurrent GCT (N, %)	10(66.7)
Recurrent GCT or pulmonary metastasis (N, %)	5 (33.3)
Location of involvement (N, %)	
Non-axial skeleton	13 (86.6)
Axial skeleton	2 (13.4)
Both	0
Campanacci staging (N, %)	
0	0
1	5 (33.3)
2	10 (66.7)
3	0
Present of tumor recurrence or pulmonary metastasis before operation (N, %)	
Yes	1 (6.7)
No	14 93.3)
Present of tumor recurrence or pulmonary metastasis after operation (N, %)	
Yes	5 (33.3)
No	10 (66.7)

DISCUSSION

Prognostication of local recurrence or pulmonary metastasis of GCT of bone after surgery is a major challenge to oncologic orthopedic surgeons.

The recurrence rate of GCT and/or pulmonary metastasis has previously been reported as ranging from 0% to 4% [26-30]. Although early detection of the patients with high risk of recurrence and aggressive GCT of bone could advance the line of management to be more aggressive, consensus on valid predictive factors has not been reached. Some predictive factors of recurrent GCT of bone have been explored, including serum alkaline phosphatase, serum calcium, serum phosphate, serum vitamin D level, and bone mineral [14-

Table 2. Independent sample T-test and number of aberrant cell along 5 passages of GCT in recurrent group vs non-recurrent group.

Sequence of cytological culture / Classification (n)		Number of aberrant cells (Mean ± SD)	p-value (95% CI of the difference)
Passage 1	- Non recurrent (10)	6.90 ± 6.67	0.002
(Primary culture)	- Recurrent (5)	22.40 ± 6.27	(-23.50 to -7.50)
Passage 2	- Non recurrent (10)	5.90 ± 6.01	0.003
	- Recurrent (5)	24.00 ± 7.91	(-27.77 to -8.43)
Passage 3	- Non recurrent (10)	6.20 ± 6.03	<0.001
	- Recurrent (5)	27.00 ± 4.36	(-26.81 to -14.79)
Passage 4	- Non recurrent (10)	8.20 ± 7.73	0.001
	- Recurrent (5)	27.20 ± 6.83	(-27.83 to -10.16)
Passage 5	- Non recurrent (10)	7.80 ± 7.86	0.002
	- Recurrent (5)	31.00 ± 9.62	(-35.02 to -11.37)

Abbreviations: SD, standard deviation; CI, confidence interval

23], yet none of these are used in clinical practice. Some centers use Campanacci staging to predict local recurrence of GCT only [13]. Nevertheless, Gorunova, et al. suggested that local recurrence probably depends more on only the adequacy of surgical treatment than the intrinsic biology of the tumors without any suitable predictive factors [32].

Conversely, some authors have proposed that the intrinsic biology of the tumors, such as DNA, may be associated with the recurrence of GCT of bone[33-35]. Jeffrey, et al. found a telomeric association and del(11)(p11) had strong associations with the local recurrence of GCT of bone[36]. Bridge, et al. also stated that telomeric fusion and cytogenetic analysis might be useful in predicting the biological behavior of GCT of bone[24]. Therefore, the present study was conducted with a cytogenetic study to identify chromosomal abnormalities that may predict recurrent GCT of bone.

The results of the cytogenetic analysis of the present study showed a significantly higher number of abnormal cells in the recurrent group compared with the nonrecurrent group. This finding could be clearly established at the primary culture or the first passage of the tumor. Furthermore, we found six patterns of consistent cytogenetic abnormalities only in our recurrent cases. The cytogenetic analysis results were also similar within the later passages (the second to fifth passages of the DNA culture). One week was usually used for each passage of cytogenetic analysis to report the aggressiveness of the tumor. These findings are very important because the mean number of cells in the primary culture was adequate for the distinct identification of aggressiveness of the intrinsic tumor biology. Therefore, we are able to provide some credible evidence that cytogenetic analysis could be a dependable prognostic factor, using a cytogenetic analysis technique that takes only one month to report its findings for final, reliable prognostication. Our study had similar results to those of Unni, et al., who reported the most common abnormal karyotypic findings in recurrence of GCT of bone were tas11p, 15p, 19q, and 21p[36].

Limitations

This study had several limitations. First of all, the present study may have been limited by inter-observer variation during the cytogenetic study due to the high technical skill required. Secondly, we did not conduct a genome sequencing study because we needed to find out the chromosomal abnormality with this cytologic study first. Finally, the sample size was small; adjusting for confounding or prognostic modeling could not be performed with adequate accuracy and precision due to the low sample size.

CONCLUSION

In conclusion, the mean number of abnormal cells and six tas patterns including tas(11;19) (p15;q13.4), tas(15;19) (q26.3;q13.4), tas(15;22) (p13;p13), tas(16;19) (p13.3;q13.4), tas(17;19) (p13;q13.4), and tas(19;22) (q13.4;q13) in cytogenetic analysis of GCT of bone primary tumor tissue were associated with local recurrence or pulmonary metastasis during 5 years of follow-up. These could be candidate prognostic factors for local recurrence or pulmonary metastasis in GCT of bone.

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Conflicts of Interest: The authors declare no conflict of interest.

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Ethical approval: This study included human participants. It had been approved by Siriraj Institutional Review Board (SIRB).

Informed Consent Statement: Research assistance obtained informed consent from all individual participants in the study.

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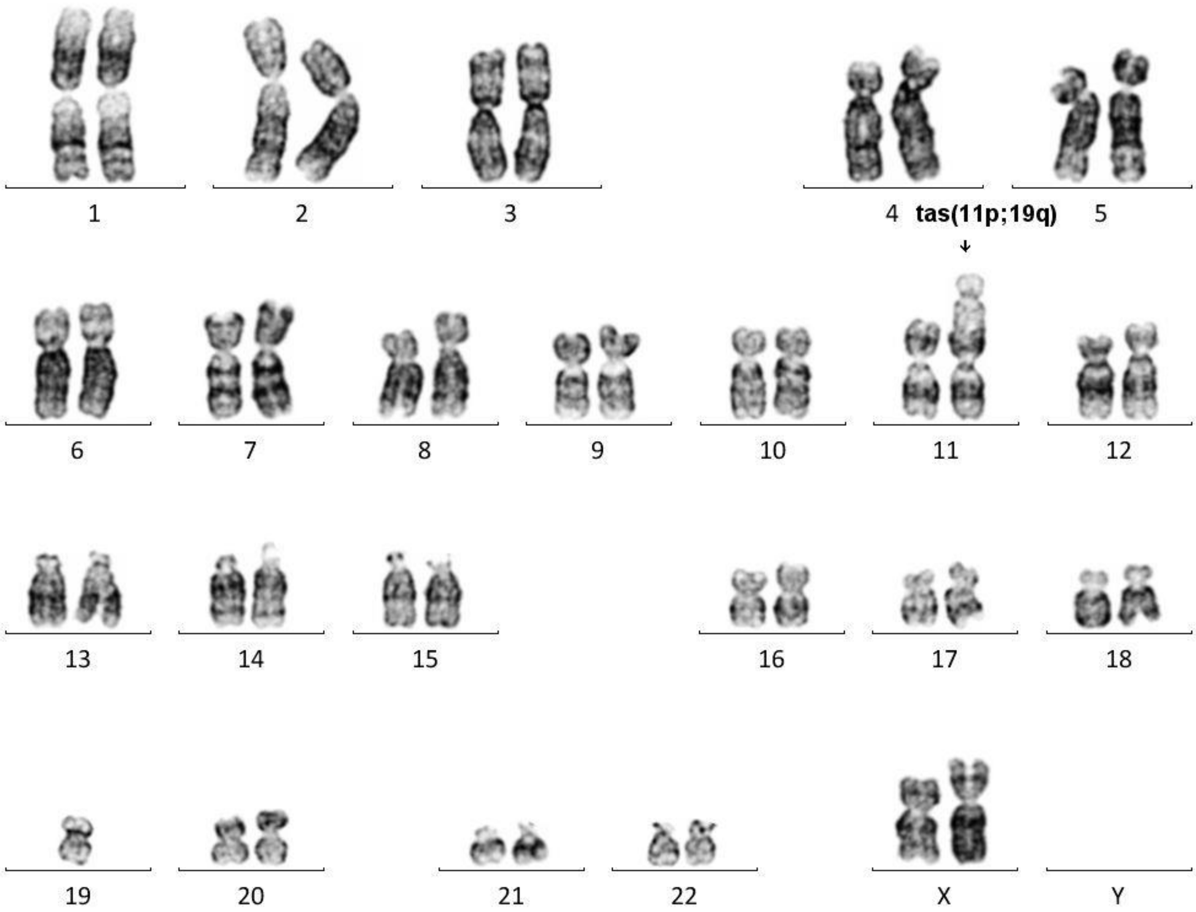

Figure 1. tas(11;19)(p15;q13.4)

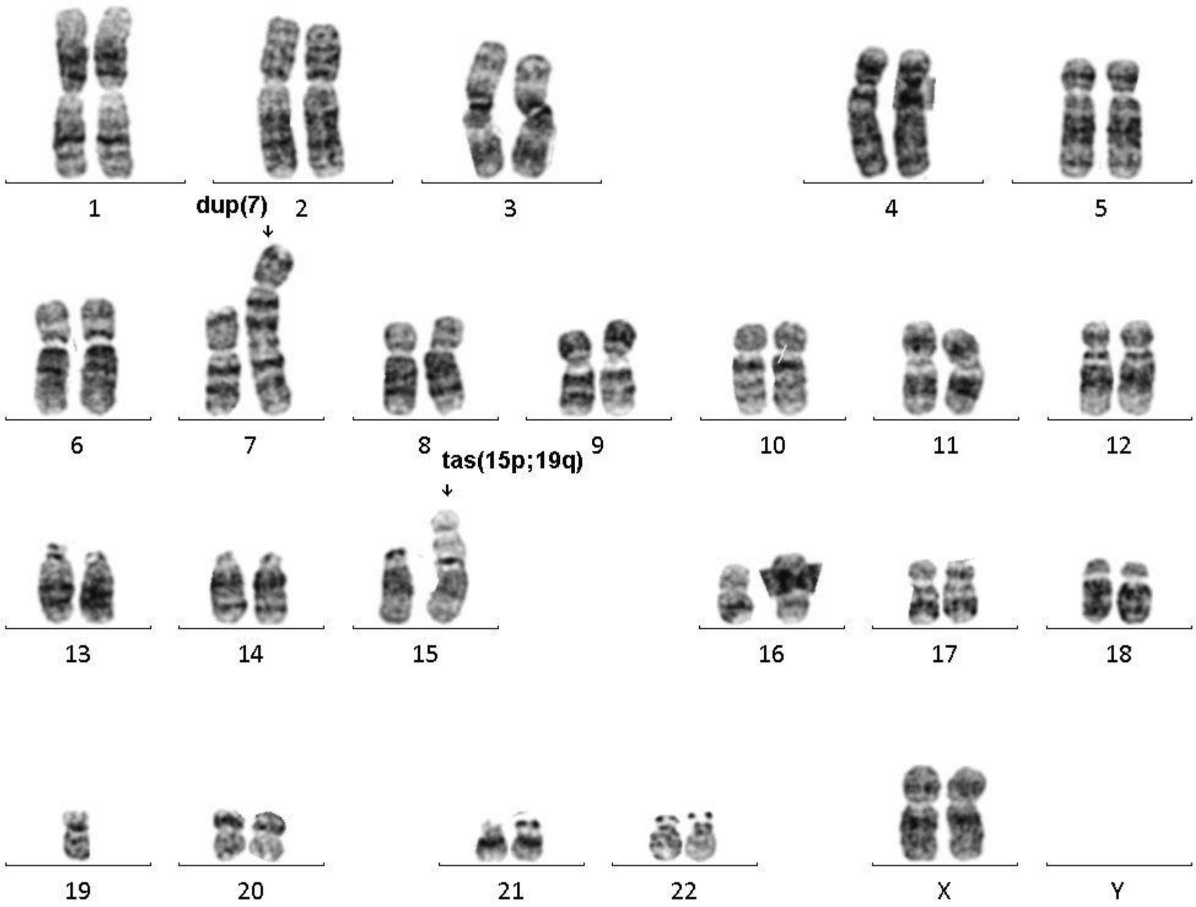

Fig2. tas(15;19)(q26.3;q13.4)

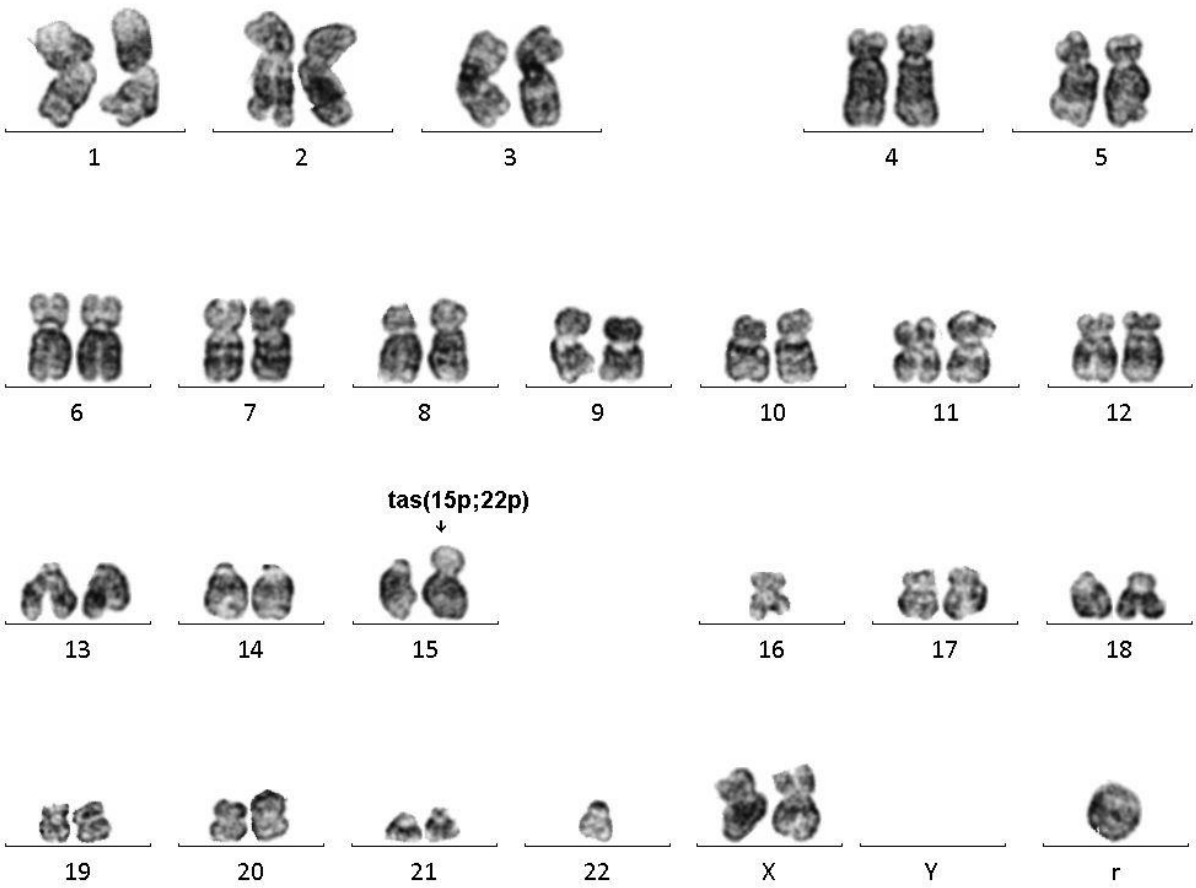

Fig3. tas(15;22)(p13;p13)

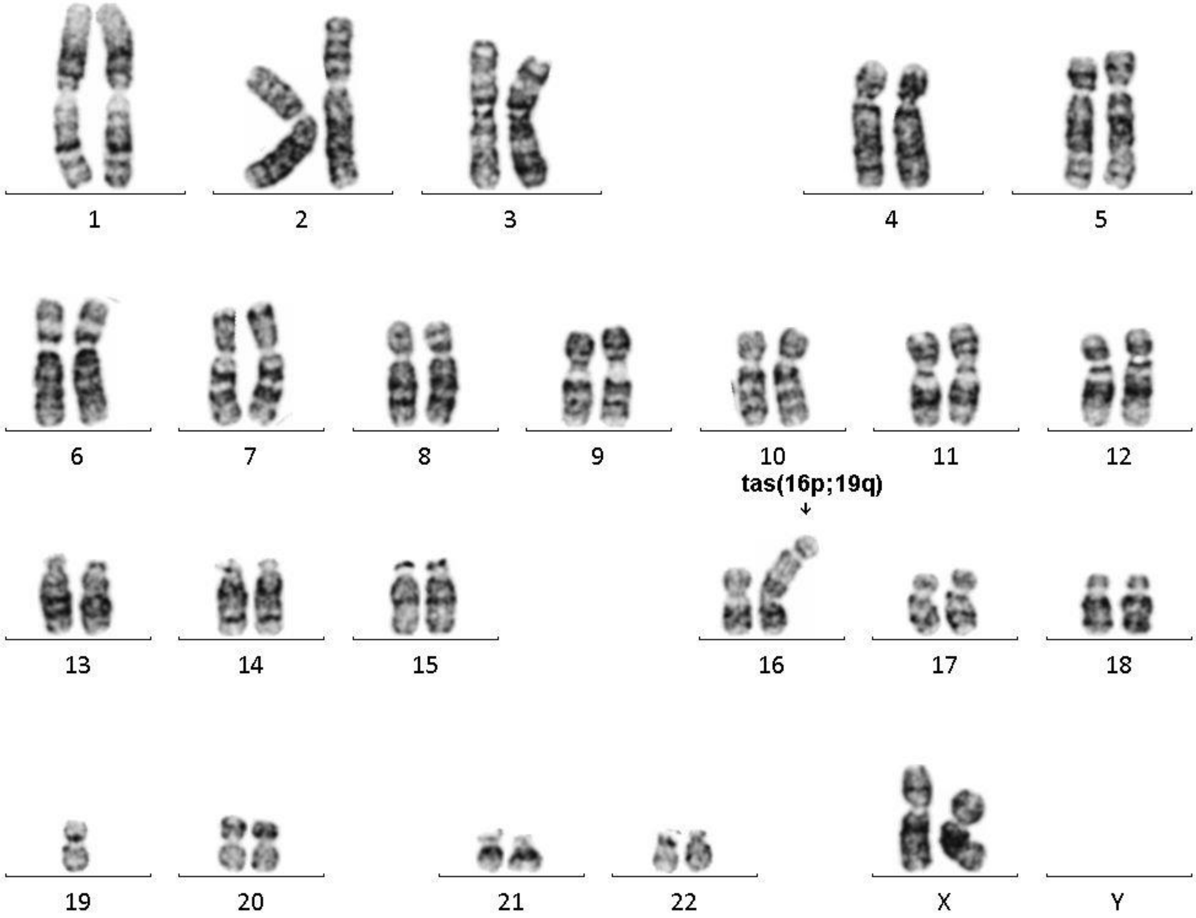

Fig4. tas(16;19)(p13.3;q13.4)

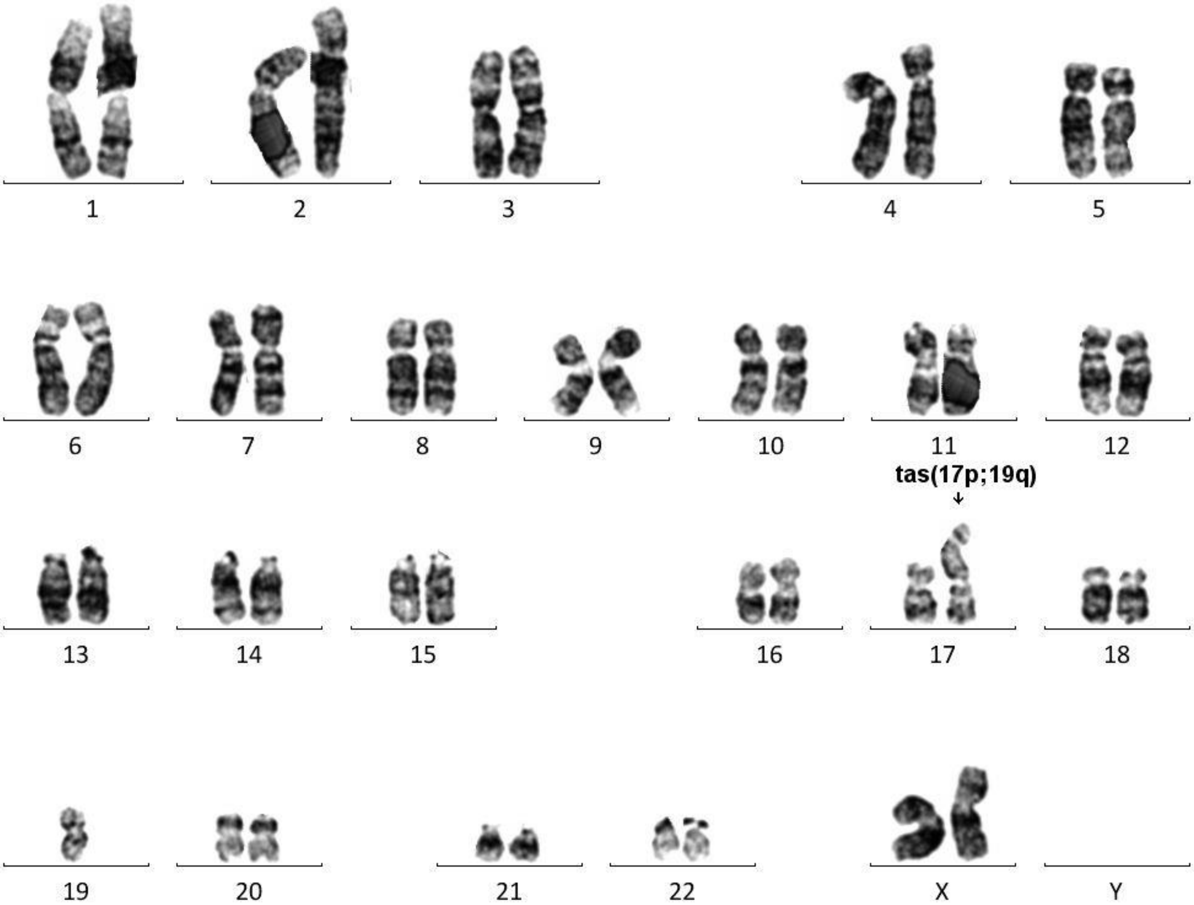

Fig5. tas(17;19)(p13;q13.4)

Fig6. tas(19;22)(q13.4;q13)