

Chromosome 10 Abnormality Predicts Prognosis of Neuroblastoma Patients With Bone Marrow Metastasis

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Research

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Abstract

Background

Neuroblastoma (NB) is the most common extracranial solid tumor in children with high heterogeneity and concealed onset. The mechanism for its occurrence and development has not been revealed. The purpose of this study was to summarize the clinical characteristics of children with NB and abnormal chromosome 10. To investigate the relationship between the number and structure of chromosome 10 abnormality and NB prognosis.

Methods

We used chromosome G-banding in the first diagnosis to evaluate the genetics of chromosomes in patients with NB, and follow up their clinical characteristics and prognosis. All participants were diagnosed with NB in Hematology Oncology Center, Beijing Children's Hospital from May 2015 to December 2018, and were followed up for at least one year.

Results

Of all 150 patients with bone marrow metastases, 42 were clearly diagnosed with chromosomal abnormalities. There were 13 patients with chromosome 10 abnormalities definitely, and the loss of chromosome 10 was the most common decrease in the number of chromosomes. These 13 patient had higher LDH, lower OS and EFS than that of children in abnormal group without chromosome 10 abnormality. Eight patients both had MYCN amplification and 1p36 deletion. Two of them had optic nerve damage and no vision, and 1 had left supraorbital metastases five months after treatment. Among the 16 children with suspected chromosome 10 abnormalities, 3 also had orbital metastases.

Conclusions

The above results showed that chromosome 10 might be a new prognostic marker. MYCN amplification and 1p36 deletion may be related with chromosome 10 abnormalities in NB. And NB patients with abnormal chromosome 10 were prone to have orbital metastases.

Background

Neuroblastoma(NB) is a malignant solid tumor of children originating in the adrenal medulla and sympathetic nervous system[1]. As the widely diagnosed disease in children, there are 8-10.2 cases of NB per million children under 15 years of age[2]. It has high heterogeneity, hidden onset and poor prognosis with less than 40% of 5-year survival rates in high risk NB[3]. Only 1-2% of the families are inherited, and most of them are sporadic[4]. However, the mechanism of occurrence, proliferation and metastasis of NB is not clear so far. With the development of chromosome karyotype analysis, whole genome sequencing and proteomics, the relationship between chromosome abnormality and apoptosis, differentiation, spontaneous regression, proliferation and metastasis of NB tumor cells is gradually revealed[5].

Chromosome 10 is a pair of 23 pairs of autosomes in humans, which contains about 135 million base pairs. There may be some important genes related to the development of NB on chromosome 10. One of the tumor suppressor genes, PTEN, is located at 10q23.3, and is a gene that is homologous to the phosphatase and the tension deleted on chromosome 10. It can influence the development of NB through PI3K/ AKT/ mTOR pathway[6]. High-risk NB children at Stage I without MYCN gene amplification whose whole chromosome aneuploidies(WCAS) factor less than 2 have poor prognosis than WCAS \geq 2. This phenomenon is most significant on chromosome 10(P=0.002)[7]. Our previous clinical study showed that 50% of the children with metastatic NB had chromosomal abnormalities, and 70% of them have chromosome number and structural abnormalities concurrently. Among them, the number abnormalities that occurred frequently were reduced on chromosome 21, 10, and 11, especially 10.

This study aimed to summarize the clinical characteristics of children with NB with abnormal chromosome 10 in a single center. And plan to explore the relationship between the abnormal number and structure of chromosome 10 and the occurrence, development and prognosis of NB. We hope to provide a new argument for chromosome genetics of neuroblastomas.

Methods

Patients Samples

This study retrospectively analyzed a total of 150 children with NB who had been diagnosed with bone marrow metastases by routine bone marrow cytology or bone marrow biopsy from May 2015 to December 2018 in Hematology Oncology Center, Beijing Children's Hospital. All the children were staged according to International Neuroblastoma Stage System (INSS)[8]. Risk stratification was conducted according to Children's Oncology Group (COG)[9]. Their bone marrow specimens were tested by chromosome G-banding when they were first hospitalized. They all regularly treated and followed up in our center until November 30, 2019.

Medical records were collected completely. Clinical data included age at diagnosis, staging, sex, MYCN gene, chromosome report and outcome. Based on detailed karyotype analysis, we found that 13 patients had abnormal chromosome 10 specifically and similar clinical characteristics. We focused on their clinical features such as primary tumor location, tumor markers at initial diagnosis, the largest diameter of the tumor, metastatic site, and so on. The Ethics Committee of Beijing Children's Hospital of Capital Medical University approved the study(2019-k-390). And we got informed consent from all participants and their parents to get samples and information.

Therapeutic regimen and follow-up

All the patients were treated according to NB protocol of Beijing Children's Hospital (BCH-NB-2007), which was developed based on Hong Kong NB protocol 7 and the European low- and intermediate-risk(IR) NB protocol[10]. For the low-risk(LR) and IR groups with favorable pathological NB, the therapy were CBVP(Carbo; etoposide) and CADO(cyclophosphamide; Adriamycin; Vincristine,) alternately for 4-6

courses and surgery. For the IR group with unfavorable pathological NB, therapy included 6–8 courses of chemotherapy combined with surgery, radiotherapy and 6 courses of cis-retinoic acid. For the high-risk(HR) group, the chemotherapy regimen was carried out sequentially for CAV(cyclophosphamide; Adriamycin; Vincristine) and CVP(etoposide; cisplatin). They were treated combined with surgery, autologous hematopoietic stem cell transplantation, local radiotherapy and 13-cis-retinoid acid. And they were followed up regularly every 3 months in the first year, every 4 months in the second year and every 6 months in the 3-4 years.

Chromosome examination

All methods were performed in accordance with the experiment guidelines and Ethical permission. Bone marrow specimens were anticoagulated with 2ml heparin, and cultured in a cell culture incubator at 37 ° C and 5.0% CO₂ for 24 hours. Before the end of culture, a certain concentration of colchicine was added to the culture medium to a final concentration of 0.1 µg / ml. Then they were continued in the incubator for 2 hours. After that, remove the culture tube, centrifuge, clear the supernatant, and add 0.075 mol/L KCL hypotonic solution. Pipette the samples and mix them in a 37 ° C water bath for 20 min. Add 1.5 ml of fixing solution (3:1 mixture of methanol and acetic acid), mix and pre-fix, and centrifuge to remove the supernatant. Then add 5 ml of Carnot's fixative, mix by pipetting, and centrifuge to remove the supernatant. Repeat the previous step to fix them twice[11].

The precipitation was prepared into a certain concentration of cell suspension with Carnot's fixed solution. Take out the slide and add a drop of suspension to each piece. Diffuse and dry them at a certain temperature and humidity. Each specimen was made into 4 pieces and baked at 60 ° C overnight. Before the examination, it was digested with trypsin and stained with Giemsa. Acquire metaphase images of cells in microscope under high magnification. Each split phase was analyzed by professional analysts through the autosoftware for karyotyping. Twenty metaphase mitotic phases were analyzed in each specimen.

The karyotype were defined based on the International System for Human Cytogenomic Nomenclature (ISCN)[12]. In tumor cells, it was considered as a meaningful clone that 2 or more cells showed the same increase or structural abnormality of chromosome, and 3 or more cells showed the same decrease. Other karyotype with fewer cells were suspected clones.

Statistical analysis

Descriptive statistics were conducted using Statistical Package for Social Scientists (SPSS) version 22. Overall survival (OS) was defined as the time from enrollment to disease-caused death or final follow-up. Event-free survival(EFS) was defined as the time to first occurrence of any event, such as disease progression, or death from any cause. The survival curves for OS or EFS were generated via the Kaplan-Meier method, and the difference between two groups was evaluated with a log-rank test. $p < 0.05$ was considered to be statistically significant.

Results

Patient cohort

All the 150 children with NB had bone marrow metastasis, and all of them underwent chromosome examination. According to the results of karyotype analysis, 42(28%) cases were identified with chromosomal abnormalities, 40(26.7%) were suspected, and 68(45.3%) were normal. As shown in Table 1, age at diagnosis in the abnormal group ranged from 8 to 105 months (mean, 38 months). In terms of tumor markers, the median lactate dehydrogenase (LDH) and median neuron specific enolase(NSE) of the abnormal group were 1088(range, 655, 3423.75) U/L and 370(range, 364.33, 370) ng/l respectively. In the normal group, the median LDH was 552.5(range, 367.75, 876.25) U/L and the median NSE was 273.5(range, 125.7, 370) ng/l. And in the suspected chromosomal abnormalities, which reported only one abnormal karyotype cell, The median LDH was 796 U/L and the median NSE was 334.15 ng/l(range, 451.5, 1248 U/L; range, 146.43, 370 ng/l).

Chromosome karyotype analysis

Among the 42 children with definite chromosomal abnormalities, the chromosome losses were more than the chromosome gains(20 vs 16; 47.6% vs 38.1%). However, the number of each detailed chromosome changes was mainly gains, as Figure 1 showed. There were 29 patients with abnormal numbers of chromosomes, including one with only gains of marked chromosomes. And in 13 patients only losses, in 6 patients only gains, while 9 patients had gains and losses at the same time. The most frequent quantity anomalies were 10 losses(30%), 17 losses(25%), 7 gains(62.5%) and 12 gains(56.25%). Chromosome karyotype analysis showed that individual chromosomes differed in the likelihood of losses or gains. For example, gains appeared frequently in chromosome 1, 6, 7, 12 and 20. Losses were more in chromosome 10, 11, 14, 17, 21 and X. The karyotype of tumor patients was so complex that it was difficult to evaluate only with abnormal quantity. Therefore, most of the above children(97.6%) also had abnormal chromosome structure. In 10 children with MYCN gene amplification, all of them were accompanied by 1p36 deletion except 2 cases without 1p36 examination.

Correlation Analysis of chromosome 10 anomalies

A total of 13 children were accompanied by chromosome 10 abnormalities, including 6 losses(46.2%), 3 gains(23.1%) and 4 with structural abnormalities(30.8%). They were all I -stage HR children with NB(Table 2). According to Figure 1, the loss of chromosome 10 was the most common decrease in the number of chromosomes. All 4 patients with structural abnormalities had changes in the 10q22 area. The median LDH and NSE of chromosome 10 abnormal group at first diagnosis were 2833(1073, 3743.5) U/L and 370(370, 445) ng/l respectively. Their LDH were higher than that of children in abnormal group without chromosome 10 abnormality (Mean, 968, 652, 2041.5 U/L), although the difference was not statistically significant($P=0.185$). And the median vanillylmandelic acid(VMA) in the abnormal group of chromosome 10 was 30.34(10.25, 183.01). Because the partial maximum of the NSE reported in our center only shows >370 , the NSE cannot be compared. And two of them had optic nerve damage and no vision. Another

child had left supraorbital metastases five months after treatment. Among 16 children with suspected chromosome 10 abnormalities, 8 had harnpan and intracranial metastases, and 3 had orbital metastases.

Outcome and prognosis

After definite diagnosis, the children were treated and followed up according to risk stratification. Among the 13 patients with a definite chromosome 10 abnormality, whose median follow-up was 17.25(7, 21.5) months, 9 had recurrences. The median progression time was 13(3.5, 17.75) months. And all these 9 children died. Thirteen NB patients with abnormal chromosome 10 had significantly lower OS than 143 patients with normal chromosome 10(66.368% vs 14.359%, $P=0.002$). However, its EFS was lower than the normal chromosome 10 group without statistically significance(40.618% vs 23.932%, $P=0.0837$, Figure 2). In detail, three-year OS was 14.359% in chromosome 10 abnormality versus 74.044% in normal group. And three-year EFS of chromosome 10 abnormality was also higher than normal group(23.932% vs 55.147%). There were statistical differences between the above two groups ($P=0.001$; $P=0.0089$, Figure 3). Three-year OS and EFS of chromosome abnormality group without abnormal no.10 were 42.248% and 25.543% respectively. As shown in Figure 4, compared with the chromosome 10 abnormal group, despite no statistical differences, the OS of the 10 abnormal group was still lower than the group without the 10 chromosomal abnormality ($P=0.2158$; $P=0.7817$).

Discussion

As a heterogeneous and occult tumor in children, most children with NB have chromosomal abnormalities when they are first diagnosed[13]. According to the Mittelman database, more than 60% of NB is aneuploidy[14]. And in WCAS, NB is prone to have 3 chromosomes 6-9, 12, 13, 17, 18, 20, and 21, and one 3, 4, 9-11, 15, 17, 19, 22 and X chromosomes [7]. NB in stages I , II , and 4S are mostly triploid, with relatively good prognosis. They often have typical chromosomes 6, 7 and 17 gains and chromosomes 3, 4, 11 and 14 losses[15]. Parodi et al. came to a preliminary conclusion that the prognosis of the whole X-chromosome-loss NB children is relatively poor, which can be used as a new prognostic indicator, and this kind of children should be treated in the IR group[16]. Marked by centromere, each chromosome is divided into long arm (q) and short arm (p). NB children without MYCN gene amplification at high risk of stage II are often accompanied by an increase in the number of chromosomes 7, 12 and 17, lost of 11q and 3p alleles, and 17q gains[17]. However, no specific association between chromosome 10 and neuroblastoma has been reported. As can be seen from Figure 1, In accordance with previous studies, the most common gains is chromosome 7, and the most common structural abnormalities are chromosomes 1 and 11. And chromosome 10 is the most frequent loss.

Previous studies have shown that there are tumor suppressor genes such as PTEN, DBMT, LGI1 on the long arm of chromosome 10, and IDI1, AKR1C3, DDH1, NET1A, PRKCQ, and the GATA-binding protein 3 on the short arm[18-19]. Among them, PTEN is the second largest deletion / mutation gene in human tumors, with a mutation rate of 50%[20]. Li Z, et al[21] have confirmed that GDNF family receptor alpha

2(GFRA2) promotes proliferation of NB cells by activating the PTEN/PI3K/AKT pathway. The allele imbalance of 10p 11.23-15.1 and 8q 21.3 appears to be specific for stage 4 tumors with MCYN amplification[22]. Loss of complete chromosome 10 is common in tumors of the brain, lungs, ovaries and skin[23]. Although there is no study of chromosome 10 and NB, it has been reported that genetic changes in chromosome 10q are common in other neurological tumors[24]. For example, members of the cysteine-rich scavenger receptor family, DMBT1(10q25.3-26.1), are heterozygously absent in oligodendrogliomas, medulloblastoma, gastrointestinal cancer, and lung cancer [25-26]. A gene that inhibits glioma, CDKN2A/B, is located on chromosome 10[27]. MGMT, located at 10q26.1, encodes a protein associated with DNA repair that can remove proto-mutant alkyl from O. It affects the development of glioblastoma by the oncogene TP53[28]. Fibroblast growth factor receptor 2(FGFR2) at 10q 26 is associated with cell proliferation, differentiation, migration, and inhibition of apoptosis. It is overexpressed in breast cancer[29], while down-regulated in prostate cancer[30].

Our study showed that the OS rate was significantly lower in NB children with abnormal chromosome 10 than children with normal chromosome 10, including children with normal chromosomes and children with abnormal chromosomes but normal chromosome 10. And the OS and EFS in the normal chromosome group were significantly higher than those in the abnormal chromosome 10 group. The sites of structural abnormalities on chromosome 10 are all 10q22, which indicate that 10q22 sites may have tumor suppressor or oncogenic genes. Other nonstatistically significant results may be due to the fact that the number of chromosome 10 abnormalities is still insufficient.

In addition, the individual effects of chromosome 10 abnormalities cannot be evaluated because the majority of chromosome karyotypes in children with NB are complex quantitative or structural abnormalities once abnormal. There may be other different chromosomes associated with neuroblastoma in the group of chromosomal abnormalities affecting prognosis.

Conclusions

In summary, although it was only a preliminary study, our study presented that chromosome 10 might be used as a new prognostic marker for NB with bone marrow metastasis. NB children with abnormal chromosome 10 were prone to have MYCN amplification and 1p36 deletion, and their outcomes were worse. MYCN amplification occurs simultaneously with deletion of 1p36 may be related with chromosome 10 abnormalities in neuroblastoma. And their NB cells were easy to metastasize to orbit. 10q22 may be the site of chromosome 10 associated with NB proliferation and metastasis. In the future we will continue to expand the sample size, focusing on 10q22 changes and 11q23 deletion.

Abbreviations

NB: Neuroblastoma; WCAS: whole chromosome aneuploidies; INSS: International Neuroblastoma Stage System; COG: Children's Oncology Group; IR: intermediate-risk; LR: low-risk; HR: high-risk; ISCN: International System for Human Cytogenomic Nomenclature; SPSS: Statistical Package for Social

Scientists; OS: Overall survival; EFS: Event-free survival; LDH: lactate dehydrogenase; NSE: neuron specific enolase; VMA: vanillylmandelic acid; FGFR2: Fibroblast growth factor receptor 2

Declarations

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We would like to thank the participating patients and their families.

Authors' Contributions

C.Y.J analyzed the data and wrote the main article. X.X and B.L.J recruited the patients and collected the data. X.Z, Z.X.Y and W.G performed Chromosome examination. X.L.M designed and guided the research. All authors read and approved the final manuscript.

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Availability of data and materials

Key data generated or analyzed during this study are included in this published article. Any additional dataset is available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The Ethics Committee of Beijing Children's Hospital of Capital Medical University approved the study(2019-k-390). We got informed consent from all participants and their parents to get samples and information.

Consent for publication

Participants provided informed consent for the publication of the study.

Competing interest

No conflict of interest or additional funding support exists in the submission of this.

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Tables

Table 1 Characteristics at diagnosis of 150 NB patients

| Characteristic | Chromosome normal | Chromosome abnormalities | Suspicious exception |
|------------------------------------|-------------------|--------------------------|----------------------|
| Sex | | | |
| Male | 37 | 25 | 24 |
| Female | 31 | 17 | 16 |
| Age | | | |
| <18 | 20 | 2 | 2 |
| ≥18 | 48 | 40 | 38 |
| Staging | | | |
| I | 66 | 42 | 40 |
| II-IV | 2 | 0 | 0 |
| Risk group | | | |
| LR | 3 | 0 | 0 |
| IR | 13 | 0 | 1 |
| HR | 52 | 42 | 39 |
| MYCN | | | |
| Amplification | 7 | 18 | 3 |
| Not amplification | 59 | 24 | 37 |
| LDH(U/L) | | | |
| ≤295 | 11 | 0 | 4 |
| 295-500 | 19 | 2 | 7 |
| 500-1500 | 30 | 23 | 23 |
| >1500 | 8 | 17 | 6 |
| NSE(ng/l) | | | |
| ≤25 | 1 | 1 | 4 |
| 25-100 | 12 | 2 | 4 |
| >100 | 55 | 39 | 32 |
| Primary tumor site | | | |
| Retroperitoneum and adrenal glands | 59 | 36 | 36 |

| | | | |
|---------------|---|---|---|
| Mediastinum | 8 | 5 | 3 |
| Pelvic cavity | 1 | 1 | 0 |
| Neck | 0 | 0 | 1 |

LR low-risk, IR intermediate-risk, HR high-risk, MYCN amplification of the MYCN gene, LDH lactate dehydrogenase, NSE neuron specific enolase

Table 2 Characteristics of NB with chromosome 10 abnormality

| Characteristic | N | % |
|--------------------|----|------|
| chromosome 10 | | |
| Gain | 3 | 23.1 |
| Loss | 6 | 46.2 |
| Textural anomaly | 4 | 30.8 |
| Sex | | |
| Male | 6 | 46.2 |
| Female | 7 | 53.8 |
| MYCN | | |
| Amplification | 10 | 76.9 |
| Not amplification | 3 | 23.1 |
| 1p36 | | |
| Lost | 8 | 61.5 |
| Not lost | 3 | 23.1 |
| None | 2 | 15.4 |
| LDH(U/L) | | |
| ≤295 | 0 | 0 |
| 295-500 | 3 | 23.1 |
| 500-1500 | 0 | 0 |
| ≥1500 | 10 | 76.9 |
| NSE(ng/l) | | |
| ≤25 | 1 | 7.7 |
| 25-100 | 0 | 0 |
| ≥100 | 12 | 92.3 |
| VMA(mg/24h urine) | | |
| ≤13.6 | 5 | 38.5 |
| ≥13.6 | 7 | 53.8 |
| None | 1 | 7.7 |
| Primary tumor size | | |

| | | |
|------------------------|----|------|
| ≤5 | 0 | 0 |
| 5-10 | 4 | 30.8 |
| ≥10 | 8 | 61.5 |
| Metastasis site | | |
| Bone | 10 | 76.9 |
| Bone marrow | 13 | 100 |
| Harnpan/Intracalvarium | 9 | 69.2 |
| Periorbital | 5 | 38.5 |
| Mediastinum | 4 | 30.8 |
| Lymph nodes | 11 | 84.6 |
| Other visceral organ | 5 | 38.5 |

MYCN amplification of the MYCN gene, 1p36 loss of 1p36, LDH lactate dehydrogenase, NSE neuron specific enolase, VMA vanillylmandelic acid

Figures

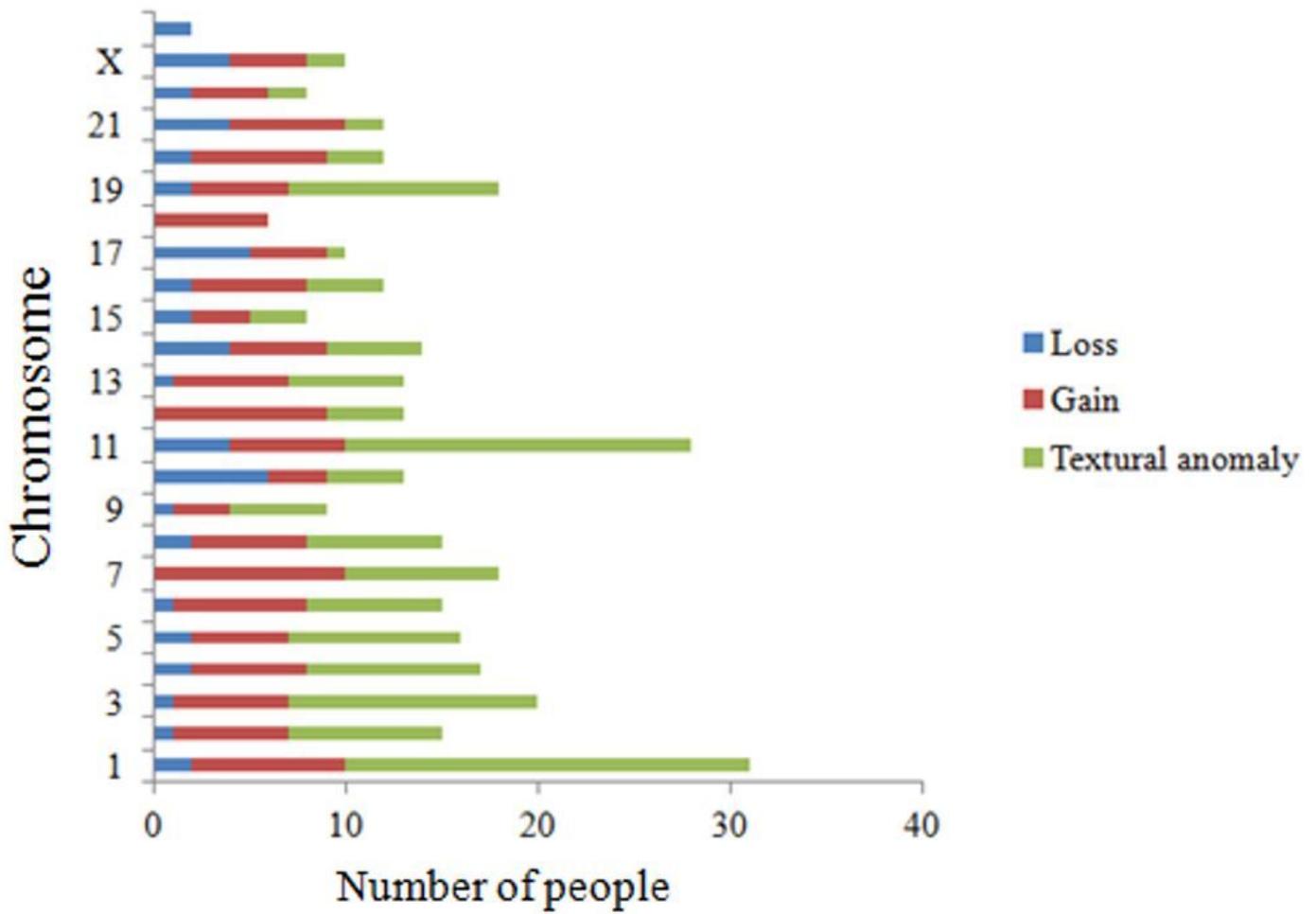


Figure 1

Distribution of abnormal number of chromosomes.

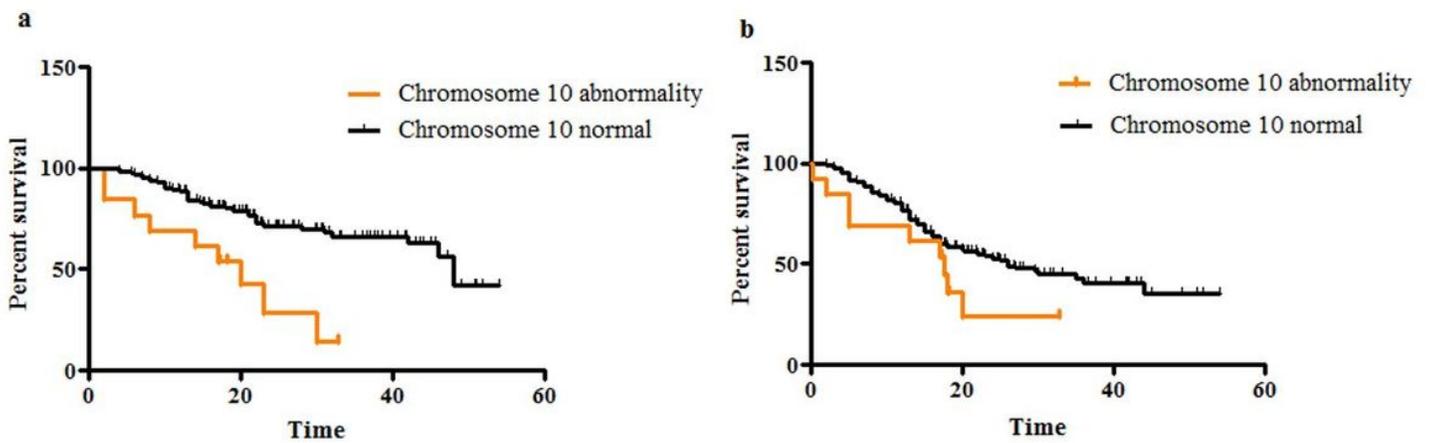


Figure 2

Kaplan-Meier estimates of survival in 150 patients with NB according to the status of chromosome 10 a Overall survival for all patients. b Event-free survival for all patients.

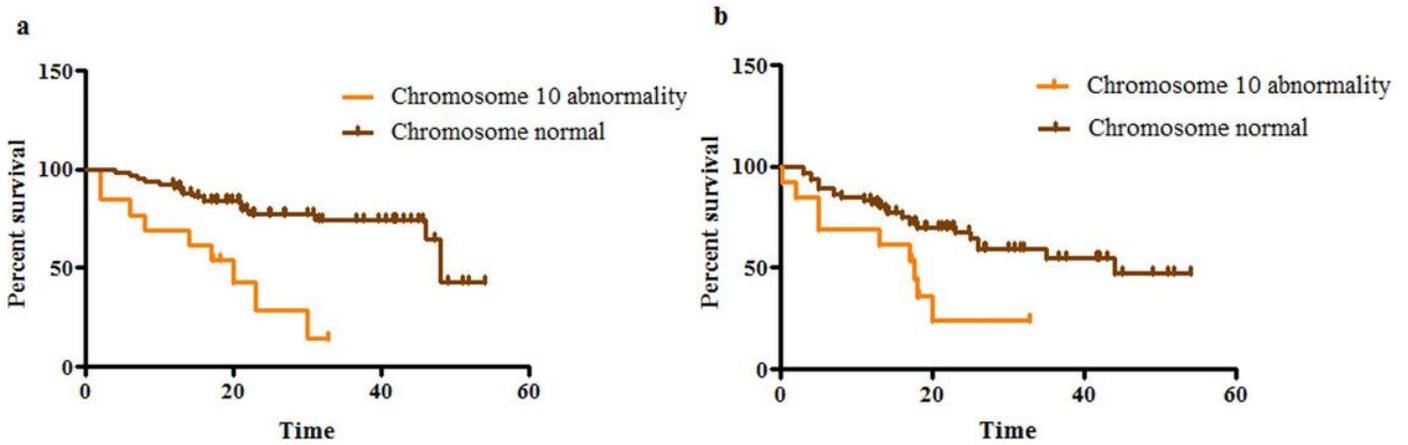


Figure 3

Kaplan-Meier estimates of survival in 110 patients with NB according to the status of chromosome 10 a Overall survival for all patients. b Event-free survival for all patients.

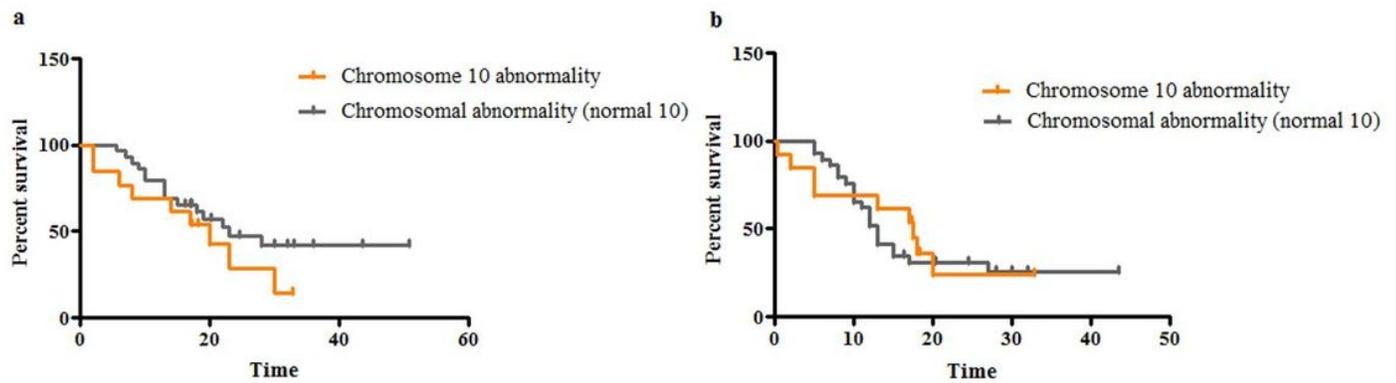


Figure 4

Kaplan-Meier estimates of survival in 42 patients with NB who had chromosome abnormality according to the status of chromosome 10 a Overall survival for all patients. b Event-free survival for all patients.