SNP array analysis facilitates the identification of novel chromosomal alterations associated with disease and SNPs related to adverse drug reactions in neuroblastoma

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Abstract. Chromosomal abnormalities are common characteristics of neuroblastoma, and have been associated with treatment, relapse and survival risk factors. The processes governing the incidence or advancement of chromosomal copy number abnormalities remain unclear, despite progress in understanding their prognostic implications. The present study aimed to provide a comprehensive understanding of genetic alterations, clinical implications, and the association between copy number aberrations (CNAs) and clinical parameters. Single nucleotide polymorphism (SNP) array analysis was performed on a set of 45 neuroblastoma samples to examine chromosomal CNAs and SNPs. Logistic regression analysis was performed to identify SNPs associated with adverse drug reactions (ADRs). In the present study, numerous CNAs were observed in 92% of neuroblastoma tumors, while CNAs were found in 15% of ganglioneuroblastoma tumors. The segmental alterations were mainly observed in stage 3 or 4 neuroblastoma cases that had tumor sizes >10 cm. The present study concentrated on analyzing entire chromosome modifications and revealed that, in contrast to gain, loss of heterozygosity (LOH) mostly occurred during stages 3 and 4 of neuroblastoma. Only stage 3 and 4 neuroblastomas with tumor sizes >10 cm were found to exhibit loss of the Y chromosome, which was associated with similar clinical characteristics as segmental alterations. LOH of the whole chromosome might be a subgroup of whole chromosome alterations, and could be a novel prognosis and treatment marker. Using a regression model, 13 SNPs were identified to be strongly associated with ADRs following chemotherapy for neuroblastoma. Although validation studies in independent cohorts are required, the present findings support the use of CNAs and SNPs for predicting neuroblastoma treatment outcomes.

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Introduction

Neuroblastoma is a tumor originating from the sympathetic nervous system and is a common extra-cranial pediatric tumor, accounting for >8% of childhood malignancies and ~15% of cancer-associated mortality in children worldwide (1,2). Neuroblastoma is a disease with broad biological and clinical heterogeneity, occurring predominantly in children <5 years old, which is characterized by a poor prognosis and high recurrence rate (3-6). Numerous factors, including age, chromosomal abnormalities, tumor grade, *MYCN* status, DNA ploidy and diagnostic category, influence the prognosis of neuroblastoma (7,8).

Cancers are genetically unstable and have numerous aberrations, including genetic mutations, gene translocations, genomic copy number aberrations (CNAs), gene expression profiles and epigenetic changes (9-12). Previous studies have reported that DNA CNAs commonly occur in neuroblastoma, including amplification of the *MYCN* oncogene and anaplastic lymphoma kinase (*ALK*) oncogene, loss of heterozygosity (LOH) of chromosomes 1p, 3p, 4p, 11q and 14q, and gains of chromosomes 1q, 2p and 17q (13-18). These chromosomal aberrations are essential to predict the outcome in neuroblastoma, and several have been incorporated into treatment stratification (19).

CNAs can be utilized to distinguish between different genetic subgroups of neuroblastoma that have certain prognostic characteristics. Whole chromosome abnormalities often accumulate in localized tumors and in children <1 year of age, and are associated with a good prognosis (20). Segmental chromosome aberrations often occur in advanced stages or older

children, and are associated with a high risk of relapse (20). In addition, *MYCN* amplification has been identified in ~20% of all neuroblastoma cases, and is associated with stage 4 neuroblastoma and a poor prognosis (21). *ALK* amplification occurs in ~4% of high-risk neuroblastoma cases and *ALK* is almost entirely co-amplified with *MYCN* (14,15). However, there is a notable issue in classifying individuals into distinct therapy groups based on their anticipated risk. Since pediatric neuroblastoma has an extensive spectrum of clinical outcomes, novel markers that could be used to build reliable prognostic classifications need to be identified.

Chemotherapy-related adverse drug reactions (ADRs) are a major factor in cancer treatment-associated mortality and are a pressing clinical problem that has to be addressed. Drug efficacy and toxicity are associated with specific single nucleotide polymorphisms (SNPs), and the analysis of SNPs could reveal novel markers to improve tailored therapy (22). Numerous studies have reported that multiple SNPs within the dihydropyrimidine dehydrogenase (*DPYD*) gene are linked to the toxicity risk and metabolism/pharmacokinetics in patients treated with fluorouracil, and the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group guidelines provide information for the interpretation of the clinical *DPYD* genotype (23-26). The relationship between toxicity of commonly prescribed chemotherapy medications, such as cisplatin, carboplatin, cyclophosphamide, doxorubicin, methotrexate and docetaxel, and different SNPs provides a reference for clinical pharmacology in adult cancer (27-30). Nevertheless, there are still numerous gaps in drug-gene interaction research on long-term clinical interventions for pediatric patients. According to the PHarmGKB database, only 633 of the 5,112 clinical annotations are related to pediatrics, and only 2,572 of the 27,452 variant annotations are applicable to pediatrics (https://www.pharmgkb.org/pediatric/dashboard). Pediatric medications are different from those for adults because of their unique characteristics of physiological development and pharmacokinetic behaviors (31,32). Children of different ages have their own specific reactions to drugs because of the continuous maturation process (33). Therefore, the treatment of pediatric neuroblastoma is different from that of adult tumors, and screening for genetic markers that predict toxicity can reduce the use of high-risk medicines in infants and children.

At present, there are few reports on the association between whole chromosome or segmental chromosomal aberrations and clinical characteristics in pediatric neuroblastoma, with a small number of studies on SNPs associated with drug-induced toxicity (34-38). In addition, the genetic characteristics of pediatric neuroblastoma are still unclear. The present study investigated the CNA profile of a cohort of 45 patients with neuroblastoma from Wuhan Children's Hospital (Wuhan, China), with the intention of assessing CNA patterns in this tumor type and validating putative CNA regions linked to clinical characteristics. Furthermore, in a small clinical cohort of neuroblastoma cases, the present study assessed the association between certain SNPs and chemotherapy-associated ADRs, which may offer novel insights into the genetic origins of ADRs, as well as some warning signs that might be utilized in clinical settings.

Materials and methods

Studied subjects. A total of 45 continuous pediatric patients with newly diagnosed neuroblastoma at Wuhan Children's Hospital were enrolled in the present study between January 2020 and January 2023, including 20 patients with ganglioneuroblastoma (GNB) and 25 patients with neuroblastoma whose formalin-fixed paraffin-embedded (FFPE) surgical specimens or bone marrow specimens were collected. All experiments and bioinformatics services were entrusted with Shanghai Cinopath Medical Laboratory Co., Ltd. The protocol of the present study was approved by the Wuhan Children's Hospital Institutional Ethics Committee. The legal guardians of all patients provided written informed consent.

Therapy and toxicity evaluation. The International Neuroblastoma Staging System (INSS) and International Neuroblastoma Risk Group (INRG) criteria were used to establish the neuroblastoma stage and risk categorization. Homogeneous treatment cohorts were defined according to the INRG based on the Image-Defined Risk Factors classification system, and patients were treated according to pediatric neuroblastoma diagnosis and treatment expert consensus in China (39). Tumors with low-risk biological characteristics were treated with carboplatin (2,800 mg/m^2), etoposide (1,440 mg/m^2), doxorubicin (120 mg/m^2) and cyclophosphamide (6,000 mg/m^2) for eight rounds of chemotherapy. Patients with intermediate-risk neuroblastoma received eight cycles of chemotherapy with cisplatin (720 mg/m^2), vincristine (12 mg/m^2), etoposide (640 mg/m^2), doxorubicin (120 mg/m^2) and cyclophosphamide (9,600 mg/m^2). Patients with high-risk biological characteristics were treated with cisplatin (600 mg/m^2), vincristine (4 mg/m^2), topotecan (18 mg/m^2), etoposide (1,800 mg/m^2), doxorubicin (150 mg/m^2) and cyclophosphamide (7,600 mg/m^2). Drug toxicity standards referred to the Common Terminology Criteria for Adverse Events v5.0 and the toxicity was divided into five categories (grades 1-5) (40). ADR grades were assessed by independent clinicians before any genotyping was performed. The highest-grade adverse reactions observed in each patient during the induction therapy were recorded.

Sample preparation and SNP array analysis. DNA was extracted from FFPE tissues or bone marrow using QIAamp DNA FFPE Advanced Kit (cat. no. 56604; Qiagen, Inc.). The DNA quality was verified based on optical density (OD)260/OD280 and OD260/OD230 ratios measured by NanoDrop One (Thermo Fisher Scientific, Inc.). Eligible samples were confirmed to have an OD260/OD280 ratio between 1.7 and 2.0 and an OD260/OD230 ratio >1.6. The integrity of the processed samples was verified by Qsep400 (BiOptic. Inc.), with DNA quality number >3 as the qualification standard. DNA concentration was determined using Qubit 4 (Thermo Fisher Scientific, Inc.). A minimum of 200 ng genomic DNA was aliquoted into 96-well plates and genotyped using the Illumina BeadStation (Illumina, Inc.). Briefly, genome-wide amplification of DNA was performed, followed by fragmentation, hybridization, fluorescence tagging and scanning according to standard Infinium protocols (41). Sample analyses were performed using the Infinium CytoSNP-850K

v1.2 BeadChip Kit (cat. no. 20103480; Illumina, Inc.), which contained ~850,000 SNP probes distributed throughout the whole genome. Samples with call rates <90% were excluded from the analysis.

Copy number analysis. In the present study, copy number variations were visually inferred from SNP arrays by GenomeStudio v2.0 (Illumina, Inc.) and normalized using MoChA v2021-05-14 (https://software.broadinstitute.org/software/mocha/) to eliminate the artifactual CNAs due to GC content across the genome. CNAs, including gain, loss, amplification and copy neutral LOH, were detected by measuring log R ratios (as assessed by aberrations in probe intensities) along with B-allele frequency (as assessed by the shift in genotype frequencies of the SNP probes) (42).

The copy number of mosaic changes was associated with the percentage of aberrant cells. The mosaicism detection limit in the present study was set at 10%. For gain, the copy number was required to be more than diploidy; for loss, the copy number was required to be less than diploidy. Furthermore, chromosome ploidy is defined as hypodiploid with 35-45 chromosomes, diploid with 46 chromosomes, hyperdiploid with 47-57 chromosomes and near-triploid with 58-80 chromosomes. *MYCN* and *ALK* amplifications were defined as regions with >10 copies. Focal changes refer to regions <5 Mb. The present study focused on CNAs with regions >5 Mb (p or q), and the frequency of CNAs was compared between neuroblastoma and GNB. Subsequently, the present study also assessed the association between CNAs and clinical features in neuroblastoma.

Correlation analysis could not be carried out in this study due to sample limitations, so frequency index was chosen as an alternative analysis. The frequency index of two variables was defined as the incidence of patients with variable A in patients with variable B; i.e., frequency index=patients with both variable A and variable B/variable B.

SNP genotyping analysis. A total of 567 SNPs potentially associated with chemotherapy drugs in neuroblastoma were analyzed through a review of the relevant literature and comparison with information from the PharmGKB database (www.pharmgkb.org). Selected SNPs were genotyped using GenomeStudio software v2.0 (Illumina, Inc.), and those with a GenCall Score <0.15 were excluded from the analysis. Therefore, 79 SNPs (Table SI) remained and were used to analyze the impact of drug-related SNPs on the toxicity of neuroblastoma treatment. The reference alleles of SNPs in in the forest plots were derived from aggregate allele frequency in the East Asian Group from the Allele Frequency Aggregator project (https://www.ncbi.nlm.nih.gov/snp/docs/gsr/alfa/).

Statistical analysis. In the present study, odds ratios (ORs) and 95% CIs were used to evaluate the risk of developing various manifestations of toxicity after therapy in neuroblastoma with specific genotypes using univariate analysis with Fisher's exact test and binary logistic regression model. To estimate the influence of the specific SNP on different manifestations of toxicity, two series of analyses were performed: The first explored SNPs associated with adverse reaction manifestations in the whole cohort and the second aimed to further evaluate whether polymorphisms were associated with toxicities during high-risk induction chemotherapy. All statistical analyses were carried out using IBM SPSS Statistics V27 (IBM Corp.). Statistical comparisons of categorical variables were performed using Fisher's exact test. For all statistical analyses, $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical samples. Among the 45 cases, 27 were male, indicating no apparent sex bias. In all but one case, patients were diagnosed for the first time. The median age at primary diagnosis was 43 months (2-132 months), and eight cases were <18 months old. Metastasis occurred in 29 patients. Tumors were classified as low-risk in 16 cases, intermediate-risk in 11 cases and high-risk in 18 cases. Most of the patients (91.1%) were in stage 2, 3 and 4, and the retroperitoneum, adrenal gland and mediastinum were the most common primary sites of neuroblastoma (88.9%). After a median follow-up time of 20 months (range, 4-42 months), 35 patients had stable disease, five patients suffered from tumor relapse or progression, three patients were not followed up and the remaining two patients died. The clinical characteristics of the 45 patients with neuroblastoma are shown in Table I.

Fig. 1 shows the number of cases included throughout the study, indicating the subsets of cases that were selected for the different analyses reported. Among the 45 newly diagnosed cases that were available in the retrospective collection, five did not have available therapy information and 40 remained for the analysis of genotypes. There were 45 patients in the entire study cohort, and the CNAs and SNP studies were performed on subgroups of the cohort. A total of 40 of the 45 patients completed the entire induction treatment at the same hospital; thus, analysis of chemotherapy toxicity associated with SNPs could be performed. Among the 45 patients, only 17 were treated with the high-risk regimen, and the association between toxicity and SNPs was analyzed.

Biological characteristics of GNB and neuroblastoma. A high number of CNAs was observed in most neuroblastoma cases (92%; 23/25); however, the incidence rate of CNAs was relatively low in GNB (15%; 3/20). The present study compared CNAs between neuroblastoma and GNB, and significant differences in CNAs were found, including differences in 1p LOH, 2 gain, 7 gain, 12 gain, 17 gain, 17q gain and Y LOH ($P<0.05$; Table SII). Furthermore, there were significant differences in age ($P=0.047$), INSS stage ($P<0.001$), risk stratification ($P<0.001$) and tumor size ($P<0.001$) between neuroblastoma and GNB. The clinical characteristics of GNB included a relatively small tumor size, low occurrence of CNAs, low INSS stage and low risk at diagnosis, suggesting slow proliferation, low invasiveness and good genomic stability as biological features of GNB. These results are summarized in Table SIII.

CNA landscape in neuroblastoma. The median total number of chromosomal aberrations per patient with neuroblastoma was 16 (range, 0-33), with a median of 8 gains (range, 0-25), four LOHs (range, 0-16) and 0 amplifications (range, 0-6). As

Table I. Clinical characteristics of the study patients.

Characteristic	n (%)
Sex	
Male	27 (60.0)
Female	18 (40.0)
Age, months	
<18	8 (17.8)
18-60	24 (53.3)
≥60	13 (28.9)
INSS stage	
Stage 1	3 (6.7)
Stage 2	18 (40.0)
Stage 3	11 (24.4)
Stage 4	12 (26.7)
Stage 4s	1 (2.2)
Risk stratification	
Low	16 (35.6)
Intermediate	11 (24.4)
High	18 (40.0)
Primary diagnosis	
Yes	44 (97.8)
No	1 (2.2)
Metastasis	
Yes	29 (64.4)
No	15 (33.3)
Data not available	1 (2.2)
Primary tumor site	
Retroperitoneum	17 (37.8)
Adrenal gland	11 (24.4)
Mediastinum	12 (26.7)
Other	5 (11.1)
State at last follow-up	
Alive	40 (88.9)
Dead	2 (4.4)
Other	3 (6.7)

INSS, International Neuroblastoma Staging System.

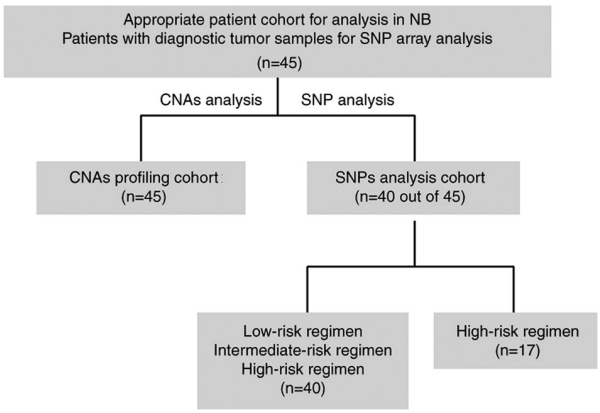

Figure 1. Flow diagram of patient inclusion. A total of 45 patients were enrolled in the present study. Among these, five patients were not included in SNP analysis, because no chemotherapy toxicity data was available. NB, neuroblastoma; SNP, single nucleotide polymorphism; CNA, copy number aberrations.

shown in Fig. 2 and Table SII, >16% of gains included 1p, 1q, 1, 2p, 2, 5, 6, 7q, 7, 8, 9, 12q, 12, 13, 17q, 17, 18, 20, 21 and 22, and >16% of LOHs were 1p, 3p, 4p, 8, 11q, 14, 15, 19q, 21q and Y, and high-frequency amplification of *MYCN* was observed. The overwhelming majority of these gains and LOHs were clonal, indicating that genomic instability is a genetic feature of neuroblastoma.

To assess CNAs associated with clinical characteristics such as INSS stage and tumor size, the gathered data were examined. The percentage of 1p LOH, 11q LOH and *MYCN* amplification in stage 4 was markedly higher, whereas 4p LOH, 7q gain, 8 LOH, 15 LOH, 12q gain, 19q LOH and 21 LOH were almost absent in stages 1, 2 and 4s. Gain in chromosome regions 1, 2, 2p, 5, 6, 7, 8, 9, 12, 13, 17, 18, 20, 21 and 22 was common in stages 1, 2 and 4s, and stage 3. Similarly, the hyperdiploid and near-triploid karyotypes were common in stages 1, 2 and 4s, and stage 3, while the diploid and hypodiploid karyotypes were more common in stage 4, which was consistent with the CNA distribution. Furthermore, the largest proportion of 1p LOH, 3p LOH, 7q gain and 12q gain was observed in neuroblastoma cases with tumor sizes >10 cm, and 8 gain, 9 gain and 15 LOH were common in patients with a tumor size <10 cm. Notably, loss of the Y chromosome (LoY) occurred only in boys with tumor sizes >10 cm. Fig. 2 provides a summary of these findings. There was >3-fold difference in these CNAs between stages 1, 2 and 4s, and stage 3 and 4 and between tumor sizes >10 cm and <10 cm, indicating that these CNAs were associated with tumor invasion and proliferation, even though the small sample size meant that the results were not significant.

Exploratory analysis of co-occurrence patterns of CNAs in neuroblastoma. Among the 25 neuroblastoma cases, 1p LOH and 17q gain accounted for 67% of *MYCN* amplifications. Previous reports have indicated positive associations between *MYCN* amplification and 1p LOH and 17q gain, and a negative association between *MYCN* amplification and 11q aberration (43-45). In accordance with this finding, if the frequency index of variable A and variable B was greater than 67%, they were considered to be positively associated (Fig. 3). Conversely, if it was less than 67%, the association between them was considered weak. Nevertheless, there was a lack of negative relationship evidence available and further investigation was not performed. The frequency index plot of CNAs in 25 neuroblastoma cases showed that *MYCN* amplification (67%), 1p LOH (78%), 3p LOH (80%), 4p LOH (100%), 11q LOH (100%), 19q LOH (86%), 1q gain (67%), 2p gain (83%), 12q gain (100%), 21 LOH (67%) and LoY (71%) were positively associated with 17q gain (Fig. 3). In addition, 4p LOH and 11q LOH (75%) or 12q gain (75%), 1p gain and 1q gain (80%), 2p gain and 1p LOH (67%), 7q gain and 11q LOH (80%) or 19q LOH (80%), and 12q gain and 11q LOH (80%) showed positive associations. In this regard, segmental chromosomal abnormalities frequently occurred together. Furthermore, segmental

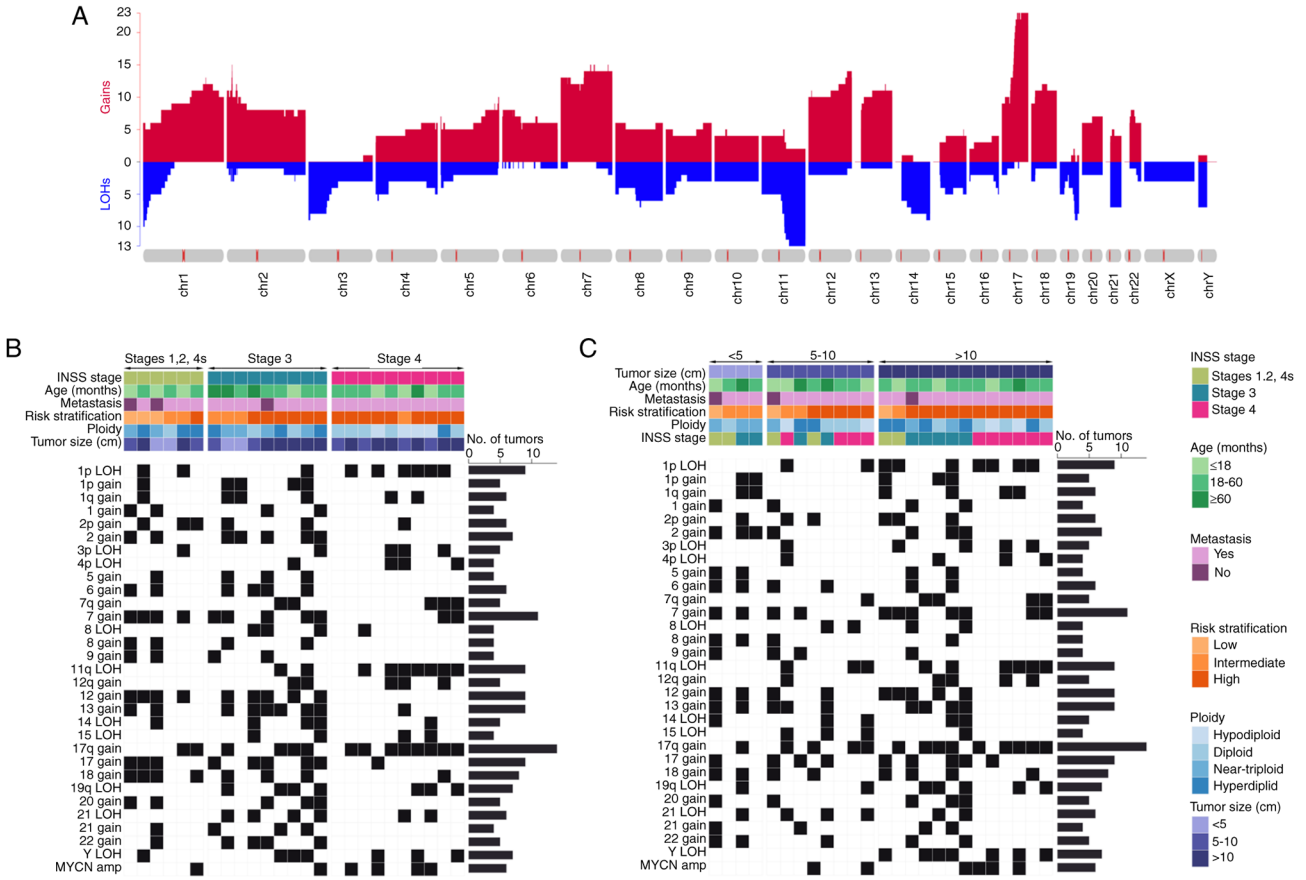

Figure 2. Copy number variants and clinical parameters characteristic of neuroblastoma. (A) Number of copy number gains/amplifications (red) and LOHs (blue) for chromosomes in neuroblastoma. (B) Recurrent copy number alterations by INSS stage group. (C) Recurrent copy number alterations by tumor size group. LOH, loss of heterozygosity; INSS, International Neuroblastoma Staging System; amp, amplification.

chromosomal abnormalities occurred in conjunction with whole chromosome abnormalities in 68% of cases.

SNPs related to toxicity during induction treatment. A total of 40 patients had clinical data regarding toxicities during induction therapy. All cases of toxicity in response to the three treatment regimens were counted and were analyzed together. The prevalence of developing toxicities (n=40) in all patients was as follows: Sepsis (n=11; 27.5%), moderate or severe hearing loss (n=9; 22.5%), pancytopenia (n=9; 22.5%), agranulocytosis (n=27; 67.5%) and thrombocytopenia (n=24; 60%). Univariate analysis was performed to assess the manifestations of toxicity of the induction chemotherapy in neuroblastoma to identify meaningful associations between SNPs and chemotherapy toxicity. Fig. 4 shows the risk assessment of various toxicity manifestations for the SNPs under investigation based on the binary logistic regression model.

A total of 11 SNPs were chosen by the binary logistic regression model based on toxicity results. As shown in Fig. 4, patients with rs6650282 CC in ATP binding cassette subfamily C member (*ABCC)4* had an increased risk of developing sepsis (P=0.034; OR, 7.71; 95% CI, 1.17-51.06) compared with patients with TT/TC. A total of three of the selected SNPs, rs6826373 TC in paired-like homeobox 2b (P=0.045; OR, 0.11; 95% CI, 0.01-0.95), rs3814057 CC in nuclear receptor subfamily 1 group I member 2 (*NR1I2;* P=0.024) and rs7624838 AA in *ABCC5* (P=0.024), were associated with a significant risk reduction of sepsis among patients. The risk of moderate or severe hearing loss was increased in patients with rs299295 TC in hyaluronan-mediated motility receptor (P=0.029; OR, 15.00; 95% CI, 1.32-169.87) and rs3824473 TC in glutamate ionotropic receptor NMDA type subunit 3A (*GRIN3A*; P=0.025; OR, 7.35; 95% CI, 1.29-41.98). Conversely, rs3745551 TC in insulin receptor was significantly associated with reduced occurrence of moderate or severe hearing loss (P=0.037). Similarly, rs7624838 AG in *ABCC5* (P=0.039; OR, 5.92; 95% CI, 1.10-31.95) predicted severe agranulocytosis, and rs10780691 TC in neurotrophic receptor tyrosine kinase 2 (P=0.038; OR, 0.22; 95% CI, 0.05-0.92) and rs17592236 TC+TT in forkhead box O1 (P=0.039; OR, 0.17; 95% CI, 0.03-0.91) were significantly associated with reduced risk of agranulocytosis. Thrombocytopenia was reduced among patients carrying rs2293347 TC in *EGFR* (P=0.017; OR, 0.19; 95% CI, 0.05-0.74). Furthermore, pancytopenia was significantly more frequent in patients with rs706713 TC in *PIK3R1* compared with those with the TT/CC genotype, and the increased risk was statistically significant (P=0.037; OR, 6.36; 95% CI, 1.12-36.08). Overall, a set of SNPs associated with ADRs was identified.

SNPs related to toxicity in the high-risk treatment group. The most homogeneously treated patients were chosen for the high-risk regimen, and all were treated with the same induction therapy at Wuhan Children's Hospital. These requirements

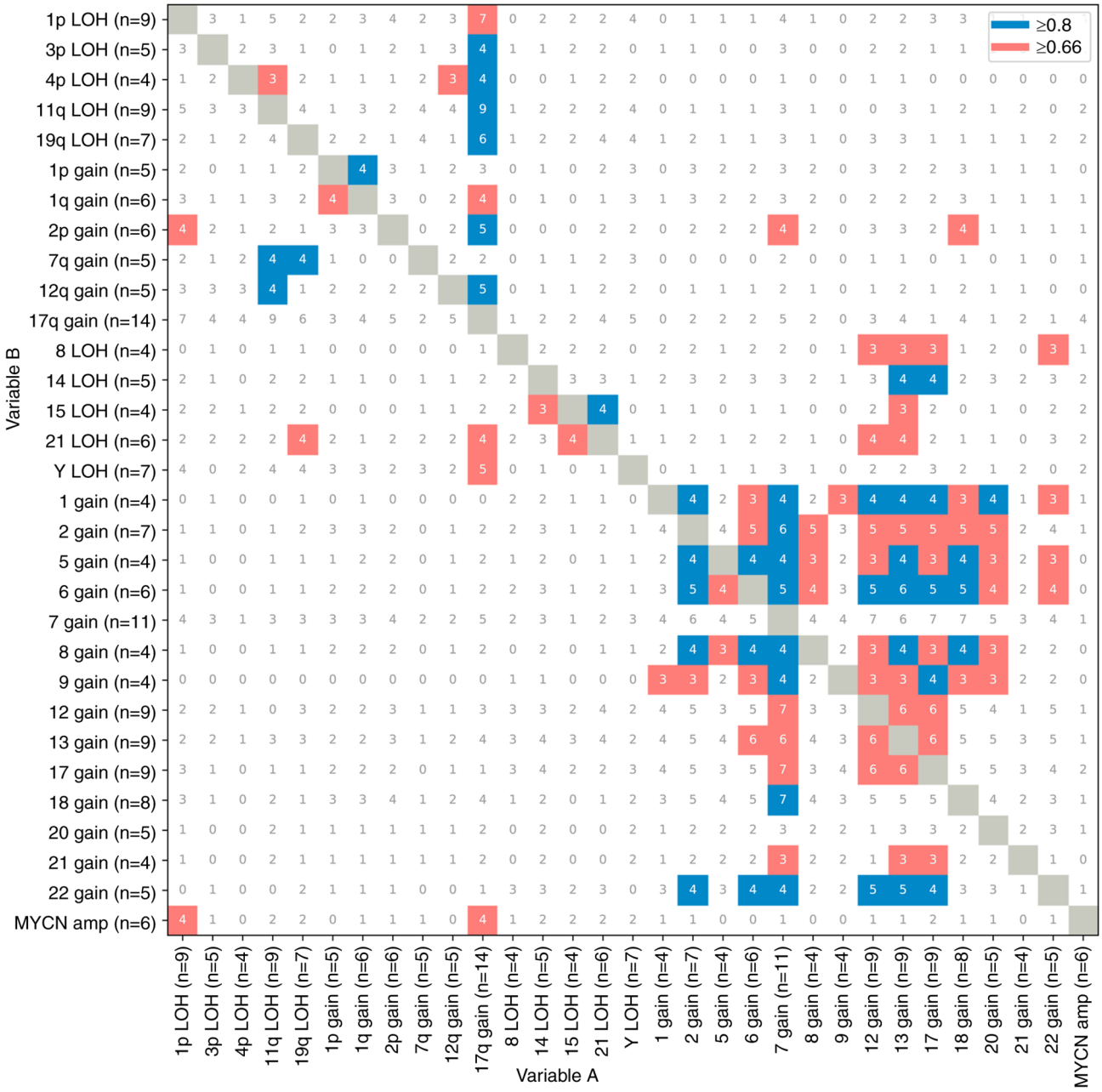

Figure 3. Frequency index among major copy number variants in 25 neuroblastoma samples. The frequency index was indicated with the ratio of variable A in variable B. Red, the frequency of variable A in variable B is ≥0.66; blue, the frequency of variable A in variable B is ≥0.8. LOH, loss of heterozygosity; amp, amplification.

were met by 17 patients. An analysis was performed to evaluate the associations between SNPs and chemotherapy toxicity after collecting the data for the high-risk treatment group. Fig. 5 shows the SNPs associated with chemotherapy toxicity according to the logistic regression model.

Two SNPs significantly reduced the risk of grade 4 adverse reactions; rs706713 TC in *PIK3R1* (P=0.011; OR, 0.02; 95% CI, 0.00-0.40) and rs7624838 AA in *ABCC5* (P=0.027; OR, 0.05; 95% CI, 0.00-0.71). Conversely, rs3824473 TC in *GRIN3A* (P=0.024; OR, 21.00; 95% CI, 1.50-293.25) predicted a significantly increased risk of grade 4 adverse reactions. The statistical model selected two SNPs, rs10228436 AG in *EGFR* and rs7624838 AA in *ABCC5*, which predicted opposite toxicity information. rs10228436 AG in *EGFR* (P=0.017; OR, 24.50; 95% CI, 1.79-336.23) was more likely to cause thrombocytopenia, while rs7624838 AA in *ABCC5* (P=0.044; OR, 0.07; 95% CI, 0.01-0.94) had the opposite effect. Additionally, rs7624838 AA in *ABCC5* (P=0.037) and rs2809244 AC in TSC complex subunit 1 (P=0.017) significantly reduced the risk of sepsis and agranulocytosis.

Discussion

Neuroblastoma causes notable mortality worldwide. Effective molecular indicators are still lacking in some cases, despite the fact that the prognosis of neuroblastoma can presently be predicted using risk markers such as INSS, *MYCN*, 11q and DNA ploidy. The potential biomarkers obtained from CNA

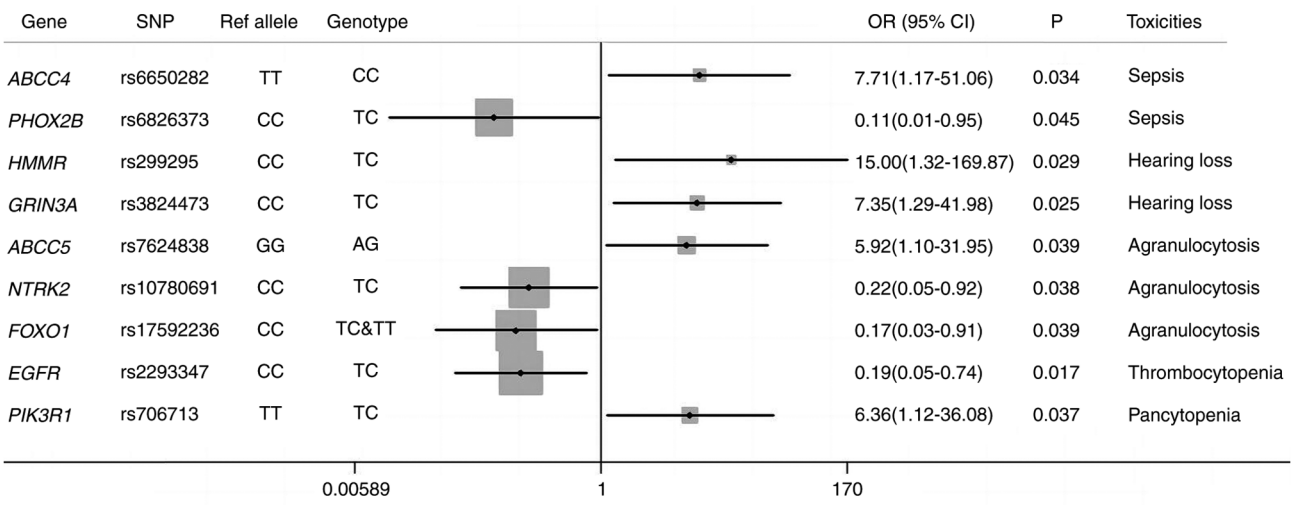

Figure 4. SNPs associated with toxicity during induction treatment. The reference allele is the aggregate allele frequency in the East Asian Group from the Allele Frequency Aggregator project. Patient cohort, n=40. Gray box, the midpoint of the box symbolizes the point estimate of the OR, and its size (area) is proportionate to the weight of the study. Vertical line, the solid vertical line corresponds to 'no effect'-an OR of 1.0. Horizontal line, if the horizontal line falls to the left of the vertical line, it can be concluded that the studied factor favors the occurrence of the outcome; if the horizontal line falls to the right of the vertical line, it can be concluded that the studied factor is detrimental to the occurrence of the outcome. OR, odds ratio; SNP, single nucleotide polymorphism.

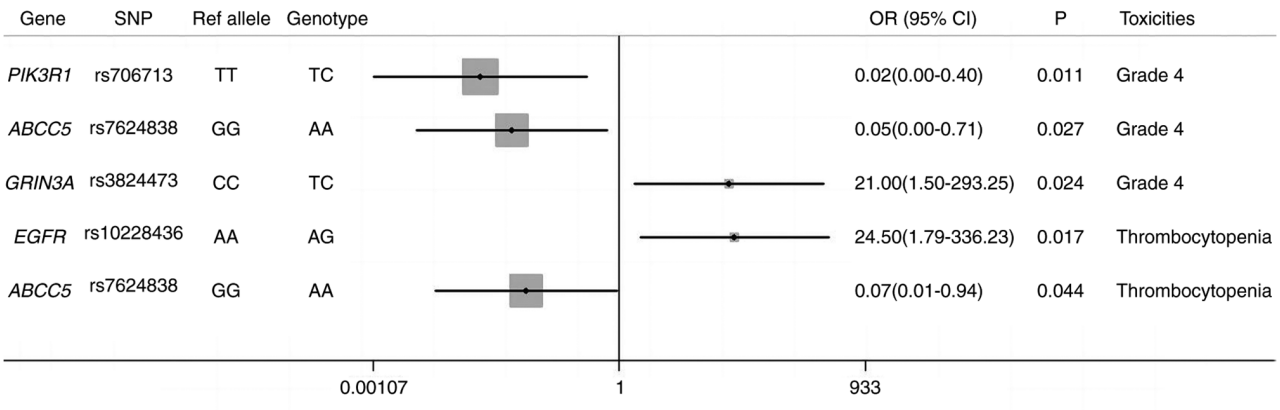

Figure 5. SNPs related to the toxicity in high-risk treatment. Reference allele, aggregate allele frequency in the East Asian Group from the Allele Frequency Aggregator project. Patient cohort, n=17. Gray box, the midpoint of the box symbolizes the point estimate of the OR, and its size (area) is proportionate to the weight of the study. Vertical line, the solid vertical line corresponds to 'no effect'-an OR of 1.0. Horizontal line, if the horizontal line falls to the left of the vertical line, it can be concluded that the studied factor favors the occurrence of the outcome; if the horizontal line falls to the right of the vertical line, it can be concluded that the studied factor is detrimental to the occurrence of the outcome. OR, odds ratio; SNP, single nucleotide polymorphism.

and SNP testing could improve precision oncology therapy, predicting patient prognosis, and predicting the response to treatments and their potential toxicities. A SNP microarray is one of the methods used to detect ADR markers, which may provide a comprehensive assay for simultaneous detection of SNP markers and tumor-associated CNAs. In the present study, a comprehensive genetic investigation relying on a SNP microarray for 45 neuroblastoma cases demonstrated the global features of the CNAs and validated the variety of CNAs in neuroblastomas. In neuroblastoma, a high incidence of CNA variation (92%) was associated with genomic instability. This suggests that the accumulation of CNAs and other genomic variations may be the primary cause of the bulk of these cancers. The therapy schedule was not always followed by all patients due to heterogeneity, and ADRs were frequent. Based on the present hypothesis, research on possible genetic markers of ADRs might help predict treatment-related toxicities. As aforementioned, SNPs may be linked to a variety of treatment-related ADRs.

Chromosome alterations are a major feature of cancer cells (46). Numerous chromosome regions of gain and LOH have been revealed in neuroblastoma; however, CNAs specific to GNB have been infrequently studied (47,48). Using a SNP array, the present study compared the CNAs in neuroblastoma and GNB in order to examine chromosomal changes. GNB exhibited a low occurrence of CNAs, smaller tumor size, lower INSS stage and lower risk at diagnosis, which indicated that GNB was characterized by slow proliferation, low invasiveness and good genomic stability, and may have a different mechanism of cancer cell evolution compared with neuroblastoma. The small number of samples that were available prevented accurate assessment of every anomaly in the present investigation.

In neuroblastoma, segmental chromosome alterations and *MYCN* amplification were almost absent in stages 1, 2 and 4s,

including LOH of 1p, 11q, 4p and 19q, and gain of 7q and 12q. Additionally, 1p LOH, 3p LOH, 7q gain and 12q gain were observed in neuroblastoma cases with tumor sizes >10 cm, indicating that segmental alterations promoted invasion and proliferation of tumor cells. The majority of the entire chromosome modifications occurred in stages 1, 2, 4s and 3, even in the presence of segmental chromosomal abnormalities. Thus, chromosomal aneuploidy may be a primary feature of localized NB. Whole chromosome aberrations are associated with an improved prognosis in patients with neuroblastoma (20,38). LOH of 8, 15, 21 and Y was mainly observed in patients with stage 4 and 3, suggesting that LOH of the whole chromosome may have similar effects on invasion as segmental alterations in neuroblastoma. LOH of the whole chromosome may be a subset of whole chromosome alterations that could help identify a novel biological subtype and may be associated with prognosis. Examining these chromosomal abnormalities might aid in clarifying the processes involved in neuroblastoma tumor growth. There was limited long-term follow-up information to identify independent associations between these copy number variations and prognosis.

In previous reports, LoY was frequently observed in elderly men; however, LoY mutations were increased in tumor tissues and associated with an overall worse prognosis and sensitization to programmed cell death protein 1-targeted immunotherapy (49,50). In the present study, LoY was only found in individuals whose tumors measured >10 cm and was markedly increased in neuroblastoma compared with in GNB. LoY was mainly present in patients diagnosed at stage 4 and 3, and was associated with larger tumor size (>10 cm), implying an association between LoY and the invasion and proliferation of tumor cells. LoY may represent a novel prognosis and treatment marker for male patients. Due to the limited sample cohort used in the current investigation, more large-scale cohort verification is still required.

According to the present findings, entire chromosome modifications exhibited a comparable likelihood for co-occurrence as segmental abnormalities. As aforementioned, segmental chromosomal abnormalities occurred in conjunction with whole chromosome abnormalities in 68% of cases. Previous studies have reported that segmental chromosome alterations may derive from an intermediate stage characterized by whole chromosome alterations (49,51,52). Co-occurrence exacerbates genomic instability during the evolution of cancer cells and promotes clonal dominance of tumor cells.

Standardized chemotherapy was administered to 40 patients in the present cohort who had neuroblastoma. Distinct toxicity symptoms were noted in equal measure across the various treatment plans. Therefore, multiple toxicity manifestations were collected for SNP analyses. The model selected 11 SNPs related to sepsis, hearing loss, agranulocytosis, thrombocytopenia and pancytopenia, including rs3814057 in the *NR1I2* gene, which has been associated with major adverse cardiovascular events in clopidogrel-treated patients and hepatotoxicity risk in anti-tuberculosis drugs-treated patients in the literature (53,54); and the rs2293347 in the *EGFR* gene, which has been reported to be associated with skin toxicity in patients with colorectal cancer receiving an antibody against EGFR (55). For high-risk regimen-treated patients, the model selected five SNPs, including *EGFR* rs10228436 which has been reported to be associated with the incidence of skin rash for imatinib in gastrointestinal stromal tumors and hepatotoxicity risk in gefitinib-treated patients with lung cancer (56,57). ADRs of the remaining polymorphisms have rarely been reported by the scientific community, suggesting that there is a need to further confirm the effects of these SNPs. In the present study, using the candidate SNPs approach, associations between multiple SNPs and chemotherapy-induced ADRs were identified. Although the mechanism to induce ADRs should be further validated in different cohorts or by molecular analysis, the present study has provided novel evidence for ADR prediction systems, which may lead to an improved outcome and quality of life for patients. If these results could be validated in different cohorts, the set of SNPs could become a predictive signature for identifying patients at risk of developing these toxicities.

The present study revealed that rs7624838 in the *ABCC5* gene, which was significantly associated with a reduced incidence of grade 4 high-risk regimen-induced ADRs, exhibited similar trends for both sepsis and thrombocytopenia, indicating that rs7624838 AA may prevent the development of severe ADRs. rs7624838 might act as an important marker associated with a lower incidence of ADRs in response to high-risk regimens. Previous studies have shown that the *ABCC5* gene can influence the disposition of endogenous metabolites, toxins and drugs in human cancer (58-60). rs7624838 is located in intron 2 of the *ABCC5* gene. It may be hypothesized that the impairment of rs7624838 in *ABCC5* could lead to a sufficient drug clearance and subsequent decrease of the drug concentration in the body. However, this hypothesis should be validated using larger samples as well as by a functional analysis of rs7624838.

The present study has certain limitations. First, selection bias was likely present, since a retrospective, single-center study was performed with a relatively small sample size. Second, the present study was not validated in conjunction with a public database. Third, there was a relatively short observation period. Further prospective, large, multi-center studies with longer follow-up periods are needed to validate the results of the present study.

According to the present study, individuals with neuroblastoma exhibited genomic instability that was noticeably higher compared with patients with GNB, and segmental changes aided the invasion and proliferation of tumor cells. LOH of whole chromosomes and segmental alterations may both be associated with invasion. In addition, LOH of the whole chromosome was associated with increased aggressiveness compared with whole chromosome gain. Furthermore, a group of SNPs linked to toxic symptoms was identified; patients with these SNPs should be assessed during the initial phase of therapy. Treatment decisions for patients with neuroblastoma should consider multiple molecular changes as predictive elements to make treatment adjustments for patients with varying probabilities of treatment success; however, the results should be validated in different comparable cohorts.

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Availability of data and materials

The microarray data generated in the present study may be found in the NCBI Gene Expression Omnibus under accession number GSE288908 or at the following URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE288908. The other data generated in the present study may be requested from the corresponding author.

Authors' contributions

KLC, YL, QX, HPL and YJL initiated the conception and design of the study, participated in the interpretation of the data and wrote the article. HCL, JQL, LC and YX performed the experiments and participated in the analysis of the data. YLT and YFY performed bioinformatics analysis including data processing, formal analysis, statistics and visualization. YLT and HPL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the local Ethics Committee of Wuhan Children's Hospital (approval no. 2024R089-E01). Written informed consent was obtained from their legal guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Gurney JG, Davis S, Severson RK, Fang JY, Ross JA and Robison L: Trends in cancer incidence among children in the US. Cancer 78: 532-541, 1996.
2. Nakagawara A, Li Y, Izumi H, Muramori K, Inada H and Nishi M: Neuroblastoma. Jpn J Clin Oncol 48: 214-241, 2018.
3. Maris J, Hogarty MD, Bagatell R and Cohn SL: Neuroblastoma. Lancet 369: 2106-2120, 2007.
4. Park JR, Kreissman SG, London WB, Naranjo A, Cohn SL, Hogarty MD, Tenney SC, Haas-Kogan D, Shaw PJ, Kraveka JM, *et al*: Effect of tandem autologous stem cell transplant vs single transplant on event-free survival in patients with high-risk neuroblastoma: A randomized clinical trial. JAMA 322: 746-755, 2019.
5. Park JA and Cheung NKV: Targets and antibody formats for immunotherapy of neuroblastoma. J Clin Oncol 38: 1836-1848, 2020.
6. Zafar A, Wang W, Liu G, Wang X, Xian W, McKeon F, Foster J, Zhou J and Zhang R: Molecular targeting therapies for neuroblastoma: Progress and challenges. Med Res Rev 41: 961-1021, 2021.
7. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, Faldum A, Hero B, Iehara T, Machin D, *et al*: The international neuroblastoma risk group (INRG) classification system: An INRG task force report. J Clin Oncol 27: 289-297, 2009.
8. Sokol E, Desai AV, Applebaum MA, Valteau-Couanet D, Park JR, Pearson ADJ, Schleiermacher G, Irwin MS, Hogarty M, Naranjo A, *et al*: Age, diagnostic category, tumor grade, and mitosis-karyorrhexis index are independently prognostic in neuroblastoma: An INRG project. J Clin Oncol 38: 1906-1918, 2020.
9. Asgharzadeh S, Pique-Regi R, Sposto R, Wang H, Yang Y, Shimada H, Matthay K, Buckley J, Ortega A and Seeger RC: Prognostic significance of gene expression profiles of metastatic neuroblastomas lacking MYCN gene amplification. J Natl Cancer Inst 98: 1193-1203, 2006.
10. Shi Y, Yuan J, Rraklli V, Maxymovitz E, Cipullo M, Liu M, Li S, Westerlund I, Bedoya-Reina OC, Bullova P, *et al*: Aberrant splicing in neuroblastoma generates RNA-fusion transcripts and provides vulnerability to spliceosome inhibitors. Nucleic Acids Res 49: 2509-2521, 2021.
11. Hiwatari M, Seki M, Matsuno R, Yoshida K, Nagasawa T, Sato-Otsubo A, Yamamoto S, Kato M, Watanabe K, Sekiguchi M, *et al*: Novel TENM3-ALK fusion is an alternate mechanism for ALK activation in neuroblastoma. Oncogene 41: 2789-2797, 2022.
12. Fetahu IS and Taschner-Mandl S: Neuroblastoma and the epigenome. Cancer Metastasis Rev 40: 173-189, 2021.
13. Brodeur GM, Seeger RC, Schwab M, Varmus HE and Bishop JM: Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 224: 1121-1124, 1984.
14. Bresler SC, Weiser DA, Huwe PJ, Park JH, Krytska K, Ryles H, Laudenslager M, Rappaport EF, Wood AC, McGrady PW, *et al*: ALK mutations confer differential oncogenic activation and sensitivity to ALK inhibition therapy in neuroblastoma. Cancer Cell 26: 682-694, 2014.
15. Bellini A, Pötschger U, Bernard V, Lapouble E, Baulande S, Ambros PF, Auger N, Beiske K, Bernkopf M, Betts DR, *et al*: Frequency and prognostic impact of *ALK* amplifications and mutations in the European neuroblastoma study group (SIOPEN) high-risk neuroblastoma trial (HR-NBL1). J Clin Oncol 39: 3377-3390, 2021.
16. Spitz R, Hero B, Ernestus K and Berthold F: Deletions in chromosome arms 3p and 11q are new prognostic markers in localized and 4s neuroblastoma. Clin Cancer Res 9: 52-58, 2003.
17. Schleiermacher G, Mosseri V, London WB, Maris JM, Brodeur GM, Attiyeh E, Haber M, Khan J, Nakagawara A, Speleman F, *et al*: Segmental chromosomal alterations have prognostic impact in neuroblastoma: A report from the INRG project. Br J Cancer 107: 1418-1422, 2012.
18. Fong C, White PS, Peterson K, Sapienza C, Cavenee WK, Kern SE, Vogelstein B, Cantor AB, Look AT and Brodeur GM: Loss of heterozygosity for chromosomes 1 or 14 defines subsets of advanced neuroblastomas. Cancer Res 52: 1780-1785, 1992.
19. Brady SW, Liu Y, Ma X, Gout AM, Hagiwara K, Zhou X, Wang J, Macias M, Chen X, Easton J, *et al*: Pan-neuroblastoma analysis reveals age-and signature-associated driver alterations. Nat Commun 11: 5183, 2020.
20. Janoueix-Lerosey I, Schleiermacher G, Michels E, Mosseri V, Ribeiro A, Lequin D, Vermeulen J, Couturier J, Peuchmaur M, Valent A, *et al*: Overall genomic pattern is a predictor of outcome in neuroblastoma. J Clin Oncol 27: 1026-1033, 2009.
21. Park JR, Eggert A and Caron H: Neuroblastoma: Biology, prognosis, and treatment. Pediatr Clin N Am 55: 97-120, 2008.
22. Olivera GG, Yáñez Y, Gargallo P, Sendra L, Aliño SF, Segura V, Sanz MÁ, Cañete A, Castel V, Mora JF, *et al*: *MTHFR* and *VDR* polymorphisms improve the prognostic value of *MYCN* status on overall survival in neuroblastoma patients. Int J Mol Sci 21: 2714, 2020.
23. Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB and Schwab M: Clinical pharmacogenetics implementation consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clin Pharmacol Ther 94: 640-645, 2013.
24. Amstutz U, Henricks LM, Offer SM, Barbarino J, Schellens JHM, Swen JJ, Klein TE, McLeod HL, Caudle KE, Diasio RB and Schwab M: Clinical Pharmacogenetics implementation consortium (CPIC) guideline for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing: 2017 update. Clin Pharmacol Ther 103: 210-216, 2018.
25. Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, Rongen GA, van Schaik RH, Schalekamp T, Touw DJ, *et al*: Pharmacogenetics: From bench to byte-an update of guidelines. Clin Pharmacol Ther 89: 662-673, 2011.

26. Lunenburg CATC, van der Wouden CH, Nijenhuis M, Crommentuijn-van Rhenen MH, de Boer-Veger NJ, Buunk AM, Houwink EJF, Mulder H, Rongen GA, van Schaik RHN, *et al*: Dutch pharmacogenetics working group (DPWG) guideline for the gene-drug interaction of *DPYD* and fluoropyrimidines. Eur J Hum Genet 28: 508-517, 2020.
27. Pu X, Hildebrandt MAT, Lu C, Lin J, Stewart DJ, Ye Y, Gu J, Spitz MR and Wu X: PI3K/PTEN/AKT/mTOR pathway genetic variation predicts toxicity and distant progression in lung cancer patients receiving platinum-based chemotherapy. Lung Cancer 71: 82-88, 2011.
28. Jabeen S, Holmboe L, Alnæs GIG, Andersen AM, Hall KS and Kristensen VN: Impact of genetic variants of RFC1, DHFR and MTHFR in osteosarcoma patients treated with high-dose methotrexate. Pharmacogenomics J 15: 385-390, 2015.
29. Sugishita M, Imai T, Kikumori T, Mitsuma A, Shimokata T, Shibata T, Morita S, Inada-Inoue M, Sawaki M, Hasegawa Y and Ando Y: Pharmacogenetic association between GSTP1 genetic polymorphism and febrile neutropenia in Japanese patients with early breast cancer. Breast Cancer 23: 195-201, 2016.
30. Bosó V, Herrero MJ, Santaballa A, Palomar L, Megias JE, de la Cueva H, Rojas L, Marqués MR, Poveda JL, Montalar J and Aliño SF: SNPs and taxane toxicity in breast cancer patients. Pharmacogenomics 15: 1845-1858, 2014.
31. Verscheijden LFM, Koenderink JB, Johnson TN, de Wildt SN and Russel FGM: Physiologically-based pharmacokinetic models for children: Starting to reach maturation? Pharmacol Ther 211: 107541, 2020.
32. Min S: Design of formulations for pediatric drugs. Prog Pharm Sci 43: 655-666, 2019.
33. Fernandez E, Perez R, Hernandez A, Tejada P, Arteta M and Ramos JT: Factors and mechanisms for pharmacokinetic differences between pediatric population and adults. Pharmaceutics 3: 53-72, 2011.
34. Ognibene M, De Marco P, Amoroso L, Fragola M, Zara F, Parodi S and Pezzolo A: Neuroblastoma patients' outcome and chromosomal instability. Int J Mol Sci 24: 15514, 2023.
35. Körber V, Stainczyk SA, Kurilov R, Henrich KO, Hero B, Brors B, Westermann F and Höfer T: Neuroblastoma arises in early fetal development and its evolutionary duration predicts outcome. Nat Genet 55: 619-630, 2023.
36. Olivera GG, Urtasun A, Sendra L, Aliño SF, Yáñez Y, Segura V, Gargallo P, Berlanga P, Castel V, Cañete A and Herrero MJ: Pharmacogenetics in neuroblastoma: What can already be clinically implemented and what is coming next? Int J Mol Sci 22: 9815, 2021.
37. Urtasun A, Olivera GG, Sendra L, Aliño SF, Berlanga P, Gargallo P, Hervás D, Balaguer J, Juan-Ribelles A, Andrés MDM, *et al*: Personalized medicine in infant population with cancer: Pharmacogenetic pilot study of polymorphisms related to toxicity and response to chemotherapy. Cancers (Basel) 15: 1424, 2023.
38. Paolini L, Hussain S and Galardy PJ: Chromosome instability in neuroblastoma: A pathway to aggressive disease. Front Oncol 12: 988972, 2022.
39. Pediatric Oncology Committee, Chinese Anti-Cancer Association, Oncology Group, Chinese Association of Pediatric Surgeons. Expert consensus on diagnosing and treating of neuroblastoma in children. Chin J Pediatr Surg 36: 3-7, 2015.
40. Department of Health and Human Services: Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, 2017.
41. Gunderson KL, Steemers FJ, Lee G, Mendoza LG and Chee MS: A genome-wide scalable SNP genotyping assay using microarray technology. Nat Genet 37: 549-554, 2005.
42. Conlin LK, Thiel BD, Bonnemann CG, Medne L, Ernst LM, Zackai EH, Deardorff MA, Krantz ID, Hakonarson H and Spinner NB: Mechanisms of mosaicism, chimerism and uniparental disomy identified by single nucleotide polymorphism array analysis. Hum Mol Genet 19: 1263-1275, 2010.
43. Attiyeh EF, London WB, Mossé YP, Wang Q, Winter C, Khazi D, McGrady PW, Seeger RC, Look AT, Shimada H, *et al*: Chromosome 1p and 11q deletions and outcome in neuroblastoma. N Engl J Med 353: 2243-2253, 2005.
44. Ambros PF, Ambros IM, Brodeur GM, Haber M, Khan J, Nakagawara A, Schleiermacher G, Speleman F, Spitz R, London WB, *et al*: International consensus for neuroblastoma molecular diagnostics: Report from the international neuroblastoma risk group (INRG) biology committee. Br J Cancer 100: 1471-1482, 2009.
45. Thompson D, Vo KT, London WB, Fischer M, Ambros PF, Nakagawara A, Brodeur GM, Matthay KK and DuBois SG: Identification of patient subgroups with markedly disparate rates of MYCN amplification in neuroblastoma: A report from the international neuroblastoma risk group project. Cancer 122: 935-945, 2016.
46. Sansregret L and Swanton C: The role of aneuploidy in cancer evolution. Cold Spring Harb Perspect Med 7: a028373, 2017.
47. Bourdeaut F, Ribeiro A, Paris R, Pierron G, Couturier J, Peuchmaur M and Delattre O: In neuroblastic tumours, Schwann cells do not harbour the genetic alterations of neuroblasts but may nevertheless share the same clonal origin. Oncogene 27: 3066-3071, 2008.
48. Toraman AD, Keser I, Lüleci G, Tunali N and Gelen T: Comparative genomic hybridization in ganglioneuroblastomas. Cancer Genet Cytogen 132: 36-40, 2002.
49. Abdel-Hafiz HA, Schafer JM, Chen X, Xiao T, Gauntner TD, Li Z and Theodorescu D: Y chromosome loss in cancer drives growth by evasion of adaptive immunity. Nature 619: 624-631, 2023.
50. Müller P, Camacho OV, Yazbeck AM, Wölwer C, Zhai W, Schumacher J, Heider D, Buettner R, Quaas A and Hillmer AM: Why loss of Y? A pan-cancer genome analysis of tumors with loss of Y chromosome. Comput Struct Biotechnol J 21: 1573-1583, 2023.
51. Ognibene M, De Marco P, Parodi S, Meli M, Di Cataldo A, Zara F and Pezzolo A: Genomic analysis made it possible to identify gene-driver alterations covering the time window between diagnosis of neuroblastoma 4s and the progression to stage 4. Int J Mol Sci 23: 6513, 2022.
52. Janssen A, Van Der Burg M, Szuhai K, Kops GJ and Medema RH: Chromosome segregation errors as a cause of DNA damage and structural chromosome aberrations. Science 333: 1895-1898, 2011.
53. Wu Y, Yu H, Tang H, Su Y, Shi TL, Liu S, Xia Q and Xu DJ: PXR polymorphisms have impact on the clinical efficacy of clopidogrel in patients undergoing percutaneous coronary intervention. Gene 653: 22-28, 2018.
54. Richardson M, Kirkham J, Dwan K, Sloan DJ, Davies G and Jorgensen AL: Association of variants within the GST and other genes with anti-tubercular agents related toxicity: A systematic review and meta-analysis. BioRxiv 9: 515817, 2019.
55. Saito R, Suzuki H, Yamada T, Endo S, Moriwaki T, Ueno T, Hirose M, Hirai S, Yamato K, Mizokami Y and Hyodo I: Predicting skin toxicity according to EGFR polymorphisms in patients with colorectal cancer receiving antibody against EGFR. Anticancer Res 33: 4995-4998, 2013.
56. Zhuang W, Qiu H, Wang X, Xin S, Huang M and Zhou Z: Impact of pharmacogenomics on imatinib toxicity in gastrointestinal stromal tumors. J Clin Oncol 35: 11043, 2017.
57. Wei F, Xi C and Shaoxing G: Relationship between MAPK1 gene polymorphism and gefitinib hepatotoxicity in NSCLC patients with activating *EGFR* mutations. Acta Pharm Sin 53: 760-764, 2018.
58. Jansen RS, Mahakena S, de Haas M, Borst P and van de Wetering K: ATP-binding cassette subfamily C member 5 (ABCC5) functions as an efflux transporter of glutamate conjugates and analogs. J Biol Chem 290: 30429-30440, 2015.
59. Chen J, Wang Z, Gao S, Wu K, Bai F, Zhang Q, Wang H, Ye Q, Xu F, Sun H, *et al*: Human drug efflux transporter ABCC5 confers acquired resistance to pemetrexed in breast cancer. Cancer Cell Int 21: 1-10, 2021.
60. Belkahla S, Khan AUH, Gitenay D, Alexia C, Gondeau C, Vo DN, Orecchioni S, Talarico G, Bertolini F, Cartron G, *et al*: Changes in metabolism affect expression of ABC transporters through ERK5 and depending on p53 status. Oncotarget 9: 1114-1129, 2018.