Analysis of the clinical characteristics of 28 children with NF1 mutation-positive acute leukemia and literature review

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Abstract

Objective: To summarize the clinical characteristics, treatment effects and survival outcomes of children with *NF1* mutation-positive acute leukemia. **Methods**: The clinical data and prognosis of 28 patients with *NF1* mutation-positive acute leukemia treated at Beijing Children's Hospital, Capital Medical University, between January 2017 and February 2024 were retrospectively analyzed. **Results**: A total of 28 patients were included in the study, with a median follow-up of 16 (1.2–85.5) months. Twelve patients had acute lymphoblastic leukemia, of whom 75.0% were at intermediate risk. Complete response (CR) was achieved in the bone marrow after induction chemotherapy, with a minimal residual disease (MRD) of 1×10^{-3} at Day 33. Fifteen patients had acute myeloid leukemia (AML), of whom 66.7% were at high risk. The CR rate in the bone marrow was 86.6% at Day 28. Eleven (73.3%) patients survived at the end of follow-up. One patient with acute promyelocytic leukemia had standard risk and a good response. The overall survival rate of children with *NF1* mutations was comparable to that of children with no mutations. However, children with germline *NF1* mutations had a poor prognosis compared with those with somatic mutations, especially in AML patients. The frequency of *NF1* mutations was 2–87.7%. The clinical manifestations of 3 patients with neurofibromatosis included café-au-lait macules, freckles, xanthogranuloma, scoliosis, and benign intracranial lesions. **Conclusion:** Most *NF1* mutations in children with acute leukemia are somatic mutations that do not affect overall survival. Children with leukemia complicated with neurofibromatosis should undergo lifelong follow-up.

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An abbreviations key

Abbreviation	The full term or phrase
JMML	juvenile myelomonocytic leukemia
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
APL	acute promyelocytic leukemia
MRD	minimal residual disease
CCLG	Chinese Children Leukemia Group
OS	Overall survival
CNS2	central nervous system leukemia
CR	complete response
D	Day
CALM	café-au-lait macules
VAF	variant allele fraction
MLPA	multiplex ligation-dependent probe amplification
OPGs	Optic pathway gliomas

Abstract

Objective: To summarize the clinical characteristics, treatment effects and survival outcomes of children with *NF1* mutation-positive acute leukemia. **Methods** : The clinical data and prognosis of 28 patients with *NF1* mutation-positive acute leukemia treated at Beijing Children's Hospital, Capital Medical University, between January 2017 and February 2024 were retrospectively analyzed. **Results** : A total of 28 patients were included in the study, with a median follow-up of 16 (1.2–85.5) months. Twelve patients had acute lymphoblastic leukemia, of whom 75.0% were at intermediate risk. Complete response (CR) was achieved in the bone marrow after induction chemotherapy, with a minimal residual disease (MRD) of 1×10^{-3} at Day 33. Fifteen patients had acute myeloid leukemia (AML), of whom 66.7% were at high risk. The CR rate in the bone marrow was 86.6% at Day 28. Eleven (73.3%) patients survived at the end of follow-up. One patient with acute promyelocytic leukemia had standard risk and a good response. The overall survival rate of children with *NF1* mutations was comparable to that of children with no mutations. However, children with germline *NF1* mutations had a poor prognosis compared with those with somatic mutations, especially in AML patients. The frequency of *NF1* mutations was 2–87.7%. The clinical manifestations of 3 patients with neurofibromatosis included café-au-lait macules, freckles, xanthogranuloma, scoliosis, and benign intracranial lesions. **Conclusion:** Most *NF1* mutations in children with acute leukemia are somatic mutations that do not affect overall survival. Children with leukemia complicated with neurofibromatosis should undergo lifelong follow-up.

Leukemia is the most common cancer and major cause of death in childhood, and chromosomal abnormalities or genetic events such as gene mutations and fusion genes occur over the course of the disease.*NF1* is a tumor suppressor gene and a negative regulator of the RAS pathway. Mutations in this gene may lead to neurofibromatosis type 1 (OMIM 613113). In addition, the risk of benign and malignant tumors is increased. Among pediatric hematological diseases, *NF1* mutations are frequently observed in juvenile myelomonocytic leukemia (JMML)[1]. Moreover, these mutations are associated with the pathogenesis of JMML and its poor

clinical prognosis. However, there are few reports on this gene in pediatric acute leukemia. Data on the clinical characteristics, treatment effects and prognosis of patients with *NF1* mutation-positive acute leukemia are lacking. In this study, the clinical and molecular biological characteristics, treatment effects and survival status of 28 children with *NF1* mutation-positive acute leukemia were retrospectively collected and analyzed to provide a reference for clinical diagnosis and treatment.

Study Subjects and Methods

1. Study subjects

A total of 28 children with *NF1* mutation-positive acute leukemia who were treated at Beijing Children's Hospital, Capital Medical University, between January 2017 and February 2024, including 12 patients with acute lymphoblastic leukemia (ALL), 15 with acute myeloid leukemia (AML), and one with acute promyelocytic leukemia (APL), were enrolled in this study. Patient information, including sex, age, immunophenotype, gene mutations, fusion genes, bone marrow, minimal residual disease (MRD), extramedullary involvement, *NF1* mutation sites and modes, and signs of neurofibromatosis, was collected. ALL patients were treated with the Chinese Children Leukemia Group (CCLG)-ALL 2018 regimen, AML patients were treated with the CCLG-AML 2015 or 2019 regimen depending on the time of the first visit, and APL patients were treated with the CCLG-APL 2016 regimen. The diagnosis of neurofibromatosis type I was made according to the standard revision of the International Consensus Group on Diagnostic Criteria of Neurofibromatosis 2021[2]. This study was approved by the Medical Ethics Committee of Beijing Children's Hospital(IEC-C-006-A04-V.07.1).

2. Gene detection methods

Bone marrow samples were collected at the first visit and sent to Beijing Haester Medical Laboratory for analysis of the mutational landscape of ALL and 248 AML-related mutations using oral mucosal epithelial DNA as a somatic tissue control.

3. Follow-up

The treatment and survival outcomes of the patients were followed up through telephone interviews and outpatient services. The follow-up period ended on June 30, 2024, and the median follow-up period was 16 (1.2–85.5) months. Overall survival (OS) was calculated starting from the time of leukemia diagnosis until the last follow-up visit or death.

4. Statistical analysis

SPSS 26.0 statistical software was used for data processing. Measurement data with a non-normal distribution are expressed as M (Q1, Q3), and enumeration data are expressed as percentages (%). Survival analysis was conducted using Kaplan-Meier curves, and the log-rank test was performed. P <0.05 was considered to indicate a statistically significant difference between two groups. A retrospective case-control design was used to compare the survival outcomes of children with *NF1* mutation-positive AML with those of children with *NF1* mutation-negative AML admitted during the same period; the patients were matched by the random number method of 1:3, and matching factors included age+-2 years, sex, risk stratification, treatment protocols, and transplantation status.

Results

1. Clinical characteristics, treatment and prognosis data of ALL patients

Of 12 ALL patients, 11 had an initial onset of ALL, and one relapsed from testicular leukemia. The clinical manifestations of these patients at the first visit included fever, fatigue and subcutaneous hemorrhage, with a median age of 7 (3.3–14.8) years and a male-to-female ratio of 7:5. At the first visit, the median white blood cell (WBC) count was 5.96×10^9/L, the median hemoglobin (HGB) level was 85 g/L, and the median platelet count was 187×10^9/L. The average proportion of myeloid progenitor cells was 89.3%. The chromosome karyotype was predominantly a normal karyotype, and all patients had B-ALL. The immunophenotype was predominantly common B cells. One patient had central nervous system leukemia (CNS2). On average, 3.6

gene mutations occurred per patient, including 5 (41.7%)*KRAS* mutations, 4 (33.3%) *NRAS* mutations, and 2 (16.7%)*CREBBP* mutations. The fusion gene mutations included 3 (25.0%)*ZNF384* mutations and one each of *TEL-AML1* and*DUX4::lgHJ6 (582).* Risk assessment revealed that 75.0% of patients were classified as having intermediate risk. All patients received chemotherapy, and two patients received the targeted drug blinatumomab as part of induction therapy. None of the patients had undergone hematopoietic stem cell transplantation. The complete response (CR) rate of bone marrow after induction chemotherapy was 100%. Two patients had MRD<1x10^{-3} at Day (D) 15, and all patients had MRD<1x10^{-3} at D33. Moreover, the patients who entered the maintenance phase were assessed as MRD-negative before consolidation therapy. Currently, one patient has stopped the medicine for 1 year, and the other patients have remained in the chemotherapy stage. See Tables 1 and 2 for detailed information.

2. Clinical characteristics, treatment and prognosis of AML and APL patients

A total of 15 AML patients had an initial onset of AML. The clinical manifestations of these patients at the first visit included fever, upper respiratory tract infection, a pale face and fatigue. Patient AML-1 was complicated with Camurati-Engelmann disease and glucose-6-phosphate dehydrogenase deficiency. The median age was 11 (0.6–15.4) years, and the male-to-female ratio was 2:1. At the first visit, the median WBC count was 7.47x10^{9}/L. Most patients had anemia and thrombocytopenia. The proportion of myeloid progenitor cells was 47%. Two patients had myeloid sarcoma, one of whom had no bone marrow invasion. The chromosome karyotype was predominantly a normal karyotype. On average, each patient had 2.27 gene mutations. There were 3 (20.0%) patients with *WT1* and *PTPN11* mutations and 2 patients with *TP53* mutations. There were 2 patients each with the fusion genes *AML1-ETO* and*ΨΒΦβ-ΜΨΗ11* each, and one patient each with *KMT2A-MLLT3, MLL-AF9, NUP98-HoxA9* and *FLT3-ITD.* The risk assessment revealed that 66.7% of patients were at high risk. With respect to chemotherapy, seven patients received the targeted drugs venetoclax and gilteritinib, and nine underwent allogeneic hematopoietic stem cell transplantation. The bone marrow CR rate was 73.3% at D21 after the induction treatment and 86.6% at D28. Four (26.7%) patients had MRD<1×10^{-3} at D21, 5 (33.3%) had MRD<1×10^{-3} at D28, and 4 (26.7%) had 1×10^{-3}[?]MRD<1x10^{-2}. Eleven (73.3%) patients survived at the end of follow-up; 2 patients died after induction therapy, one died due to severe infection and multiple organ failure after transplantation, and one died due to recurrence. There was one APL patient who was an 8-year-old girl, with 91% bone marrow blast cells, a normal karyotype, *PML-RARA* fusion genes and standard risk. She took arsenicals and retinoic acid throughout treatment, which has been stopped for 3 years. See Tables 1 and 3 for details.

3. Survival analysis of leukemia patients with *NF1* gene mutations

The range of mutation frequencies of the *NF1* gene was wide, ranging from 1.8% to 87.7%. The *c.4676G>A* site had the highest mutation frequency, and the *c.6855C>A* site had the lowest mutation frequency. Three children had multisite mutations. No duplicate or novel mutation sites were identified. The mutation types included nonsense mutations, missense mutations, frameshift mutations, and in-frame insertions. See Table 4 for details. Among the detected mutations, the median variant allele fraction (VAF) of the *NF1* mutation was 0.27 in ALL patients and 0.39 in AML patients, suggesting that it plays a role in driving mutations in the leukemic clones but is not dominant. Children with germline *NF1* mutations carried other adverse genetic factors such as *TP53, KMT2A-MLLT3, MLL-AF9,* and *NUP98-HoxA9* . Children with somatic mutations carried mutations in *FLT3, c-KIT,* and *TP53* . The overall treatment and outcomes of the 28 children with *NF1* gene mutations are shown in Figure 1. The 5-year OS rate of the ALL group was better than that of the AML group; however, the difference was not statistically significant [100% vs. (67.7+-14.8)%, $X2$ =2.32,P =0.127; Figure 2]. In contrast, the difference in the 5-year OS rate between patients with germline and somatic *NF1* mutations was significant [(33.3+-25.5)% vs. (95.5+-4.4)%, $X2$ =6.34,P =0.012; Figure 3]. All the children with AML with germline*NF1* mutations died. The OS rate of children with AML was 73.3%. There was no difference in the 5-year OS rate between the *NF1* mutation and non-mutation groups [(64+-16.5)% vs. (66.6+-7.9)%,P =0.994; Figure 4]. All ALL and APL patients survived at the end of follow-up, suggesting that the *NF1* gene did not affect the OS prognosis of childhood leukemia patients.

4. Clinical characteristics of the *NF1* gene and neurofibromatosis type 1

Six (21.4%) patients had germline *NF1* mutations; all patients had ALL with a definite family history, and two patients were complicated with neurofibromatosis. Figure 5 displays the gene mutation sites of Patient ALL-2, showing germline mutations inherited from the mother. The remaining patients had somatic mutations, and one patient was complicated with neurofibromatosis. Patient ALL-1 had cafe-au-lait macules (CALM) (Figure 6), scoliosis (Figure 7) and intracranial involvement (Figure 8: right basal ganglia, bilateral thalamus). Patient ALL-2 had CALM, xanthogranuloma and intracranial involvement (left basal ganglia, right thalamus, midbrain, pons, brachium pontis, and bilateral cerebellar hemispheres). Patient ALL-3 had CALM, freckles and intracranial involvement (Figure 9: bilateral basal ganglia, thalamus, hypothalamus, bilateral temporal poles, hippocampus, cerebral peduncle, pons and cerebellum). The patient carried a nonsense mutation, and the sites were verified to be the wild type in the parents. The patient's father had signs of CALM and suffered from leukemia in 2014; he carried an *MLL* fusion gene and *WT1* mutations but no *NF1* mutation. Therefore, the patient was considered to have somatic*NF1* gene mutations, but the possibility of a chimeric mutation was not excluded. Three of the remaining patients had signs of CALM or freckles, but none met the diagnostic criteria for neurofibromatosis.

Discussion

Neurofibromatosis type I caused by *NF1* mutations is classified as an RAS disease (RASopathy)[3], with unique phenotypic characteristics, such as CALM, skin neurofibroma, bone dysplasia and Lisch nodules of the iris. Patients have a high risk of benign and malignant tumors[4], with an estimated 59.6% lifetime cancer risk and a low long-term survival rate[5]. A follow-up study of 2,427 patients with neurofibromatosis type I revealed that 9 (0.6%) patients had comorbid ALL, 2 had AML, 2 had chronic myeloid leukemia, and one had chronic lymphocytic leukemia[6]. A study including 1,021 adult patients with newly developed AML revealed that 5% of patients had *NF1* mutations, and one-third of the gene mutation loci were located at threonine 676 (p.Thr676fs*24). This locus is associated with a lower CR rate and shorter OS[7]. RAS pathway mutations occur in more than 40% of pediatric ALL patients,[8] but the proportion of *NF1* mutations is minimal, and no relevant large-scale study findings are available.

The *NF1* gene is located at chromosome 17q11.2, which spans 350 kb, including 57 constitution exons and 4 alternative spliced exons (9a, 10a2, 23a, and 48a), and encodes neurofibromin. Neurofibromin is a RAS-GTP-activating protein that inactivates RAS-GTP by accelerating its hydrolysis to RAS-GDP. Loss of *NF1* gene expression may cause abnormal activation of RAS and downstream signaling pathways, mainly the RAF-MEK-ERK pathway. However, this gene also interacts with the PI3K-AKT-mTOR pathway to influence the secondary regulation of cell proliferation, differentiation, migration and apoptosis[9] and increases the genetic susceptibility to benign and malignant tumors. In addition, it has been reported that*NF1* splicing site variation may trigger exon skipping, which in turn may affect the structure and function of proteins[10]. The vast majority of *NF1* mutations are intragenic, including point mutations (85% -90%) and single or multiple exon deletions or duplications (2%). Fewer than 10% of deletions involve the entire gene and flanking genomic regions[11]. There is a strong genotype-phenotype correlation with 17q11.2 microdeletion syndrome[12]. Detecting these mutations is dependent on multiplex ligation-dependent probe amplification (MLPA)[13]. Specific mutation types were not distinguished in this study. Thus, further research and improvements are needed.

This study revealed that *NF1* mutations do not affect the overall effect of treatment on pediatric acute leukemia patients. Survival outcomes are associated with adverse cytogenetic features, risk assessment, treatment protocols, and disease progression. Patients with germline *NF1* gene mutations had a poorer prognosis than those with somatic mutations, especially children with AML. However, all three children with AML in this study had a high risk and concurrent adverse genetic variants, such as *KMT2A-MLLT3, MLL-AF9,* and*NUP98-HoxA9* . These patients had a poor remission rate after induction therapy. Moreover, only a small number of patients were included in this study. Therefore, the associations with prognosis should be further investigated. ALL patients mainly had intermediate risk, whereas AML patients mostly had high risk. Stratified therapy based on MRD guidance has been adopted, and targeted drugs such as blinatumomab, venetoclax and gilteritinib have been administered simultaneously. Both the low 5-year survival rate and

high recurrence rate of AML are bottlenecks in the treatment of acute leukemia. High-risk children should actively undergo transplantation or use targeted drugs and new treatment protocols. In terms of fusion genes, some ALL patients carried a *ZNF384* fusion gene rearrangement, which has not been reported previously. The transcription factor zinc finger protein 384 gene is located at chromosome 12q13 and can fuse with a variety of upstream partner genes, causing defects in the integrin signaling pathway. This fusion gene is associated with premature migration of bone marrow hematopoietic stem cells into the peripheral blood and may be one of the mechanisms of ALL.

In this study, the clinical manifestations of neurofibromatosis included CALM, freckles, xanthogranuloma, scoliosis, and benign intracranial lesions. No other benign or malignant tumors were discovered. Neurofibromatosis was more common in patients with germline *NF1* gene mutations, which is consistent with the findings of previous studies[14,15]. Somatic *NF1* gene mutations were mostly acquired cytogenetic mutations secondary to leukemic tumor cells. However, the possibility of chimeric mutations should be considered when patients present with clinical symptoms of neurofibromatosis. The clinical symptoms of neurofibromatosis are atypical, variable and appear gradually over time because of incomplete penetrance of the *NF1* gene in children and adolescents. When germline *NF1* gene mutations are identified or the patient exhibits relevant clinical symptoms, the French Neurofibromatosis Type I Guidelines 2020 should be followed for clinical follow-up[16].

In the CNS, bright signals in the cerebellum, brainstem, thalamus and basal ganglia occur in 70% of children with neurofibromatosis; these signals indicate benign brain lesions that appear at approximately 3 years of age and gradually disappear between 20 and 30 years of age[17]. In this study, abnormal head nuclear magnetic resonance signals were observed in all three neurofibromatosis patients at the first visit, and these signals were slightly reduced during chemotherapy. Patient ALL-3 had more abnormal signals after drug withdrawal than before withdrawal. These lesions do not affect brain function or intellectual development. Optic pathway gliomas (OPGs) are the most common CNS tumors in children under 7 years of age with*NF1* mutations, with an incidence of 15–20%. However, these patients are mostly asymptomatic,[18] and no OPGs were found in this study.

Patients with neurofibromatosis mainly receive symptomatic treatment. The MEK inhibitor selumetinib is approved by the US Food and Drug Administration for the treatment of children with *NF1* -associated plexiform neurofibromas. Interestingly, some studies have shown that the TKI inhibitors imatinib and nilotinib may decrease the volume of plexiform neurofibromas[19,20]. These two drugs are also used in Philadelphia chromosome-positive ALL patients, suggesting that the potential value of targeted drugs deserves further investigation in these patients.

In conclusion, this study is a single-center retrospective study with the largest number of children with *NF1* mutation-positive acute leukemia available in China. However, most children have not stopped drug treatment. Further follow-up studies are needed to determine the long-term prognosis of these patients.

Conflict of Interest statement: The manuscript has no conflicts of interest.

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Ethics statement: We have affirm that informed consent of the case patients has been properly documented.

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Table 1 Clinical characteristics of patients with *NF1* mutation-positive acute leukemia

Patients' clinical characteristics		ALL(n=12)	AML(n=15)	APL(n=1)
Sex: Male/Female		7:5	2:1	0:1
Age:		7(3.3-14.8)	11(0.6-15.4)	8
Chromosomal karyotype				
	Normal karyotype	10(83.3%)	13(86.6%)	1(100%)
	Hyperdiploid	2(16.7%)	0	0
	-7	0	1(6.7%)	0
	t(7; 11)(p15; p15)	0	1(6.7%)	0
Level of risk				
	Low risk	1(8.3%)	3(20.0%)	1(100%)
	Intermediate risk	9(75.0%)	2(13.3%)	0
	High risk	2(16.7%)	10(66.7%)	0
Treatment protocols				
	Targeted drugs	3(25.0%)	7(46.7%)	0
	Transplantation	0	9(60.0%)	0

Table 2 Genes, MRD and survival of patients with *NF1* mutation-positive ALL

No.	Level of Risk	Gene Mutations	Gene Fusion	Treatment Methods
ALL-1	Intermediate risk	No	No	Chemotherapy
ALL-2	Low risk	*IKZF1, KMT2C, ASXL3, SMC1A*	No	Chemotherapy
ALL-3	Intermediate risk	*TP53, RB1, PIK3CD, USH2A*	No	Chemotherapy
ALL-4	High risk	*INRAS, KRAS, CSMD1, SOS1*	No	Chemotherapy
ALL-5	Intermediate risk	*CREBBP, KRAS, IRF8, TBL1XR1*	*SPI1::ZNF384*	Chemotherapy + blinatumomab
ALL-6	Intermediate risk	*NRAS, FLT3, IKZF3*	*DUX4::lgHJ6(582)*	Chemotherapy
ALL-7	Intermediate risk	No	No	Chemotherapy
ALL-8	Intermediate risk	*FLT3, PTPN11, KRAS, ETV6*	*EP300-ZNF384*	Chemotherapy
ALL-9	Intermediate risk	*NRAS, KRAS*	No	Chemotherapy
ALL-10	High risk	*NRAS, KRAS, CREBBP*	No	Chemotherapy + blinatumomab
ALL-11	Intermediate risk	No	*TEL-AML1*	Chemotherapy
ALL-12	Intermediate risk	*ASXL1, ATRX, CHD2*	*EP300-ZNF384*	Chemotherapy

Table 3 Genes, MRD and survival of patients with *NF1* mutation-positive AML + APL

No.	Regimens	Level of Risk	Gene Mutations	Gene Fusion	Treatmen
AML-1	CCLG-2019	High risk	*PTPN11*	*KMT2A-MLLT3, MLL-AF9*	Chemothe
AML-2	CCLG-2015	High risk	*GATA2, WT1*	*NUP98-HoxA9*	Chemothe
AML-3	CCLG-2019	High risk	No	No	Chemothe
AML-4	CCLG-2019	High risk	No	No	Chemothe
AML-5	CCLG-2019	High risk	*IDH2, NRAS, WT1*	No	Chemothe
AML-6	CCLG-2019	Intermediate risk	*IDH1, NPM1, PTPN11*	*Low-level FLT3-ITD*	Chemothe
AML-7	CCLG-2019	High risk	No	No	Chemothe

No.	Regimens	Level of Risk	Gene Mutations	Gene Fusion	Treatmen
AML-8	CCLG-2019	Standard risk	No	*ʺBΦβ-MΨH11*	Chemothe
AML-9	CCLG-2019	High risk	*RUNX1, ASXL1, SETBP1, EZH2*	No	Chemothe
AML-10	CCLG-2019	High risk	No	No	Chemothe
AML-11	CCLG-2015	Standard risk	*PHF6*	*AML1-ETO*	Chemothe
AML-12	CCLG-2015	Intermediate risk	*PTPN11*	No	Chemothe
AML-13	CCLG-2015	High risk	*WT1*	No	Chemothe
AML-14	CCLG-2015	Standard risk	*TP53*	*AML1-ETO*	Chemothe
AML-15	CCLG-2015	High risk	*c-KIT,TP53*	*ʺBΦβ-MΨH11*	Chemothe
APL-1	APL-2016	Standard risk	No	*PML-RARA*	Chemothe

Table 4 Summary of the symptoms of patients with neurofibromatosis and their *NF1* gene loci

No.	Clinical Symptoms	*NF1* Gene Variation Loci (Variation Frequency%)
ALL-1	CALM, scoliosis, intracranial involvement	*exon3: c.247C>T(p.Q83*)(70.2%)*
ALL-2	CALM, xanthogranuloma, intracranial involvement	*exon38(c.5749+5G>A)*
ALL-3	CALM, scoliosis, intracranial involvement	*exon44:c.6733C>T:p.Q2245X(36.8%)*
ALL-4	No	*exon28:c.3827 G>A:p.R1276Q(3.6%)*
ALL-5	No	*exon2:c.128_147delinsAACGG:p.L43_Y49delinsQR(4.8%) e*
ALL-6	No	*exon18:c.2206_2208delinsGCCACTCCTGGGG:p.N736fs*3*
ALL-7	CALM	*exon20:c.2334_2336dup:p.E778dup (82.8%)*
ALL-8	No	*exon10:c.1074dup:p.N359X (8.8%)*
ALL-9	No	*exon41:c.6185G>A:p.R2062H (13.1%)*
ALL-10	No	*exon12:c.1391C>T:p.P464L (8.9%) exon34:c.4537C>T:p.I*
ALL-11	No	*exon17:c.1996_1997insAGGCTACC:p.S666X (17.3%)*
ALL-12	No	*exon48:c.7153_7154insGGCCTG:p.I2384_V2385insGP(2%)*
AML-1	No	*c.2033C>T(35.9%)*
AML-2	CALM	*c.1933A>G(47.7%)*
AML-3	No	*c.888+5G>A(51.99%)*
AML-4	No	*c.6007-1G¿C(1.9%) c.6855C¿A p.Tyr2285Ter(1.8%)*
AML-5	No	*c.6927_6941delinsCCCAGA(30%)*
AML-6	No	*c.3168_3169ins(16.7%)*
AML-7	No	*c.2033dup(21.4%)*
AML-8	No	*c.4676G>A(87.7%)*
AML-9	No	*exon18:c.2033dupC:p.I679fs*21 (78.9%)*
AML-10	No	*c.1885G>A(35.12%)*
AML-11	CALM, freckles	*c.910C>T(39.2%)*
AML-12	No	*c.5902C>T(37.84%)*
AML-13	No	*c.654+44A>C(50.65%)*
AML-14	No	*c.730+48A>G(54.66%)*
AML-15	No	*c.6921+40A>G(54.08%)*
APL-1	No	*c.6427+10A>G(50.3%)*

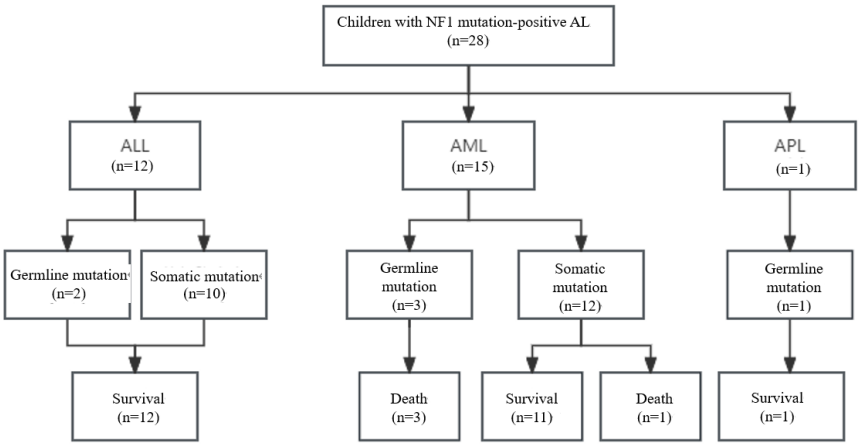

Figure 1 Treatment and outcomes of 28 children with *NF1* mutation-positive acute leukemia

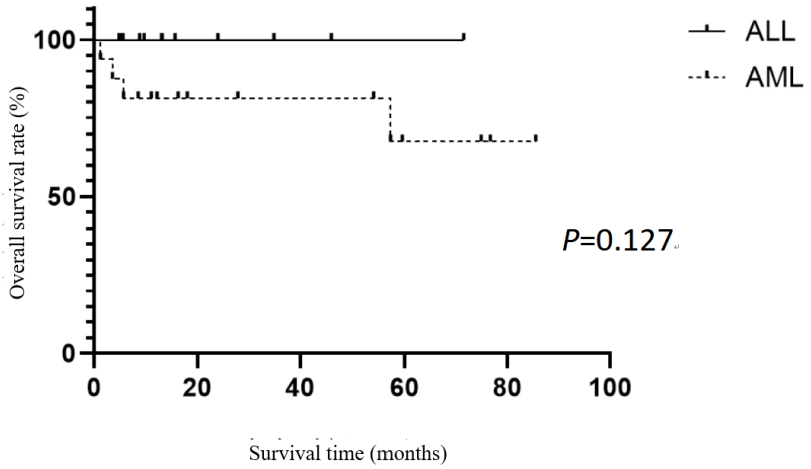

Figure 2 Survival curves of the *NF1* mutation-positive ALL and AML subgroups

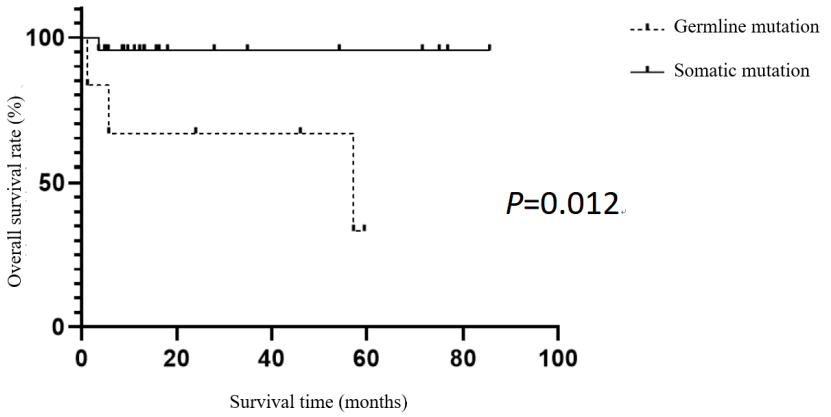

Figure 3 Survival curves of the germline and somatic *NF1* mutation subgroups

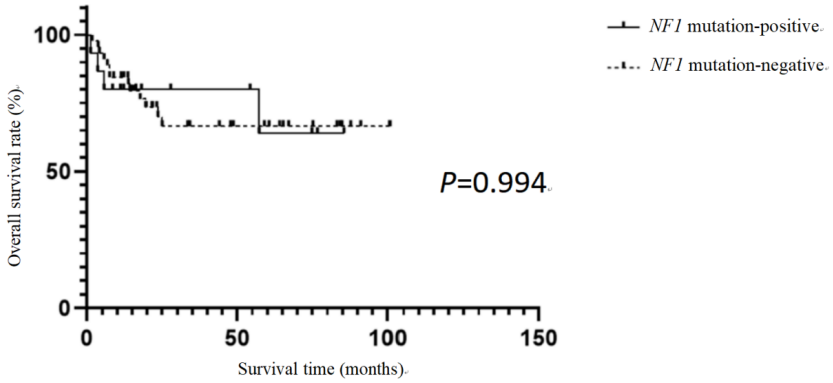

Figure 4 Survival curves of the *NF1* mutation-positive and mutation-negative AML groups

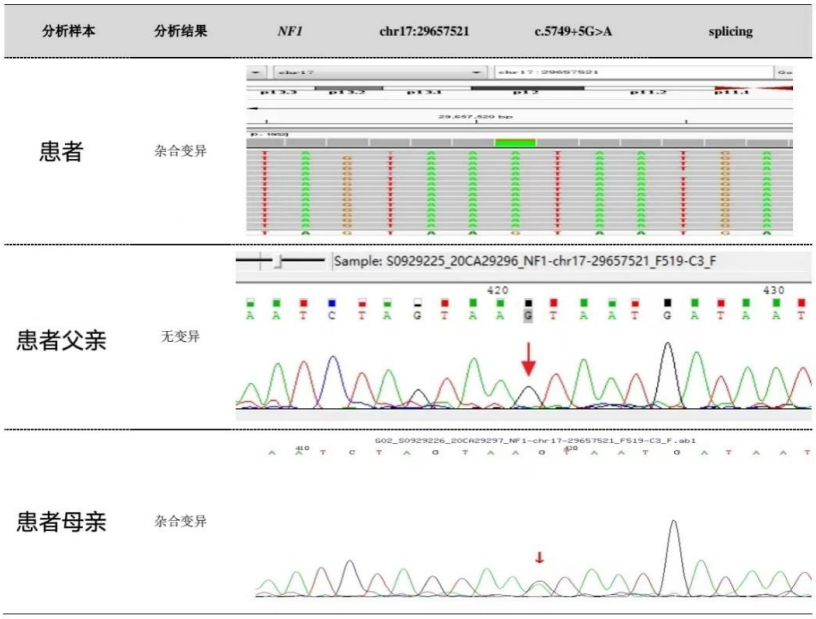

Figure 5 *NF1* gene loci of Patient ALL-2

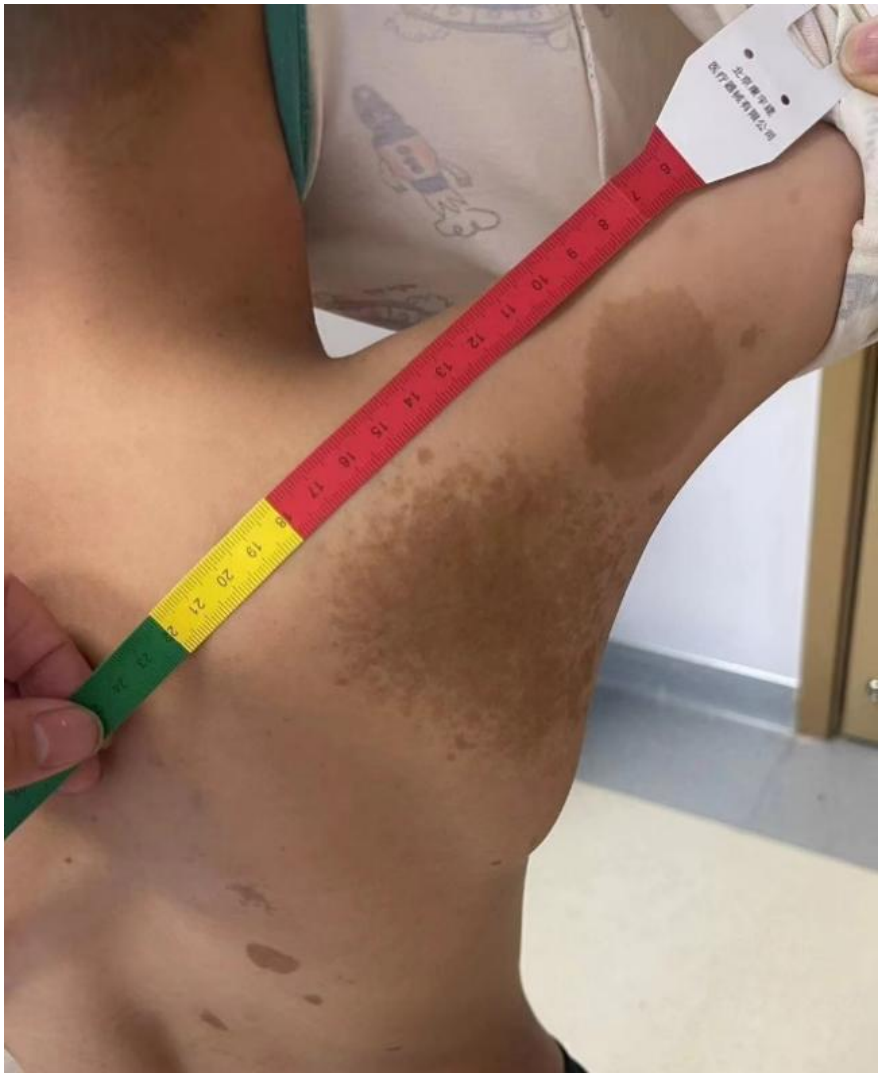

Figure 6 Cafe-au-lait-spot photos of Patient ALL-1

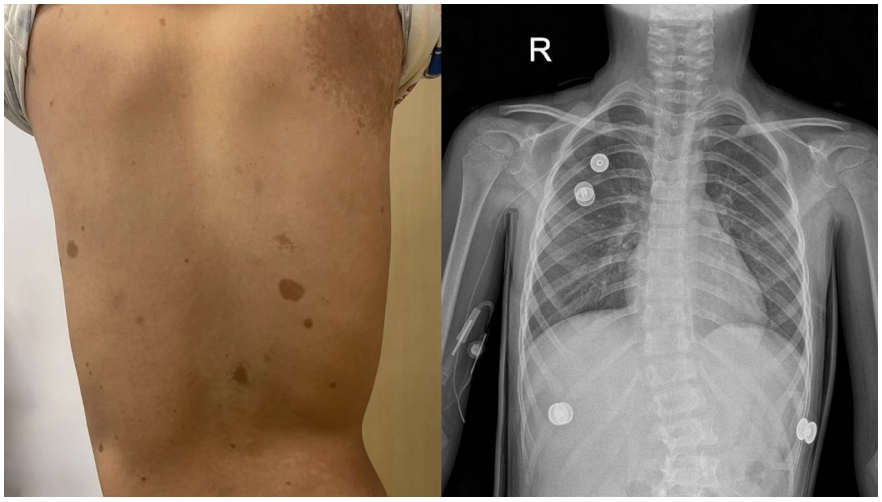

Figure 7 Scoliosis photos and X-ray images of Patient ALL-1

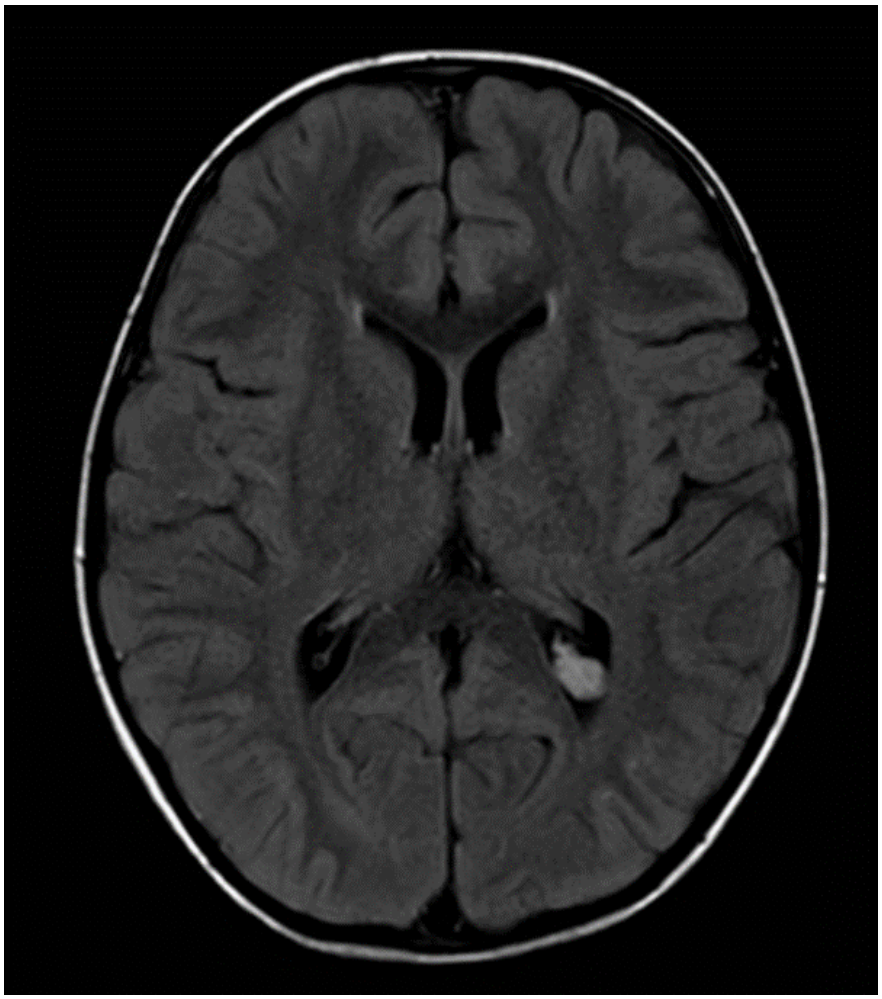

Figure 8 Intracranial involvement image of Patient ALL-1

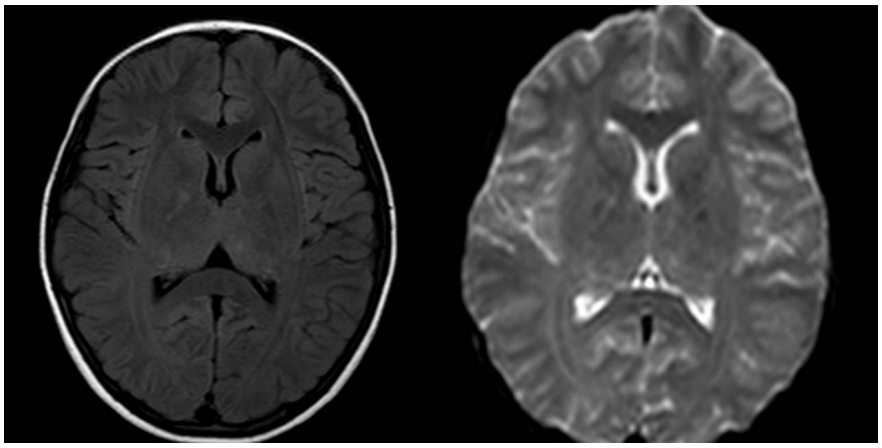

Figure 9 Intracranial involvement images of Patient ALL-3

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`Table.docx` available at https://authorea.com/users/824428/articles/1220743-analysis-of-the-clinical-characteristics-of-28-children-with-nf1-mutation-positive-acute-leukemia-and-literature-review