Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

# Clinical Study of *MAP2K1*-Mutated Langerhans Cell Histiocytosis in Children

**Ying Yang**
Beijing Children's Hospital Capital Medical University https://orcid.org/0000-0001-7111-3641
**Chanjuan Wang**
Beijing Children's Hospital Capital Medical University
**Dong Wang**
Beijing Children's Hospital Capital Medical University
**Lei Cui**
Beijing Children's Hospital Capital Medical University
**Na Li**
Beijing Children's Hospital Capital Medical University
**Hongyun Lian**
Beijing Children's Hospital Capital Medical University
**Honghao Ma**
Beijing Children's Hospital Capital Medical University
**Yunze Zhao**
Beijing Children's Hospital Capital Medical University
**Liping Zhang**
Beijing Children's Hospital Capital Medical University
**Wei Liu**
Children's Hospital Affiliated of Zhengzhou University: Zhengzhou Children's Hospital
**Yizhuo Wang**
Beijing Tongren Hospital
**Wanshui Wu**
Capital Medical University Affiliated Beijing Shijitan Hospital
**Rui Zhang** ( ruizh1973@126.com )
Beijing Children's Hospital Capital Medical University
**Zhigang Li**
Beijing Children's Hospital Capital Medical University
**Tianyou Wang**
Beijing Children's Hospital Capital Medical University

**Research Article**

**Keywords:** MAP2K1, mutation, Langerhans cell histiocytosis, children

**Posted Date:** August 10th, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-773172/v1

**License:** This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

## Abstract

# Purpose

To analyze the genetic and clinical features of children with *MAP2K1*-mutated Langerhans cell histiocytosis (LCH).

# Methods

We compared the clinical features of 37 children with *MAP2K1*-mutated LCH with those of the $BRAF^{V600E}$ mutation group (n = 133) and no known mutation group (n = 59) in the same period.

# Results

We found 13 mutations of the *MAP2K1* gene, which were mainly concentrated at p.53–62 and p.98–103. The most common mutation site was c.172_186del (12/37). Compared with the $BRAF^{V600E}$ mutation group, the patients with *MAP2K1* mutations were mainly characterized by single system multiple bone involvement ($P = 0.022$), with later disease onset ($P = 0.029$) as well as less involvement of risk organs, especially liver ($P = 0.024$). There was no significant difference in clinical features compared with the no known mutation group. The 2-year progression-free survival rate of first-line treatment (ChiCTR1900025783, 07/09/2019) in *MAP2K1*-mutated patients was 65.6% ± 9.5%. The prognosis of patients with lung involvement was poor [HR (95% CI) = 6.312 (1.769–22.526), $P = 0.005$]. More progression or relapses could be found in patients with bony thorax involvement (8/17 vs. 2/20, $P = 0.023$), yet involvements in other sites of bones, such as craniofacial bone involvement (8/26 vs. 2/11, $P = 0.688$) and limb bone involvement (5/12 vs. 5/25, $P = 0.240$), were not correlated to disease progression or relapse.

# Conclusion

The children with *MAP2K1*-mutated LCH have specific clinical features requiring clinical stratification and precise treatment. *MAP2K1*-mutated patients with lung involvement (especially with bony thorax involvement) had poor prognosis.


## 1. Introduction

Langerhans cell histiocytosis (LCH) is a rare clonal disease characterized by the expansion of Langerhans cells derived from myeloid precursors and is more common in children than in adults (Allen et al. 2018). Various clinical manifestations of LCH can be found with many systems involved, such as bone, skin, lung, liver, spleen etc. Among them, the prognosis of patients with risk organ (RO) involvement,

including the liver, spleen and hematopoietic system, is generally poor. The risk of sequelae of the central nervous system (CNS) and disease reactivation is higher in patients with CNS-risk lesions (including craniofacial bone, eye, ear and oral involvement) (Chow et al. 2017).

At present, some studies have found that there is a clear correlation between the MAPK pathway and the pathogenesis of LCH. *BRAF*$^{V600E}$ is the most common gene mutation, identified in more than 60% of LCH patients. *MAP2K1* is another common mutant gene whose mutation frequency varies from 8.6% to 46% (Lian et al. 2019). The activated coding product of the *MAP2K1* gene, MAP2K1 kinase, can activate its downstream ERK kinase, which in turn affects the proliferation, differentiation and other functions of cells (Hayase et al. 2020).

In this study, the gene mutation sites, clinical features and prognosis of patients with the *BRAF*$^{V600E}$ mutation or no known mutation of the MAPK pathway were compared with that of newly treated patients with *MAP2K1*-mutated LCH to provide evidence for the clinical diagnosis and treatment of *MAP2K1*-mutated LCH.

# 2. Materials And Methods

## 2.1 Patients

### 2.1.1 *MAP2K1* mutation group

This was a single-center retrospective case-control study. All children newly diagnosed with *MAP2K1*-mutated LCH who were admitted to Beijing Children's Hospital, Capital Medical University, between July 1, 2017, and October 31, 2020, were enrolled. The inclusion criteria were as follows: (1) the age of disease onset was less than 18 years old; (2) diagnosed with LCH according to the pathological examinations of biopsied tissue: positive staining of the lesion cells with CD1a and/or CD207 (Langerin), or Birbeck granules could be found under an electron microscope; (3) *MAP2K1* mutation was detected in biopsied tissue before treatment; (4) newly treated patients; (5) accepted regular follow-up and evaluation of treatment response on time. The exclusion criteria were as follows: (1) the clinical data at the time of initial diagnosis could not be obtained, and (2) they had received chemotherapy or targeted drug treatment before enrolment or did not follow the treatment regimen of our center.

### 2.1.2 *BRAF*$^{V600E}$ mutation group and no known mutation of the MAPK pathway group

In the same period, all LCH patients with the *BRAF*$^{V600E}$ mutation or no known mutation of the MAPK pathway in biopsied tissue were included as the control groups. Except for the gene mutation, the other inclusion and exclusion criteria were the same as those for the *MAP2K1* mutation group.

## 2.2 Regimen of treatment and follow-up

### 2.2.1 First-line treatment

Before September 2019, all patients diagnosed with LCH were treated with the LCH-III regimen developed by Histiocyte Society (HS) (Histiocyte Society. 2009) except that methotrexate was not used in this regimen, and those diagnosed after September 2019 were treated with the CCHG-LCH-2019 regimen of our center (ChiCTR1900025783), in which patients were treated with the same dosage and course of prednisone as the LCH-2009 regimen combined with vincristine 1.5 mg/$m^2$, vindesine 3 mg/$m^2$ or vinblastine 6 mg/$m^2$. Patients with isolated disease located in functionally critical anatomical sites, such as vertebral lesions with intraspinal soft tissue extension or craniofacial bone involvement with intracranial soft tissue extension, were also treated with systemic therapy (Histiocyte Society. 2009). The treatment response was evaluated at the $6^{th}$, $12^{th}$, $25^{th}$ and $52^{nd}$ weeks during chemotherapy and at the $3^{rd}$ month, $6^{th}$ month, $1^{st}$ year, $2^{nd}$ year and $3^{rd}$ year after drug withdrawal.

### 2.2.2 Second-line treatment

The patients who met one of the following criteria were given second-line treatment: (1) the patients without RO involvement ($RO^-$) who were evaluated as having disease progression (active disease-worse, AD-Worse) at the $6^{th}$ week of first-line chemotherapy; or were evaluated as AD-Worse or intermediate response (active disease-mixed, AD-Mixed) at the $12^{th}$ week of first-line chemotherapy; (2) patients with pulmonary or pituitary involvement who had no improvement of pulmonary and pituitary lesions at the $6^{th}$ or $12^{th}$ week of first-line chemotherapy compared with the previous response assessment; (3) patients with RO involvement ($RO^+$) who had no significant improvement in risk organs (the enlarged part of the liver and spleen reduced less than half, or there was still primary disease-related fever or hematocytopenia), primary disease-related organ dysfunction, or whose overall response was evaluated as AD-Worse or AD-Mixed at the $6^{th}$ and $12^{th}$ week of first-line chemotherapy; and (4) the lesions of the risk organs, CNS risk sites, CNS system or lung were aggravated during maintenance chemotherapy or after drug withdrawal. Before September 2019, patients received HS-LCH salvage treatment if they met one of the criteria above; after September 2019, patients were treated with second-line chemotherapy with CCHG-LCH-2019 (cytarabine +/- cladribine, with the same dosages and courses as HS-LCH salvage treatment). The treatment response was evaluated every 4 courses during second-line chemotherapy. The evaluation after withdrawal was the same as that with first-line chemotherapy.

Each evaluation was conducted according to the Histiocyte Society Evaluation and Treatment Guidelines (2009). Follow-up was continued until December 1, 2020, or until the patient died.

### 2.3 Determination of mutations in biopsied tissue

Genomic DNA was extracted from 10×5 μm unstained sections of formalin-fixed paraffin-embedded tissue using the QIAamp DNA formalin-fixed paraffin-embedded Tissue Kit (Qiagen, Hilden, Germany). Next-generation sequencing of whole exomes was used to detect mutations in the MAPK pathway (using the HiSeq 4000 sequencing platform before 2018 and the Illumina NovaSeq 6000 sequencing platform after 2018). The genomic DNA of oral mucosal cells was taken as a normal control from 61 patients, and the remaining patients had only biopsied tissues sampled.

### 2.4 Statistical Analysis

IBM SPSS (23.0) software was used for the statistical analysis. The measurement of data with normal distribution was expressed as`X±SD, while the measurement of data with nonnormal distribution was expressed as the median (upper and lower quartile). For continuous variables, data were analyzed using the t-test and Mann-Whitney U-test, depending on the data distribution. The count data were expressed as the number of cases or percentages, and the chi-square test was used for the categorical variables. The objective response rate (ORR) after 6 weeks of first-line chemotherapy was defined as the percentage of patients with nonactive disease (NAD) and AD-Better among all patients at the 6-week evaluation of first-line chemotherapy, and the disease control rate (DCR) after 6 weeks of first-line chemotherapy was defined as the percentage of patients with NAD, AD-Better and AD-Stable among all patients at the 6-week evaluation of first-line chemotherapy. Progression-free survival (PFS) was estimated from the date of diagnosis to the date of one of the following events: AD-Mixed or AD-Worse; relapse or reactivation after drug withdrawal or death, whichever came first; the last contact with the patient. Survival curves were estimated using the Kaplan-Meier method, and the log-rank test was used to estimate the differences among the groups. A Cox proportional hazards model was used to analyze the independent prognostic factors of the *MAP2K1* mutation group. A P-value of <0.05 was considered statistically significant.

## 3. Results

### 3.1 Characteristics of *MAP2K1* gene mutation

In this study, thirteen *MAP2K1* mutations were detected in 37 patients with LCH (Table 1), accounting for 11.1% (37/334) of all patients. The most common mutation was c.172_186del (p.Q58_E62del), accounting for approximately 32.4% (12/37); the nucleotide mutations of c.165_179del (p.Q56_V60del), c.166_180del (p.Q56_V60del), c.294_311del (p.I99_K104del), and c.389A>G (p.Y130C) have not been reported in the literature, but the amino acid mutation of p.Q56_V60del caused by the first two nucleotide mutations has been reported (Hayase et al. 2020).

In 81.1% (30/37) of the patients, the mutations were concentrated in amino acids 53-62, which were located in the negative regulatory region of MAP2K1 kinase; in the other 7 cases (18.9%), the mutations were concentrated in amino acids 98-103, which were located in the catalytic core of MAP2K1 kinase. There were two point mutations in one patient, p.I103N and p.Y130C; the latter was not reported.

### 3.2 Clinical and laboratory characteristics of patients with *MAP2K1* mutation

The male-to-female ratio of the 37 patients with a *MAP2K1* mutation was 1.31:1, and the median age at disease onset was 4.56 (1.54, 9.42) years old. The median follow-up time was 17.67 (4.82, 24.92) months. The *BRAF*$^{V600E}$ mutation group and no known mutation of the MAPK pathway group included 133 patients and 59 patients, respectively. There were more boys than girls in the three groups, but there was no significant difference in the sex ratio among the groups (Table 2). The age at disease onset in the

*MAP2K1* mutation group was higher than in the *BRAF*$^{V600E}$ mutation group (Z=-2.179, *P*=0.029, Table 2), but there was no difference compared with the no known mutation group (*P*>0.05, Table 2).

A significantly lower incidence of multisystem (MS) involvement (with or without RO involvement, MS-RO$^{+}$/MS-RO$^{-}$) and a higher incidence of single-system (SS) involvement were found in the *MAP2K1* mutation group than in the *BRAF*$^{V600E}$ mutation group ($\chi^2$=5.892, *P*=0.015). Only one *MAP2K1*-mutated patient (2.7%) had RO involvement, with hemophagocytic lymphohistiocytosis (HLH). The incidence of RO involvement was significantly lower than that of the *BRAF*$^{V600E}$ mutation group ($\chi^2$=7.653, *P*=0.006), especially in liver involvement (2.7% vs. 17.3%, *P*=0.024). In addition, more patients with SS-multiple bone involvement (48.6% vs. 28.6%, *P*=0.022) and fewer patients with skin involvement (2.7% vs. 25.6%, *P*=0.002) were found in the *MAP2K1* mutation group than in the *BRAF*$^{V600E}$ mutation group. There was no significant difference in other clinical features between the two groups (*P*>0.05, Table 2).

Compared with the *BRAF*$^{V600E}$ mutation group, the levels of C-reactive protein (CRP), IFN-γ, IL-10 and IL-6 in the *MAP2K1* mutation group were significantly lower (*P*<0.05). There was no significant difference in other laboratory examinations (*P*>0.05, Table 2).

No significant difference in clinical characteristics or laboratory examinations was found between the *MAP2K1* mutation group and the no known mutation of the MAPK pathway group (*P*>0.05, Table 2).

### 3.3 Analysis of treatment response and prognosis in the *MAP2K1* mutation group

In the *MAP2K1* mutation group, one patient, in whom only the left femur was involved, did not require chemotherapy according to the regimen and got improvement during the follow-up period, while the other 36 patients received first-line chemotherapy after diagnosis. Except for the patient who was not evaluated at the 6$^{th}$ week as planned, the ORR was 48.6% (17/35), and the DCR was 85.7% (30/35) after 6 weeks of first-line chemotherapy. In the same period, the ORR and DCR of the *BRAF*$^{V600E}$ mutation and the no known mutation of the MAPK pathway groups were 52.8% (65/123) and 84.6% (104/123) and 56.4% (31/55) and 94.5% (52/55), respectively. There was no significant difference in ORR and DCR after 6 weeks of first-line chemotherapy among the three groups (*P*>0.05, Table 2).

We compared the prognosis of the three groups after first-line treatment and analyzed the prognosis of the patients who received second-line chemotherapy in the three groups. Although the 2-year PFS of the *BRAF*$^{V600E}$ mutation group was significantly lower than that of the no known mutation of MAPK pathway group (56.2% ± 4.8% vs. 70.1% ± 6.6%, $\chi^2$=4.545, *P*=0.033, Log-rank test), there was no significant difference in 2-year PFS between the *MAP2K1* mutation group and the other two groups after first-line treatment (*P*>0.05, Table 2, Fig. 1A). In terms of the efficacy of second-line chemotherapy, there was no significant difference in 2-year PFS between the *MAP2K1* mutation group and the *BRAF*$^{V600E}$ mutation group, but the PFS of both groups were significantly lower than that of the no known mutation of the MAPK pathway group ($\chi^2$=6.531, *P*=0.011; $\chi^2$=7.409, *P*=0.006; log-rank test, Table 2, Fig. 1B).

### 3.4 Clinical characteristics, treatment response and prognosis between different mutated domains of the *MAP2K1* mutation group

The 37 patients with *MAP2K1* mutations were divided into two groups: the mutation of the negative regulatory domain group (n=30) and the mutation of the catalytic core domain group (n=7). We compared the clinical and laboratory characteristics of the two groups and found that there was no significant difference in clinical features (including age, sex, and organs involved) or laboratory examinations ($P$>0.05). In addition, no significant difference in 2-year PFS was found between the two groups after first-line treatment (61.7% ± 10.7% vs 85.7% ± 13.2%, $\chi^2$=0.528, $P$=0.468, log-rank test). A total of 7 patients in the negative regulatory domain group received second-line treatment, yet three of them had progression or relapse, and the 2-year PFS was 35.7% ± 26.7%. No patient in the catalytic core domain mutation group received second-line chemotherapy. However, there was no statistical difference in the rate of switching to second-line treatment between the two groups (23.3% vs. 0%, $P$=0.306).

### 3.5 Analysis of prognostic factors in the *MAP2K1* mutation group

We further explored the prognostic factors of *MAP2K1*-mutated patients. Except for age at disease onset, which was grouped according to the median, other clinical features were grouped according to presence/absence, and laboratory examinations were grouped according to the normal reference range. Statistical differences in 2-year PFS after first-line treatment among different groups were compared. Univariate analysis showed that MS-LCH, lung involvement and elevated CRP were associated with poor prognosis in the *MAP2K1* mutation group ($P$ < 0.05, Table 3).

The statistically significant factors above were included in the multivariate Cox proportional hazards model. After adjusting for confounding factors, lung involvement was an independent risk factor for poor prognosis in patients with *MAP2K1*-mutated LCH [HR (95% CI) = 6.312 (1.769-22.526), $P$ = 0.005] (Table 3). It was noteworthy that lung involvement was significantly associated with bony thorax involvement (5/17 vs. 0/20, $P$=0.014, Table 4). In addition, more progression or relapses could be found in patients with bony thorax involvement (8/17 vs. 2/20, $P$=0.023), yet involvements in other sites of bones, such as craniofacial bone involvement (8/26 vs. 2/11, $P$=0.688) and limb bone involvement (5/12 vs. 5/25, $P$=0.240), were not correlated to disease progression or relapse. Thus, the independent prognostic significance of lung involvement in *MAP2K1* positive patients may be possibly due to the co-occurrence of bony thorax involvement.

## 4. Discussion

MAP2K1 kinase is an important component of the MAPK pathway, which is activated by upstream RAF kinase and further activates downstream ERK kinase to regulate the growth and proliferation of cells. *MAP2K1* mutations occur in more than 1% of neoplastic diseases, with a higher mutation frequency in diseases such as histiocytic disorders, melanoma and hairy cell leukemia. Studies have shown that point mutations in *MAP2K1* are predominant in melanoma and hairy cell leukemia (Williams et al. 2020), while

deletion mutations are common in LCH (Nann et al. 2019; Chakraborty et al. 2014). The study presented here included the largest number of patients with *MAP2K1*-mutated LCH to date. The results showed that *MAP2K1* mutations were concentrated at p.53-62 and p.98-103, located in the negative regulatory region and catalytic core domain of MAP2K1 kinase, respectively. Therefore, these mutations lead to abnormal activation of MAP2K1 kinase, which further activates ERK kinase and induces LCH (Alayed et al. 2016).

In this study, two point mutations in *MAP2K1* were detected in one patient in the same biopsied tissue. Kamionek *et al* (Kamionek et al. 2016) reported that two point mutations were also found in the same pulmonary nodule in a patient with LCH (p.A26T, p.S24N), but only one point mutation (p.A26T) was found in another pulmonary nodule in the same patient, which may indicate clonal evolution of independent nodules arising from a common progenitor cell. Because single-cell sequencing of the biopsied tissue of this patient was not performed and the mutations of other lesions were not detected, it is not clear whether the two mutations originated from one or two clones.

By comparing the clinical features of patients with *MAP2K1* mutation and the *BRAF*$^{V600E}$ mutation, it was found that *BRAF*$^{V600E}$-mutated LCH occurred earlier with a younger age of disease onset, and most of the patients had RO (especially liver) and skin involvement, while the patients with *MAP2K1* mutation mostly had SS-multiple bone involvement. These results are similar to those of previous studies (Cai et al. 2019).

The differences in the above clinical features are related to the functional characteristics of the *MAP2K1* and *BRAF* mutation sites. The *BRAF*$^{V600E}$ mutation is a RAS-independent kinase activation mutation (class I *BRAF* mutation), causing high-level *BRAF* kinase activity that is not regulated by upstream signals and highly activates downstream ERK kinase (Yaeger et al. 2019). Mutations of amino acids 53-62 of MAP2K1 kinase are located in the negative regulatory region and are RAF-regulated mutations (class II *MAP2K1* mutations). Thus, the activity of MAP2K1 mutated at these sites is regulated by upstream RAF kinase, and the phosphorylation level of ERK kinase is not as high as that which results from the *BRAF*$^{V600E}$ mutation. Mutations of amino acids 98-103 are located in the catalytic core of MAP2K1 kinase and are RAF-independent mutations (class III *MAP2K1* mutations). MAP2K1 mutated at these sites is not regulated by upstream signals and highly activates downstream ERK kinase (Gao et al. 2018). Therefore, patients with the *BRAF*$^{V600E}$ mutation often have multiple system or RO involvement, a stronger inflammatory response and disease activity, such as high levels of CRP, IFN-γ, IL-10 and IL-6, and fever, all of which are related to the poor efficacy of chemotherapy (Kobayashi et al. 2018; Heritier et al. 2016). Unfortunately, due to the influence of the small sample size and short follow-up time, we did not find a difference in prognosis between the *MAP2K1* mutation group and the *BRAF*$^{V600E}$ mutation group or the relationship between the two mutated domains of MAP2K1 kinase and the clinical features and prognosis. In addition, BRAF kinase is located upstream of MAP2K1 kinase, and its substrate also includes MAP2K2 kinase, which may affect the clinical characteristics and prognosis and needs further study. However, it was noted that 7 out of 30 patients with mutations in negative regulatory region had progression during first-line chemotherapy and switched to second-line chemotherapy, moreover three patients had progression or relapse. Although no statistical significance was found between patients with

mutations in the two domains possibly due to small sample size, it still suggested the association of mutations in negative regulatory region with tolerance to chemotherapy. MEK inhibitors may be novel choices for these patients (Gao et al. 2018).

In this study, the clinical and laboratory characteristics, response rate after 6 weeks of first-line treatment and 2-year PFS of first-line treatment in the *MAP2K1* mutation group were not significantly different from those in the no known mutation of the MAPK pathway group, which may be related to the greater heterogeneity of the latter. Thus, although these patients do not carry known mutations of the MAPK pathway, they are likely to carry other unknown genetic variations that induce LCH, which may affect the clinical features and prognosis. Our findings suggest that further identification of new genetic mutations in LCH is of great significance for clinical stratification and precise treatment.

In LCH, somatic and heterogenic mutations within the MAPK pathway affect myeloid progenitor and lead to the activation of kinases, which affects the recruitment of monocytes/macrophages and the differentiation of T cells by regulating the activities of many transcription factors and enzymatic proteins and can ultimately lead to the formation of pulmonary lesions (Radzikowska et al. 2017). There is a clear correlation between lung involvement and tobacco exposure in adolescents and adults with LCH, and more than 2/3 of these patients have respiratory symptoms, such as cough and dyspnea (Rodriguez-Galindo et al. 2020). However, only 10% of children with lung involvement have respiratory symptoms, which are diagnosed mainly by high-resolution computed tomography (Wang et al. 2018). This lack of symptoms may delay diagnosis in the absence of routine radiological monitoring. In this study, the five *MAP2K1*-mutated LCH patients with lung involvement had no history of tobacco exposure, and only 1 of them had a mild cough. The rest were diagnosed by high-resolution computed tomography, manifesting as scattered/bilateral cystic changes or reticulonodular changes. Interestingly, in this study, lung involvement is an independent risk factor for poor prognosis in patients with *MAP2K1* mutations. As lung involvement may occur mostly in patients with bony thorax involvement and more progression or relapses could be found in patients with bony thorax involvement, the involvement of bony thorax may contribute to the poor prognosis of patients with lung involvement, the underlying mechanisms of which remaining to be further studied. In addition, the sample size of this study is small and does not meet the requirements of the events per variable, so the results may not be robust enough, and the reliability needs to be confirmed by further research.

In this study, the efficacy of second-line chemotherapy with cytarabine as the main drug in the *MAP2K1* mutation group was significantly worse than that in the no known mutation of the MAPK pathway group. Three of 7 patients with *MAP2K1*-mutated LCH had progression or relapse during the third course of second-line chemotherapy, 3 months after drug withdrawal, or maintenance chemotherapy. This suggests the application value of MEK-targeted inhibitors such as trametinib in patients with *MAP2K1* mutations, especially in patients with poor response to conventional chemotherapy (Baiocchi et al. 2014). Lorillon *et al* (Lorillon et al. 2018) reported a patient with *MAP2K1*-mutated LCH (p.E102_I103del) with lung involvement. The pulmonary lesion progressed consistently during the treatment with cladribine and improved after treatment with trametinib with good tolerance. Papapanagiotou *et al* (Papapanagiotou et

al. 2017) reported a pituitary-involved patient with *MAP2K1*-mutated LCH (p.E102_I103delinsEI). During treatment with trametinib, the dosage of desmopressin to control diabetes insipidus was reduced, and the relapsed lesion could be quickly reduced by the reuse of trametinib. However, it has been found that p.Q56_G61delinsR, p.C121S/G128D, and p.98_104delinsQ mutations (of which only the first was found in this study) may be related to resistance to trametinib (Nelson et al. 2015; Azorsa et al. 2018), so the efficacy and safety of targeted therapy need to be further studied.

In conclusion, *MAP2K1* mutations are concentrated at amino acids 53-62 and 98-103, and c.172_186del is the most common mutation site in pediatric LCH. Compared with patients with the *BRAF*$^{V600E}$ mutation, patients with the *MAP2K1* mutation were mainly characterized by more SS-multiple bone involvement, less RO and skin involvement and later disease onset. The prognosis of patients with lung involvement is poor and should be given more attention in future studies.

# Abbreviations

| | |
|---|---|
| AD | active disease |
| ALT | alanine aminotransferase |
| AST | aspartate aminotransferase |
| CNS | central nervous system |
| CRP | C-reactive protein |
| CT | computed tomography |
| DCR | disease control rate |
| FFPE | formalin-fixed paraffin-embedded |
| HLH | hemophagocytic lymphohistiocytosis |
| LCH | Langerhans cell histiocytosis |
| MS | multisystem |
| NAD | nonactive disease |
| ORR | objective response rate |
| PFS | progression-free survival |
| PLT | platelet |
| RO | risk organ |
| SS | single-system |
| WBC | white blood cell |

# Declarations


## Acknowledgments

The authors would like to thank all study patients and their family members.

## Funding

This work was supported by the National Natural Science Foundation of China (No.82070202), the Capital's Funds for Health Improvement and Research (No. 2020-2-2093 and 2020-2-1141), the Special Fund of the Pediatric Medical Coordinated Development Center of Beijing Hospitals Authority (No. XTZD20180201), National Science and Technology Key Projects (No. 2017ZX09304029004), and Funding for Reform and Development of Beijing Municipal Health Commission (Genetic and immunological pathogenesis of pediatric histiocytosis and its guiding role in clinical diagnosis and treatment).


## Conflicts of interest

No potential conflicts of interest were disclosed.

## Availability of data and material

The data that support the findings of this study are available on request from the corresponding authors. CCHG-LCH-2019 regimen is available from http://www.chictr.org.cn/index.aspx.

## Code availability

Not applicable

## Authors' contributions

Rui Zhang, Zhigang Li and Tianyou Wang participated in the study concept and design, revising this article critically for important intellectual content, and final approval of the version to be published. Ying Yang, Chanjuan Wang and Dong Wang carried out study design, the acquisition of data, analysis and interpretation of data, and drafted the manuscript. All authors contributed to revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

## Ethics approval

Written informed consent was obtained from the parents or guardians of the children who served as subjects of the study. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (IRB) of Beijing Children's Hospital, Capital Medical University (Number: [2021]-E-035-R). CCHG-LCH-2019 regimen has been registered as a clinical trial (ChiCTR1900025783).

# References


1. Allen CE, Merad M, McClain KL (2018) Langerhans-Cell Histiocytosis. N Engl J Med 379:856–868. https://doi.org/10.1056/NEJMra1607548 ()
2. Chow TW et al ( (2017) Late outcomes in children with Langerhans cell histiocytosis. Arch Dis Child 102:830–835. https://doi.org/10.1136/archdischild-2016-312185 )
3. Lian T, Li C, Wang H (2019) Trametinib in the treatment of multiple malignancies harboring MEK1 mutations. Cancer Treat Rev 81:101907. https://doi.org/10.1016/j.ctrv.2019.101907 ()
4. Hayase T et al ( (2020) Analysis of the BRAF and MAP2K1 mutations in patients with Langerhans cell histiocytosis in Japan. Int J Hematol. https://doi.org/10.1007/s12185-020-02940-8 )
5. Histiocyte Society (2009). LCH Evaluation and Treatment Guidelines [Internet]. Available from https://histiocytesoc.thankyou4caring.org/document.doc?id=290
6. Williams EA et al ( (2020) Melanoma with in-frame deletion of MAP2K1: a distinct molecular subtype of cutaneous melanoma mutually exclusive from BRAF, NRAS, and NF1 mutations. Mod Pathol 33:2397–2406. https://doi.org/10.1038/s41379-020-0581-5 )
7. Nann D et al ( (2019) Pediatric Langerhans cell histiocytosis: the impact of mutational profile on clinical progression and late sequelae. Ann Hematol 98:1617–1626. https://doi.org/10.1007/s00277-019-03678-y )
8. Chakraborty R et al ( (2014) Mutually exclusive recurrent somatic mutations in MAP2K1 and BRAF support a central role for ERK activation in LCH pathogenesis. Blood 124:3007–3015. https://doi.org/10.1182/blood-2014-05-577825 )
9. Alayed K et al ( (2016) BRAF and MAP2K1 mutations in Langerhans cell histiocytosis: a study of 50 cases. Hum Pathol 52:61–67. https://doi.org/10.1016/j.humpath.2015.12.029 )
10. Kamionek M et al ( (2016) Mutually exclusive extracellular signal-regulated kinase pathway mutations are present in different stages of multi-focal pulmonary Langerhans cell histiocytosis supporting clonal nature of the disease. Histopathology 69:499–509. https://doi.org/10.1111/his.12955 )
11. Cai J et al ( (2019) A novel fusion gene PLEKHA6-NTRK3 in langerhans cell histiocytosis. Int J Cancer 144:117–124. https://doi.org/10.1002/ijc.31636 )
12. Yaeger R, Corcoran RB (2019) Targeting Alterations in the RAF-MEK Pathway. Cancer Discov 9:329–341. https://doi.org/10.1158/2159-8290.CD-18-1321 ()
13. Gao Y et al ( (2018) Allele-Specific Mechanisms of Activation of MEK1 Mutants Determine Their Properties. Cancer Discov 8:648–661. https://doi.org/10.1158/2159-8290.CD-17-1452 )
14. Kobayashi T et al ( (2018) Fever and Skin Involvement at Diagnosis Predicting the Intractable Langerhans Cell Histiocytosis: 40 Case-Series in a Single Center. J Pediatr Hematol Oncol 40:e148–e153. https://doi.org/10.1097/MPH.0000000000001080 )
15. Heritier S et al ( (2016) BRAF Mutation Correlates With High-Risk Langerhans Cell Histiocytosis and Increased Resistance to First-Line Therapy. J Clin Oncol 34:3023–3030.

https://doi.org/10.1200/JCO.2015.65.9508 )

16. Radzikowska E (2017) Pulmonary Langerhans' cell histiocytosis in adults. Adv Respir Med 85:277–289. https://doi.org/10.5603/ARM.a2017.0046 ()
17. Rodriguez-Galindo C, Allen CE (2020) Langerhans cell histiocytosis. Blood 135:1319–1331. https://doi.org/10.1182/blood.2019000934 ()
18. Wang D **et al (** (2018) Clinical Research of Pulmonary Langerhans Cell Histiocytosis in Children. Chin Med J (Engl) 131:1793–1798. https://doi.org/10.4103/0366-6999.237400 )
19. Baiocchi RA (2014) Driving toward targeted therapy for LCH. Blood 124:1546–1548. https://doi.org/10.1182/blood-2014-07-587378 ()
20. Lorillon G **et al (** (2018) Response to Trametinib of a Pulmonary Langerhans Cell Histiocytosis Harboring a MAP2K1 Deletion. Am J Respir Crit Care Med 198:675–678. https://doi.org/10.1164/rccm.201802-0275LE )
21. Papapanagiotou M **et al (** (2017) Trametinib-Induced Remission of an MEK1-Mutated Langerhans Cell Histiocytosis. JCO Precis Oncol 1:1–5. https://doi.org/10.1200/PO.16.00070 )
22. Nelson DS **et al (** (2015) MAP2K1 and MAP3K1 mutations in Langerhans cell histiocytosis. Genes Chromosomes Cancer 54:361–368. https://doi.org/10.1002/gcc.22247 )
23. Azorsa DO **et al (** (2018) Clinical resistance associated with a novel MAP2K1 mutation in a patient with Langerhans cell histiocytosis. Pediatr Blood Cancer 65:e27237. https://doi.org/10.1002/pbc.27237 )

# Tables

Table 1
Mutation sites of MAP2K1

| Nucleotide position (number of cases) | Amino acid position |
|---|---|
| Negative regulatory domain | |
| c.159_173del (4) | p.F53_Q58delinsL |
| c.165_179del (1) | p.Q56_V60del |
| c.166_180del (2) | p.Q56_V60del |
| c.167_181del (3) | p.Q56_G61delinsR |
| c.168_182del (2) | p.K57_G61del |
| c.169_183del (1) | p.K57_G61del |
| c.170_184del (5) | p.Q58_E62del |
| c.172_186del (12) | p.Q58_E62del |
| Kinase domain | |
| c.294_311del (1) | p.I99_K104del |
| c.302_307del (4) | p.E102_I103del |
| c.304_309del (1) | p.E102_I103del |
| c.308T > A; c.389A > G (1) | p.I103N; p.Y130C |

Table 2

Comparison of clinical features, laboratory examinations and prognosis among the MAP2K1 mutation group, $BRAF^{V600E}$ mutation group and no known mutation of the MAPK pathway group

| | MAP2K1 mutation group (n = 37) | $BRAF^{V600E}$ mutation group (n = 133) | P value | No known mutation of the MAPK pathway group (n = 59) | P value |
|---|---|---|---|---|---|
| Sex (boy) | 21 (56.8%) | 73 (54.9%) | 0.840[a] | 37 (62.7%) | 0.561[a] |
| Age at disease onset | 4.56 (1.54,9.42) | 2.20 (1.08, 5.09) | 0.029[b] | 3.75 (1.44, 5.68) | 0.297[b] |
| Fever before treatment | 3 (8.1%) | 28 (21.1%) | 0.071[a] | 8 (13.6%) | 0.626[c] |
| Clinical classification | | | | | |
| $MS\text{-}RO^{+}$ LCH | 1 (2.7%) | 30 (22.6%) | 0.014[a] | 1 (1.7%) | 0.726[a] |
| $MS\text{-}RO^{-}$ LCH | 7 (18.9%) | 28 (21.1%) | | 8 (13.6%) | |
| SS LCH | 29 (78.4%) | 75 (56.4%) | | 50 (84.7%) | |
| Liver involvement | 1 (2.7%) | 23 (17.3%) | 0.024[a] | 1 (1.7%) | > 0.999[d] |
| Spleen involvement | 1 (2.7%) | 19 (14.3%) | 0.100[c] | 0 (0%) | 0.385[d] |
| Hematopoietic system involvement (complicated with HLH) | 1 (2.7%) | 12 (9.0%) | 0.352[c] | 0 (0%) | 0.385[d] |
| Lung involvement | 5 (13.5%) | 15 (11.3%) | 0.932[c] | 4 (6.8%) | 0.458[c] |
| Bone involvement | | | | | |
| Single bone | 12 (32.4%) | 43 (32.3%) | 0.428[a] | 22 (37.3%) | 0.198[a] |
| Multiple bone | 24 (64.9%) | 78 (58.6%) | | 30 (50.8%) | |
| SS-multiple bone involvement | 18 (48.6%) | 38 (28.6%) | 0.022[a] | 26 (44.1%) | 0.661[a] |
| Skin involvement | 1 (2.7%) | 34 (25.6%) | 0.002[a] | 8 (13.6%) | 0.157[c] |
| Lymph node involvement | 4 (10.8%) | 9 (6.8%) | 0.639[c] | 2 (3.4%) | 0.201[d] |
| Pituitary involvement | 1 (2.7%) | 9 (6.8%) | 0.593[c] | 1 (1.7%) | > 0.999[d] |

| | MAP2K1 mutation group (n = 37) | $BRAF^{V600E}$ mutation group (n = 133) | P value | No known mutation of the MAPK pathway group (n = 59) | P value |
|---|---|---|---|---|---|
| CNS-risk sites involvement | 15 (40.5%) | 71 (53.4%) | 0.167[a] | 18 (30.5%) | 0.314[a] |
| AST (U/L) | 33.20 (24.95, 40.33) | 31.80 (25.90, 42.30) | 0.701[b] | 32.60 (23.40, 40.60) | 0.987[b] |
| ALT (U/L) | 15.35 (12.83, 21.90) | 14.20 (11.30, 21.90) | 0.561[b] | 13.70 (11.30, 18.90) | 0.178[b] |
| WBC ($10^9$/L) | 8.36 (7.11, 10.05) | 8.66 (6.90, 10.35) | 0.526[b] | 8.73 (7.40, 10.49) | 0.360[b] |
| PLT ($10^9$/L) | 338.5 (300.5, 422.3) | 361.0 (290.0, 456.0) | 0.847[b] | 349.0 (295.0, 424.0) | 0.878[b] |
| CRP (mg/L) | 2.24 (1.24, 7.67) | 8.00 (5.00, 23.00) | < 0.001[b] | 1.95 (1.34, 4.39) | 0.976[b] |
| IFN-γ (pg/ml) | 0.22 (0, 1.27) | 0.90 (0.26, 2.81) | 0.009[b] | 0.30 (0, 1.01) | 0.825[b] |
| TNF-α (pg/ml) | 0.74 (0.04, 9.05) | 1.40 (0.45, 8.32) | 0.299[b] | 0.66 (0, 3.89) | 0.647[b] |
| IL-10 (pg/ml) | 2.43 (1.30, 4.32) | 3.81 (2.44, 7.64) | 0.004[b] | 3.08 (2.06, 5.09) | 0.320[b] |
| IL-6 (pg/ml) | 5.03 (2.37, 56.26) | 24.10 (6.46, 118.40) | 0.003[b] | 17.64 (3.36, 81.53) | 0.158[b] |
| Response rate after 6 weeks of first-line chemotherapy [e] | | | | | |
| ORR | 17 (48.6%) | 65 (52.8%) | 0.655[a] | 31 (56.4%) | 0.470[a] |
| DCR | 30 (85.7%) | 104 (84.6%) | 0.866[a] | 52 (94.5%) | 0.253[d] |
| 2-year PFS of first-line treatment | 65.6%±9.5% | 56.2%±4.8% | 0.269[f] | 70.1%±6.6% | 0.407[f] |
| 2-year PFS of second-line treatment [g] | 35.7% ±26.7% | 45.1%±9.7% | 0.920[f] | 100% | 0.011[f] |

a. Pearson Chi-Square; b. Mann-Whitney U test; c. continuity correction; d. Fisher's exact test; e. the number at the 6th week evaluation of first-line chemotherapy: 35 patients in the *MAP2K1* mutation group, 123 patients in the *BRAF*$^{V600E}$ mutation group, 55 patients in the no known mutation of the MAPK pathway group; f. log-rank test; g. the number of patients treated with second-line chemotherapy: 7

patients in the *MAP2K1* mutation group, 34 patients in the *BRAF*$^{V600E}$ mutation group and 12 patients in the no known mutation of the MAPK pathway group. AST, aspartate transaminase; ALT, alanine transaminase; WBC, white blood cell; PLT, platelet.

Table 3
Analysis of prognostic factors in patients with MAP2K1 mutations

| | Univariate analysis | | Cox regression | | |
|---|---|---|---|---|---|
| | 2-year PFS of first-line chemotherapy | P value | Hazard ratio | 95% confidence interval | P value |
| Age at disease onset | | | | | |
| <4.56 years old | 56.2%±13.2% | 0.263 | | | |
| ≥4.56 years old | 79.1%±11.1% | | | | |
| Fever before treatment | | | | | |
| Presence | 66.7%±27.2% | 0.682 | | | |
| Absence | 65.7%±9.9% | | | | |
| Clinical classification | | | | | |
| MS-LCH | 25.0%±19.8% | 0.011 | - | - | 0.554 |
| SS-LCH | 77.2%±9.2% | | | | |
| Lung involvement | | | | | |
| Presence | 0% | 0.002 | 6.312 | 1.769–22.526 | 0.005 |
| Absence | 76.2%±8.7% | | | | |
| SS-multiple bone involvement | | | | | |
| Presence | 70.2%±11.3% | 0.713 | | | |
| Absence | 62.2%±16.0% | | | | |
| CRP (mg/L) | | | | | |
| Elevated (> 10mg/L) | 33.3%±19.2% | 0.011 | - | - | 0.116 |
| Normal (≤ 10mg/L) | 73.2%±10.1% | | | | |

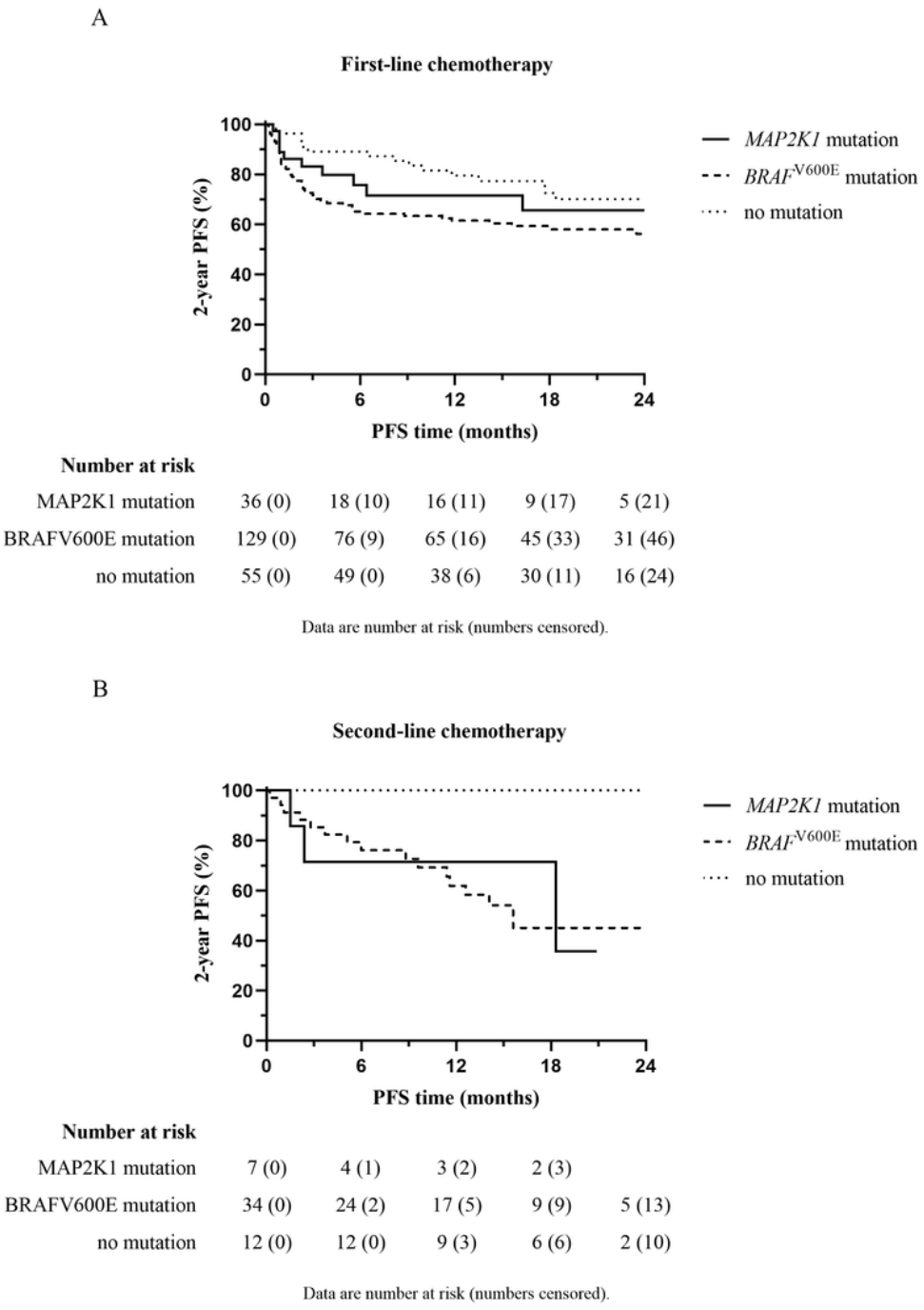
Table 4

Details of MAP2K1-mutated patients with lung involvement

| | Age | Gender | Clinical classification | Lesions | Amino acid position | Sites of progression or relapsed |
|---|---|---|---|---|---|---|
| Patient 1 | 1.48 | girl | MS-RO$^{-}$ | Lung, left parietal bone, C1, C4, C6, T1, T2, T10, L5, S2, left femur | p.F53_Q58delinsL | C6, T1 |
| Patient 2 | 0.20 | boy | MS-RO$^{-}$ | Lung, right parietal bone, maxilla, mandible, frontal bone, occipital bone, sphenoid bone, bilateral temporal bone, sternum, multiple ribs, bilateral clavicle, C1, C2, T11, L2, bilateral scapula, left humerus, skin, bilateral cervical and inguinal lymph nodes | p.Q58_E62del | Parietal bone, occipital bone |
| Patient 3 | 0.73 | boy | MS-RO$^{+}$ | Liver, spleen, hematopoietic system (complicated with HLH), lung, frontal bone, bilateral parietal bone, bilateral temporal bone, occipital bone, sphenoid bone, bilateral orbital wall, right external auditory canal, mandible, right clavicle, right scapula, bilateral humerus, multiple ribs, T9-10, bilateral ilium, left ischium, left pubis, bilateral femur, left fibula, bilateral cervical lymph nodes | p.Q58_E62del | N/A |

| | Age | Gender | Clinical classification | Lesions | Amino acid position | Sites of progression or relapsed |
|---|---|---|---|---|---|---|
| Patient 4 | 0.83 | girl | MS-RO$^{-}$ | Lung, bilateral temporal bone, left maxilla, left zygomatic bone, left orbital wall, sternum, bilateral cervical lymph nodes | p.Q58_E62del | Lung |
| Patient 5 | 2.50 | boy | MS-RO$^{-}$ | Lung, C2, T2-4, T9, T11, L1, L3, left fifth rib, frontal bone, left orbital wall, left sphenoid bone, bilateral parietal bone | p.I103N; p.Y130C | Parietal bone |

# Figures




## Figure 1

Prognosis of patients with LCH with different genetic characteristics. (A) 2-year PFS after first-line treatment (χ2=5.040, P=0.080, log-rank test). (B) 2-year PFS after second-line treatment (χ2=7.553, P=0.023, log-rank test).