Association between clinicopathologic characteristics and BRAF<sup>V600E</sup> expression in Chinese patients with Langerhans cell histiocytosis

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Keywords
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Abstract
Background: The identification of V-raf murine sarcoma viral oncogene homolog B1 (BRAF)<sup>V600E</sup> mutations has been recommended in patients with Langerhans cell histiocytosis (LCH) with difficult diagnosis and failure of first-line treatment. The reported frequencies of BRAF<sup>V600E</sup> mutations vary in Chinese patients with LCH.

Methods: We conducted a retrospective analysis of LCH patients with a definitive pathological diagnosis who were hospitalized between 2013 and 2017. The BRAF<sup>V600E</sup> mutations were detected with the human BRAF<sup>V600E</sup> amplification refractory mutation system-PCR (ARMS-PCR) kit from the collected tissue samples.

Results: This study consisted of 46 male (68.7%) and 21 female (31.3%) patients, with a mean age of 29.1 years (range, 2–76 years). Most were adults (45/67.2%) with the multisystem-LCH (MS-LCH) disease subtype (49/61.3%). The overall frequency of BRAF<sup>V600E</sup> mutations was 22.4% (15 of 67 patients), confirmed by PCR analysis. These mutations were not closely correlated with age (nonadults vs. adults = 5/22.7% vs. 10/22.2%, P = 0.54), gender (female vs. male = 9/19.6% vs. 6/28.6%, P = 0.61), LCH classification type (single system: MS-risk organ+: MS-risk organ− = 3/16.7%: 12/28.6%, P = 0.19) or prognosis (cured: improved/stable: exacerbated: died = 4/44.4%: 19.2%: 20%: 0, P = 0.37). There were 33 patients (49.2%) with lung involvement, and 12 patients (36.3%) underwent lung biopsies; after screening, four patients were diagnosed with solitary pulmonary LCH, all of whom were negative for BRAF<sup>V600E</sup> mutations.

Conclusion: The BRAF<sup>V600E</sup> mutation rate in patients with LCH was lower than those reported in other studies. In addition, BRAF<sup>V600E</sup> mutations might not be correlated with age, gender, LCH classification type or prognosis for Chinese cases.

Key points
Significant findings of the study
The overall frequency of BRAF<sup>V600E</sup> mutations in our study was lower than in some other reports. All four of our pulmonary Langerhans cell histiocytosis (PLCH) cases had negative BRAF<sup>V600E</sup> mutation. BRAF<sup>V600E</sup> mutations might not be correlated with age, gender, LCH classification type or prognosis for Chinese cases.
What this study adds

The BRAFV600E mutation rate in LCH varies.

Introduction

As V-rf murine sarcoma viral oncogene homolog B1 (BRAF)V600E mutations are present in approximately half the samples from patients with Langerhans cell histiocytosis (LCH), and treatment with BRAF inhibitors has been reported to be effective for some patients with LCH, the identification of BRAFV600E mutations is recommended for all patients with LCH with difficult diagnosis and failure of first-line treatment (grade C2).1 The reported frequencies of BRAFV600E mutations in patients with LCH vary among different ethnicities or countries.2 No BRAFV600E mutations were reported in adults with LCH in the study by Tong et al.,3 but in the studies by Wei et al. and Zeng et al.,4,5 the frequencies of BRAFV600E mutations were 17.3% and 15.6% for Chinese adult patients with LCH and 46.4% and 32% for all recruited patients with LCH, respectively. Here, we retrospectively analyzed BRAFV600E mutations and the clinical features of patients with LCH with a positive pathological diagnosis at our hospital over the last five years.

Methods

Patients

A computer-assisted search for patients hospitalized at Peking Union Medical College Hospital from January 2013 to December 2017 identified 167 patients diagnosed with LCH according to the 2016 World Health Organization criteria.1 Most patients were diagnosed by our pathologist in consultation with biopsy/surgical samples from other hospitals. Finally, 67 patients with complete medical records, radiologic images and pathological specimens were retrospectively recruited into this study. All patients were followed-up every one to six months, depending on disease activity and treatment. The mean follow-up period was 36.8 months, ranging from seven to 59 months.

All patients underwent chest CT scans, cerebral magnetic resonance imaging, bone scintigraphy scans, whole body bone plain films, and bone marrow biopsies, and 26 patients underwent 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) scans. The involved sites were defined according to the typical imaging scans and/or the pathological manifestations.

The following information was analyzed: age, sex, clinical manifestations, serological results, radiologic findings, pathological manifestations, treatments and outcomes. Hematoxylin-eosin (HE) staining and immunohistochemical (IHC) staining analysis of CD68, CD1a, CD207, and S100 were performed for all enrolled patients. All glass slides were reviewed and scored by two pathologists (R.E.F. and J.L.), who were blinded to the molecular results. The two pathologists independently came to a consensus diagnosis based on the WHO recommendations for all enrolled patients.6

All patients and/or their relatives provided written informed consent. This study was approved by the ethics committee of Peking Union Medical College Hospital (JS-1127, ZS-1058), in accordance with the Declaration of Helsinki.

DNA extraction

Tumor DNA was extracted from formalin-fixed, paraffin-embedded tissues from our pathological sample bank. Following HE and immunohistochemical staining, the samples with the highest CD1a-positive histiocyte density were selected for further BRAF mutation analysis.

DNA was extracted from the collected tissue samples using the QIAGEN QIAamp DNA FFPE Tissue Kit (154051332, QIAGEN China (Shanghai) Co. Ltd., Shanghai, China), following the manufacturer's protocol.

Detection of BRAFV600E mutations by PCR analysis

The BRAFV600E mutations were detected with the human BRAFV600E ARMS-PCR kit (P216010801Y, Amoy Diagnostics Co. Ltd., Xiamen, China), which has been approved by the China Food and Drug Administration. The extracted DNA quality was evaluated by amplification of a housekeeping gene following the instructions in the HEX channel. PCR was performed on the PCR System 7500 (ABI) system for 47 cycles according to the instructions supplied with the BRAFV600E ARMS-PCR kit. Both negative and positive controls were included in each set of amplifications. The sequencing results were analyzed and interpreted according to the manufacturer’s protocol.

Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS) version 9.0 software package. Quantitative variables are presented as the mean ± standard deviation (SD), and categorical data are presented as the frequency and percentage in the text and figures.

Results

General clinical characteristics

The general clinical characteristics of the 67 enrolled patients are summarized in Table 1. The study group
| Case | Gender | Age (years) | Involved organ/tissue | Subtypes | Biopsy site | BRAF V600E status | Treatment | Outcomes |
|------|--------|-------------|-----------------------|----------|-------------|-------------------|-----------|----------|
| 1    | M      | 43          | Lung                  | SS       | VATS lung biopsy | WT               | Quit smoking | Cured    |
| 2    | M      | 15          | Pituitary             | SS       | surgical pituitary biopsy | WT               | Radiotherapy | Improved |
| 3    | M      | 17          | Maxillary sinus       | SS       | surgical maxillary sinus mass biopsy | WT               | Surgery | Cured    |
| 4    | M      | 22          | Lung, pituitary, mandible, vertebrae | MS-RO− | VATS lung biopsy, mandible biopsy | WT               | Chemotherapy | Relapse |
| 5    | F      | 36          | Pituitary, mandible   | MS-RO−   | Surgical pituitary biopsy | WT               | Radiotherapy | Improved |
| 6    | F      | 2           | Skull, pituitary      | MS-RO−   | Surgical skull biopsy | V600E           | Chemotherapy | Improved |
| 7    | F      | 12          | Lung, liver, pituitary | MS-RO+   | Surgical hepatic biopsy | WT               | Chemotherapy | Improved |
| 8    | M      | 51          | Lung, pituitary       | MS-RO−   | VATS lung biopsy | WT               | Chemotherapy | Improved |
| 9    | M      | 73          | Lymph nodes, skin     | MS-RO−   | Surgical lymph node biopsy | WT               | Chemotherapy | Improved |
| 10   | M      | 12          | Left humerus          | SS       | Surgical bone biopsy | WT               | Surgery, radiotherapy | Improved |
| 11   | M      | 55          | Lung, oral mucosa, lymph node | MS-RO− | Surgical mucosa biopsy | V600E           | Chemotherapy | Improved |
| 12   | M      | 37          | Femur                 | SS       | Surgical bone biopsy | WT               | Surgery | Cured    |
| 13   | M      | 26          | Lung, vertebrae, liver, pituitary | MS-RO− | Surgical bone and liver biopsy | WT               | Chemotherapy, liver transplantation | Relapse |
| 14   | M      | 15          | Lung, pituitary, thyroid | MS-RO− | Surgical thyroid resection | WT               | Surgery, chemotherapy | Improved |
| 15   | M      | 39          | Pituitary             | SS       | Surgical pituitary biopsy | WT               | Radiotherapy, surgery | Improved |
| 16   | M      | 13          | Pituitary             | SS       | Surgical pituitary biopsy | WT               | Surgery | Improved |
| 17   | M      | 37          | Lung, thyroid         | MS-RO−   | Thyroid fine needle biopsy | WT               | Chemotherapy | Improved |
| 18   | M      | 5           | Vertebrae             | SS       | Surgical bone biopsy | V600E           | Surgery | Cured    |
| 19   | F      | 6           | Vertebrae             | SS       | Surgical bone biopsy | V600E           | Surgery | Cured    |
| 20   | F      | 9           | Pituitary             | SS       | Surgical pituitary biopsy | WT               | Surgery, radiotherapy | Improved |
| 21   | F      | 14          | Pituitary, vertebrae, skin | MS-RO− | Surgical bone biopsy | WT               | Surgery, chemotherapy | Improved |
| 22   | F      | 25          | Pituitary             | SS       | Surgical pituitary biopsy | WT               | Surgery, radiotherapy | Improved |
| 23   | F      | 43          | Skull                 | SS       | Surgical bone biopsy | WT               | Surgery, chemotherapy | Improved |
| 24   | M      | 76          | Multiple lymph nodes  | MS-RO−   | Surgical lymph node biopsy | WT               | Chemotherapy | Exacerbation |
| 25   | M      | 42          | Lung, mandible        | MS-RO−   | Surgical mandible biopsy | V600E           | Chemotherapy | Improved |
| 26   | M      | 17          | Skull, femur, pituitary | MS-RO− | Surgical bone biopsy | V600E           | Chemotherapy | Improved |
| 27   | M      | 12          | Skull, femur, vertebrae, pituitary | MS-RO− | Surgical bone biopsy | WT               | Chemotherapy | Improved |
| 28   | M      | 43          | Pituitary, vertebrae, tibia | MS-RO− | Surgical bone biopsy | V600E           | Chemotherapy | Improved |
Table 1 Continued

| Case | Gender | Age (years) | Involved organ/tissue | Subtypes | Biopsy site | BRAF\textsuperscript{V600E} status | Treatment | Outcomes |
|------|--------|-------------|-----------------------|----------|-------------|-------------------------------|-----------|----------|
| 29   | M      | 12          | Vertebrae             | SS       | Surgical bone biopsy | WT              | Surgery, radiotherapy   | Improved |
| 30   | F      | 49          | Lung, pituitary, liver, lymph node | MS-RO\textsuperscript{*} | VATS lung biopsy, skin biopsy | WT | Chemotherapy | Improved |
| 31   | M      | 28          | Lung, pituitary, skin | MS-RO\textsuperscript{*} | VATS lung biopsy | WT | Chemotherapy | Improved |
| 32   | F      | 17          | Lung, pituitary, skin, lymph node | MS-RO\textsuperscript{*} | Skin biopsy | WT | Chemotherapy | Relapse |
| 33   | M      | 21          | Lung, thyroid, pituitary, liver, lymph node | MS-RO\textsuperscript{*} | Thyroid fine needle biopsy | WT | Chemotherapy, thyroid radiotherapy | Improved |
| 34   | M      | 15          | Pituitary, liver, lymph node | MS-RO\textsuperscript{*} | Liver fine needle biopsy | WT | Chemotherapy | Improved |
| 35   | M      | 8           | Lung, thyroid, pituitary, lymph node | MS-RO\textsuperscript{*} | Thyroid fine needle biopsy | WT | Chemotherapy | Improved |
| 36   | M      | 24          | Lung, skull, pituitary, skin, stomach | MS-RO\textsuperscript{*} | Stomach and skin biopsy | V600E | Chemotherapy | Exacerbation |
| 37   | F      | 14          | Skull, pituitary      | MS-RO\textsuperscript{*} | Surgical bone biopsy | WT | Surgery, chemotherapy | Improved |
| 38   | M      | 20          | Pituitary, skin       | MS-RO\textsuperscript{*} | Skin biopsy | WT | Surgery | Improved |
| 39   | M      | 31          | Lung, skin, muscle, lymph node | MS-RO\textsuperscript{*} | Surgical muscle biopsy | WT | Chemotherapy | Improved |
| 40   | M      | 7           | Skull, radius, vertebrae, pituitary | MS-RO\textsuperscript{*} | Surgical bone biopsy | WT | Surgery, chemotherapy | Improved |
| 41   | M      | 39          | Skull, vertebrae, lung | MS-RO\textsuperscript{*} | Surgical bone biopsy | WT | Chemotherapy | Improved |
| 42   | F      | 32          | Skin, lymph node, liver, Lung, pituitary | MS-RO\textsuperscript{*} | Lymph node biopsy | WT | Chemotherapy | Improved |
| 43   | M      | 14          | Lung, pituitary       | MS-RO\textsuperscript{*} | Surgical pituitary biopsy | WT | Chemotherapy | Improved |
| 44   | F      | 23          | Skull                  | MS-RO\textsuperscript{*} | Surgical bone biopsy | V600E | Surgery | Cured |
| 45   | F      | 52          | Rib                    | MS-RO\textsuperscript{*} | Surgical bone biopsy | V600E | Surgery | Cured |
| 46   | M      | 38          | Lung, skin, pituitary, thyroid | MS-RO\textsuperscript{*} | Thyroid fine needle biopsy | WT | Chemotherapy | Relapse |
| 47   | M      | 28          | Lung, alveolar bone   | MS-RO\textsuperscript{*} | VATS lung biopsy, bone biopsy | WT | — | Died |
| 48   | F      | 12          | Lung                   | MS-RO\textsuperscript{*} | Surgical pituitary biopsy | WT | Chemotherapy | Improved |
| 49   | M      | 42          | Lung                   | SS        | VATS lung biopsy | WT | Quit smoking | Cured |
| 50   | F      | 32          | Lung, pituitary        | MS-RO\textsuperscript{*} | VATS lung biopsy | WT | — | Stable |
| 51   | F      | 25          | Skull, vertebrae, skin | MS-RO\textsuperscript{*} | Skin biopsy | WT | Chemotherapy | Improved |
| 52   | M      | 16          | Lung, pituitary        | MS-RO\textsuperscript{*} | Transbronchial lung biopsy | V600E | Chemotherapy | Improved |
| 53   | M      | 23          | Lung, skull, pituitary, thyroid | MS-RO\textsuperscript{*} | Thyroid fine needle biopsy | WT | Chemotherapy | Improved |
| 54   | M      | 54          | Vertebrae, pituitary   | MS-RO\textsuperscript{*} | Surgical bone biopsy | WT | Chemotherapy | Improved |
| 55   | F      | 47          | Lung, femur, pituitary, thyroid | MS-RO\textsuperscript{*} | Surgical thyroid resection | V600E | Surgery, chemotherapy | Improved |
| 56   | M      | 31          | Lung, skull, pituitary, skin | MS-RO\textsuperscript{*} | Skin biopsy | V600E | Chemotherapy | Improved |
| 57   | F      | 31          | Femur, skull, vertebrae, rib, oral mucosa, pituitary | MS-RO\textsuperscript{*} | Oral mucosa biopsy | V600E | Chemotherapy | Improved |
| 58   | M      | 25          | Pituitary              | SS        | Surgical pituitary biopsy | WT | Surgery, radiotherapy | Improved |
| 59   | F      | 24          | Lung, thyroid, pituitary | MS-RO\textsuperscript{*} | Surgical bone biopsy | WT | Chemotherapy | Improved |
consisted of 46 male (68.7%) and 21 female (31.3%) patients, with a mean age of 29.1 years (range, 2–76 years). Most were adults, with 22 (32.8%) younger than 18 years and two (3.0%) older than 60 years. According to the 2016 WHO classification criteria for LCH, 18 patients (28.7%) had Single system-LCH (SS-LCH) type, seven (10.4%) had multisystem-LCH (MS-LCH) risk organ (RO)+ type and 42 (62.9%) had MS-LCH-RO− type.

The BRAFV600E molecular analysis was successful for all 67 enrolled patients. The overall frequency of BRAFV600E mutations was 22.4% (15 of 67 patients) according to the PCR analysis. The main involved sites included the pituitary gland (42/62.7%), lung (33/49.3%), skull (16/23.9%), vertebrae (14/20.9%), lymph nodes (11/16.4%), skin (11/16.4%), thyroid gland (8/11.9%), limb bone (8/11.9%), and liver (7/10.4%). Most patients (50/74.6%) improved

| Case | Gender | Age (years) | Involved organ/tissue | Subtypes | Biopsy site | BRAFV600E status | Treatment | Outcomes |
|------|--------|-------------|-----------------------|----------|-------------|-----------------|-----------|----------|
| 60   | M      | 57          | Lung, liver, lymph nodes | MS-RO+ | Surgical lymph node biopsy | WT | Chemotherapy | Improved |
| 61   | M      | 24          | Skull, vertebrae, pituitary | MS-RO− | Surgical bone biopsy | WT | Chemotherapy | Improved |
| 62   | M      | 27          | Lung, pituitary | MS-RO− | VATS lung biopsy | WT | Chemotherapy | Improved |
| 63   | M      | 47          | Lung | SS | VATS lung biopsy | WT | Quit smoking | Cured |
| 64   | M      | 47          | Skull | SS | Surgical bone biopsy | V600E | Chemotherapy | Improved |
| 65   | M      | 29          | Skull, pituitary | MS-RO− | Surgical bone biopsy | WT | Chemotherapy | Improved |
| 66   | M      | 35          | Lung | SS | VATS lung biopsy | WT | Quit smoking | Improved |
| 67   | F      | 48          | Lung, pituitary | MS-RO− | Surgical pituitary biopsy | WT | Chemotherapy | Improved |

Table 1: The clinical characteristics of patients with different BRAFV600E mutations

F, female; M, male; MS, multisystem; RO, risk organ; SS, single system; VATS, video-assisted thoracic surgery; WT, wild type.
after surgery, chemotherapy and/or radiotherapy. Nine patients (13.4%) were cured after surgical resection (6/9%) or after quitting smoking (3/4.5%). Four patients (4/6%) relapsed, and two patients (2/3%) experienced exacerbation of the disease, although they underwent chemotherapy. One patient (1/1.5%) was stable without treatment, and one (1/1.5%) died of respiratory failure.

Clinical characteristics of patients with different BRAFV600E statuses

The clinical characteristics of patients with different BRAFV600E mutation statuses are shown in Table 2. These mutations were not closely correlated with age (nonadults vs. adults = 5/22.7% vs. 10/22.2%, \( P = 1.00 \)), gender (female vs. male = 9/19.6% vs. 6/28.6%, \( P = 0.61 \)), LCH classification type (SS: MS-RO+: MS-RO− = 3/16.7%: 12/28.6%: 0, \( P = 0.19 \)), or prognosis (cured/improved/stable: exacerbated: died = 4/44.4%: 19.2%: 20%: 0, \( P = 0.37 \)). As the pituitary gland, lung and thyroid and patients with other involved sites. In addition, all the LCH patients with liver involvement were negative for BRAFV600E mutations. There were no correlations between BRAFV600E mutations status and the prognosis.

Patients with LCH with pulmonary involvement

Pulmonary involvement is frequently present in systemic forms of LCH. In addition, pulmonary LCH (PLCH) is restricted to the lungs. Among the 67 patients with LCH in this study, there were 33 patients (49.2%) who had lung involvement. In addition, 12 patients (36.3%) underwent lung biopsies. After multiple screening tests, only four patients (12.1%) were diagnosed with PLCH, and none were positive for BRAFV600E mutations. The clinical characteristics of patients with or without lung involvement LCH are shown in Table 3. Lung involvement was more common in patients with the MS-LCH subtype (\( P = 0.006 \)), and patients with thyroid gland involvement (100% vs. 36.2%, \( P = 0.0073 \)). After either surgical or fine needle biopsy, eight patients were diagnosed with LCH with thyroid involvement. The imaging studies of these eight patients showed classic lung shadows indicating diffuse cysts with bizarre shapes.
In our cohort, most of the patients with LCH with lung involvement were patients with systemic LCH. All four patients with PLCH were smokers. Three were cured after quitting smoking and avoiding secondhand smoke for four to six months, without medications. The fourth patient with PLCH was diagnosed three months prior to this manuscript being written, and his lung infiltrations improved after quitting smoking, without taking medication. Although all patients with PLCH had good prognoses, there was no difference in prognosis between patients with LCH with and without lung involvement.

**Discussion**

The BRAF gene is located on chromosome 7q34, and is a member of the RAF kinase family. Although mutations of BRAF have been identified in a large number of solid tumors, it was first reported by Badalian-Very et al. in 2010 that BRAFV600E expression was identified in 57% of samples from patients with LCH. Following this study, there were several studies that focused on the mitogen-activated protein kinase (MAPK) pathway signal transmission, including BRAFV600E, mitogen-activated protein kinase 1 (MAP2K1) and NRAS. It was reported that the activating BRAFV600E mutation rate ranged from 35% to 60% in different studies using PCR or immunohistochemistry (IHC) staining in patients with LCH. According to the review by Selway et al., the BRAFV600E mutation rate was 51.13% in 397 patients with LCH. The mutation rate varies across different studies and different races. The BRAFV600E mutation rates reported in Chinese patients with LCH were 0 in the study by Tong et al., 56% in the study by Wei et al., and 22.5% in our study.

The patient age distribution, involved sites, stage, and different detection tests might influence the BRAFV600E subtype. Badalian-Very et al. and Héritier et al. reported that younger LCH patients tended to be positive for BRAFV600E mutations, and most patients with BRAFV600E mutations in the study by Wei et al. had bone involvement or the MS-LCH subtype; in the study by Tong et al., skin and lung were the most commonly involved sites, and 77.8% of the patients had the LCH-SS subtype. However, according to the meta-analysis of existing LCH BRAFV600E studies by Selway et al., there was no difference in the presence of BRAFV600E mutations between adults and children, between those with the SS-LCH and MS-LCH subtypes, and those with different involved sites. In our cohort, BRAFV600E mutations were not correlated with age, gender, LCH classification type, or prognosis. According to the meta-analysis by Selway et al. and the study by Héritier et al. on the BRAFV600E mutation in patients with LCH, the rate of the BRAFV600E mutation was increased in patients who experienced involvement of higher risk organs, such as the liver and spleen. In the study by Selway et al., although 75% of the biopsied liver samples were positive for BRAFV600E mutations, none of our patients with LCH with liver involvement had BRAFV600E mutations.

The lung is a commonly involved site in patients with LCH, and PLCH has been commonly reported in previous studies. However, only four patients (6%) in our cohort were diagnosed with solitary PLCH. Most of the previous studies did not show the completed screening tests for the enrolled patients with LCH. In our study, all 67 patients underwent strict screening tests including chest CTs, cranial MRIs, bone scans and bone marrow biopsies, with the exception of detailed serum tests. In addition, 26 patients underwent 18FDG-PET-CT scans, which is a useful and sensitive tool for the identification of active lesions, the stratification of disease stages, and the monitoring of a therapeutic response in patients with LCH. Most of our patients with lung involvement were diagnosed with the MS-LCH subtype after these screening tests, and only four patients were diagnosed with solitary PLCH.

Solitary PLCH is a smoking-related non-neoplastic disease, and the study by Yousem et al. failed to show clonality in patients with PLCH. However, some studies reported BRAFV600E mutation rates ranging from 28% to 89% in patients with PLCH. In our study, all four PLCH patients smoked, and all were negative for BRAFV600E mutations. Three of them were cured, and the fourth patient experienced improvement after quitting smoking and avoiding secondhand smoke, without medications. The natural history of PLCH varied widely. Some patients with PLCH may remit or stabilize after quitting smoking. However, others may develop pulmonary fibrosis, pulmonary hypertension, and respiratory failure, even after chemotherapy. There has been no further analysis of the BRAFV600E mutation status for those self-cured PLCH patients in most studies.

There were several limitations in our study. First, all enrolled patients had a definitive diagnosis of LCH and had complete clinical records, radiological images and pathological specimen, which could cause a selection bias. The BRAFV600E mutations in our study were detected by PCR analysis, and the DNA was extracted from formalin-fixed paraffin-embedded tissues, which could minimize the sensitivity. Second, not all enrolled patients underwent lung biopsies. There were 33 patients (49.2%) who had lung involvement, but only 12 patients (36.3%) underwent lung biopsies. LCH was diagnosed with extrapulmonary tissue biopsies, even for some cases with diffuse pulmonary infiltrations. However, for these cases, the clinical characters and extrapulmonary pathological manifestations were sufficient for the diagnosis of LCH, and to avoid excessive damage, lung biopsies were not performed. Third, peripheral...
blood BRAF\(^{V600E}\) mutations for LCH cases had been previously reported, and the mutation status might be associated with the disease burden and therapy response.\(^{23–26}\) As our study was retrospective, we were unable to obtain samples from all enrolled cases, and most of them were treated when we connect with them. Peripheral blood BRAF\(^{V600E}\) mutation status will be analyzed in our future prospective studies, especially for MS-LCH cases.

In conclusion, the BRAF\(^{V600E}\) mutation rate in patients with LCH was lower than in some reported studies. In addition, BRAF\(^{V600E}\) mutations might not correlated with age, gender, LCH classification type, or prognosis in our patients with LCH.

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Disclosure

The authors do not have any competing interests and/or bias with regard to this publication.

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