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High Prevalence of *BRAF V600E* Mutations in Langerhans Cell Histiocytosis of Head and Neck in Chinese Patients

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Abstract

Langerhans cell histiocytosis (LCH) is characterized by clonal proliferation of Langerhans cells and has been classified as a hematolymphoid tumor. *BRAF V600E* mutation was found to be frequent in LCH; however, it has also been reported that Asia patients with LCH tend to show a lower rate of *BRAF V600E* mutation. In this study, we found LCH from the head and neck region often involved bone especially the posterior of the mandible and presented a high prevalence of *BRAF V600E* mutation in Chinese patients. Our findings also showed immunohistochemical detection correlated very well to DNA sequencing of BRAF alterations, which may be useful in the diagnosis of LCH, especially in cases with a low proportion of Langerhans cells, and BRAF inhibitors might be a treatment option for patients with LCH harboring *BRAF V600E* mutation.

Keywords

Langerhans cell histiocytosis, *BRAF V600E* mutation, head and neck Sanger sequencing, immunohistochemistry

Introduction

Langerhans cell histiocytosis (LCH) is a well-known but relatively rare disease, characterized by clonal proliferation of Langerhans cells, which are the primary antigen-presenting cells mainly existing in the skin and mucosal surfaces.[1] These cells express S100, CD1α, and CD207 (langerin).[2,3] This disease presents a broad spectrum of clinical features ranging from self-limited, unifocal diseases to rapidly progressive, disseminated even lethal types, which always involve an inflammatory component. It has been classified into 3 independent syndromes: eosinophilic granuloma, characterized by unifocal or multifocal lytic bone lesions; Hand-Schuller-Christian disease with a typical clinical triad of bone defects, exophthalmos, and diabetes insipidus; and Letterer-Siwe disease, identified by the disseminated involvement of multiple organ systems with the worst prognosis occurring mostly in children younger than 3 years of age.[4]

Head and neck regions are commonly involved sites of LCHs with a proportion of 60% to 80%,[5-7] which include bone as the most favorable site with involvement of the skull vault, temporal bone, orbit, or jaw bone.[8] Paranasal sinus involvement is rare.

The etiology of this disease remains unclear. There has been a prolonged, ongoing debate about whether LCH is a neoplasm or a reaction. Approximately 20 years earlier, the discovery of clonal proliferation of Langerhans cells in LCH provided evidence of its neoplastic process.[9] Currently, the World Health Organization has officially classified the condition as a hematolymphoid tumor. The B-Raf proto-oncogene, serine/threonine kinase gene (*BRAF*), located on chromosome 7q34, serves as a serine/threonine kinase and plays a pivotal role in activating the mitogen-activated protein kinase (MAPK) pathway, regulating a series of cellular functions including cell survival, proliferation, differentiation, and apoptosis.[10] The *BRAF V600E* mutation was found to be a frequent genetic alteration in papillary thyroid cancer, ameloblastomas, and LCH.

In this study, we investigated the *BRAF V600E* mutation in LCH originating from the head and neck regions using Sanger sequencing and immunohistochemistry and analyzed the relationship of this gene alteration with the clinicopathological features and prognosis of LCHs.

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Materials and Methods

Patients and Tissue Samples

Cases diagnosed as LCH were reviewed from the files of Peking University School and Hospital of Stomatology during 2005 to 2017, and 36 cases were confirmed and selected for this study. Patients' follow-up data were obtained by clinical interviews or reviewing the medical records after surgery. The corresponding 36 paraffin-embedded samples were obtained from the archives of the Department of Oral Pathology, Peking University School of Stomatology, following the approval of the University Institutional Ethics Committee.

DNA Extraction and Polymerase Chain Reaction

DNA was isolated from Langerhans cell–rich areas of unstained, 10-μm paraffin slides using standard protocols of the TIANamp FFPE DNA kit (DP331, TIANGEN). Polymerase chain reaction (PCR) was performed following a touchdown protocol as follows: 94°C for 5 minutes, followed by 22 cycles at 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 30 seconds, decreasing by 0.5°C every cycle, then 16 regular cycles at a fixed annealing temperature of 50°C. The final extension was 7 minutes at 72°C. Specific primers were used to amplify a 230 bp base sequence in exon 15 of the *BRAF* gene: sense primer, 5′-TGCTTGCTCTGATAGGAAAATG-3′, and antisense primer, 5′-CCACAAAATGGATCCAGACA-3′. The PCR products were analyzed using agarose gel electrophoresis and then sent to BGI Tech Solutions Co, Ltd (Beijing) for sequencing.

Immunohistochemistry

Four-micrometer-thick serial sections were cut, mounted on poly-L-lysine–coated slides, deparaffinized in xylene, and sequentially rehydrated using a graded ethanol series. Endogenous peroxidase activity was quenched by incubation with fresh 3% hydrogen peroxide (H_2O_2) for 10 minutes at room temperature, and then, antigen retrieval was performed with 10% ethylenediaminetetraacetic acid buffer (pH 8.0). Immunostaining was performed by incubating tissue sections with antibodies specific for CD1α, S100, and CD207 (ready-to-use reagent; ZSGD-BIO, Beijing, China) overnight at 4°C followed by a 30-minute incubation with the secondary antibodies. The immunocomplexes were visualized using liquid 3,3′-diaminobenzidine (DAB) plus substrate plus chromogen system (Dako, Glostrup, Denmark). The sections were lightly counterstained with hematoxylin and mounted.

In addition, to evaluate the presence of BRAF protein in LCH, we performed immunohistochemistry using specific mouse monoclonal antibody, VE1 (Ventana Medical Systems, Inc, Oro Valley. AZ; Cat. Number 790-4855). Immunostaining was performed on 4-μm sections including cell conditioning with Ventana (Ventana Medical Systems) for 80 minutes, followed by preprimary peroxidase inhibition, sequential incubation with the primary antibody (12 μg/mL) for 32 minutes at 37°C, OptiView HQ Universal Linker for 12 minutes, and OptiView HRP Multimer for 12 minutes. Subsequently, the tissues were counterstained with hematoxylin II (Ventana Medical System, Inc) and Bluing Reagent (Ventana Medical System, Inc) for 4 minutes. The primary antibody was replaced with phosphate-buffered saline in the negative control sections. Colorectal cancer tissue sections were used as positive controls. The immunohistochemistry results were analyzed by 2 independent pathologists who were blinded to the information of each patient. As previously reported,[11] a positive result was defined as cytoplasmic staining apparent at ×400 magnification and the percentage of positive cells was up to 10%.

Statistical Analysis

All statistical analyses were performed using the IBM statistical package for the social sciences (SPSS) statistical software version 23. Categorical variables were compared by using Fisher's exact test. Differences at $P < .050$ were considered significant.

Results

Clinicopathologic Features

There were 36 LCHs in the head and neck, and 31 cases involved the bone with 22 in the mandible, 2 in the maxillary, 1 in temporal bone, and 6 in both the mandible and maxillary, among which 2 also involved the lymph nodes. In 28 cases with mandible involvement, 13 were located in the ramus of the mandible, 6 in the angle of the mandible, and 9 in the body of the mandible. Five cases involved the soft tissue with 2 lesions in the gingiva, and 1 each in the floor of the mouth, the tempus, and the parotid gland. Furthermore, 22 cases were unifocal lesions, and 13 cases showed multifocal lesions with 1 case uncertain. Patients' age ranged from 20 days to 56 years with a mean age of 16.5 years. In addition, 23 and 13 cases were male and female patients, respectively (as shown in Table 1).

The radiographic examination revealed 2 types of lesions: intraosseous and lesions adjacent to involved mucosa including the alveolar process, tempus, and palate. Intraosseous lesions usually presented solitary irregular or oval bone destruction. The periphery of the lesions varied

Table 1. Clinicopathologic Features and *BRAF V600E* Genotype of 36 Langerhans Cell Histiocytosis.

				BRAF Genotype			
Case	Age (Years)	Sex	Site	Extension	PCR/Seq	IHC	Status (Months)
1	1	Male	Bone/Man	Unifocal	WT	−	Rec/1 NED/48
2	1	Male	Bone/Man	Unifocal	V600E	+	NED/44
3	4	Female	Bone/Man	Unifocal	V600E	+	NED/42
4	45	Female	Bone/Man	Multifocal	WT		Rec/12 NED/36
5	3	Male	Bone/tempus	Unifocal	WT	−	NED/14
6	31	Male	Bone/Man, Max	Multifocal	V600E	+	NA
7	2	Male	Bone/Man	Unifocal	V600E	+	NED/108
8	5	Male	Bone/Man	Unifocal	V600E	+	NED/105
9	47	Male	Bone/Man, Max	Multifocal	V600E	+	Rec/8 NED/62
10	44	Male	Floor of the mouth	Unifocal	V600E	+	Rec/12 NED/60
11	51	Female	Parotid gland	Multifocal	WT	−	NA
12	2	Male	Bone/Man	Unifocal	V600E	+	NA
13	5	Female	Bone/Man	Multifocal	V600E	+	Rec/24 NED/225
14	12	Male	Bone/Man, LN	Multifocal	WT	−	Rec/120 NED/140
15	2	Female	Bone/Man, LN	Multifocal	V600E	+	NA
16	56	Male	Gingival	Multifocal	WT		NA
17	7	Female	Bone/Man	Unifocal	V600E	+	NED/123
18	1	Male	Bone/Man	Unifocal	V600E	+	DOD/122
19	26	Female	Bone/Man	Unifocal	V600E	+	NED/121
20	3	Male	Bone/Man	Unifocal	V600E	+	NA
21	10	Male	Tempus	Unifocal	V600E	−	NA
22	8	Male	Bone/Man	Unifocal	V600E	+	NED/70
23	1	Male	Bone/Man	Multifocal	V600E	+	Rec/2 NED/93
24	13	Male	Bone/Man	Unifocal	V600E	−	NED/89
25	5	Male	Bone/Man	Unifocal	V600E	+	NED/84
26	5	Male	Bone/Man	Unifocal	V600E	+	NED/76
27	0	Male	Bone/Man	Unifocal	WT	+	NA
28	46	Male	Bone/Max, Man	Multifocal	WT		NED/63
29	24	Male	Bone/Max, Man	Multifocal	V600E	+	NA
30	38	Female	Gingival	NA	V600E	+	NA
31	24	Female	Bone/Max, Man	Multifocal	V600E	+	Rec/36 NA/96
32	2	Male	Bone/Man	Unifocal	V600E	+	NED/92
33	3	Female	Bone/Man	Unifocal	V600E	+	NED/71
34	1	Female	Bone/Max	Unifocal	WT		NA
35	48	Female	Bone/Max	Unifocal	WT		NED/13
36	19	Female	Bone/Max, Man	Multifocal	V600E	+	NED/13

Abbreviations: PCR, polymerase chain reaction; Seq, result of sequencing; IHC, result of immunohistochemistry; Man, mandibular; WT, wide-type; Rec, recurrence; NED, no evidence of disease; V600E, *BRAF V600E* mutation; Max, maxillary; NA, not available; LN, lymph node; DOD, dead of disease.

from moderately to well-defined but without cortication in 18 cases. The alveolar lesions commonly presented severe alveolar bone resorption (13 cases), and occasionally, the periphery exhibited a moth-eaten form (Figure1A and B). Overall, the imaging features of this disease are generally similar to malignant neoplasms. Histopathologically, the

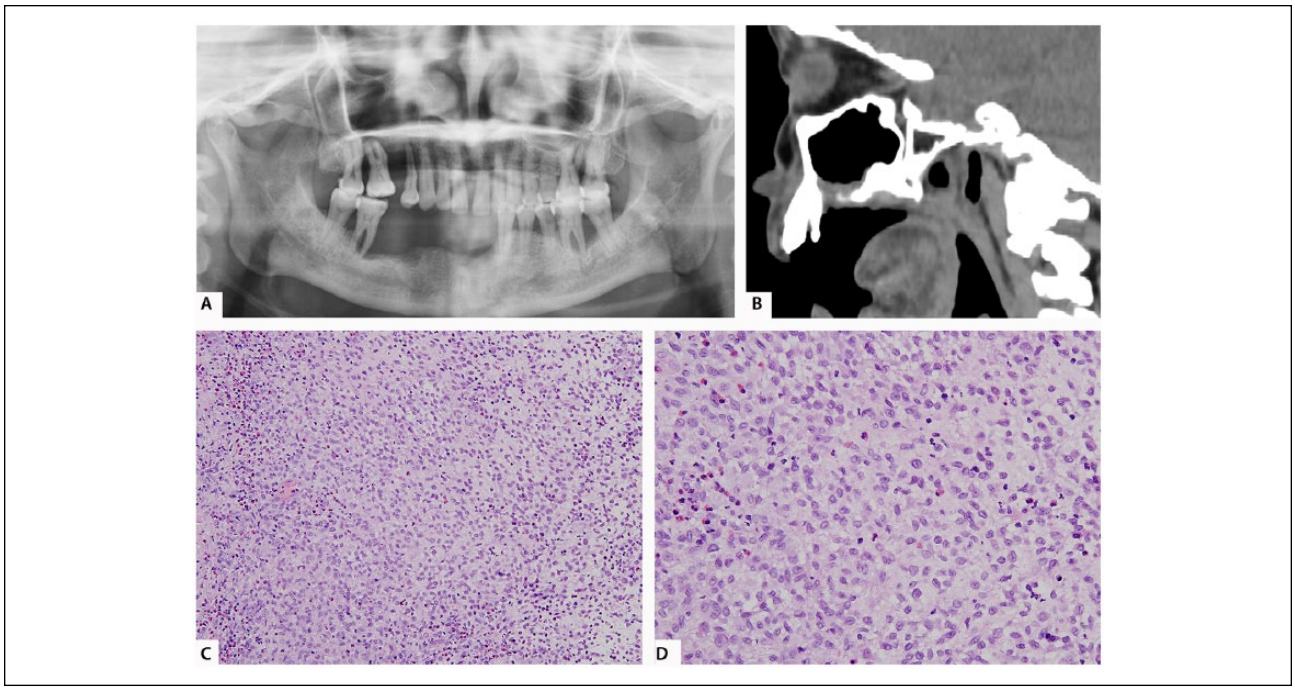

Figure 1. Representative radiographic and pathologic photos of Langerhans cell histiocytosis in head and neck. (A and B) Radiographic examinations revealed osteolytic presentation with maxillary and mandible involved. (C) Tumor consisted of Langerhans cells and chronic inflammatory infiltrations with typical eosinophils identified (hematoxylin and eosin [H&E], original magnification ×200). (D) High-power view reveals typical Langerhans cell with irregular nuclei, some of which have nuclear grooves or folds (H&E, original magnification ×400).

lesions showed a classical feature of Langerhans cells with distinct cell margin, abundant pink cytoplasm, and lobulated, reniform, or oval nuclei with deep nuclear grooves (Figure 1C and D). Langerhans cells were labeled with a panel of markers including CD1α, S100, and CD207 (Figures 2 and 3).

BRAF V600E Alteration in LCH

BRAF V600E mutation was analyzed in all 36 cases using DNA sequencing, and BRAF V600E protein was detected in 31 cases using immunohistochemistry with the VE1 antibody (Figures 2D and 3D). Twenty-six cases (26/36, 72.2%) were identified with *BRAF V600E* mutation using DNA sequencing (c.1799T > A, Figure 4). Immunostaining detected BRAF mutant protein in 25 cases (25/31, Figure 4). One lesion showing positive immunostaining for VE1 antibody did not exhibit the *BRAF V600E* mutation in the sequencing analysis. Two lesions with *BRAF V600E* mutation did not show positive immunoreactivity (as shown in Table 1).

Relationship Between BRAF V600E *Mutation and Clinical Parameters of LCH*

Follow-up data were obtained for 25 cases, while 11 cases were lost. One patient died less than 1 year after diagnosis without any treatment. Seven cases relapsed after curettage and chemotherapy from 1 month to 10 years, which all involved regions other than the head and neck. Seventeen cases exhibited complete remission after initial treatment, which lasted until this article was submitted. The relationship of *BRAF V600E* mutation to the clinicopathologic features of the LCH cases is shown in Table 2. Briefly, no statistically significant correlations were observed between *BRAF V600E* expression and clinicopathologic features including age, sex, anatomic site, stage, and outcome ($P \geq .050$).

Discussion

In the present study, we found that LCH of the head and neck regions mainly involved the mandible (28/36, 77.8%), especially the posterior (67.9%), with only 8 cases involving the maxillary and one with the temporal bone involvement. It also showed a comparatively significant male predominance, with a male-to-female ratio of 1.8:1. Of all the follow-up patients, 1 patient died, 7 relapsed, and 15 cases were free of disease, which suggested LCH in the head and neck comparatively had a good prognosis.

Then, we investigated the prevalence of *BRAF V600E* mutation in LCH of the head and neck region. Twenty-six cases (26/36, 72.2%) were identified with *BRAF V600E* mutation using DNA sequencing in the present series, and

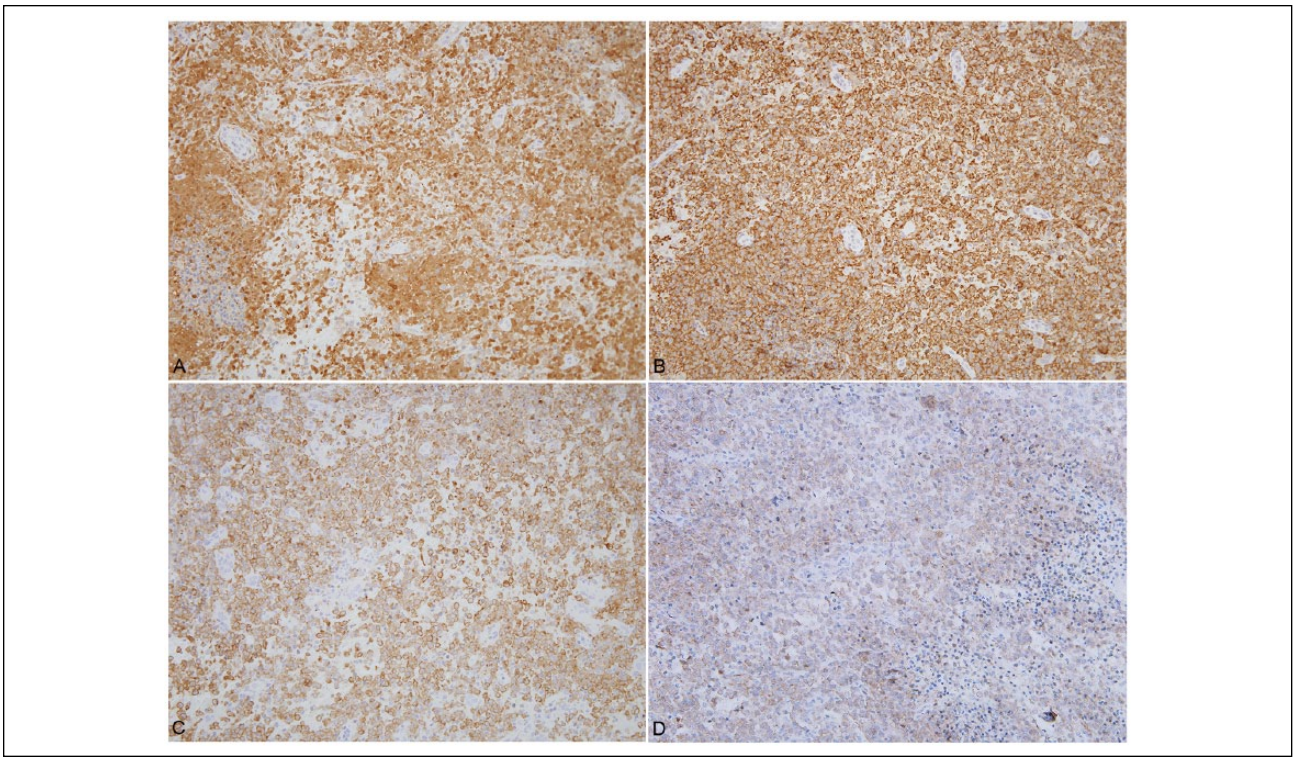

Figure 2. One case showing Langerhans cell histiocytosis lesion positive for S100 (A), CD1α (B), CD207 (C), and VE1 (D). All images, original magnification, ×200.

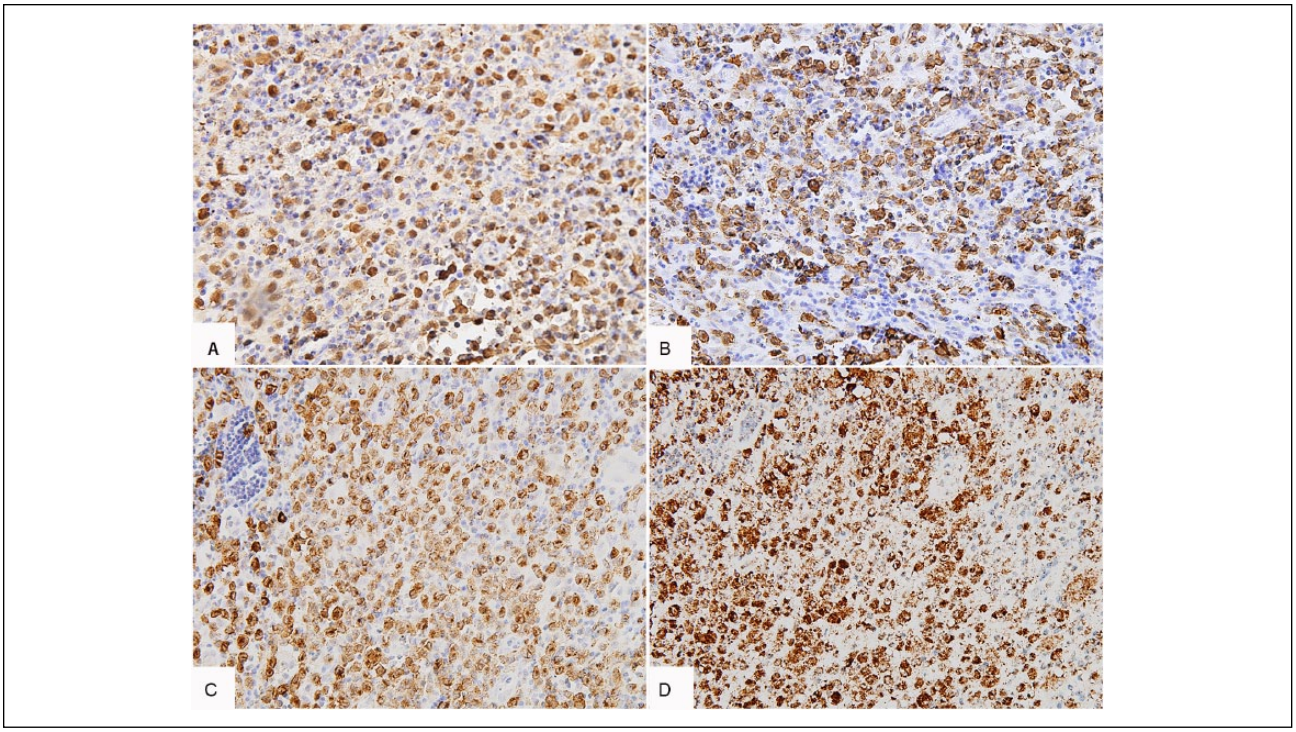

Figure 3. Another case of Langerhans cell histiocytosis showing positivity for S100 (A), CD1α (B), CD207 (C), and VE1 (D). All images, original magnification, ×400.

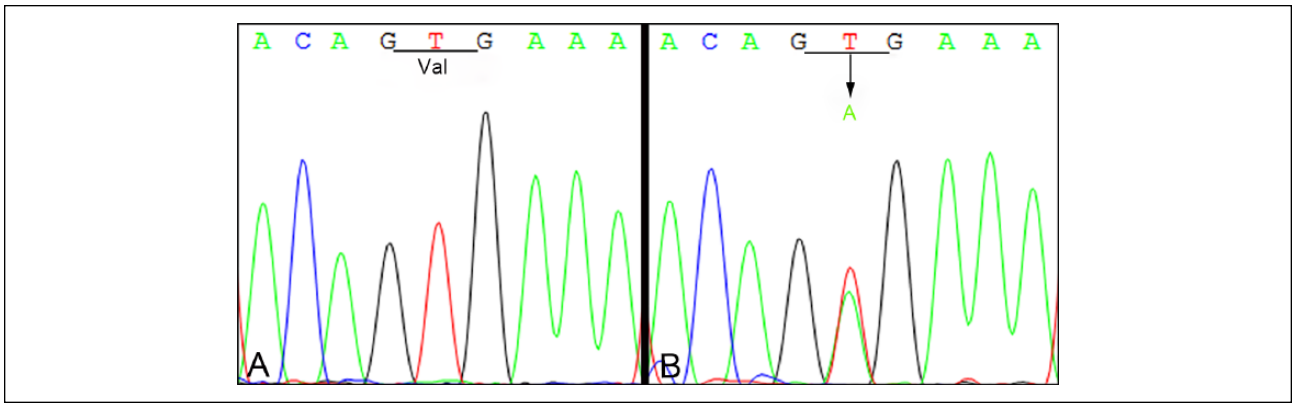

Figure 4. Somatic *BRAF V600E* mutation in Langerhans cell histiocytosis. (A) Wild-type *BRAF V600E* genes. (B) *BRAF V600E* mutation, GTG > GAG (valine > glutamic acid).

Table 2. Correlation Between the Clinicopathologic Features With *BRAF V600E* Mutation.

Variables	Total	*BRAF*-Mutated	Wide-Type	*P*[a]
Age (years)	36	26	10	.440
<18	23	18	5	
≥18	13	8	5	
Sex	36	26	10	.716
Male	23	17	6	
Female	13	9	4	
Anatomic sites	36	26	10	.154
Bone	31	23	8	
Soft tissue	5	3	2	
Stage	36	26	10	.444
Focal	22	17	5	
Multifocal	13	8	5	
Data not available	1	1	0	
Outcome	36	26	10	.362
Recurrence	8	5	3	
Recovery	16	13	3	
Died	1	1	0	
Lost	11	7	4	

[a]$P < .05$ statistically significant.

the prevalence seemed to be a little higher than that of previous reports, which showed *BRAF V600E* mutations in 21% to 65% of LCH cases.[4,12,13] Furthermore, we performed immunostaining in our series using VE1 antibody specific for BRAF V600E protein, and the results were basically consistent with the sequencing data in all the LCH cases except for one that showed positive VE1 staining but no *BRAF V600E* mutation based on the sequencing, and 2 cases with *BRAF V600E* mutation showing negative staining. PCR analysis may cause false negative because of the low tumor cell quantities as previously reported.[14-16]

Although the frequency of *BRAF V600E* mutation was reported to be almost 50% in LCH,[3,14] some studies showed much lower rates of this genetic alteration. Sasaki et al[13] demonstrated *BRAF V600E* mutations in 4 of the 19 Japanese patients (21%) and proposed the possibility that different genetic backgrounds were involved in the pathogenesis of LCH across various ethnicities. In addition, other associated reports such as that of Go et al[17] in South Korea showed 22.2%, and Tong et al[18] in China showed no detected cases. However, our findings were not in line with those of the other studies as well as recently published articles.[19,20] Tong et al[18] mentioned that LCH cases with bone and multisystem involvements might present higher *BRAF V600E* mutant rates. In addition, only 5 LCH cases involved the bone in the report of Sasaki et al,[13] and *BRAF V600E* mutation was found in 3 of the 5 cases (60%). The present series were all located in the head and neck region and, especially, involved the bone (86.1%) and presented a high prevalence of *BRAF V600E* mutation (72.2%). Therefore, the low rates of *BRAF V600E* mutation detected in some studies was likely because they mainly involved the soft tissue, whereas LCHs with bone involvement present a high prevalence of *BRAF V600E* mutation.

Notably, BRAF is the protein kinase with the most frequently occurring mutation in human cancers. Mutations at the V600E position of the *BRAF* gene are induced by the base transversion from thymine to adenine (T>A), leading to the amino acid substitution of valine by glutamic acid, which occurs within the activation portion of the kinase domain.[21] Furthermore, the *BRAF V600E* mutation leads to the constitutive activation of the MAPK pathway. *BRAF V600E* mutations have also been found in diverse solid tumors, involving gliomas and the thyroid, colon, ovarian, and hepatobiliary cancers.[4] Some studies[3,15,22,23] showed that *BRAF V600E* mutations were not relevant to the clinical characteristics or

outcomes of LCHs, whereas others demonstrated that it defined an aggressive group of LCH.[14] The present study was in line with a former study that showed no significant relationship between the clinical parameters and *BRAF V600E* alteration.

The *MAP2K1* mutation was detected in *BRAF V600E*–negative LCHs. *MAP2K1* encodes MEK1 as a core MAPK pathway member downstream of BRAF.[24] The identification of *MAP2K1* mutation explains the difference in the clinical manifestations between *BRAF V600E*–mutated and wide-type LCH, which further emphasizes the importance of the MAPK pathway in LCH pathogenesis. However, the studies focused on *BRAF V600E* mutation had a good bearing on both molecular diagnosis and high specifically targeted therapy. Presently, the clinical diagnosis of LCH mainly depends on the detection of CD1α, S100 protein, and CD207 expression in LCH lesions, along with the characteristic pathologic presentation. BRAF-mutant protein (VE1) expression might provide additional assistance to clinical pathologists.[14] Notably, molecular-targeted therapies with fewer side effects should be developed at a broad range. A *BRAF V600E* inhibitor has been described as effective in several other diverse *BRAF V600E*–mutated cancers such as metastatic melanoma and refractory hairy cell leukemia.[25,26] It might serve as another treatment option for patients with LCH with *BRAF V600E* mutations.

In conclusion, we found that LCH originating from the head and neck region often involved the bone especially the posterior mandible and presented a high prevalence of *BRAF V600E* mutation. We immunohistochemically detected *BRAF* alterations, which might be useful in the diagnosis of LCH, especially in cases with a low proportion of Langerhans cells. Furthermore, *BRAF* inhibitors might also be a potential treatment option for patients with LCH harboring *BRAF V600E* mutations.

Declaration of Conflicting Interests

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Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Informed Consent

Not applicable, because this article does not contain any studies with human or animal subjects.

Trial Registration

Not applicable, because this article does not contain any clinical trials.

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