ORIGINAL ARTICLE

BRAFV600E mutation in cutaneous lesions of patients with adult Langerhans cell histiocytosis

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Abstract

Background Langerhans cell histiocytosis (LCH) is characterized by the proliferation of pathologic Langerhans cells. The disease can develop in any age and can affect almost any organ. Cutaneous involvement is frequent in LCH. The recent demonstration of the activating, oncogenic BRAFV600E gene mutation in LCH samples strongly supports the neoplastic origin of the disease.

Objectives Our aim was to analyse the clinical data of the patients and whether BRAFV600E mutation is present in skin lesions of patients with adult onset LCH, and to investigate whether the BRAFV600E mutation status has any effect on the clinical presentation and the outcome of the disease.

Methods We diagnosed and treated 15 adult LCH patients in the period of 1987–2012 and collected their clinical data. Three of our patients suffered from skin involvement and 12 patients had multiorgan disease (five patients out of the multisystem group died). Eleven formalin-fixed paraffin-embedded skin samples from 10 patients were available for BRAFV600E mutation analysis.

Results Among the 11 examined samples, 6 contained the BRAFV600E mutation (54.5%). Our results indicate that in the adult group of LCH patients the presence of BRAFV600E mutation is similar to what was previously suggested in case of the childhood forms, at least as far as skin lesions are concerned. The BRAF mutation status of our patients does not seem to correlate with the extent and/or the outcome of the disease.

Conclusion Our results support the neoplastic origin of LCH and suggest that skin lesions of LCH are sufficient for the diagnosis of the disease and for assessing its BRAF status. In addition, analysis of BRAF status of patients with LCH can lead to the administration of new targeted therapies which may provide better disease control and prognosis.

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Conflict of interest

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Introduction

Langerhans cell histiocytosis (LCH) is characterized by the proliferation of S100 protein, CD1a and Langerin-positive cells containing electron-microscopically detectable Birbeck granules. These cells resemble or are similar to epidermal antigen presenting dendritic Langerhans cells (LC) and thought to be derived from haematopoietic, histiocytic precursors or from LCs.[1–8] LCH is a rare disease, the incidence of childhood and adult forms is estimated to 1-9 per million but the number of adult cases is significantly lower.[4,7–9] The clinical appearance of LCH is highly variable, it can develop at any age and in almost any organ. According to the recommendation of the 'Histiocytic Society' LCH can be classified into three main forms depending on the involved organs: single system disease and multisystem disease with or without 'risk organ' (lung, liver, spleen, haematopoietic) dysfunction.[1,7–11]

Cutaneous involvement is frequently present in LCH. Skin manifestation is more common in children, but numerous adult patients are also presented with skin lesions.[9,12] As up to 30–80% of LCH patients develop skin symptoms, the diagnosis is frequently provided by histopathological examination of the skin biopsy.[1,7,12]

The prognosis depends on the dissemination of the disease, the presence or absence of organ dysfunction, the patient's age of onset and the response to the initial therapy.

The aetiology and pathogenesis of the disease has not been fully understood yet, and even the fact whether LCH is a true malignant or a reactive, inflammatory process has been debated in the literature.[2–4,6–9] Recently, however, a new possible molecular genetic background has been suggested with the demonstration of the activating, oncogenic BRAFV600E gene mutation in a subset (38–68%) of LCH samples. This strongly supports the neoplastic origin of the disease.[2,3,5,6,13,14]

Our first aim was to collect adult onset LCH patients and to analyse the characteristics of their disease. The second goal of our study was to detect the presence and rate of BRAFV600E mutation solely from skin lesions. Previous literature data evaluated the incidence of this mutation in all kinds of LCH lesions, but among the analysed samples the ratio of skin lesion was relatively low. Based on the presented data it can be estimated that so far approximately not more than 30 skin samples were examined.[3,5,13–15] As skin is a frequently involved organ and is an easily accessible site for biopsy to establish the histopathological diagnosis of LCH patients it is very important to obtain data on the availability of BRAF mutation status in such samples too.[1,7,12]

Materials and methods

Patients and samples

Fifteen adult LCH patients were diagnosed and treated between 1987 and 2012 at the Department of Dermatology and Allergology, Albert Szent-Györgyi Clinical Center University of Szeged, Hungary. Clinical and treatment characteristics of the patients are summarized in Tables 1 and 2.

The available archived formalin-fixed paraffin-embedded blocks were also retrieved. Eleven skin samples from 10 LCH patients could be examined. One patient had two biopsies (a punch biopsy and an excision) of the trunk's skin in 1 month interval to establish the diagnosis (Table 2). The other 5 patients' skin samples were also examined at our department earlier, but the samples originated from other laboratories and the paraffin blocks were sent back after examination and were not available for our current analysis.

BRAFV600 mutation detection

Five μm sections were obtained from formalin-fixed paraffin-embedded tissue biopsy specimens. After deparaffinization genomic DNA samples were isolated using the cobas® DNA Sample Preparation Kit (Roche Molecular Systems Inc., Roche Molecular Diagnostics, Branchburg, NJ, USA) according to the users' manual. BRAFV600E mutation was detected on the cobas z 480 Analyzer with the cobas® 4800 BRAF V600 Mutation Test (Roche Molecular Systems Inc., Roche Molecular Diagnostics) following the instructions of the manufacturer.

Results

The development of skin symptoms drew the attention to the underlying LCH in most of our 15 LCH patients. The male: female ratio was 5:10 (1:2), the median age at the time of the presentation of the skin lesions was 56 years (age range 24–91 years). Three patients had single system disease, exhibiting only skin symptoms, whereas 12 patients suffered from multisystem disease with or without organ dysfunction. Diabetes insipidus was present in six patients, the lymphoreticular system was affected in nine cases and one patient had idiopathic thrombocytopenic purpura. Four patients had pulmonary involvement and three patients developed bone lesions.

Most of the skin lesions were papules, plaques and nodules, situated at the predilection sites. The flexural areas and the head were involved in most cases and three patients suffered from genital skin lesions. Thirteen patients had more than one affected region. Pruritus, erosion and ulceration also complicated the lesions very frequently (Fig. 1). Clinical data are shown in Table 1. Diagnosis was confirmed by the examination of skin biopsy specimens, showing the characteristic microscopic morphology and the proliferation of CD1a positive cells (Fig. 2).

BRAF mutation detection was performed in 11 skin samples from 10 LCH patients (patient no. 15 had two biopsies). Among the 11 samples, 6 contained BRAFV600E mutation which represents 54.5%. Although the case number in our study is relatively low and two BRAF positive biopsies came from one patient (patient no. 15), we can conclude that approximately half of the examined samples contained the mutation.

Two of our BRAF positive patients with multisystem disease died. One of them died in spite of a combined therapy, whereas the other patient refused treatment. The remaining three patients positive for the examined BRAF mutation also had a multisystem disease with organ dysfunction and they showed significant improvement or became symptom free upon topical and/or systemic steroid treatment and supportive therapy for diabetes insipidus (Table 2, Fig. 3).

Two patients died of the disease in the BRAF negative group suffering from the multisystem form of the disease. The remaining three patients in the same group improved in response to the treatment, even though two of them had multisystem involvement with organ dysfunction, whereas the third patient had a single system disease affecting only the skin. Although our case number is too low to draw statistical conclusions, our observations strongly suggest that the BRAF mutation status may not correlate with the extent and/or the outcome of this disease.

Discussion

The clinical manifestation of LCH is highly variable. Almost any organ can be involved and the disease can develop at any age.

Table 1 Clinical data, treatment and outcome of the 15 adult LCH patients

Patient	Age/ gender	Skin					Systemic involvement				Treatment					Outcome
		Head	Flexural areas	Glabrous areas	Genital area	Skin symptoms	Bone	Lung	Endocrine system	Lymphoreticular system	Surgery	Topical steroid	Systemic steroid	PUVA	Cytostatic drugs	
1	24/♀	–	+	–	–	Pruritus, papules	–	–	–	–	–	+	–	–	–	Improved
2	40/♀	–	+	+	–	Pruritus, papules, nodules	+	+	–	+	+	+	+	–	–	Improved
3	82/♀	+	+	+	–	Pruritus, papules, plaques, erosions	–	–	–	+	–	+	–	–	–	Deceased
4	47/♀	–	–	+	+	Indurated, ulcerated plaques, nodules	–	–	–	–	+	+	–	–	–	Improved
5	72/♂	+	+	+	–	Pruritus, erythema, plaques, scaling	–	+	–	+	–	+	+	–	+	Deceased
6	68/♀	–	+	+	–	Papules, plaques, Erosions	–	–	+Diabetes insipidus	+	–	+	+	–	+	Deceased
7	91/♀	–	+	+	–	Pruritus, papules	+	–	–	–	–	–	–	–/+	–	Deceased
8	51/♀	–	–	–	+	Erosion of the vulva, ulcer of the oral mucosa	+	–	+Diabetes insipidus	–	–	+	+	–	–	Improved
9	36/♂	+	+	+	+	Papules, plaques, Erosions	–	+	+Diabetes insipidus	+	–	–	+	–	+	Improved
10	50/♂	–	+	+	–	Pruritus, erythema, plaques, nodules	–	–	+Diabetes mellitus	+	–	+	–	–	+	Improved
11	65/♀	+	+	+	–	Erythema, plaques, Erosion	–	–	–	+ITP	–	+	+	–	–	Improved

Table 1 (Continued)

Patient	Age/ gender	Skin					Systemic involvement				Treatment					Outcome
		Head	Flexural areas	Glabrous areas	Genital area	Skin symptoms	Bone	Lung	Endocrine system	Lymphoreticular system	Surgery	Topical steroid	Systemic steroid	PUVA	Cytostatic drugs	
12	66/♀	–	+	+	–	'burning' tongue, ulcer of the oral mucosa papules	–	–	+Diabetes insipidus	–	–	+	–	–	–	Improved
13	56/♂	+	–	+	–	Papules, nodules, erosions	–	+	+Diabetes insipidus	+	–	+	–	–	+	Improved
14	54/♀	+	–	+	–	Pruritus, papules pustules	–	–	–	–	–	+	+	–	+	Impoved
15	71/♂2 bx	+	+	+	–	Pruritus, papules, pustules, erosions	–	–	–	Hepato-spleno-megaly	–	+	–	–	–	Deceased

Childhood cases are thought to have a tendency to be multisystem disease, whereas adult patients are more prone to develop a single system disease, although there are some controversies in the literature concerning these data.[1]

The most frequently involved organs are the bone (which is also the most common site of the single system disease), skin, pituitary gland (leading to the development of diabetes insipidus), liver, spleen, lung, bone marrow.[1,7,9]

Cutaneous involvement can be frequently present in the affected individuals. According to the available literature, 30–80% of the patients develop skin symptoms and it is often the skin biopsy that provides the histopathological diagnosis.[1,7,12] Although skin manifestation is more common in children, adult forms can be presented also with skin lesions. These symptoms can affect any part of the body but typically involve flexural, genital, perianal and glabrous areas. Papules, plaques, eczema-like or seborrhoeic dermatitis-like rash with scales and scab along with pruritus are the most common skin manifestations.[9,12]

Adult forms are almost similar to the childhood ones, but the single lung involvement seems more frequent (up to 20–30%) in adults. The isolated pulmonary disease is strongly associated with smoking and polyclonal LCs can be detected in these lesions quite frequently.[4,7]

The prognosis of LCH depends on its dissemination, the presence or absence of organ dysfunction, the patient's age at disease onset and the response to initial therapy.[1,7,8] Patients with widespread, high-risk disease have poor prognosis with a 20–50% mortality rate in spite of combined therapy,[2,3,7,8,16] and the 5-year event free survival ratio of adult forms is much better even in the disseminated forms.[7] Treatment of LCH depends mainly on the dissemination of the disease. Single system disease – mainly bone lesions – frequently requires surgical removal (curettage) maybe combined with intralesional steroids while multisystem forms, even with organ dysfunction needs combined chemotherapy (vinblastine, methotrexate) and/or prednison.[1,4,7–9,16] Localized cutaneous lesions can be treated with topical steroid or tacrolimus while disseminated skin disease can improve upon thalidomide, interferon, PUVA or methotrexate and chemotherapy.[7,9,10] Treatment of LCH in adults is more challenging than in childhood because experience is more limited due to the low incidence in this age group.[16]

The aetiology and exact pathogenesis of this disease is still not fully understood. It has been debated in the literature whether LCH is a true malignant or a reactive, inflammatory process.[2–4, 6–9] It has been suggested that LCH is a clonal disease although there is a significant inflammatory component in the infiltrate (eosinophils, macrophages, lymphocytes, giant cells) with elevated levels of cytokines and chemokines.[3,4,9]

The recent demonstration of the activating, oncogenic BRAFV600E gene mutation in 38–68% of LCH samples and the fact that the neoplastic LC cases often carry this mutation strongly supports the neoplastic origin of the disease.[2,3,5,6,13,14]

Table 2 BRAF mutation status of 11 lesions from 10 patients with their disease type, treatment modalities and outcomes

Patient no.	BRAF mutation	Multisystem disease	Treatment	Outcome
5	No	Yes, with organ dysfunction	Topical and systemic steroid, cytostatic drugs	Deceased
6	Yes	Yes, with organ dysfunction	Topical and systemic steroid, cytostatic drugs	Deceased
7	No	Yes	PUVA	Deceased
8	Yes	Yes, with organ dysfunction	Topical and systemic steroid	Improved
9	No	Yes, with organ dysfunction	Systemic steroid, cytostatic drugs	Improved
10	No	Yes, with organ dysfunction	Topical steroid, cytostatic drugs	Improved
11	Yes	Yes, with organ dysfunction	Topical and systemic steroid	Improved
12	Yes	Yes, with organ dysfunction	Topical steroid	Improved
14	No	No	Topical and systemic steroid, cytostatic drugs	Improved
15 (2 bx)	Yes Yes	Yes, with organ dysfunction	No treatment	Deceased

Patient no. refers to patient's numbers in Table 1.

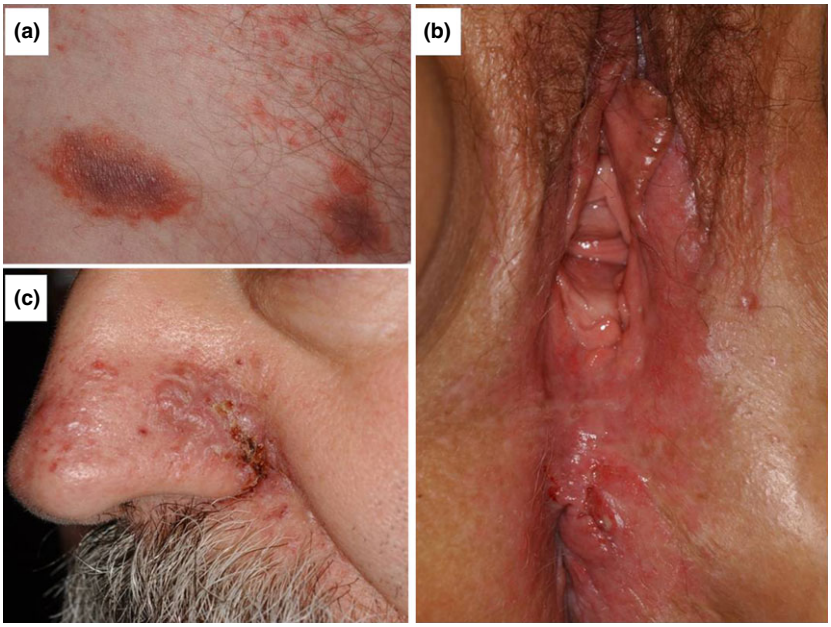

Figure 1 Various skin lesions in LCH: (a) erythematous plaques on the trunk – Patient 10, (b) erosions on the vulva – Patient 8, (c) scaling papules and plaques on the nose – Patient 13 (for patient numbers, refer Table 1).

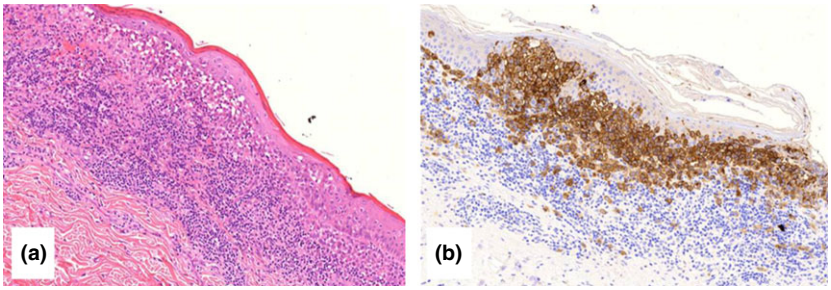

Figure 2 The characteristic histopathological picture of skin lesion in LCH – Patient 15 (a) HE, (b) CD1a staining.

Recent data suggest that there is an activation of the RAS-RAF-MEK pathway in the background of LCH but in BRAF negative cases, however, the precise activating factors are not known yet.[4,6,9] Better understanding of the molecular background of the disease is very important because in disseminated and treatment refractory cases the possibility of a new targeted therapy based on molecular pathology can be a promising option in the therapeutic repertoire. It has also been demonstrated that among

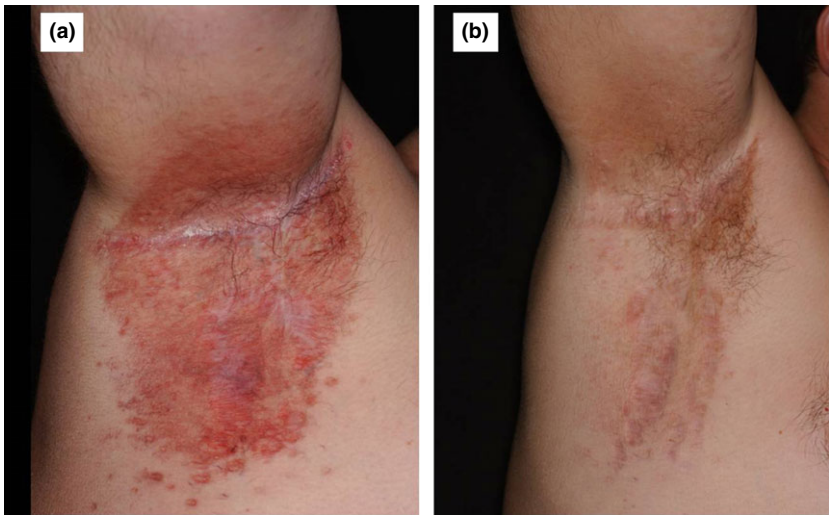

Figure 3 Significant improvement upon systemic steroid and methotrexate treatment in Patient 9.

other histiocytic disorders (both neoplastic and reactive conditions) only nearly half of Erdheim-Chester diseases carry the same BRAF mutation.[13] There is even a recent report on effective vemurafenib treatment in disseminated combined Erdheim-Chester disease and LCH carrying the BRAFV600E mutation.[15,17,18]

In the present work we were able to prove the presence of the BRAFV600E mutation in 6 among 11 LCH skin samples (54.5%). The frequency of BRAFV600E mutation in our series correlates well with the initial data of Badalian-Very et co-workers (57%)[3] and the recent study of Satoh and his colleagues (68%),[14] whereas was higher than the results demonstrated by two other authors' Sahm[5] and Haroche and their groups (38%).[13]

It should be also emphasized that the previous studies analysed the BRAF mutation status from lesions of various anatomical sites (bone, lung, etc.) and only a few cutaneous lesions were involved in these studies. Ours is the first one that was focusing especially to skin LCH lesions. Taking into account the literature data of 57% BRAF positivity in various LCH lesions[3], our results strongly suggest that skin lesions of LCH are eligible for the diagnosis of the disease and for the assessment of the BRAF mutation status, too. Knowing the high proportion of skin involvement in LCH patients this observation highlights the importance of skin biopsy as an easily accessible diagnostic material.

According to the previous data it was suspected that the presence of this particular BRAF mutation might correlate with younger age,[3,4,19] but another studies could not prove this hypothesis.[14] The results on our adult group of LCH patient, however, strongly suggest that the occurrence of BRAFV600E mutation can be as frequent in childhood as in the adult forms at least as far as skin lesions are concerned. Although the case number in our study is too low to make statistical analysis, our observations also suggest that the BRAF mutation status may not present a clear correlation with the extent and/or the outcome of the disease.

Conclusion

Our results support the neoplastic origin of LCH and correlates well with the previous data on BRAFV600E mutation ratio in this rare disease. These data suggest that the occurrence of the mutation in adult forms can be as frequent as in childhood cases, and underline the importance of the early recognition of skin symptoms and the high impact of skin biopsy in the diagnosis of the disease. Currently it is not clear whether the carriage of the BRAF mutation has any effect on the extent and/or the outcome of the disease, and further studies are required to answer these questions. However, our results underlie the importance of molecular pathway analyses that can potentially lead to the administration of promising new targeted therapies in affected individuals, providing better disease control and prognosis for the LCH patients.[15]

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