Genetic homogeneity of adult Langerhans cell histiocytosis lesions: Insights from BRAF^{V600E} mutations in adult populations

JOANNE LOUISE SELWAY[1,2], PARVATHY ELACODE HARIKUMAR[2], ANTHONY CHU[2,3] and KENNETH LANGLANDS[1,2]

[1]Medical School; [2]Buckingham Institute for Translational Medicine, University of Buckingham, Buckingham MK18 1EG; [3]Faculty of Medicine, Imperial College London, Dermatology Department, London W12 0NN, UK

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Abstract. Langerhans cell histiocytosis (LCH) is a heterologous disease with a recognized disparity in incidence, affected sites and prognosis between adults and children. The recent identification of BRAF^{V600E} mutations in LCH prompted the investigation of the frequency of these mutations in adult and childhood disease with the involvement of single or multiple sites in the present study. The study analysed the BRAF^{V600E} status in a cohort of adult LCH patients by DNA sequencing, and performed a broader meta-analysis of BRAF^{V600E} mutations in LCH in order to investigate any association with disease site and severity. A review of the literature revealed that ~47% of lesions from cases of adult disease (patient age, >18 years) were V600E-positive compared with 53% in those under 18 years. When single and multiple site disease was compared, there was a slight increase in the former (61 vs. 51%, respectively). A greater difference was observed when high- and low-risk organs were compared; for example, 75% of liver biopsies (a high-risk organ) were reported to bear the mutation compared with 47% of lung biopsies. In the adult LCH population, DNA sequencing identified mutations in 38% of 29 individuals, which is slightly lower than the figure identified from the meta-analysis (in which a total of 132 individuals were sampled), although we this value could not be broken down by clinical status. Thus, V600E status at presentation in itself is not predictive of tumour course, but a considerable proportion of LCH patients may respond to targeted V600E therapies.

Introduction

Langerhans cell histiocytosis (LCH) is a rare disorder affecting adults and children, characterised by an abnormal accumulation of epidermal Langerhans-like cells in various sites (1-3). The aetiology and pathogenesis of LCH remains to be established, but it is hypothesized to be a clonal disorder (4,5). A diagnosis of LCH is made following the detection of lesional cluster of differentiation (CD)1a$^+$ Langerhans-like cells, whereas non-Langerhans histiocytoses, including Erdheim Chester disease, are CD1a$^-$. LCH is a heterogeneous disease, affecting all ages and ethnicities, and disease may take a variety of courses: Certain disease cases may spontaneously remit, while other cases may lead to fatality. Risk factors include the time to diagnosis and the site of lesions (6).

A study from the Histiocyte Society recognised a disparity in the site, age of onset and incidence of LCH between adults and children (7). The age of onset in children is between 1-3 years, whereas adult disease is more heterogeneous, with a higher incidence in young (18-30 years) and older adults (>70 years) (7). The reported incidence of LCH ranges from 0.5-5.4 cases per million persons per year (8-10), but these figures are largely associated with childhood disease. The incidence of adult disease is likely to be underreported, as LCH is frequently treated according to the affected system, without a secondary referral. Childhood disease is more likely to be referred to an oncologist, providing more accurate statistics.

Although childhood LCH lesions tend to predominate in bone (11), adult LCH is more widespread, commonly presenting in the skin, bone and lung. Table I lists the common sites of LCH in both adults and children reported in several previous studies (6,7,12). This heterogeneity, coupled with relative scarcity, renders it challenging to implement randomized control trials in adults or children to optimize therapy. Notwithstanding the Histiocyte Society's LCH protocol that provides a therapeutic framework, treatment for LCH is variable across centres (13).

Despite an unknown aetiology, the misguided myeloid dendritic cell precursor model provides one underlying mechanism to explain the abnormal localization and accumulation of dendritic cells (14,15), and this is consistent with the treatment of LCH as a haematological disease (at least in paediatric cases). Haematopoietic forms of cancer occur as a consequence of arrest at a discrete stage of an ordered developmental pathway, frequently associated with distinct patterns

Correspondence to: Dr Joanne Louise Selway, Medical School, University of Buckingham, Hunter Street, Buckingham MK18 1EG, UK
E-mail: joanne.selway@buckingham.ac.uk

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of mutation. This is in contrast to primary and metastatic solid tumours, which tend to exhibit a higher level of heterogeneity at the genetic level (16). The most significant recent advance in the understanding of LCH has been provided by studies describing the high incidence of BRAF^{V600E} mutations in childhood disease (17-19). This observation, along with the immature phenotype of LCH cells, is consistent with the discrete patterns of mutation and arrested development observed in haematopoietic types of cancer. In order to further investigate whether this mutation demonstrated a discrete pattern in LCH, the current study aimed to investigate the BRAF^{V600E} status in a broader range of LCH cases (with respect to age and site) in order to establish whether there is commonality in the aetiology of a disease with heterologous presentation. In addition, the assessment of numerous biopsies from patients with LCH presenting at multiple sites may provide further evidence for a clonal origin of this disorder.

Materials and methods

Meta-analysis. PubMed (www.ncbi.nlm.nih.gov/pubmed) was searched for manuscripts referencing LCH biopsies that had been subject to BRAF^{V600E} screening by direct sequencing, reverse transcription-polymerase chain reaction (PCR) or immunohistochemistry. Relevant patient information (disease classification and BRAF status) from 10 published manuscripts were isolated for meta-analysis (Table II) (15,17-25). The search criteria used were 'BRAFV600E' and 'Langerhans cell histiocytosis'. Results were filtered for adults (>18 years) and paediatric disease (<18 years), and secondly according to disease site, including bone or skin.

BRAF^{V600E} sequencing. Research Ethics Committee (REC) approval at Hammersmith Hospital (London, UK) was obtained for the present study (REC reference no. 60/Q0406/107). In total, 33 adult LCH samples, representing 30 patients were available to screen for the BRAF^{V600E} mutation. DNA was extracted from 21 archival paraffin embedded LCH biopsies and 12 fresh biopsies enriched for CD1a positive cells using an AllPrep DNA/RNA FFPE kit and AllPrep DNA/RNA Mini kit from Qiagen, Inc. (Valencia, CA, USA), respectively. The extractions were performed according to the manufacturer's protocol. CD1a-positive cell selection was performed using MACS CD1a MicroBeads kit purchased from Miltenyi Biotec, Inc. (Cambridge, MA, USA). Cells were selcted using magnetic beads coated with a human anti-mouse IgG monoclonal antibody and a magnetic particle concentrator according to the manufacturer's instructions. DNA quality was assessed using a NanoDrop (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Amplification and sequencing of exon 15 of the BRAF gene was performed by Source BioScience (Nottingham, UK). Source BioScience designed the primer pairs and performed region-specific PCR optimization, amplification and sequencing. The oligonucleotide sequences were as follows: PCR forward primer (5'AAC ACATTTCAAGCCCCAAA); PCR reverse primer (5'AGC ATCTCAGGGCCAAAAAT); forward sequencing primer (5'TCATAATGCTTGCTCTGATAGGA); reverse sequencing primer (5'GGCCAAAAATTTAATCAGTGGA). PCR reaction mixes contained 25 ng DNA (or a no template control), 0.6 μM of each primer and Roche High-Fidelity master mix at a 1X final concentration with the following conditions: Initial denaturation at 94˚C for 279 sec; followed by 35 cycles of 94˚C for 20 sec, 55˚C for 15 sec and 65˚C for 30 sec; followed by a final elongation step at 65˚C for 60 sec. PCR products were cleaned up using Zymo ZR-96 clean and concentrator kit (Zymo Research Corp, Irvine, CA, USA). Products were sequenced as standard using an ABI 3730 machine (Thermo Fisher Scientific, Inc.).

Table I. Most common sites of Langerhans cell histiocytosis in children and adults with their frequency, where reported.

	Frequency in adults, %		Frequency in children, %	
Site	SS	MS	SS	MS
Bone	52	77	70-80	
Skin	6	65	10	50
Ear, nose and throat			>15	
Pituitary	0.9	44	5-50	
Orbits			<20	
Mouth			6	
Gastrointestinal tract			2-13	
Lungs	40	43	<5	12
Liver		5	4	
Lymph nodes			<10	
Thyroid		9		

Adapted from (6,7,12). SSl, single-system; MS, multi-system.

Statistical analysis. Statistical analysis was performed using Prism statistics software (version 4; GraphPad Software, Inc., La Jolla, CA, USA). Analysis was performed using χ^2 and Fisher's exact tests. P<0.05 was considered to indicate a statistically significant difference.

Results

A meta-analysis of existing LCH BRAF^{V600E} studies was performed to invesitigate the heterogeneity of LCH at the genetic level. Fig. 1A reveals no difference in the prevalence of BRAF^{V600E} mutations between adult and paediatric LCH. Similarly, when the incidence of the BRAF^{V600E} mutation in various clinical classifications of LCH was evaluated, no significant differences were identified between multi-system (MS)-LCH and focal or single system (SS) LCH (Fig. 1B). In addition, investigation of the mutation in varying sites revealed no consistent pattern (Fig. 1C).

The results of BRAF^{V600E} sequencing in adult LCH cases are presented in Table III. Of the 29 patients analysed, 11 patients exhibited a BRAF^{V600E} mutation, which corresponds to 38% of patients with LCH being BRAFV600E-positive for the mutation. There were 3 patients for whom multiple samples were analysed. Lesional gum and bone samples from patient 16 exhibited differential status with respect to the BRAF^{V600E} mutation. However, follow up with PCR revealed the two

Table II. Summary of Langerhans cell histiocytosis published data used for meta-analysis.

Authors, year	Age, years (no. of patients)	Total no. of patients	Classification	$BRAF^{V600E}$ screening	V600E no. of patients	WT no. of patients	P-value	Refs.
Bates *et al*,	<18 (1)	1	SS (0)	Pyrosequencing	-	-		(20)
2013	>18 (N/A)		MS (1)		1	-		
Yousem *et al*,	<18 (N/A)	5	SS (5)	Next generation sequencing and Sanger sequencing	2	3		
2013	>18 (5)		MS (-)		-	-		
Satoh *et al*,	<18 (16)	16	SS (9)	Next generation pyrosequencing	6	3	0.615	(21)
2012	>18 (N/a)		MS (7)		3	4		
Badalian-Very *et al*,	<18 (27)	52	SS (44)	Pyrosequencing	27	17	0.700	(18)
2010	>18 (17)		MS (8)		4	4		
Chilosi *et al*,	<18 (11)	38	SS (33)	Pyrosequencing and VE1 immunoreactivity	17	16	0.343	(17)
2014	>18 (27)		MS (5)		1	4		
Haroche *et al*,	<18 (N/A)	29	SS (N/A)	Pyrosequencing	-	-		(22)
2012	>18 (N/A)		MS (N/A)		-	-		
Sahm *et al*,	<18 (49)	89	SS (85)	Direct sequencing and VE1 immunoreactivity	31	54	0.154	(23)
2012	>18 (40)		MS (4)		3	1		
Wei *et al*,	<18 (36)	52	SS (43)	Direct sequencing	25	18	0.684	(15)
2013	>18 (16)		MS (7)		3	4		
Berres *et al*,	<18 (97)	100	SS (45)	Qiagen $BRAF^{V600E}$ qPCR mutation assay and	27	18	0.532	(24)
2014	>18 (3)		MS (55)	Sanger sequencing	37	18		
Go *et al*,	<18 (19)	27	SS (N/A)	Direst Sanger sequencing, Peptide nucleic acid	6	21		(19)
2014	>18 (8)		MS (N/A)	clamp qPCR (PNAcqPCR) assay and Anyplex™ qPCR assay				

SS, single system; MS, multi system; N/A, information not available; qPCR, quantitative-polymerase chain reaction; PNAcqPCR, peptide nucleic acid clamp real-time polymerase chain reaction; WT, wild type.

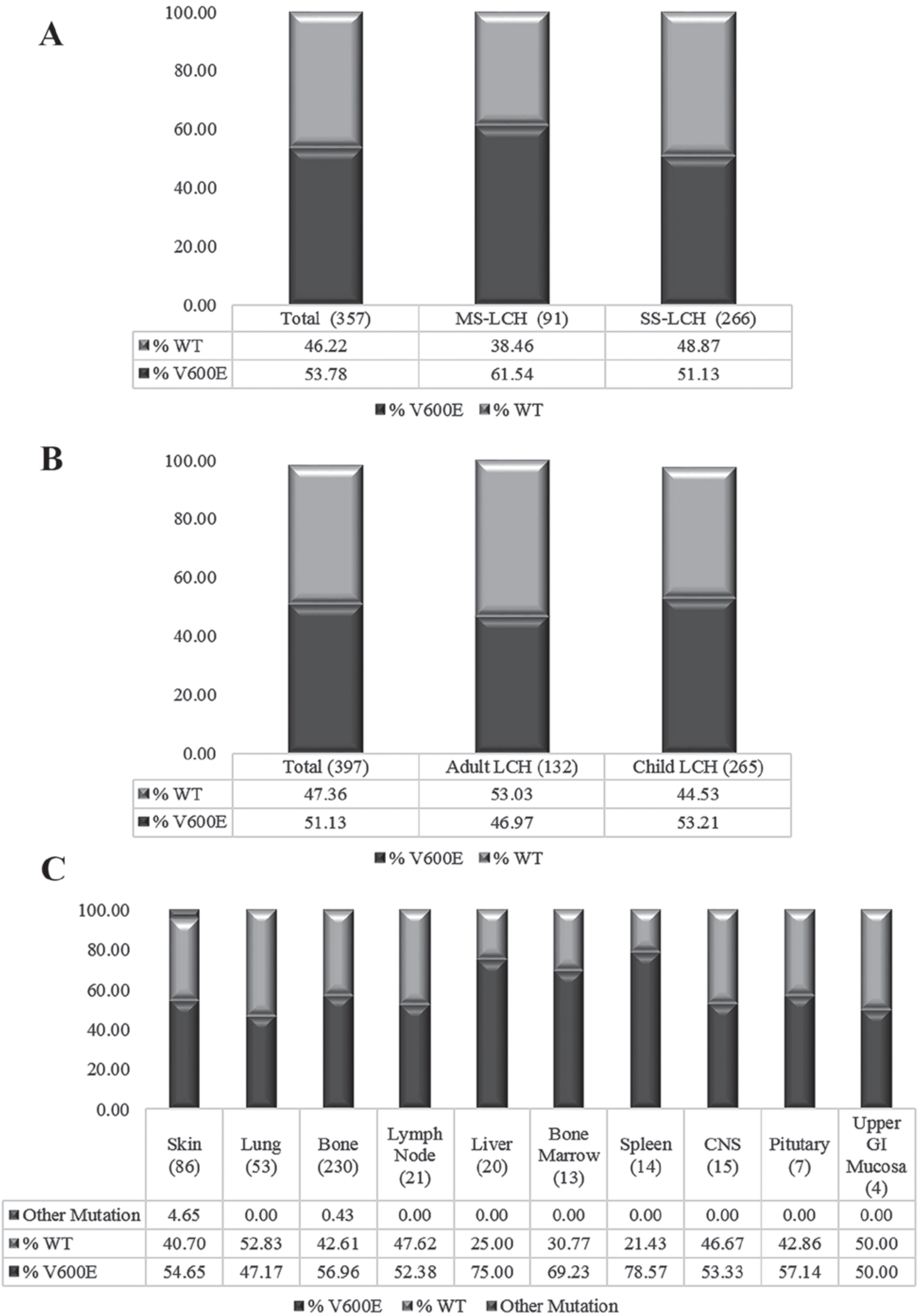

Figure 1. Meta-analysis of $BRAF^{V600E}$ mutations in published LCH work. Comparison of $BRAF^{V600E}$ mutation status in LCH between (A) adult vs. paediatric LCH, (B) MS-LCH vs. SS-LCH and (C) in various sites. Graphs B and C represent data from adult and paediatric biopsies. LCH, Langerhans cell histiocytosis; WT, wild type; MS-LCH, multi-system Langerhans cell histiocytosis; SS-LCH, single system Langerhans cell histiocytosis; CNS, central nervous system; GI, gastrointestinal.

Table III. $BRAF^{V600E}$ mutation screening in adult LCH cases using Sanger Sequencing.

Patient no.	Type	Tissue type	Clinical status	BRAF status
1	Cells	N/A	LCH	WT
2	Cells	N/A	LCH	WT
3	Cells	Skin	MS	WT
4	Cells	N/A	LCH	WT
5	Cells	N/A	LCH	WT
6	Cells	N/A	LCH	V600E
7	Cells	BALF	SS	WT
8	Cells	BALF	MS	WT
9	Cells	Skin	SS	V600E
10	Cells	BALF	SS	WT
11	Cells	Skin	LCH	V600E
12	Cells	BALF	SS	WT
13	FFPE	Skin	MS-HR	WT
14	FFPE	Skin	MS-HR	WT
15	FFPE	Skin	MS-HR	WT
16a	FFPE	Gum	MS	WT
16b	FFPE	Bone		V600E
17	FFPE	Skin	SS	WT
18	FFPE	Skin	SS	WT
19	FFPE	Skin	SS	WT
20a	FFPE	LN	LCH	WT
20b	FFPE	LN		V600E
21	FFPE	LN	LCH	V600E
22	FFPE	N/A	LCH	WT
23	FFPE	Liver	LCH	WT
24	FFPE	N/A	LCH	WT
25	FFPE	Skin	LCH	V600E
26	FFPE	Skin	MS	V600E
27	FFPE	Skin	SS	V600E
28a	FFPE	Lung		V600E
28b	FFPE	Lung	LCH	V600E
28c	FFPE	Lung		V600E
29	FFPE	Thyroid	LCH	V600E

FFPE, formalin-fixed paraffin-embedded; N/A, information not available; BALF, bronchoalveolar lavage fluid; LN, lymph node; SS, single system; MS, multisystem; MS-HR, multisystem high risk; WT, wild type; LCH, Langerhans cell histiocytosis of unknown status.

samples to be $BRAF^{V600E}$-positive (data not shown), suggesting a low level of mutated cells in the gum sample below the sensitivity threshold of sequencing. It is of note that PCR analysis did not identify V600E mutations in any other biopsies identified as wild-type by sequencing. A total of 3 lung samples were available for patient 28, all of which were $BRAF^{V600E}$-positive. There were two lymph node samples available for patient 20, of which one was $BRAF^{V600E}$-positive. It is possible that one of these lymph node samples was obtained from a node not involved in the lesion; however, the clinical history of these samples is not available.

Discussion

The $BRAF^{V600E}$ mutant was present in the adult population in the current study at a slightly lower frequency than that indicated by the meta-analysis (38 vs. 47%, respectively), albeit with no discernible pattern linking the mutation to lesional site (skin or bone) or disease severity (SS or MS-LCH). While the meta-analysis revealed a higher prevalence of mutations in high-risk organs, $BRAF^{V600E}$ status by itself does not necessarily identify high-risk disease. The present findings are broadly comparable with those reported by Berres *et al* (25).

Consistency in $BRAF^{V600E}$ mutation status in more than one lesion from the same individual (with the exception of one lymph node biopsy) is consistent with a clonal origin of the disease (4,5). Moreover, the fact that dendritic cells derive from circulating myeloid precursors, and that lesional Langerhans cells have an immature phenotype, suggests that LCH can be considered to be a haematological tumour (14,19).

Haematopoietic forms of cancer typically exhibit arrested cell development at a discrete stage of an ordered developmental pathway, frequently associated with distinct patterns of mutation. In addition, the increased prevalence of $BRAF^{V600E}$ mutations in higher risk organs including the liver and spleen (Fig. 1C) was concordant with results from Héritier *et al* (26) suggesting that the expression of this mutation in at-risk organs increases the aggressiveness of LCH, particularly in younger patients. Clinically, LCH is currently treated as a haematological disease in paediatric cases.

BRAF mutations in haematological malignancy are relatively rare (27,28). The $BRAF^{V600E}$ mutation has a high prevalence in in hairy cell leukaemia and has been suggested to be the disease-defining event in this disorder (29). It is, however, rare in other B-cell or associated lymphoproliferative disorders (28) and is notably absent from chronic and acute myeloid neoplasms (30,31).

BRAF mutation-targeting therapy, including the BRAF inhibitors vemurafenib and dabrafenib, have demonstrated evidence of therapeutic activity in several $BRAF^{V600E}$-mutated cancer types, including hairy cell leukaemia (29,32-34). However, the results of the current study suggest that, prior to administering BRAF therapy, clinicians must be aware that the mutation characterizes a subset of LCH and administration of such therapies should be predicted upon genotyping. Furthermore, the resistance-profile of BRAF inhibitors in melanoma (35) must be considered to ensure LCH is treated and eliminated, rather than driving drug resistance and limiting future clinical options.

The present study has demonstrated that $BRAF^{V600E}$ mutations are present within a sub-population of LCH patients. The haematological tumour profile exhibited by LCH suggests that certain treatments that are currently undertaken in paediatric LCH cases and for other haematopoietic types of cancer may aid the treatment of LCH. An investigation of the effective treatments for hairy cell leukaemia may offer more therapeutic options for this disease.

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