# Genetic studies yield clues to the pathogenesis of Langerhans cell histiocytosis

Li Zhang and Joel Moss

**Affiliation**: Cardiovascular and Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA.

**Correspondence**: Joel Moss, Cardiovascular and Pulmonary Branch, National Heart, Lung, and Blood Institute, Room 6D05, Building 10, National Institutes of Health, Bethesda, MD, 20892-1590, USA.
E-mail: mossj@nhlbi.nih.gov

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**Identifying new therapeutic targets of interstitial lung disease from genetic studies**
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Interstitial lung diseases (ILDs) comprise a heterogeneous group of rare respiratory disorders characterised by diffuse pulmonary infiltrates and destruction of lung parenchyma [1]. With growing evidence that mutations in genes encoding proteins that participate in critical regulatory pathways have roles in the pathogenesis of some forms of ILD, it appears that some diseases under the ILD umbrella may be neoplastic and associated with the presence of cancer-like cells, not found in healthy individuals [2]. For example, lymphangioleiomyomatosis (LAM) results from the proliferation of LAM cells with mutation of *tuberous sclerosis* (*TSC*)*1* and *TSC2* genes. LAM can be sporadic or occur in association with TSC, an autosomal dominant disorder [3]. *TSC2* mutations were detected in seven (50%) out of 14 and a *TSC1* mutation in one (7.1%) out of 14 TSC-LAM patients [4]. Sporadic LAM appears to be driven by *TSC2* mutation [5]. The proteins encoded by *TSC1* and *TSC2* are upstream of the mammalian target of rapamycin pathway, which has been targeted in LAM treatment (Multicenter International Lymphangioleiomyomatosis Efficacy and Safety of Sirolimus (MILES) trial) [6].

Pulmonary Langerhans cell histiocytosis (PLCH) is a rare ILD that occurs predominantly in young smokers [7–10]. PLCH is considered to be a neoplastic process, which results from the proliferation of tumour cells exhibiting aberrant expression of CD1a on their surface and *BRAF-V600E* oncogenetic mutations [11].

In this issue of the *European Respiratory Journal*, Mourah *et al.* [12] present the remarkable finding that *NRAS* mutations occur in PLCH lesions. This is the first documentation of *NRAS* mutations in patients with Langerhans cell histiocytosis (LCH).

Activating mutations are seen at high frequency in genes encoding proteins of the mitogen-activated protein kinase (MAPK) pathway, such as *BRAF-V600E* in cancer [13]. Extracellular growth factors bind to cell surface transmembrane tyrosine kinase receptors, resulting in activation of RAS, a member of the GTP-binding superfamily of 20-kDa proteins, which then activates a protein kinase cascade (RAS, RAF, MAPK kinase (MEK), extracellular signal-regulated kinase (ERK)) comprising the MAPK signalling pathway, which regulates cell proliferation [14]. BRAF, a member of the RAF protein family, is a serine-threonine kinase. Activating mutations in the *BRAF* gene have been associated with cancers,

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including melanomas, colorectal carcinomas and ovarian neoplasms [15]. The *BRAF-V600E* mutation results in constitutive activation of the serine-threonine kinase and increased cell proliferation [16]. The discovery of the activating mutation in *BRAF* is consistent with the conclusion that LCH is a neoplastic process with inflammatory manifestations [17]. ERK activation appears to be a universal event in LCH, resulting from dysregulation of upstream signalling proteins. The RAF/MEK/ERK pathway is involved in several cellular responses including cell cycle regulation, cell proliferation and differentiation, and cell survival and apoptosis [18]. Because activation of the RAF/MEK/ERK signalling pathway was observed in all cases [11], additional activating mutations in other members of this cascade were expected.

*MAP2K1* mutations were reported in 2014 in two independent studies. Brown *et al.* [19] observed that 18 (45%) out of 40 LCH patients had *BRAF* mutations whereas 11 (27%) out of 40 had a *MAP2K1* mutation. Results of whole exome sequencing of samples from LCH lesions and normal tissues obtained from 41 patients, presented by Chakraborty *et al.* [20], revealed that 20 (50%) out of 41 cases had a somatic *BRAF* mutation, while seven other cases harboured *MAP2K1* mutations. The *MAP2K1* mutations were associated with phosphorylation of ERK. In addition, somatic mutations of the MAPK pathway genes, *ARAF* and *ERBB3*, were detected in two individuals [20]. Nelson *et al.* [21] later reported detection of *MAP2K1* and *MAP3K1* mutations in LCH lesions. In all of these studies, mutations in *BRAF* or in *MAP2K1* were mutually exclusive, with *MAP2K1* involved in a minority (25–35%) of LCH patients [20].

Mourah *et al.* [12] again confirmed activation of the MAPK signalling pathway and found the *BRAF-V600E* mutation in 13 (50%) out of 26 PLCH biopsies, with a *MAP2K1* mutation in three (20%) out of 13 *BRAF* wild-type PLCH lesions. A recent study found that 50–60% of Erdheim–Chester disease (ECD) patients and LCH patients harboured *BRAF* mutations in the diseased tissue [22]. Thus, LCH and ECD may derive from a common cellular progenitor [22, 23]. Emile *et al.* [24] focused on ECD, providing evidence of important similarities to and differences from LCH. In particular, *NRAS* mutations were detected in three out of 17 ECD *BRAF-V600E* wild-type patients [24]. Mourah *et al.* [12] further explored gene mutations in LCH. They used both standard pyrosequencing and highly sensitive E-*ice*-COLD PCR to discover mutations that underlie LCH pathogenesis. 11 (40%) out of 26 flow-sorted CD1a-positive cells isolated from PLCH lesions had an *NRAS* mutation. Importantly, *NRAS* mutations were found only in CD1a-positive cells, demonstrating the genetic lesion in PLCH. In contrast to other forms of LCH, smoking may be the stimulus for PLCH [8, 10]. Phosphatidylinositol 3-kinase (PI3K)/AKT is one of the main downstream effectors of the RAS family, regulating metabolism, growth, proliferation, survival, transcription and protein synthesis [14, 25]. Activating mutations of *NRAS* have been associated with lung cancer [26], melanoma [27], colorectal cancer [28] and acute myeloid leukaemia [29]. Mourah *et al.* [12] also showed activation of the AKT pathway in all PLCH cases, a functional consequence of the *NRAS* mutation. This finding raises the possibility of combined therapy in LCH targeting the RAS/RAF/PI3K/AKT and BRAF/MEK/ERK pathways.

Hutter *et al.* [30] reported that the NOTCH signalling pathway was involved in an LCH lesion. Activated NOTCH1 was detected by Western blot in protein lysates from 10 (80%) out of 12 LCH biopsies (bone, skin and mucosa). Molecular analysis revealed that both isolated and in-tissue LCH cells selectively expressed the NOTCH ligand Jagged 2 (*JAG2*). They further showed that *JAG2* signalling induced key LCH-cell markers in monocyte-derived dendritic cells, suggesting a role of NOTCH signalling in LCH oncogenesis. Therefore, in selected patients, interference with NOTCH signalling might be a potential strategy for LCH treatment [30].

Mourah *et al.* [12] also identified concurrent *BRAF-V600E* and *NRAS* mutations in seven (27%) out of 26 PLCH lesions, concluding that they were derived from different clonal populations. Using univariate analyses, they found that clinical outcomes were better for patients with only single *BRAF* or *NRAS* mutations than for those with both. Although more studies are needed, a trend for worse pulmonary function is observed for patients with *BRAF-V600E* mutations than those with *NRAS* mutations and *BRAF* wild type.

In summary, the identification of new therapeutic targets may lead to successful treatments for more members of this family of lung diseases. The effectiveness of sirolimus in stabilising lung function, reducing the sizes of angiomyolipomas, and chylous effusions, as well as clearing circulating LAM cells is proof of concept that therapy targeting defective genetic and biochemical pathways can be successful [3, 6, 31–33]. Targeted therapy is currently used in the management of patients with advanced nonsmall cell lung cancer (NSCLC). Mutations in the epidermal growth factor receptor (EGFR) tyrosine kinase are observed in NSCLC adenocarcinomas; the presence of an *EGFR* mutation confers a more favourable prognosis and strongly predicts sensitivity to EGFR tyrosine kinase inhibitors [34]. Personalised medical therapies may similarly be found for other lung diseases. Here, Mourah *et al.* [12] highlight *NRAS* as an important target in the search for novel molecular therapies. Discovering additional different mutations beyond *BRAF-V600E* is no doubt a worthwhile means to improve patient care.

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