Targetable driver mutations in multicentric reticulohistiocytosis

Multicentric reticulohistiocytosis (MRH) is a very rare systemic disease, characterized by multiple destructive arthritic and papulonodular skin lesions that can also affect other organs including the lungs and heart.[1] MRH is classified as a group C histiocytosis (cutaneous and mucocutaneous histiocytosis)[2] with typical histopathological findings such as histiocytic infiltration, particularly of multinucleated giant cells with eosinophilic cytoplasms. From the first report in 1937, only 200-300 cases of MRH have been reported, and the molecular pathogenesis of MRH remains poorly understood. Given that its clinical manifestations are similar to those of rheumatoid arthritis (RA), it has been suspected that MRH is an autoimmune or inflammatory disease, and treatments similar to those for RA, including administration of corticosteroids, methotrexate, bisphosphonates, and several biological anti-inflammatory agents (etanercept, adalimumab, and infliximab), have been tried.[3,4] Although spontaneous remission is occasionally observed during the first ten years after diagnosis, functional prognosis is usually poor; joint replacement surgery has often been required because of the progression of destructive arthritis, and current treatment is inadequate, especially in severe cases.[4] In this study, we performed a comprehensive genetic analysis in two MRH patients to help elucidate its molecular pathogenesis.

We studied specimens from two patients with MRH and from 13 patients with Langerhans cell histiocytosis (LCH). One of our patients with MRH has been reported elsewhere.[5] Patients provided written informed consent. The Ethics Committee of the Nagoya University Graduate School of Medicine approved this study, which was conducted in accordance with the principles of the Declaration of Helsinki. We performed whole-exome sequencing (WES) and RNA sequencing in two patients with MRH. We performed PCR–amplicon-based targeted deep sequencing covering *BRAF* and *MAP2K1*, and RNA sequencing in 13 patients with LCH. Details of these analyses are provided in the *Online Supplementary Methods*.

A 60-year old female (unique patient number 1, UPN1) visited our hospital because of multiple skin lesions that had first appeared when she was 52 years old. She had a history of breast cancer at the age of 48. Her family history was unremarkable. Fluorodeoxyglucose (FDG) positron-emission tomography (PET) showed the accumulation of FDG in the polyarticular lesions of bilateral upper and lower extremities (Figure 1A). The histological findings of periarticular lesions showed infiltration of histiocytes and multinucleated giant cells (Figure 1C). Immunohistochemical analysis showed CD68 was positive in these cells, whereas LCH markers such as CD1a and Langerin were negative. No abnormalities were revealed in her blood test results, and no blasts appeared in her peripheral blood (*Online Supplementary Table S1*). We diagnosed the patient as having MRH based on the clinical and pathological features.

UPN2 was a 39-year old male who visited our hospital because of disturbances in his daily activities caused by polyarticular nodular lesions and arthralgia in addition to multiple skin lesions (erythematous papules) (*Online Supplementary Figure S1*).[5] He had no remarkable family history. At the age of 30, abnormal chest shadows were found on X-ray images at routine medical examination. A bronchoscopic biopsy revealed the infiltration of histiocytic cells. The disease was followed up without any intervention because of the lack of subjective symptoms. FDG-PET showed abnormal accumulation of FDG in the large and small joints of the body (including phalangeal joints) (Figure 1). We performed a biopsy of a periarticular lesion of the left elbow and diagnosed him as having MRH based on the typical histological findings similar to those of UPN1 (Figure 1C).

We performed WES using biopsy specimens containing histiocytic cells and peripheral blood mononuclear cells derived from the two patients with MRH. We identified eight non-synonymous somatic mutations in the histiocytic cells of each specimen, which were suggestive of clonal expansion (Table 1). While to the best of our

Table 1. Somatic mutations in multicentric reticulohistiocytosis.

UPN	Gene	Nucleic acid change	Amino acid change	VAF
UPN1	*TRPV3*	c.916G>A	p.V306M	0.15
UPN1	*BEND2*	c.1069G>A	p.V357I	0.13
UPN1	*PANK4*	c.512C>T	p.P171L	0.12
UPN1	*NOX1*	c.107C>T	p.A36V	0.12
UPN1	*CLUH*	c.713A>T	p.Y238F	0.11
UPN1	*ADH7*	c.811G>A	p.G271S	0.09
UPN1	*AKAP1*	c.335C>G	p.P112R	0.09
UPN1	*DOCK1*	c.689A>G	p.K230R	0.02
UPN2	*CDK2*	c.431C>G	p.P144R	0.13
UPN2	†*TET2*	c.2890C>T	p.Q964*	0.12
UPN2	SYNE1	c.12137C>T	p.T4046M	0.11
UPN2	†*MAP2K1*	c.305_310delAGATCA	p.103_104delIK	0.07
UPN2	*LIPG*	c.1276C>A	p.L426M	0.04
UPN2	*ZNF233*	c.16-2A>T	(exon 3)	0.04
UPN2	*LRIG2*	c.1576G>C	p.D526H	0.02
UPN2	*OTOF*	c.3614C>T	p.A1205V	0.02

†Mutations for which identical mutations were reported as drivers in the literature or databases. UPN: unique patient number; VAF: variant allele frequency.
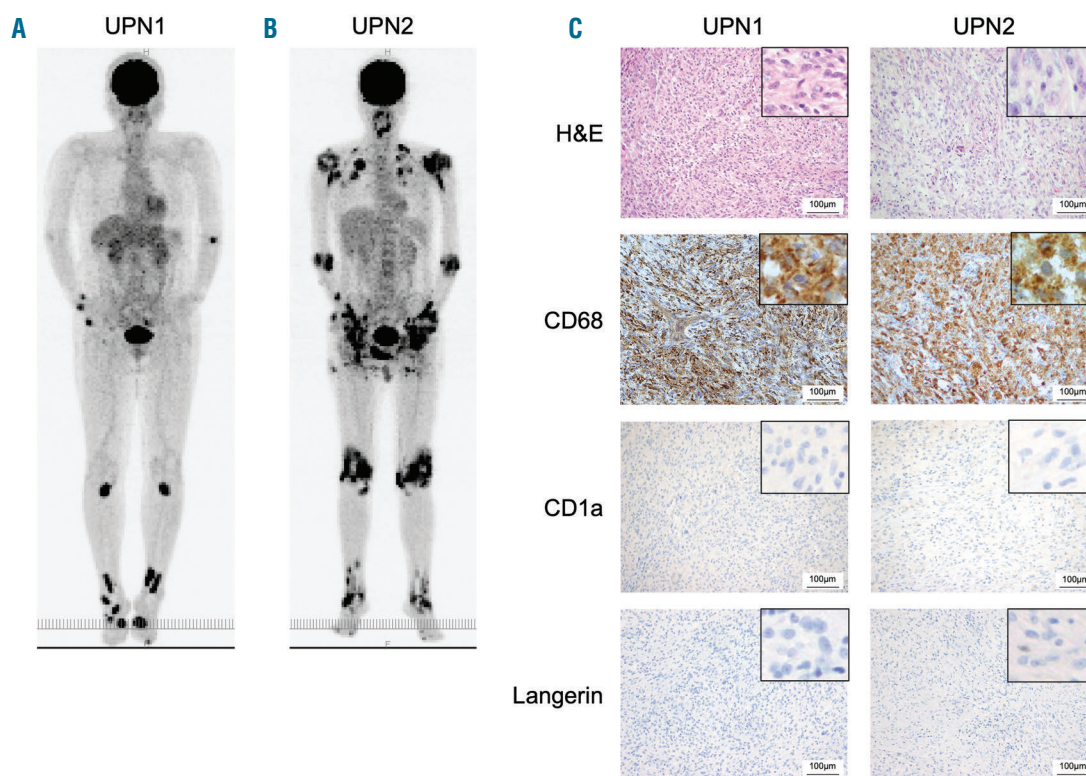
Figure 1. Fluorodeoxyglucose positron-emission tomography (FDG-PET) and histopathological findings of patients with multicentric reticulohistiocytosis (MRH). (A and B) FDG-PET images of Patient 1 (UPN1) (A) and Patient 2 (UPN2) (B). (C) Histopathological and immunohistochemical analyses of biopsy samples. Each inset shows higher magnification. H&E: Hematoxylin & Eosin.

knowledge UPN1 carried no identifiable driver point mutations, we detected an in-frame deletion of *MAP2K1* (encoding dual specificity mitogen-activated protein kinase 1, or MEK1, c.305_310delAGATCA, p.103_104delIK) and a nonsense mutation of *TET2* (encoding methylcytosine dioxygenase TET2, c.2890C>T, p.Q964*) in UPN2. The *MAP2K1* mutation was located in the protein kinase domain of *MAP2K1*, and identical mutations have been reported as gain-of-function driver mutations in melanoma.[6] Taken together, the presence of driver and non-driver mutations suggested neoplastic clonal proliferation of histiocytic cells in MRH.

We identified a novel in-frame fusion involving *KIF5B* (encoding kinesin-1 heavy chain) and *FGFR1* (encoding fibroblast growth factor receptor 1 tyrosine kinase) in UPN1 (Figure 2A, *Online Supplementary Table S2* and *Online Supplementary Figure S3*) by RNA sequencing. The fusion protein contained the dimerization domain derived from KIF5B, which is known to activate RET (*KIF5B-RET*)[7] or ALK (*KIF5B-ALK*)[8] tyrosine kinases, and the tyrosine kinase domain derived from FGFR1, which drives breast cancer (*ERLIN2–FGFR1*),[9] lung squamous cell carcinoma (*BAG4-FGFR1*),[9] and hematologic malignancies (*BCR-FGFR1*).[10] This protein structure strongly suggests gain-of-function tyrosine kinase activity, and we analyzed the differential gene expressions between UPN1 and UPN2 to examine the function of the KIF5B-FGFR1 fusion protein. We found 69 and 191 genes significantly over-expressed in UPN1 and UPN2 with adjusted *P*-values <0.1, respectively (*Online Supplementary Table S3*). A gene set enrichment analysis revealed marked enrichment of genes up-regulated in response to tyrosine

kinase activation in UPN1, suggesting upregulation of tyrosine kinase activity of the KIF5B-FGFR1 fusion protein. On the other hand, UPN2 showed enrichment of genes up-regulated in KRAS signaling, indicating the activation of RAS-MAPK signal transduction pathway by the mutated *MAP2K1* (Figure 2B and C).

Because patients with MRH harbored a *MAP2K1* mutation similar to that observed in patients with LCH, and an *FGFR1* tyrosine kinase fusion, which had not been reported in LCH, we guessed that the tyrosine kinase fusions like the *KIF5B-FGFR1* might be present in LCH. To test this hypothesis, we performed a mutational analysis including targeted deep sequencing and RNA sequencing in 13 patients with LCH (*Online Supplementary Table S4*). Among 13 patients, eight and two harbored a *BRAF* p.V600E mutation and a *MAP2K1* p.102_103delEI mutation, respectively. The remaining three patients carried no *BRAF* mutations, *MAP2K1* mutations, or fusion genes.

When the expression profiles were compared between MRH and LCH, LCH showed upregulation of the genes associated with maturation of dendritic cells in response to inflammatory signals (LINDSTEDT_DENDRITIC_CELL_MATURATION_A and LINDSTEDT_DENDRITIC_CELL_MATURATION_B) (*Online Supplementary Table S5* and *Online Supplementary Figure S4*). This finding suggests that the source of tumor cells in MRH might be different from that in LCH, in which marked enrichment of genes associated with DC progenitors and late DC has been reported.[11]

The mutational analysis in our study suggests that MRH should be considered a neoplastic disease caused by the activation of the RAS-MAPK pathway similar to
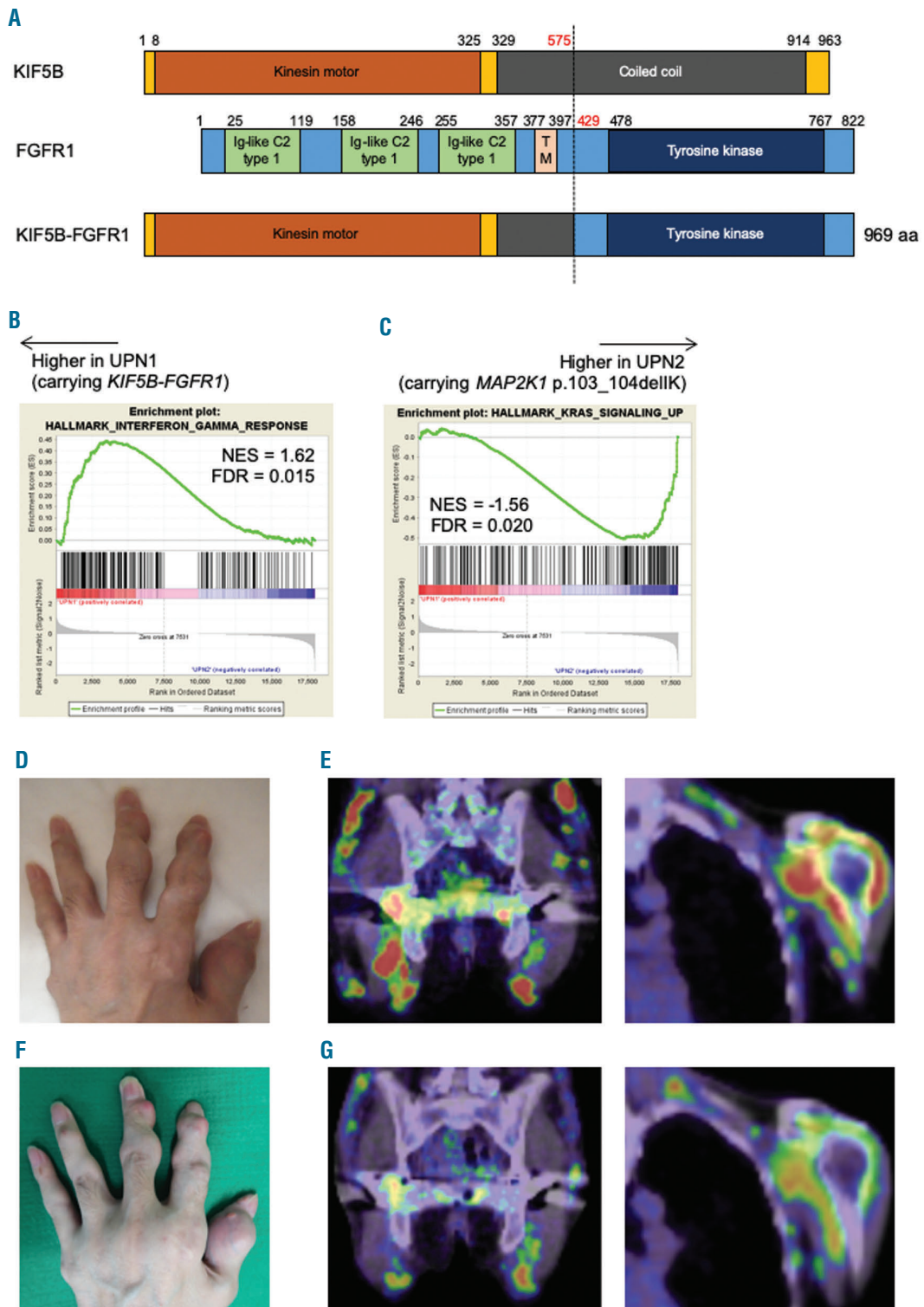
Figure 2. Genetic lesions and treatment response in patients with multicentric reticulohistiocytosis (MRH). (A) Structure of predicted KIF5B-FGFR1 fusion protein. Numbers indicate amino acid residues. Kinesin motor: kinesin motor domain; coiled coil: coiled coil domain; Ig-like C2 type1: immunoglobulin-like C2-type1 domain; TM: transmembrane domain. (B and C) Gene set enrichment analysis comparing the expression profiles obtained from Patient 1 (UPN1) and Patient 2 (UPN2). The genes in the HALLMARK_INTERFERON_GAMMA_RESPONSE gene set were up-regulated in UPN1, whereas those in the HALLMARK_KRAS_SIGNAL-ING_UP were up-regulated in UPN2. NES: normalized enrichment score; FDR: false discovery rate. (D and F) Picture of the left hand of UPN2 before (D) and after (F) chemotherapy showing shrinkage of the periarticular masses, a reduced redness of the skin, and increased wrinkles in the joints. (E and G) Positron emission tomography-computed tomography findings of periarticular lesions of pelvis and left shoulder in UPN2 before (E) and after (G) chemotherapy.

that present in patients with LCH, Erdheim-Chester disease (ECD), and juvenile xanthogranuloma, all of which are classified as L group or C group histiocytosis.[11] We hypothesized that chemotherapy resembling that for LCH might be effective in these patients, and upon approval by our institutional review board, we performed chemotherapy used for LCH (JLSG-02 induction A chemotherapy) (*Online Supplementary Table S6*) to patient UPN2 who harbored *MAP2K1* and *TET2* mutations in his histiocytes.

The patient's disease had been resistant to RA-like immunosuppressive therapies, and he had undergone total joint replacements of the left knee and bilateral hips because of intractable pain emanating from destructive arthritis. After starting chemotherapy, the periarticular masses gradually decreased in size and became soft (Figure 2D and F) and the subjective symptoms including the right knee pain improved. As a result of this response, the patient's right knee did not require the joint replacement surgery that had been considered inevitable before starting chemotherapy. Moreover, positron emission tomography-computed tomography (PET-CT) scan after the treatment showed decreased metabolic activity of tumorous and inflammatory cells (Figure 2E and G). The maximum standardized uptake value (SUVmax) of the right femoral mass decreased from 15.11 to 6.89. However, we had to stop the treatment course because of peripheral neuropathy and reversible cerebral vasoconstriction syndrome (RCVS) that were thought to be chemotherapy adverse-associated events.

Our results suggest that MRH is not an autoimmune or an inflammatory disease but a neoplastic one that may be caused by an aberrant activation of the RAS-MAPK pathway or tyrosine kinases. Both pathways are implicated in the molecular pathogenesis of several histiocytic neoplasms.[11] Both genetic alterations detected in the patients with MRH (*KIF5B-FGFR1* fusion and *MAP2K1* deletion) are druggable, and targeted therapies using FGFR1 or MEK inhibitors may be effective treatments for patients with MRH, as their efficacies have been recently demonstrated in other histiocytic diseases such as ECD.[11]

In current clinical practice, the diagnosis of MRH is completely based on clinical presentation and not on genetic abnormalities. Because our study is limited in terms of the number of patients, the genetic background of MRH still needs to be explored. Further identification of mutations in MRH will clarify the relationships with other hematologic malignancies that share similar mutations with MRH, including myeloproliferative neoplasm or acute leukemia with *FGFR1* rearrangement.[12]

On the basis of the results of our mutational analysis, we administered chemotherapy therapy similar to that used to treat LCH in a patient with the *MAP2K1* mutation. Although we had to stop the chemotherapy because of adverse events (peripheral neuropathy and RCVS), especially attributed to vincristine,[13] we observed improvement in both subjective and objective symptoms, and we think our patient had a partial but clinically substantial good response.

In summary, our results indicate that MRH should be considered a neoplastic disease and suggest promising effects of chemotherapy for its treatment. Further studies are warranted to contribute to the development of optimal therapeutic approaches for MRH, possibly including molecular targeted therapies.

Norihiro Murakami,[1,] Tomohisa Sakai,[2,*] Eisuke Arai,[2] Hideki Muramatsu,[1] Daisuke Ichikawa,[1] Shuji Asai,[2] Yoshie Shimoyama,[3] Naoki Ishiguro,[2] Yoshiyuki Takahashi,[1] Yusuke Okuno[4,**] and Yoshihiro Nishida[5,**]*

*NM and TS contributed equally to this work. **YO and YN contributed equally to this work as co-senior authors.

[1]*Department of Pediatrics, Nagoya University Graduate School of Medicine;* [2]*Department of Orthopedic Surgery, Nagoya University Graduate School of Medicine;* [3]*Department of Pathology and Laboratory Medicine, Nagoya University Hospital;* [4]*Center for Advanced Medicine and Clinical Research, Nagoya University Hospital and* [5]*Department of Rehabilitation, Nagoya University Hospital, Nagoya, Japan*

References

1. Luz FB, Gaspar TAP, Kalil-Gaspar N, Ramos-e-Silva M. Multicentric reticulohistiocytosis. J Eur Acad Dermatol Venereol. 2001;15(6):524-531.
2. Emile JF, Abla O, Fraitag S, et al. Revised classification of histiocytoses and neoplasms of the macrophage-dendritic cell lineages. Blood. 2016;127(22):2672-2681.
3. Islam AD, Naguwa SM, Cheema GS, Hunter JC, Gershwin ME. Multicentric Reticulohistiocytosis: a Rare Yet Challenging Disease. Clin Rev Allergy Immunol. 2013;45(2):281-289.
4. Tariq S, Hugenberg ST, Hirano-Ali SA, Tariq H. Multicentric reticulohistiocytosis (MRH): case report with review of literature between 1991 and 2014 with in depth analysis of various treatment regimens and outcomes. Springerplus. 2016;5:180.
5. Nishida Y, Asai S, Arai E. Multicentric reticulohistiocytosis misdiagnosed as tenosynovial giant cell tumour. Rheumatology (Oxford). 2017 Aug 11. [Epub ahead of print]
6. de Unamuno Bustos B, Murria Estal R, Perez Simo G, et al. Towards Personalized Medicine in Melanoma: Implementation of a Clinical Next-Generation Sequencing Panel. Sci Rep. 2017;7(1):495.
7. Kohno T, Ichikawa H, Totoki Y, et al. KIF5B-RET fusions in lung adenocarcinoma. Nat Med. 2012;18(3):375-377.
8. Takeuchi K, Choi YL, Togashi Y, et al. KIF5B-ALK, a novel fusion oncokinase identified by an immunohistochemistry-based diagnostic system for ALK-positive lung cancer. Clin Cancer Res. 2009;15(9):3143-3149.
9. Wu YM, Su F, Kalyana-Sundaram S, et al. Identification of targetable FGFR gene fusions in diverse cancers. Cancer Discov. 2013;3(6):636-647.
10. Demiroglu A, Steer EJ, Heath C, et al. The t(8;22) in chronic myeloid leukemia fuses BCR to FGFR1: transforming activity and specific inhibition of FGFR1 fusion proteins. Blood. 2001;98(13):3778-3783.
11. Diamond EL, Durham BH, Haroche J, et al. Diverse and Targetable Kinase Alterations Drive Histiocytic Neoplasms. Cancer Discov. 2016;6(2):154-165.
12. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-2405.
13. Sankhe S, Kamath N, Sahu A. A rare case of chemotherapy induced reversible cerebral vasoconstriction syndrome in a patient of acute lymphocytic leukemia. J Cancer Res Ther. 2015;11(4):1012-1014