RESEARCH LETTER

blood advances

TO THE EDITOR:

A patient-derived CABIN1 mutation recapitulates features of idiopathic multicentric Castleman disease in a mouse model

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Idiopathic multicentric Castleman disease (iMCD) is a rare and life-threatening condition characterized by episodic proinflammatory hypercytokinemia and multicentric lymphadenopathy.[1] Characteristic histopathologic features include dysmorphic germinal centers (GCs), follicular dendritic cell hyperproliferation, hypervascularization, and polytypic plasmacytosis.[2] Systemic hypercytokinemia during iMCD flares is the main driver of patients' symptoms. To date, interleukin-6 (IL-6) is thought to be a key cytokine, given that neutralization of IL-6 can be effective at controlling the disease in one-third of patients.[3-7] Further studies utilizing patient samples during iMCD flare revealed heightened T-cell activation and mammalian target of rapamycin signaling.[8,9] Accordingly, mammalian target of rapamycin inhibition with sirolimus is effective in treating a subset of patients with iMCD.[10] Still, one-third of patients do not respond to any available targeted therapy, and cytotoxic chemotherapy is their only option. Better understanding of iMCD etiology and pathogenesis is urgently needed to identify additional treatments, but research using patient biospecimens is confounded by the treatments that patients receive and disease heterogeneity. An animal model that can be used to answer key questions of the early events in iMCD would be highly valuable.

Although no genetic etiology has been identified thus far, genetic variants that regulate inflammation may serve as causative or predisposing factors to iMCD. To test this possibility, whole-exome sequencing on an iMCD-index (IC) patient[9] and their parents was performed (supplemental Figure 1). Based on the Natioanl Institutes of Health and Broad algorithms, 5 germ line variants were predicted to be biologically relevant to iMCD. Among these genes, only heterozygous missense mutations (L816F and V2185M) in CABIN1 displayed a high conservation score and were predicted to have a deleterious impact on protein function (Figure 1A; supplemental Figure 2). Given that CABIN1 plays a role in T-cell activation as a negative regulator of calcineurin and myocyte enhancer factor 2 (MEF2),[11] we hypothesized that these CABIN1 mutations could be contributing to iMCD pathogenesis.

To test this hypothesis, we utilized CRISPR/Cas9 to introduce germ line single-nucleotide changes in CABIN1 at p.L816F and p.V2185M (referred to CABIN1^{L816F} and CABIN1^{V2185M} hereafter) and generated CABIN1$^{L816F/L816F}$ homozygous, CABIN1$^{V2185M/V2185M}$ homozygous, and L816F/V2185M compound heterozygous (CABIN1$^{V2185M/L816F}$) mice (Figure 1B). At baseline, characterization of multiple lymphoid organs of CABIN1 mutant mice did not reveal any striking difference in the overall frequency of immune cell populations such as B cells, T cells, and regulatory T cells, compared to wild-type (WT) control mice (supplemental Figure 3). Moreover, no spontaneous morbidity or mortality was apparent in CABIN1 mutant mice followed for at least 1 year of age.

To immunologically challenge the mice, we next infected the CABIN1 mutant mice with lymphocytic choriomeningitis virus clone 13 (LCMV CL13), a strain of LCMV that establishes chronic infection

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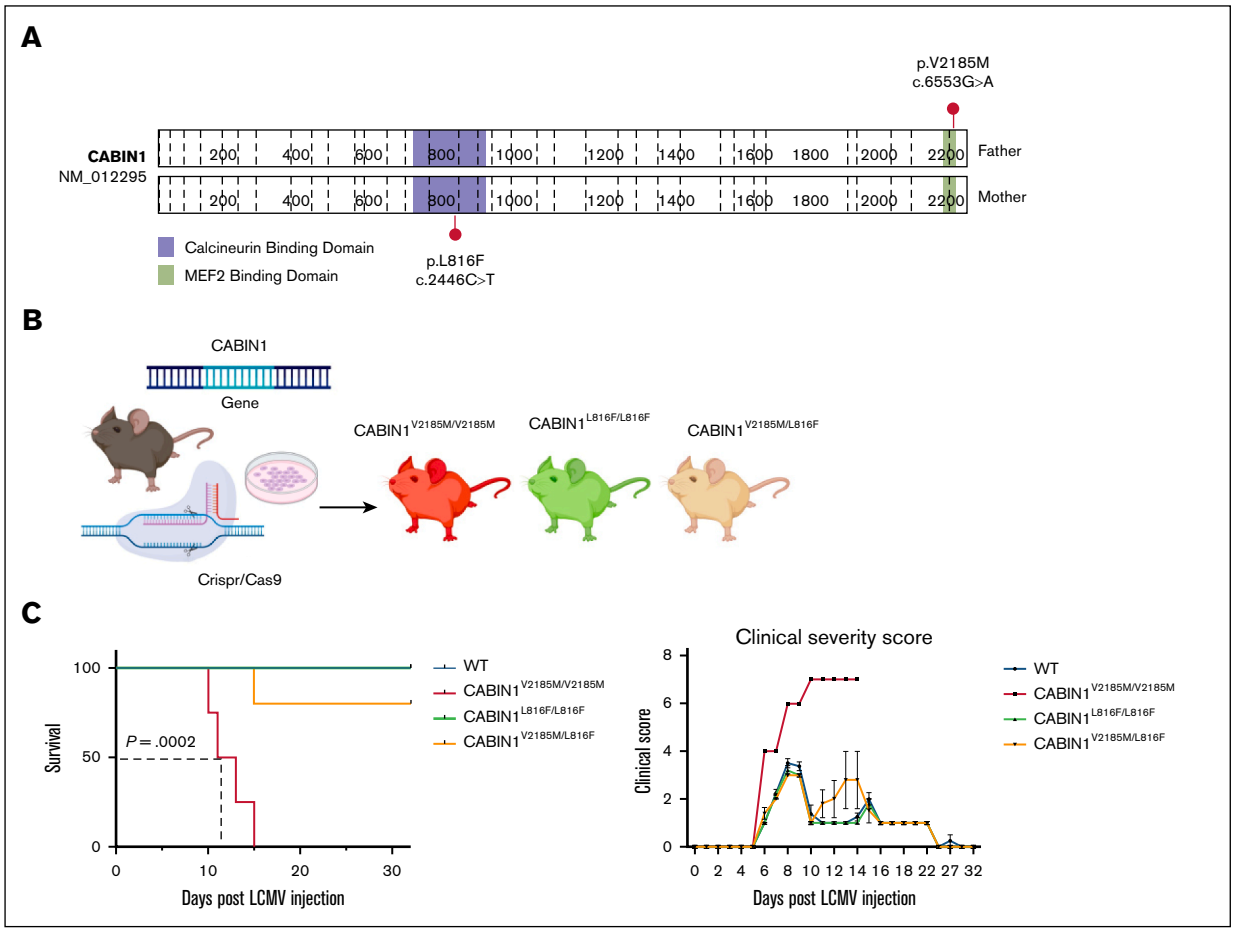

Figure 1. Generation of CABIN1-mutant mouse model and phenotypic characterization. (A) CABIN1 compound heterozygous mutations in the iMCD-IC patient identified by whole-exome sequencing. (B) Generation of mutant mice harboring germ line CABIN1 gene mutations using CRISPR/Cas9 technique. (C) Kaplan-Meier survival curve and clinical score after LCMV CL13 infection. WT and CABIN1-mutated mice were infected with LCMV CL13. Mortality (left) and morbidity (right) were assessed over time. Data from N = 4 to 8 mice from 2 independent experiments are shown.

(supplemental Figure 4). CABIN1$^{V2185M/V2185M}$ mice displayed significantly higher morbidity and mortality compared to WT mice following LCMV CL13 infection (Figure 1C-D). Of importance, compound heterozygous CABIN1$^{V2185M/L816F}$ (mimicking the patient) mice also showed increased morbidity/mortality, however, with a less robust and more variable presentation (Figure 1C-D). Therefore, further studies to understand the impact of the mutation were carried out with homozygous CABIN1$^{V2185M/V2185M}$ mice.

Next, we tested whether LCMV CL13–infected CABIN1$^{V2185M/V2185M}$ exhibited pathological features observed in iMCD. All key cytokines/chemokines during iMCD flares, including IL-6, vascular endothelial growth factor A, monocyte chemoattractant protein 1, and chemokine (C-X-C motif) ligand 13 (CXCL13) were significantly elevated in the serum of LCMV CL13–infected CABIN1$^{V2185M/V2185M}$ compared to WT controls, with the most robust increase in CXCL13 levels (Figure 2A-B). Importantly, this increase was not only observed following LCMV CL13 but also after infection with the LCMV Armstrong strain, which causes a less severe infection (Figure 2B).

As abnormal GC formation is required for the diagnosis of iMCD, we immunized CABIN1$^{V2185M/V2185M}$ mice with a messenger RNA vaccine (ovalbumin [OVA] messenger RNA combined with lipid nanoparticles) to assess lymph node histologic changes. Similar to LCMV, immunization with OVA-lipid nanoparticles led to elevated serum CXCL13 levels in CABIN1$^{V2185M/V2185M}$ compared to WT mice (Figure 2B). Moreover, CABIN1$^{V2185M/V2185M}$ mice displayed fewer and smaller reactive GCs with an expansion of stromal elements (Figure 2C; supplemental Figure 5), reminiscent of lymph node histopathology in human patients with iMCD.

To test how the V2185M mutation impacted downstream signaling pathways, we examined the expression of target proteins downstream of MEF2 and calcineurin. Upon T-cell receptor activation by anti-CD3/CD28, expression of a MEF2-induced gene, Nur77, was significantly increased in CABIN1$^{V2185M/V2185M}$ compared to WT T cells (supplemental Figure 6A). Interestingly, no increase in IL-2 production (target of calcineurin/NFAT pathway) was detected in activated T cells following phorbol-12-myristate-13-acetate/ionomycin treatment (supplemental Figure 6B). These data suggest that the disease phenotype seen in CABIN1$^{V2185M/V2185M}$ mice may be related to MEF2 rather than calcineurin dysregulation.

In this study, to our knowledge, we present the first demonstration that a germ line genetic mutation may be able to contribute to

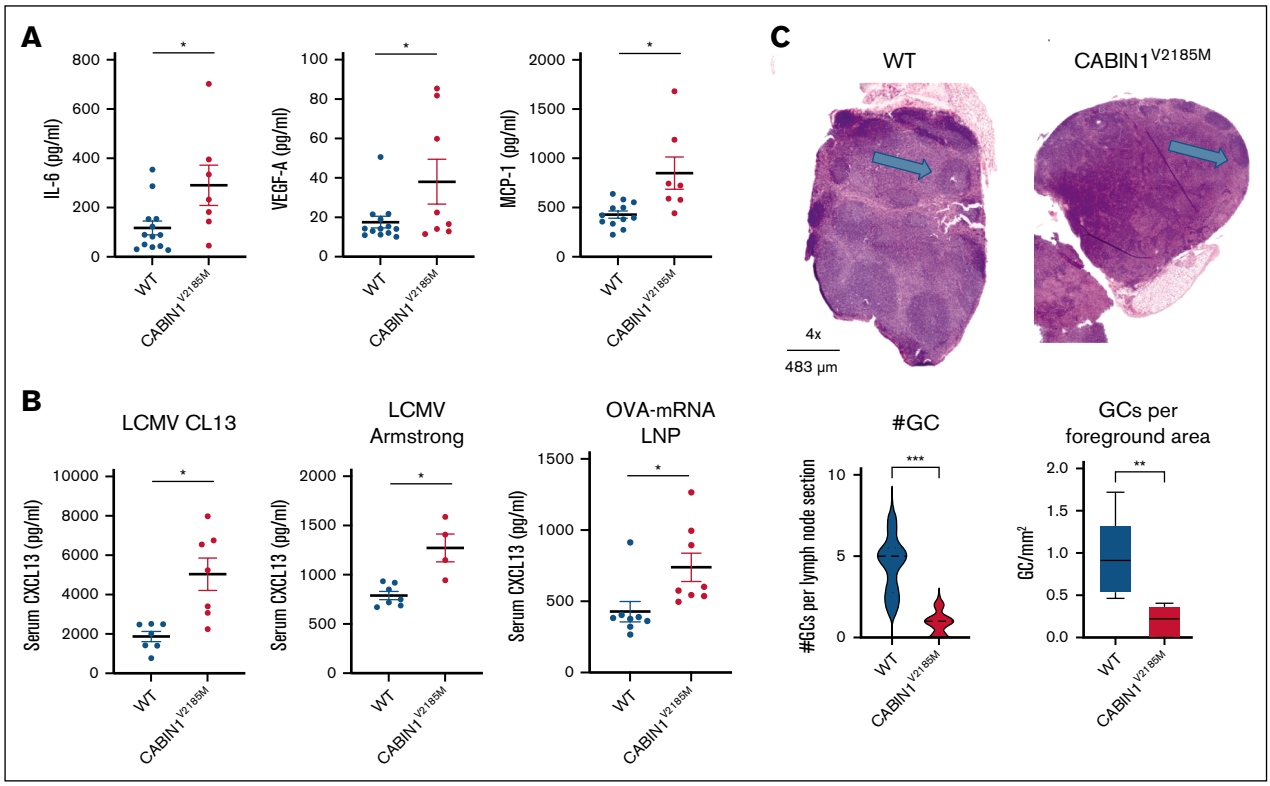

Figure 2. Cytokine profile and histological changes in CABIN1$^{V2185M/V2185M}$ mice following viral and antigenic stimulation. (A) Serum cytokine levels after LCMV CL13 infection of CABIN1$^{V2185M/V2185M}$ mutant mice. WT (blue) and CABIN1^{V2185M} mice (red) were infected with LCMV CL13, and serum IL-6, vascular endothelial growth factor A (VEGF-A), and monocyte chemoattractant protein 1 (MCP-1) levels were measured on day 7 post LCMV CL13 infection. Data from N = 7 to 12 mice per group from 2 independent experiments are shown. *$P < .05$ by Student *t* test. (B) Serum CXCL13 levels after viral (LCMV CL13 and Armstrong) and antigenic (OVA-messenger RNA [mRNA] lipid nanoparticles [LNP]) challenge. WT (blue) and CABIN1^{V2185M} mice (red) were injected with either LCMV CL13, LCMV Armstrong, or OVA mRNA-LNP model antigen. Serum CXCL13 levels were measured on day 7 (after LCMV infection) or day 12 (after OVA mRNA-LNP). Data from N = 4 to 11 mice per group from 2 independent experiments. *$P < .05$ by Student *t* test. (C) Histological changes after OVA-LNP immunization of CABIN1$^{V2185M/V2185M}$ mutant mice. Regional lymph nodes from WT and CABIN1$^{V2185M/V2185M}$ mice were analyzed by hematoxylin and eosin staining on day 12 post OVA mRNA-LNP injection. The number of GCs (blue arrows) per lymph node and the number of GCs per area were assessed. Data from N = 10 mice from 2 experiments are shown. *$P < .05$; **$P < .01$; ***$P < .001$.

iMCD-related symptoms. Our data show that upon pathogenic infection or immunization, CABIN1$^{V2185M/V2185M}$ mice recapitulate the cytokine/chemokine milieu and abnormal lymph node morphology seen in human iMCD. Over the past several years, many theories have emerged regarding the etiology and pathogenesis of iMCD.[12] However, detailed knowledge of the key cell types and signaling pathways in iMCD is still lacking. Therefore, studies like this one that uncover potential pathogenic mechanisms and treatment approaches are crucially important. Mechanistically, we showed that CABIN1 mutations could affect GC response. We favor that CABIN1 dysregulation in T cells is responsible for this effect, because a recent study showed that aberrant MEF2 signaling leads to alterations in T follicular helper-cell differentiation and function.[13] Identifying the responsible cell type(s) and mechanism will be a focus of future studies.

It was surprising to observe that there was no obvious phenotype with just the L816F mutation. This could be because the L816F mutation in mice may behave differently than in humans. Alternatively, the V2185M heterozygosity could have predisposed rather than drive the patient to iMCD. This is supported by our data showing that the compound heterozygous mice displayed increased mortality and morbidity, albeit to a lesser extent. In addition, based on the relatively prevalent mean allele frequencies of L816F and V2185M mutations, it is more likely that they are disease-predisposing rather than driver mutations (supplemental Figure 2). Although the exact prevalence of the CABIN1 mutation in iMCD patients is unknown, this study provides proof-of-concept that genetic mutations that cause dysregulation of immunoregulatory proteins leading to hypercytokinemia could predispose to iMCD. Our study also provides a potential mouse model to further unravel the pathogenesis of iMCD.

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References

1. Sumiyoshi R, Koga T, Kawakami A. Candidate biomarkers for idiopathic multicentric Castleman disease. *J Clin Exp Hematop*. 2022;62(2):85-90.
2. Fajgenbaum DC, Uldrick TS, Bagg A, et al. International, evidence-based consensus diagnostic criteria for HHV-8-negative/idiopathic multicentric Castleman disease. *Blood*. 2017;129(12):1646-1657.
3. Beck JT, Hsu SM, Wijdenes J, et al. Brief report: alleviation of systemic manifestations of Castleman's disease by monoclonal anti-interleukin-6 antibody. *N Engl J Med*. 1994;330(9):602-605.
4. Waterston A, Bower M. Fifty years of multicentric Castleman's disease. *Acta Oncol*. 2004;43(8):698-704.
5. van Rhee F, Casper C, Voorhees PM, et al. A phase 2, open-label, multicenter study of the long-term safety of siltuximab (an anti-interleukin-6 monoclonal antibody) in patients with multicentric Castleman disease. *Oncotarget*. 2015;6(30):30408-30419.
6. van Rhee F, Fayad L, Voorhees P, et al. Siltuximab, a novel anti-interleukin-6 monoclonal antibody, for Castleman's disease. *J Clin Oncol*. 2010;28(23):3701-3708.
7. van Rhee F, Wong RS, Munshi N, et al. Siltuximab for multicentric Castleman's disease: a randomised, double-blind, placebo-controlled trial. *Lancet Oncol*. 2014;15(9):966-974.
8. Arenas DJ, Floess K, Kobrin D, et al. Increased mTOR activation in idiopathic multicentric Castleman disease. *Blood*. 2020;135(19):1673-1684.
9. Fajgenbaum DC, Langan RA, Japp AS, et al. Identifying and targeting pathogenic PI3K/AKT/mTOR signaling in IL-6-blockade-refractory idiopathic multicentric Castleman disease. *J Clin Invest*. 2019;129(10):4451-4463.
10. Bayram E, Pehlivan UA, Fajgenbaum DC, Paydas S. Refractory idiopathic multicentric Castleman disease responsive to sirolimus therapy. *Am J Hematol*. 2023;98(2):361-364.
11. Esau C, Boes M, Youn HD, Tatterson L, Liu JO, Chen J. Deletion of calcineurin and myocyte enhancer factor 2 (MEF2) binding domain of Cabin1 results in enhanced cytokine gene expression in T cells. *J Exp Med*. 2001;194(10):1449-1459.
12. Dispenzieri A, Fajgenbaum DC. Overview of Castleman disease. *Blood*. 2020;135(16):1353-1364.
13. Kim YJ, Oh J, Jung S, et al. The transcription factor Mef2d regulates B:T synapse-dependent GC-T(FH) differentiation and IL-21-mediated humoral immunity. *Sci Immunol*. 2023;8(81):eadf2248.