**ONLINE SUPPLEMENTARY DATA**

## Supplementary Data 1. Monogenic autoinflammatory bone disorders

### 1.1 Familial CNO: Majeed syndrome

Familial cases of autosomal-recessively inherited CNO (Majeed syndrome) have been reported in consanguineous families (1). Affected individuals develop severe early-onset CNO within the first two years of life, a Sweet syndrome-like skin rash, and dyserythropoietic anemia. Majeed syndrome has been linked to homozygous mutations in the *LPIN2* gene resulting in a loss of functional activity (1, 2). The lipin2 protein functions as phosphatidate phosphatase, which seems to play a role in lipid metabolism (3). It is expressed by various tissues, including the liver, kidneys, gastrointestinal tract, lymphoid tissues, and bone marrow. The exact function and involvement of lipin2 in signaling pathways remains to be determined. Based on findings from studies on obesity-induced chronic inflammation, it has been claimed that fatty acid accumulation may induce pro-inflammatory phenotypes through toll like receptor (TLR)2 and TLR4-stimulation (4). TLR ligation subsequently results in the activation of mitogen activated protein kinases (MAPK), particularly Jun kinase (JNK)1 which mediates pro-inflammatory cytokine expression (pro-IL-1β, pro-IL-18, IL-6, TNF, etc.), and the induction of NLRP3 inflammasome assembly and subsequent pro-inflammatory cytokine secretion (active IL-1β, IL-18) (Supplementary Figure 1) (5). TLR4 stimulation with lipopolysaccharide (LPS) of human or murine monocytes with altered lipin2 expression resulted in disturbed expression of TNF-α, IL-6, or the chemokine CCL-2. Monocytes with increased levels of lipin2 failed to produce pro-inflammatory cytokines, whereas reduced lipin2 expression was associated with pro-inflammatory phenotypes. Thus, lipin2 has been discussed as a negative regulator of fatty acid-induced pro-inflammatory signaling in monocytes and macrophages through impaired MAPK pathways (3, 6, 7). This is of particular

interest, since patients with sporadic CNO also exhibit impaired MAPK activation in monocytes (8). Interestingly, heterozygous mutations in family members from Majeed syndrome patients develop psoriatic skin lesions, which can also occur in CNO patients (9, 10). Of note, the *LPIN2* gene falls within a chromosomal region identified as a psoriasis susceptibility locus, further suggesting a potential pathophysiological relationship between psoriasis and CNO/CRMO (1, 11).

Since the exact pathophysiology is unknown, treatment of Majeed syndrome is empirical. Patients only moderately respond to treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) and/or corticosteroids. Based on altered cytokine expression in the aforementioned experiments in lipin2-deficient cells, IL-1 blockade with recombinant IL-1 receptor antagonist anakinra has been performed in two patients. Anakinra induced remission of bone inflammation and mildly improved anemia (2, 6, 7). However, since bone marrow biopsies have not been performed, it remains unclear whether dyserythropoiesis resolved or not.

### 1.2 Inflammasome-associated monogenic disorders

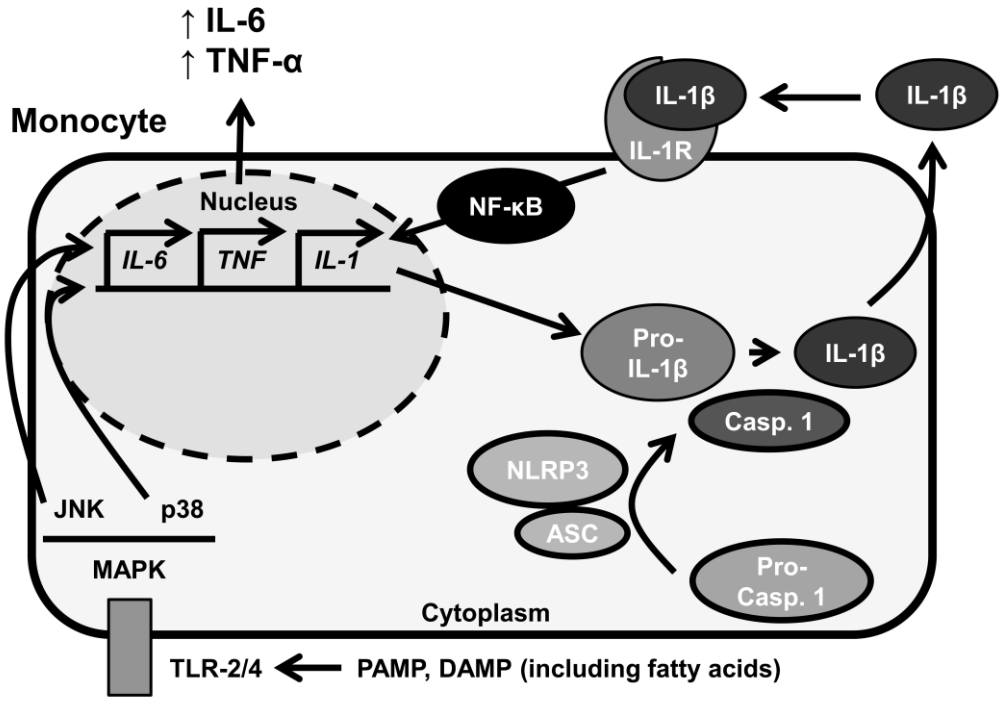
Inflammasomes are multi-protein complexes that, in response to endogenous or exogenous stimuli, assemble and mediate the cleavage and subsequent release of pro-inflammatory effector cytokines, including IL-1β and IL-18. Several monogenic autoinflammatory syndromes are caused by uninhibited activation of inflammasome assembly or defective post-translational control of inflammasome-dependent cytokines. Two of these inflammasome-associated disorders are DIRA and PAPA syndrome (4-7).

***DIRA*** is an autoinflammatory bone disorder, clinically manifesting within the first days of life. Characteristic findings are pustular rash, oral ulcers, rib widening with periostal inflammation, multifocal osteolytic lesions, and heterotopic ossification. DIRA is caused by autosomal recessively inherited mutations in the *IL1RN* gene, encoding for

the IL-1 receptor antagonist, a post-translational regulator of IL-1 signaling, resulting in uncontrolled pro-inflammatory cytokine and chemokine expression. Life-long substitution with recombinant IL-1 receptor antagonist anakinra results in complete remission (12-14).

***PAPA*** syndrome is another severe autoinflammatory bone disorder. Affected children present with sterile erosive arthritis, cystic acne, and pyoderma gangrenosum-like ulcerative lesions (15, 16). Symptoms frequently persist into adulthood and result in joint destruction and physical disability (6, 17). Individuals with PAPA syndrome carry autosomal dominant mutations in the *PSTPIP1* gene, encoding for the CD2-binding protein 1 (CD2BP1). Through its carboxyl-terminal SH3 domain, PSTPIP1/CD2BP1 interacts with the inflammasome-related protein pyrin that negatively regulates caspase-1 activation and subsequent IL-1β activation and release (18). Interaction of PSTPIP1/CD2BP1 with pyrin requires phosphorylation. Dephosphorylation of PSTPIP1/CD2BP1 is mediated by the protein tyrosine phosphatase PEST and altered by PAPA-associated mutations in the *PSTPIP1* gene, resulting in increased phosphorylation and increased interaction of PSTPIP1/CD2BP1 with pyrin. Similarly to patients with Familial Mediterranean Fever (FMF), who carry loss-of-function mutation in *MEFV*, encoding for pyrin, PAPA patients exhibit uncontrolled inflammasome activity and IL-1β release, reflected by systemic inflammation and inflammatory lesions characterized by infiltrates of neutrophilic granulocytes and monocytes (16). Treatment of PAPA syndrome is challenging. Corticosteroids, thalidomide, cyclosporine, tacrolimus and IVIG have been used with variable success. Data on biologic treatment are rare, with case reports suggesting both efficacy and inefficacy of TNF-inhibitors and IL-1 blockade with recombinant IL-1 receptor antagonist anakinra (6, 17).

**Supplementary Figure 1. TLR signaling through MAPK activation results in proinflammatory cytokine expression and secretion.**




Activation of TLR-2 and TLR-4 through PAMP or DAMP result in the activation of MAPK, including JNK and p38. As a results, proinflammatory cytokine genes, including IL-1, IL-6, and TNF, are trans-activated. While IL-6 and TNF-α proteins are secreted “right away,” IL-1 is expressed in its inactive form pro-IL-1β and requires activation through caspase-1. So-called inflammasomes are multiprotein complexes that result in caspase-1 activation. Active caspase-1 mediates cleavage of pro-IL-1β into its active form IL-1β, which is subsequently secreted from cells. IL-1β is a potent proinflammatory cytokine that, in response to binding to its receptor complex, activates NF-κB pathways, which feed into proinflammatory cytokine expression (including IL-1, IL-6, and TNF-α). TLR: Toll-like receptor; MAPK: mitogen-activated protein kinases; PAMP: pathogen-associated molecular patterns; DAMP: danger-associated molecular patterns; JNK: Jun kinases; IL-1: interleukin 1; TNF-α: tumor necrosis factor-α; NF-κB: nuclear factor-κB; Casp. 1: caspase-1.

**Supplementary Data 2. Nonbacterial Osteomyelitis in mice**

Two well-characterized murine models exist, both resembling severe CRMO. Chronic multifocal osteomyelitis (cmo) mice carry a spontaneous homozygous mutation, lupo mice carry a chemically induced homozygous mutation in the proline-serine-threonine phosphatase-interacting protein 2 (*Pstpip2*) gene (cmo mice: p.L98P; lupo mice: p.I282N) (19, 20). Both strains develop severe systemic cytokine and chemokine dysregulation, contributing to systemic inflammation, extramedullary hematopoiesis, inflammatory skin lesions, and sterile osteomyelitis. Though the exact contribution of *Pstpip2* mutations remains unknown, two groups recently demonstrated a central contribution of enhanced IL-1ß expression and secretion in cmo mice. Both groups showed that IL-1 receptor I-deficient animals were protected from the development of osteomyelitis. Furthermore, they demonstrated that IL-1β but not IL-1α was responsible for bone inflammation in osteomyelitis-prone cmo animals (21, 22). This is in line with our findings in monocytes from humans with CRMO, in which a lack of anti-inflammatory cytokines IL-10 and IL-19 resulted in increased IL-1β mRNA expression, and NLRP3 inflammasome activation with subsequently increased cleavage and secretion of mature IL-1β (23). Furthermore, Greenhill et al. recently documented that IL-10-deficient mice exhibit increased expression of inflammasome components (including Nlrp3, Aim2, caspase 1, and caspase 12) in the synovium in an induced joint destruction and bone erosion model (24). The underlying mechanisms in human monocytes from CRMO patients and IL-10-deficient mice on the one end vs. cmo mice on the other, however, largely differ. While monocytes from human CRMO patients express increased amounts of IL-1ß mRNA and exhibit increased inflammasome activity, neutrophils are the main source of IL-1ß in cmo mice (21-23). Furthermore, cmo mice that are also deficient of the inflammasome components NLRP3, ASC, or caspase-1 and mice treated with caspase-1 inhibitors

were not protected from the development of osteomyelitis, indicating that IL-1ß release in these animals is independent of the NLRP3 inflammasome. Thus, additional proteases other than caspase-1 appear to be responsible for pro-inflammatory IL-1β activation in cmo mice (21, 22, 25). Promising candidates are caspase-8, which has been claimed to cleave pro-IL-1β into its active form, and other proline serine proteases. Indeed, cmo mice deficient of both caspase-1 and 8 were protected, suggesting redundant roles of those enzymes. However, additional studies are warranted to finally answer the question of which mechanisms are responsible for IL-1β release in cmo mice (21, 22, 25). Furthermore, additional mechanisms seem to contribute to the pro-inflammatory phenotype in cmo mice. In agreement with most recent finding of our group, macrophages from cmo mice respond to M-CSF or LPS stimulation with increased expression of macrophage inflammatory protein-1α (MIP-1α) or IL-6 (19, 20). Furthermore, serum MIP-1α and IL-6 levels are increased in cmo mice, suggesting that they may be involved in the induction of osteomyelitis. The exact contribution of altered cytokine expression to bone inflammation and subsequent bone-loss, however, remains subject to speculation.

## REFERENCES

1. Ferguson PJ, Chen S, Tayeh MK, Ochoa L, Leal SM, Pelet A, et al. Homozygous mutations in LPIN2 are responsible for the syndrome of chronic recurrent multifocal osteomyelitis and congenital dyserythropoietic anaemia (Majeed syndrome). J Med Genet 2005;42:551-7.
2. Herlin T, Fiirgaard B, Bjerre M, Kerndrup G, Hasle H, Bing X, et al. Efficacy of anti-IL-1 treatment in Majeed syndrome. Ann Rheum Dis 2013;72:410-3.
3. Valdearcos M, Esquinas E, Meana C, Pena L, Gil-de-Gomez L, Balsinde J, et al. Lipin-2 reduces proinflammatory signaling induced by saturated fatty acids in macrophages. J Biol Chem 2012;287:10894-904.
4. Ferguson PJ, El-Shanti HI. Autoinflammatory bone disorders. Curr Opin Rheumatol 2007;19:492-8.
5. Vega MI, Huerta-Yepaz S, Garban H, Jazirehi A, Emmanouilides C, Bonavida B. Rituximab inhibits p38 MAPK activity in 2F7 B NHL and decreases IL-10 transcription: pivotal role of p38 MAPK in drug resistance. Oncogene 2004;23:3530-40.
6. Morbach H, Hedrich CM, Beer M, Girschick HJ. Autoinflammatory bone disorders. Clin Immunol 2013;147:185-96.
7. Stern SM, Ferguson PJ. Autoinflammatory bone diseases. Rheum Dis Clin North Am 2013;39:735-49.
8. Hofmann SR, Morbach H, Schwarz T, Rosen-Wolff A, Girschick HJ, Hedrich CM. Attenuated TLR4/MAPK signaling in monocytes from patients with CRMO results in impaired IL-10 expression. Clin Immunol 2012;145:69-76.
9. El-Shanti HI, Ferguson PJ. Chronic recurrent multifocal osteomyelitis: a concise review and genetic update. Clin Orthop Relat Res 2007;462:11-9.
10. Ferguson PJ, Bing X, Vasef MA, Ochoa LA, Mahgoub A, Waldschmidt TJ, et al. A missense mutation in pstpip2 is associated with the murine autoinflammatory disorder chronic multifocal osteomyelitis. Bone 2006;38:41-7.
11. Asumalahti K, Laitinen T, Lahermo P, Suomela S, Itkonen-Vatjus R, Jansen C, et al. Psoriasis susceptibility locus on 18p revealed by genome scan in Finnish families not associated with PSORS1. J Invest Dermatol 2003;121:735-40.
12. Aksentijevich I, Masters SL, Ferguson PJ, Dancey P, Frenkel J, van Royen-Kerkhoff A, et al. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. N Engl J Med 2009;360:2426-37.
13. Morbach H, Dick A, Beck C, Stenzel M, Muller-Hermelink HK, Raab P, et al. Association of chronic non-bacterial osteomyelitis with Crohn's disease but not with CARD15 gene variants. Rheumatol Int 2010;30:617-21.
14. Reddy S, Jia S, Geoffrey R, Lorier R, Suchi M, Broeckel U, et al. An autoinflammatory disease due to homozygous deletion of the IL1RN locus. N Engl J Med 2009;360:2438-44.
15. Lindor NM, Arsenault TM, Solomon H, Seidman CE, McEvoy MT. A new autosomal dominant disorder of pyogenic sterile arthritis, pyoderma gangrenosum, and acne: PAPA syndrome. Mayo Clin Proc 1997;72:611-5.
16. Smith EJ, Allantaz F, Bennett L, Zhang D, Gao X, Wood G, et al. Clinical, Molecular, and Genetic Characteristics of PAPA Syndrome: A Review. Curr Genomics 2010;11:519-27.
17. Almeida de Jesus A, Goldbach-Mansky R. Monogenic autoinflammatory diseases: concept and clinical manifestations. Clin Immunol 2013;147:155-74.

18. Wise CA, Gillum JD, Seidman CE, Lindor NM, Veile R, Bashiardes S, et al. Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder. Hum Mol Genet 2002;11:961-9.
19. Chitu V, Nacu V, Charles JF, Henne WM, McMahon HT, Nandi S, et al. PSTPIP2 deficiency in mice causes osteopenia and increased differentiation of multipotent myeloid precursors into osteoclasts. Blood 2012;120:3126-35.
20. Grosse J, Chitu V, Marquardt A, Hanke P, Schmittwolf C, Zeitlmann L, et al. Mutation of mouse Mayp/Pstpip2 causes a macrophage autoinflammatory disease. Blood 2006;107:3350-8.
21. Cassel SL, Janczy JR, Bing X, Wilson SP, Olivier AK, Otero JE, et al. Inflammasome-independent IL-1beta mediates autoinflammatory disease in Pstpip2-deficient mice. Proc Natl Acad Sci U S A 2014;111:1072-7.
22. Lukens JR, Gross JM, Calabrese C, Iwakura Y, Lamkanfi M, Vogel P, et al. Critical role for inflammasome-independent IL-1beta production in osteomyelitis. Proc Natl Acad Sci U S A 2014;111:1066-71.
23. Hofmann SR, Kubasch AS, Ioannidis C, Rosen-Wolff A, Girschick HJ, Morbach H, et al. Altered expression of IL-10 family cytokines in monocytes from CRMO patients result in enhanced IL-1beta expression and release. Clin Immunol 2015;161:300-7.
24. Greenhill CJ, Jones GW, Nowell MA, Newton Z, Harvey AK, Moideen AN, et al. Interleukin-10 regulates the inflammasome-driven augmentation of inflammatory arthritis and joint destruction. Arthritis Res Ther 2014;16:419.
25. Ferguson PJ, Laxer RM. New discoveries in CRMO: IL-1beta, the neutrophil, and the microbiome implicated in disease pathogenesis in Pstpip2-deficient mice. Semin Immunopathol 2015;37:407-12.