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P2RX7 gene variants associate with altered inflammasome assembly and reduced pyroptosis in chronic nonbacterial osteomyelitis (CNO)

Amandine Charras [a], Sigrun R. Hofmann [b], Allison Cox [c], Felix Schulze [b], Susanne Russ [b], Sarah Northey [a], Xuan Liu [d], Yongxiang Fang [d], Sam Haldenby [d], Hella Hartmann [e], Alexander G. Bassuk [c], Ana Carvalho [a], Francesca Sposito [a], Lev Grinstein [f], Angela Rösen-Wolff [b], Almut Meyer-Bahlburg [g], Michael W. Beresford [a,h], Elke Lainka [i], Dirk Föll [j], Helmut Wittkowski [j], Hermann J. Girschick [k], Henner Morbach [l], Steffen Uebe [m], Ulrike Hüffmeier [m], Polly J. Ferguson [c,1], Christian M. Hedrich [a,h,*]

[a] *Department of Women's and Children's Health, Institute of Life Course and Medical Sciences, University of Liverpool, UK*
[b] *Department of Pediatrics, Medical Faculty Carl Gustav Carus, Technische Universität Dresden, Germany*
[c] *Stead Family Department of Pediatrics, University of Iowa Carver College of Medicine, Iowa City, USA*
[d] *Centre of Genome Research, Institute of Infection, Veterinary & Ecological Sciences, University of Liverpool, UK*
[e] *Light Microscopy Facility, Centre for Regenerative Therapies, Technische Universität Dresden, Germany*
[f] *Department of Pediatrics, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany*
[g] *Pediatric Rheumatology and Immunology, Department of Pediatrics, University Medicine Greifswald, Greifswald, Germany*
[h] *Department of Rheumatology, Alder Hey Children's NHS Foundation Trust, Liverpool, UK*
[i] *Department of Pediatrics II, University Hospital Essen, University of Duisburg-Essen, Essen, Germany on behalf of the German Autoinflammatory Disease Network (AID Net), Germany*
[j] *Department for Pediatric Rheumatology & Immunology, University Hospital Münster, Germany on behalf of the German Autoinflammatory Disease Network (AID Net), Germany*
[k] *Department of Pediatrics, Vivantes Hospital Friedrichshain, Berlin, Germany*
[l] *Department of Pediatrics, University Hospital Würzburg, Germany*
[m] *Institute of Human Genetics, University Hospital Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany*

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ABSTRACT

Chronic nonbacterial osteomyelitis (CNO), an autoinflammatory bone disease primarily affecting children, can cause pain, hyperostosis and fractures, affecting quality-of-life and psychomotor development. This study investigated CNO-associated variants in *P2RX7*, encoding for the ATP-dependent *trans*-membrane K^+ channel P2X7, and their effects on NLRP3 inflammasome assembly.

Whole exome sequencing in two related transgenerational CNO patients, and target sequencing of *P2RX7* in a large CNO cohort (N = 190) were conducted. Results were compared with publicly available datasets and regional controls (N = 1873). Findings were integrated with demographic and clinical data. Patient-derived monocytes and genetically modified THP-1 cells were used to investigate potassium flux, inflammasome assembly, pyroptosis, and cytokine release.

Rare presumably damaging *P2RX7* variants were identified in two related CNO patients. Targeted *P2RX7* sequencing identified 62 CNO patients with rare variants (32.4%), 11 of which (5.8%) carried presumably damaging variants (MAF <1%, SIFT *"deleterious"*, Polyphen *"probably damaging"*, CADD >20). This compared to 83 of 1873 controls (4.4%), 36 with rare and presumably damaging variants (1.9%). Across the CNO cohort, rare variants unique to one (Median: 42 versus 3.7) or more (≤11 patients) participants were over-represented when compared to 190 randomly selected controls. Patients with rare damaging variants more frequently experienced gastrointestinal symptoms and lymphadenopathy while having less spinal, joint and skin involvement (psoriasis). Monocyte-derived macrophages from patients, and genetically modified THP-1-derived macrophages reconstituted with CNO-associated *P2RX7* variants exhibited altered potassium flux, inflammasome assembly, IL-1β

* Corresponding author. Department of Women's and Children's Health, Institute of Life Course and Medical Sciences, University of Liverpool, UK.
E-mail address: chedrich@liverpool.ac.uk (C.M. Hedrich).
[1] Equal contribution.

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and IL-18 release, and pyroptosis. Damaging *P2RX7* variants occur in a small subset of CNO patients, and rare *P2RX7* variants may represent a CNO risk factor. Observations argue for inflammasome inhibition and/or cytokine blockade and may allow future patient stratification and individualized care.

1. Introduction

Chronic nonbacterial osteomyelitis (CNO) is an autoinflammatory bone disease that primarily affects children and adolescents [1]. The clinical spectrum ranges from singular, sometimes self-limited bone lesions to chronically active or recurring inflammation of multiple bones, also referred as chronic recurrent multifocal osteomyelitis (CRMO) [2]. Due to its variable presentation and limited awareness among healthcare providers, diagnosis and treatment can be delayed [3]. This is particularly worrying, because untreated CNO can result in bone sclerosis, pathological fractures (mainly affecting vertebrae), growth anomalies, chronic pain, and psychosocial problems [4,5]. Thus, CNO can significantly influence patients' psychomotor development and quality-of-life [6].

Recent discoveries have contributed to a better understanding of molecular pathomechanisms driving inflammation in CNO, including reduced expression of immune regulatory IL-10, increased expression of pro-inflammatory IL-1β, IL-6, and TNF, increased production of NLRP3 inflammasome components, and enhanced inflammasome assembly. The resulting pro-inflammatory environment likely contributes to increased osteoclast differentiation and activation, bone remodelling and inflammatory bone loss [1,7]. However, the exact underlying molecular mechanisms remained unknown.

Following the identification of *P2XR7* variants in affected individuals from a family with CNO, this study investigated the prevalence of gene variants in a large national cohort of CNO patients and matched healthy controls. The *P2XR7* gene encodes for the membrane-bound P2X7 receptor, a key regulator of NLRP3 inflammasome assembly [8,9]. Recent studies suggested P2X7 to be involved in inflammatory bone and joint diseases, including osteoarthritis [10,11]. Thus, the study furthermore functionally investigated three selected CNO-associated *P2XR7* variants in genetically modified THP-1-derived macrophage-like cells to understand their functional impact on inflammasome assembly, pyroptosis and inflammatory cytokine release.

2. Methods

2.1. Cohorts

European patients were diagnosed by rheumatologists experienced with CNO [1]. DNA was collected alongside clinical and demographic information at the Department of Paediatrics, Medical Faculty Carl Gustav Carus, Technische Universität Dresden (N = 18, including mother of index patient), the Department of Paediatrics, University Hospital Würzburg (N = 66), the Department of Medicine, University of Greifswald (N = 2), and through the German AID-Net (N = 106) [12] (Supplement Table 1). Ethnicity-matched-healthy individuals (N = 1873) were accessed through the Institute of Human Genetics, University Hospital Erlangen. Patients/controls and/or their legal guardians gave written informed consent. Research was permitted by local ethics committees and conducted in accordance with the Declaration of Helsinki.

2.2. Whole blood assays

Whole blood collected in hirudin coated tubes (Sarstedt) was primed with 1 μg/ml ultra-pure LPS (Invivogen), incubated with ATP for 30 min, and used for IL-1β quantification using cytometric beads (Becton Dickinson) [13].

2.3. Sequencing and variant identification

Sequencing with 150bp paired-end reads was performed in N = 190 CNO patients (index patient and her mother were previously sequenced (**Supplement Methods**)) using the QIAseq Targeted DNA Panels kit (Qiagen, MiSeq_2500, Illumina). Demultiplexing, adaptor and quality trimming was performed, and reads were aligned to the human reference genome (hg38). Variants were called, filtered, and annotated as described in **Supplement Methods**. Publicly available databases were used in combination to predict pathogenicity of variants. A rarity filter considered SNPs according to their population frequency in Genome Aggregation Database (gnomAD V3.1.2) dataset, 1000 Genomes Project, and ethnicity matched healthy controls (N = 1873). A population allele frequency threshold of 1% (MAF) was applied. Next, a pathogenicity filter was applied, retaining variants within the *"deleterious"* SIFT category, a *"probably damaging"* Polyphen category, and a CADD score of >20. To this list of variants, additional three variants that did not have associated information, but were predicted to cause a premature stop codon were retained (c.763G > T/p.Glu255*; c.417G > A/p.Trp139*; c.1465G > T/p.Glu489*).

2.4. ASC speck quantification

$CD14^+$ monocytes were isolated from peripheral blood by negative selection (Stemcell Technologies). For ASC speck imaging, primary monocytes or THP-1 cells were stimulated with LPS, LPS/ATP, LPS + BzATP or LPS + nigericin. ASC specks were stained and quantified by microscopy (**Supplement Methods**). Extracellular specks in cell culture supernatants were quantified following the adjusted protocol from Rowczenio et al. [14] on the Guava flow cytometer, analysed by FlowJo. Results are displayed using the formula: ASC ratio = Absolute value [((particles percentage after LPS + BzATP or LPS + nigericin treatment)-(particles percentage after PMA overnight for the same cell)/(-particles percentage after LPS priming-particles percentage after PMA overnight for the same cells))].

2.5. Genetically modified THP-1 cells

The *P2RX7* gene was deleted in THP-1 cells on one (clone 3F2) or both alleles (clone 4G8) using CRISPR/Cas9 technology (Cyagen Biosciences INC). The pL.SSVF_XbaI_GFP_MluI.i_gb3.*Bsp*EI.Hygro.str vector was kindly donated by S. Thieme (Department of Pediatrics, Universitätsklinikum Carl Gustav Carus, TU Dresden, Dresden, Germany). From these, pL.SSVF_XbaI_*P2RX7*_MluI.i_gb3.*Bsp*EI.Hygro.str plasmids comprising the RNA coding sequence of *P2RX7* were generated. Three *P2RX7* variants were investigated: c.349C > T (index family, rare gain-of-function/GOF), c.489C > T (common_gain-of-function/GOF), c.920G > A (rare_loss-of-function/LOF) (Supplement Figures 1,2, Supplement Table 4**, Supplement Methods**). Virus vector particle production was performed as previously described [15]. Human THP-1 cells were cultured for 2–3 d in 24-well plates before and 3–4 d after lentiviral vector transduction in RPMI1640 cell culture medium supplemented with 10% FBS and 2 mM L-glutamine. After transduction, 2000 μg/ml hygromycin was added to RPMI1640 medium. Copy numbers were tested with quantitative PCR, and the adjusted numbers of the *P2RX7* transgene were two per cell.

2.6. Cell culture

THP-1 cells were treated overnight with PMA (2.5 ng/ml, Sigma-

Aldrich) to generate macrophage-like cells, followed by priming (1 μg/ml LPS, Sigma-Aldrich) for 3h and treated with LPS, LPS/ATP (1 mM, Invivogen), LPS/BzATP (150/300 μM, Alomone), or LPS/nigericin (5/10 μM) as indicated.

2.7. Pyroptosis

LDH release, a pyroptosis surrogate, was measured using cytotoxicity detection kits (Roche) following manufacturer's instructions. Results are displayed using the formula: Cytotoxicity %= ((experimental value-negative control)/(positive control-negative control)) X100 (negative control: untreated (overnight PMA) and positive control: cells treated with Lysis solution).

2.8. Cytokine release

Human IL-1β and IL-18 were quantified following the manufacturer's protocol (U-PLEX/Meso Scale Discovery).

2.9. Intracellular K+ concentrations

THP-1-derived monocytes were stained with PBFI-AM 5 μM plus Pluronic F-127 (ThermoFisher), washed and stimulated with LPS (1 μg/ml), LPS/BzATP (150/300 μM) or LPS/nigericin (5/10 μM).

3. Statistics

Data were analysed using parametric or non-parametric statistical tests (as indicated) after checking for homoscedasticity and normal distribution (Shapiro-Wilk test), using Prism, version 9.0 (GraphPad software), displayed as mean and standard error (SEM) and p-values <0.05 were considered statistically significant.

4. Results

4.1. The rare P2RX7 c.349C > T variant segregates with CNO in a family

Using whole exome sequencing, we identified the rare *P2RX7* variant c.349C > T/p.Arg117Trp/rs28360445 in heterozygous state in a mother and daughter affected by CNO (Supplement Figures 1,2,3B). *P2RX7* encodes for the ATP-sensitive P2X7 transmembrane K^+ channel that regulates NLRP3 inflammasome assembly [16]. To understand c.349C > T/p.Arg117Trp variant's consequences, whole blood was collected from both affected individuals and five adult healthy controls [7]. CNO patients exhibited increased release of IL-1β and IL-18 after priming (LPS) and P2X7 activation (LPS/ATP) (Fig. 1A and B). IL-1β release was reduced in presence of MCC950, a "small molecule" inhibiting NLRP3 activation (Fig. 1C) [13]. After clinical remission was achieved in the paediatric CNO patient (naproxen and methotrexate; >6 months of treatment), experiment was repeated and showed IL-1β release profiles similar to those in an age, sex and ethnicity-matched-healthy control (Fig. 1D). Next, NLRP3 inflammasome assembly was quantified (intracellular ASC "specks") in $CD14^+$ monocyte-derived macrophages [17]. CNO patient exhibited higher numbers of ASC specks (Fig. 1E and F), increased IL-1β and IL-18 release (Fig. 1G) after priming (LPS) and stimulation (LPS/ATP) compared to matched control. Data suggest that CNO-associated *P2RX7* c.349C > T/p.Arg117Trp contributes to increased inflammasome assembly and cytokine release.

4.2. P2RX7 variants associate with CNO in a large national cohort

To investigate whether *P2RX7* variants are common among CNO patients, 190 additional German CNO patients (192 total, all White European) were enrolled in this study, and targeted sequencing of *P2RX7* was performed. The cohort included 122/186 (65.6%) girls (Table 1, Supplement Table 1, Supplement Fig. 3A); median age at disease-onset was 10 years [IQR, 2–18], with boys being slightly older (12 versus 9; p = 0.001).

A total of 70 SNPs in *P2RX7* were identified, 32 (46%) of which had previously been reported and referenced (dbSNP) [18]; 23 (33%) exhibited *in-silico* predicted "high impact" or "modifier" effects. Fourteen SNPs were identified as rare, possibly damaging/pathogenic variants. Rare damaging SNPs, defined by a combination of *in silico* prediction tools, were more common in the CNO patient cohort compared to publicly available databases (3/14 identified; 21%) or the healthy control cohort (2/14; 14%), or not present in either (9/14; 64%) (Supplement Table 2, Supplement Figures 1,2,3B). One or a combination of these rare possibly damaging variants were present in 11 CNO patients (5.8% of the CNO cohort). A total of 10/11 (91%) patients carried common variants (MAF >0.05) in addition to at least one rare variant.

The majority of *P2RX7* SNPs identified in the CNO patient cohort were rare (MAF >1%) or had not previously been reported (53/70, 75.7%). Most rare SNPs identified CNO patients (48/53, 90.6%) were absent in control cohort (N = 1873) (Supplement Table 2). To determine differences in frequencies of CNO-associated SNPs between patients and controls, a Fisher's exact test was performed comparing 190 CNO patients with 1873 bone-healthy controls from the same region (Germany, White European participants), which delivered higher frequencies of some SNPs in patients ($p < 0.05$; FDR<0.05) (Supplement Table 3). As mentioned above, 53 rare *P2RX7* SNPs were identified in 62/190 CNO patients (32.3%); only 5 of these rare variants were identified across 83/1873 controls (4.4%), 2 of which (rs28360445 and rs28360457, Supplement Table 2), presumably rare damaging variants, were identified in 36/1873 controls (1.9%). In addition, October 1873 controls (0.5%) carried one of 43 additional rare variants that were not present in the CNO patient cohort.

Next, using a bioinformatic simulation, the number of unique variants across the CNO cohort (N = 190, included in *P2RX7* panel sequencing) was compared against randomly selected healthy controls (N = 190), which was repeated 1000 times. Across randomly selected 190 (of 1873) controls (repeated 1000 times), a median of 3.7 distinct SNPs were found unique to one sample, 0.002 rare SNPs were present in two of 190 controls, 0.002 rare SNPs were present in three samples. From 190 CNO patients sequenced (*P2RX7* panel sequencing), 42 rare SNPs were found unique to one patient sample, four distinct rare SNPs were present in two CNO patients, three rare SNPs were present in three of 190 CNO samples, one rare SNP was present in five CNO samples, and 11 CNO patients carried the same rare SNP (Supplement Table 4). This comparison indicated a significantly higher number of genetic variants among CNO patients compared to healthy controls.

Genotype:phenotype correlations revealed that patients carrying rare variants had joint involvement (p = 0.0004), psoriasis (p = 0.02) and/or spinal lesions (p = 0.007) less frequently than the remaining cohort. Carriers of rare damaging *P2RX7* variants more frequently experienced abdominal symptoms (p = 0.008), lymphadenopathy (p = 0.02), and/or, though not reaching significance level, mandibular involvement (p = 0.06). Furthermore, while not statistically significant, patients with rare damaging variants more frequently required 2nd-line treatments (disease-modifying anti-rheumatic drugs/DMARDs, bisphosphonates; p = 0.08) (Table 1).

4.3. Generation of genetically modified THP-1 cell lines

To assess gene dose effects and the activity of selected *P2RX7* variants, P2X7-deficient THP-1 monocyte were generated by mono- (3F2) or bi-allelic (4G8) deletion of *P2RX7* using CRISPR-Cas9 genome editing (Supplement Fig. 3C). Next, 4G8 cells were stably transduced to reconstitute P2X7 expression (pLenti: empty vector; WT: *wild-type P2RX7*; CNO-associated *P2RX7* variants: c.349C > T (rare/GOF (*in silico* prediction)), c.920G > A (rare/LOF (*in silico* prediction)), c.489C > T (common/GOF (*in silico* prediction)) (Supplement Figures 3,4, Supplement Table 5). All 4G8 cells with reconstituted P2X7 expression carried

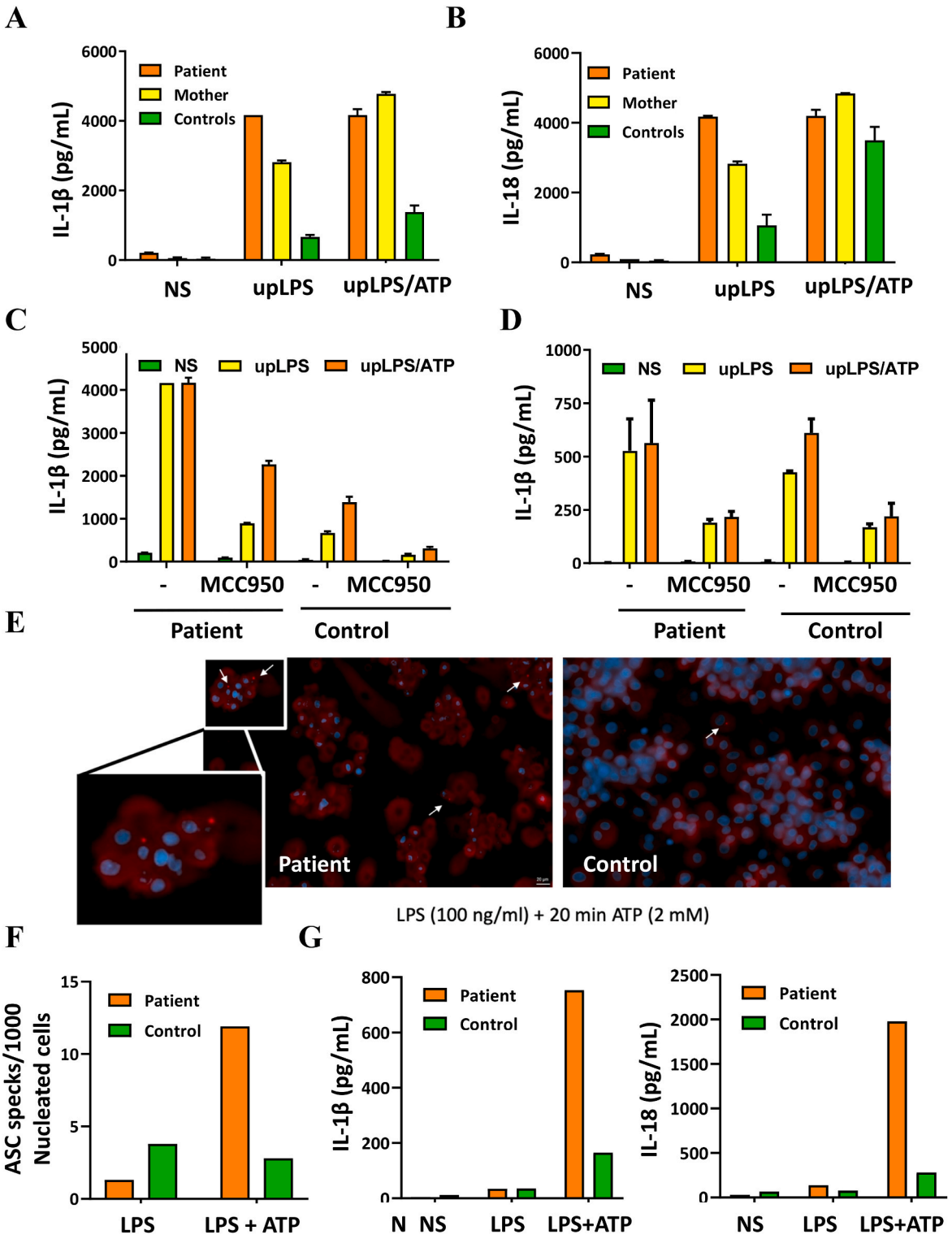

Fig. 1. CNO patients with the *P2RX7* c.349C > T variant exhibit increased inflammasome activity. We identified the rare *P2RX7* variant c.349C > T/p.Arg117Trp (rs28360445) in heterozygous state in two affected family members both with a history of CNO. Using whole blood stimulation assays, both CNO patients exhibited increased IL-1β (A) and IL-18 (B) release from PBMCs when compared to healthy young adult controls (N = 5). C&D) Release of mature IL-1β was suppressed in response to inhibition of NLRP3 inflammasome assembly with MCC950 in the paediatric patient and a matched control. However, effects of MCC950 were incomplete in patients' cells (C). After the induction of clinical remission (naproxen and methotrexate), differences in IL-1β expression between patient and control cells were marginal (D). E) In $CD14^{+}$ monocyte-derived macrophages, NLRP3 inflammasome assembly is represented by the presence of ASC "specks" (Hoechst (blue nuclei), mCherry (red), arrows in white indicate ASC "specks"). F) In response to priming and stimulation (LPS + ATP), macrophages from the CNO patient exhibited a higher number of ASC specks when compared to control cells. G) This was accompanied by increased IL-1β and IL-18 release. NS: Not stimulated, up: ultrapure, MCC950: small molecular NLRP3 inhibitor.

Table 1
CNO patient characteristics.

	Global CNO cohort	Rare damaging variants	Remaining cohort	stat/p value
Number of patients	191 + 1 adult	11 (+1 adult)	180	–
Female	65.2% (122/186 + 1 adult)	81.8% (9 + 1 adult)	64.6% (113/175)	**0.01**
Median age at disease onset [IC]	10 [2–18] (excluding adult, where age at onset was not known)	9 [2–12] (excluding adult, where age at onset was not known)	10 [2–18]	0.1
Pattern of bone inflammation				
Multifocal bone involvement	71% (120/168)	70% (7/10)	71.5% (113/158)	1
Mandibular involvement	2.2% (4/185)	9.1% (1/11)	1.7% (3/174)	0.06
Spinal involvement	22.7% (42/185)	9.1% (1/11)	23.5% (41/174)	**0.007**
Joint manifestations				
Joint manifestations	84% (89/106)	62.5% (5/8)	85.7% (84/98)	**0.0004**
Arthritis	4.7% (5/106)	0% (0/8)	5.1% (5/98)	0.07
Arthralgia	79.2% (84/106)	62.5% (5/8)	80.6% (79/98)	**0.007**
Gastrointestinal manifestation				
Abdominal pain and or diarrhoea	17.5% (21/120)	33.3% (3/9)	16.2% (18/111)	**0.008**
Ulcerating colitis or Crohn disease	3.2% (6/186)	0% (0/11)	3.4% (6/175)	0.25
Others manifestations				
Lymphadenopathy	2.5% (3/117)	11.1% (1/9)	1.8% (2/108)	**0.02**
Psoriasis	6.7% (12/180)	0% (0/11)	7.1% (12/169)	**0.02**
Treatment				
Need for c/b DMARDs or bisphosphonates	51.6% (96/186)	63.6% (7/11)	50.8% (89/175)	0.08

IC: interquartile range; bDMARDs: Biologic disease-modifying anti-rheumatic drugs; cDMARDs: conventional disease-modifying anti-rheumatic drugs. Mann Whitney and Chi square with Yates correction. Number of patients included vary as indicated because of missing data.

two *P2RX7* copies. Expression of P2X7 mRNA and surface protein was reduced in 4G8 *P2RX7*$^{-/-}$ compared to control THP-1 cells, while expression was comparable across 4G8 cell lines reconstituted with *WT* or after introducing *P2RX7* variants (Supplement Figure 5).

4.4. *P2RX7 variants associate with altered cytokine release and pyroptosis*

We observed reduced pyroptosis (LDH release) in P2X7-deficient cells (3F2, 4G8) compared to control THP-1 cells, which was accompanied by reduced IL-1β and IL-18 release in response to priming (LPS) and stimulation (LPS/BzATP) (Fig. 2A–C). THP-1 derived macrophages reconstituted with *WT P2RX7* exhibited increased pyroptosis and IL-1β release when compared to controls (pLenti) (Fig. 2D and E). IL-18 release remained slightly reduced (Fig. 2F). Compared to *WT* P2X7 expressing cells, cells expressing the rare c.920G > A variant exhibited reduced pyroptosis in response to priming (LPS) and stimulation (LPS/ATP) across investigated time points (Fig. 3A). This was accompanied by reduced IL-1β and IL-18 release when compared to *WT* controls and other *P2RX7* variants tested (Fig. 3B and C). Both remaining variants (c.349C > T, c.489C > T) exhibited reduced pyroptosis after 2h and 4h compared to controls (Fig. 3A) in the presence of sustained IL-1β release. Notably, compared to controls, c.349C > T variants exhibited increased IL-18 release (Fig. 3C). As a positive control, stimulation with LPS and nigericin was used, which delivered no differences in pyroptosis or cytokine release between *WT* and *P2RX7* variants (Supplement Figure 6). Compared to *WT* P2X7 expressing cells, THP-1-derived macrophages expressing the rare LOF variant showed reduced pyroptosis after prolonged LPS stimulation at 30min and 1h, and reduced IL-1β release at 1h (Supplement Figure 7).

4.5. *P2RX7 variants affect K+ flux*

NLRP3 inflammasome assembly involves P2X7 mediated K^+ efflux [13]. While mono-allelic presence of *P2RX7* (3F2) controlled intracellular K^+, bi-allelic deletion (4G8) resulted in K^+ accumulation (Fig. 4A).

Activation of P2X7 (LPS/BzATP) reduced intracellular K^+ concentrations in *WT* but not in *P2RX7*-deficient cells, suggesting that P2X7 activation in 3F2 cells over basal activity was not possible (Supplement Figure 8A, Supplement Table 6). Intracellular K^+ contents remained higher in 4G8 cells than in *WT* THP-1 cells during stimulation (Fig. 4B). As expected, K^+ flux was restored by reconstitution with *P2RX7* (Fig. 4C, Supplement Fig. 8B and C, Supplement Table 7). Compared to *WT P2RX7*, K^+ efflux was reduced in cells expressing the rare c.920G>A-LOF variant which contained the highest intracellular K^+ level compared to the remaining cells tested. It was increased in cells with the rare c.349C>T variant, and unaltered in cells with the common c.489C > T-GOF variant (Fig. 4D, Supplement Fig. 8D and E, Supplement Table 7).

4.6. *P2RX7 variants affect inflammasome assembly and release*

To investigate effects of *P2RX7* variants on inflammasome assembly (ASC specks), we used fluorescence microscopy. Reconstitution of P2X7 expression in 4G8 cells with *WT P2RX7* expression plasmids increased ASC speck formation over controls (pLenti) (Supplement Figure 9A). Compared to *WT P2RX7*, the c.349C > T variant associated with increased, the c.920G > A-LOF variant with lower, and the common c.489C > T-GOF variant with comparable speck counts (Supplement Figures 9C, 10). Because ASC particles can be released from cells, contributing to propagation of inflammation [19], we monitored extracellular specks in cell culture supernatants [14]. Consistent with the presence of intracellular ASC specks, extracellular speck counts were reduced in P2X7-deficient 4G8 THP-1 macrophage-like cells when compared to cells expressing *WT* P2X7 (Supplement Figure 9B). Furthermore, when compared to *WT P2RX7*, the c.920G > A LOF variant associated with reduced speck release (Supplement Figure 9D). Both GOF variants tested associated with comparable ASC speck counts that, at 2h and 4h, were above the *WT* control.

5. Discussion

This study links rare and common CNO-associated *P2RX7* variants with altered inflammasome assembly, pro-inflammatory cytokine release, and reduced pyroptosis. Notably, because of the presence of familial clusters, associations with other inflammatory diseases, and up to 50% of CNO patients having a family history of systemic inflammatory disease, a genetic component to CNO had been suspected [1] but not been proven in large patient cohorts.

We and others previously reported increased expression of pro-inflammatory cytokines and NLRP3 inflammasome components, as well as increased activation of inflammasomes in monocytes from CNO patients [7]. This is of particular interest in the context of here identified variants in *P2RX7*, encoding for the P2X7 transmembrane protein that controls K^+ efflux, thereby triggering NLRP3 inflammasome assembly, caspase-1 activation, and cleavage and release of pro-inflammatory IL-1β and IL-18 [20]. Inflammasome assembly results in pyroptosis, inflammatory cell death that contributes to the termination of inflammatory responses [16].

Dysregulated expression, release and post-translational control of IL-

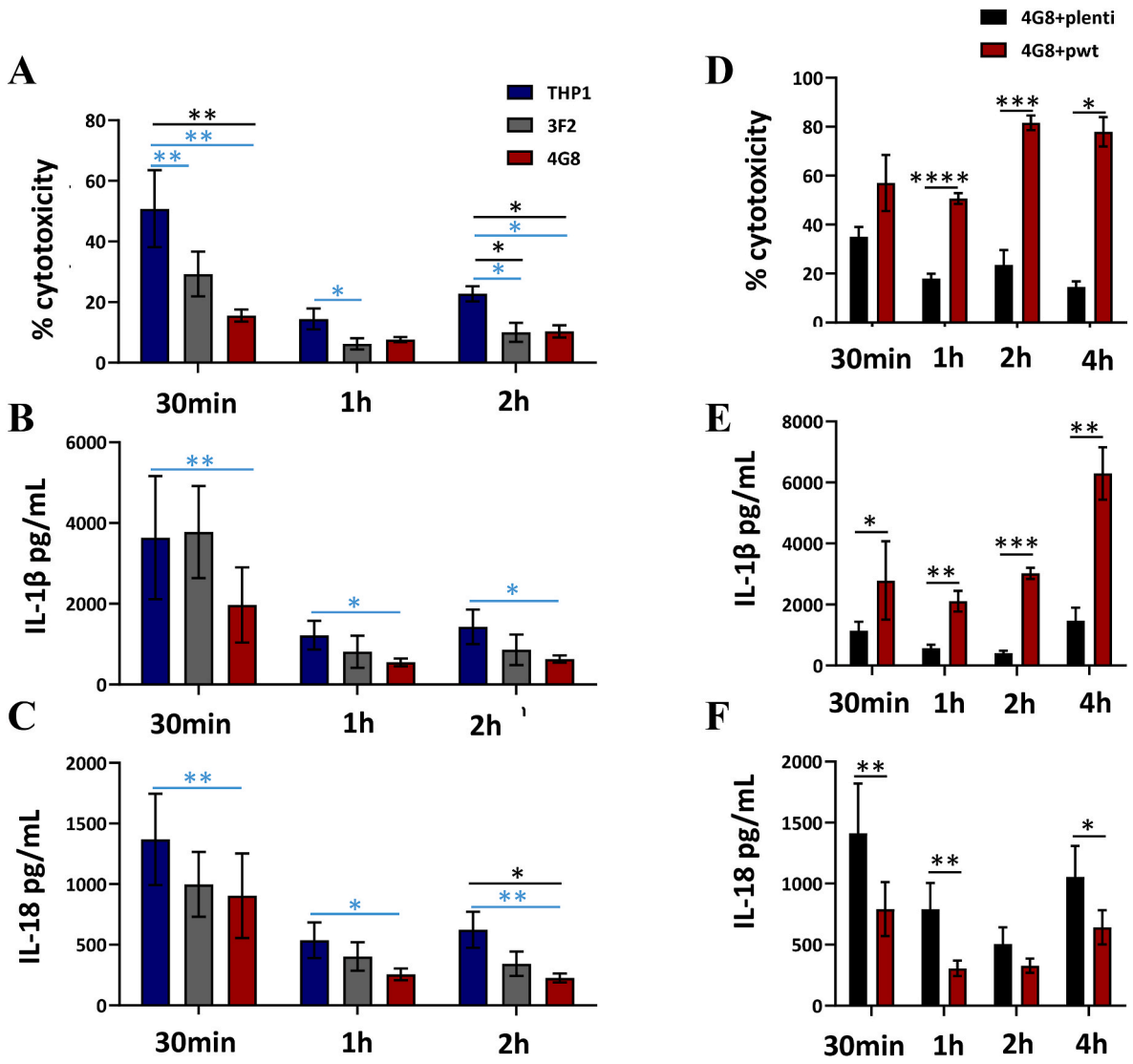

Fig. 2. P2X7 determines pyroptosis and cytokine release. Pyroptosis, IL-1β and IL-18 release in response to priming (LPS) and stimulation (LPS + BzATP) were investigated in P2X7-deficient cells (mono- 3F2 and bi-allelic 4G8 deletion; A-C) and in THP-1 derived 4G8 macrophages reconstituted with *wild-type* P2X7 expression vectors (D–F). A-C) Kruskal Wallis test and Dunn's multiple comparison (result shown in black print; A: $N \geq 6$, B/C: $N \geq 8$). A-C) Wilcoxon tests comparing THP1 to 3F2 cells and THP1 to 4G8 cells (result shown in blue print). D-F) Wilcoxon tests (D: $N = 6$, E/F: $N \geq 8$); *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.001$.

1β have previously been linked with bone inflammation in monogenic systemic autoinflammatory diseases, namely Deficiency of IL-1 Receptor Antagonist (DIRA), Pyogenic Arthritis Pyoderma gangrenosum and Acne (PAPA), and Majeed syndrome (MS) [6,21]. DIRA is caused by LOF mutations in *IL1RN*, encoding the endogenous IL-1 receptor antagonist (IL-1RA) [1,22,23]17,22. PAPA is an autosomal dominant auto-inflammatory disease caused by mutations *PSTPIP1*, encoding a protein involved in the autoinflammatory cascade [1,24]. Majeed Syndrome, previously called "familial CNO", is caused by autosomal recessive mutations in *LPIN2*, a gene involved in lipid metabolism [1,25]. Recently, Lordén et al. demonstrated that lipin-2, via the regulation of cholesterol levels, controls the activation of P2X7, underscoring patho-mechanistic links between MS and CNO [16]. Dysregulation of the P2X7: IL-1β axis had been suggested in a patient with SAPHO (Synovitis Acne Pustulosis Hyperostosis Osteitis), a (usually) adult-onset systemic inflammatory condition resembling CNO [6]. The reported patient was successfully treated with the recombinant IL-1 receptor antagonist anakinra [26]. While reports of paediatric CNO patients treated with anakinra are limited to small case series, a subset of otherwise treatment resistant patients responded [27].

CNO-associated variants in *P2RX7* represent a combination of reported and previously unknown, mostly GOF but also LOF variants. Eleven patients in this cohort (11/191, 5.7%) exhibited one or more rare *P2RX7* variants. The ultra-rare variant rs28360445 (c.349C > T/p.Arg117Trp) identified by WES in the index family, was predicted to cause LOF through reduced channel/macropore function [28]. However, functional work in *ex vivo* isolated blood cells from CNO patients and in genetically modified THP-1 cells showed increased K^+ flux, inflammasome assembly, and IL-1β and IL-18 release, but reduced pyroptosis. This suggests GOF and impaired inflammatory cell death, a mechanism to control damage. The exact molecular mechanisms contributing to disassociation between K^+ flux and inflammasome assembly on the one hand and pyroptosis on the other remain currently unknown and will be the focus of future studies.

Functional testing of rs28360457 (c.920G > A/p.Arg370Gln), identified in one CNO patient, confirmed predicted LOF by introduction a positively charged arginine at residue 370 [29]. Reduced K^+ flux associated with impaired inflammasome assembly, pro-inflammatory cytokine release and pyroptosis. This suggests that reduced cytokine release in the context of reduced pyroptosis may result in a cumulative

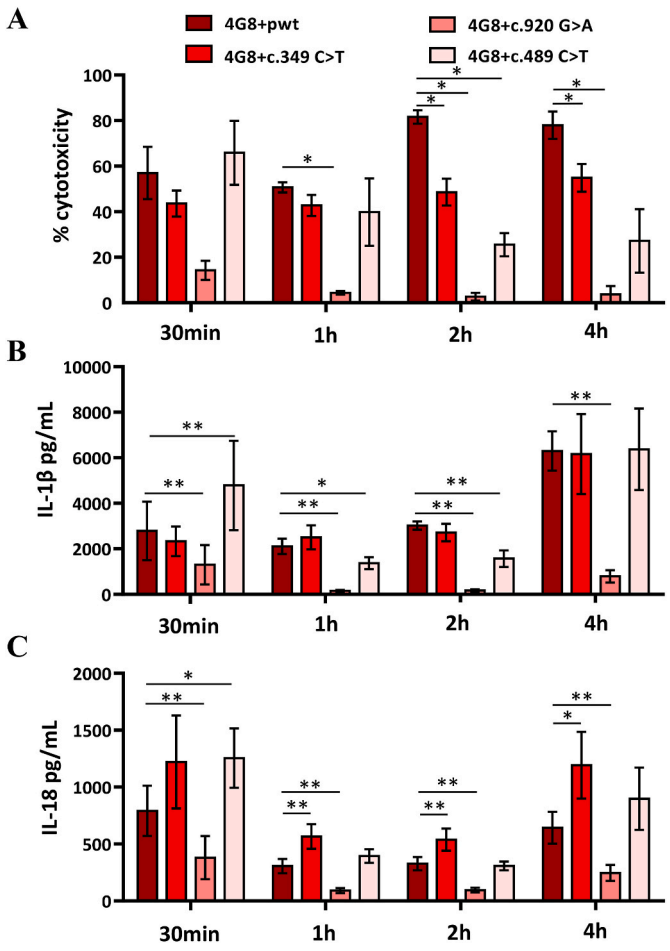

Fig. 3. Macrophages carrying CNO-associated *P2RX7* variants show prolonged cell survival and variable cytokine expression. The impact of *P2RX7* variants identified in CNO patients on NLRP3 inflammasome activation, pyroptosis and cytokine release were tested in 4G8 THP-1 derived macrophages (*P2RX7*$^{-/-}$) stably expressing wild-type (*WT*) or variant P2X7. A) All variants tested displayed prolonged survival when compared to cells expressing *WT* P2X7. This was most pronounced in cells with the c.920G > A LOF variant. Both remaining variants exhibited reduced pyroptosis after 2 h when compared to *WT* controls (N = 6). B, C) Cells with the c.920G > A variant exhibited reduced IL-1β and IL-18 release when compared to *WT* controls and other variants (N ≥ 8). Wilcoxon tests comparing *WT* against individual *P2RX7* variants with Bonferroni correction for multiple testing, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.001.

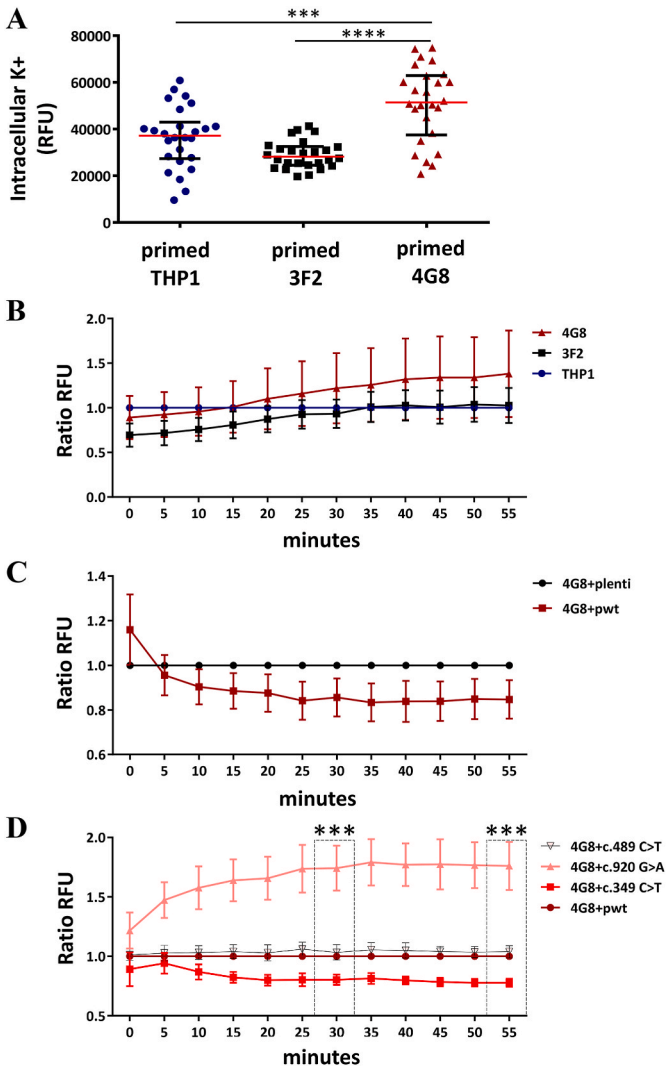

Fig. 4. *P2RX7* variants affect K^+ flux. In the following, potassium concentrations in each cell line across time points are based on the K^+ concentration in wild-type controls at the same time point. A) Under resting conditions, monoallelic presence of *P2RX7* sufficiently controls intracellular K^+ concentrations, while bi-allelic deletion results in intracellular accumulation of K^+ (N = 26). Anova and Tukey's multiple comparison tests. B) The intracellular K^+ content is higher in 4G8 cells when compared to wild-type THP1 cells. At each time point, fluorescence of variant cell-lines was compared to the fluorescence of the reference cell line (THP-1) (N = 4). C) K^+ flux can be restored by reconstitution of 4G8 cells with *P2RX7* expression vectors. At each time point, fluorescence of reconstituted cells was compared to the fluorescence measured in the reference cell line (4G8+plenti) (N = 4). D) When compared to wild-type (*WT*) P2X7 expressing cells, K^+ content is higher in cells carrying the rare c.920G>A LOF variant; it is slightly lower in cells carrying the rare c.349C > T variant, and unaltered in cells with the common c.489C > T GOF variant. At each time point, fluorescence of reconstituted cells was compared to the fluorescence measured in the reference cell line (4G8+plenti) (N = 4). Friedman test and Dunn's multiple comparison test; ***p < 0.001, ****p < 0.001.

pro-inflammatory phenotype. Supporting this hypothesis, rs28360457 has previously been linked with bone loss [30], osteoporosis [31], and increased risk of hepatocellular carcinoma [32], conditions characterized by inflammation. Alternatively, rs28360457 may prolong cell survival in the presence of additional GOF variants. Indeed, the here reported CNO patient carrying rs28360457 was also a homozygous carrier of 2 additional, more common, GOF variants: the here also functionally tested rs208294 (see below), and rs7958311(c.809G > A, p.Arg270His). Recently, rs7958311 has been reported in the context of chronic pain, particularly chronic pelvic pain [33], a relatively common long-term complication of CNO [3]. Furthermore, the here included patient presented with a multifocal form of CNO including the pelvis [34].

Further rare variants associated with CNO remain currently functionally untested. An unreported variant at chromosome 12: 121,154,840 (c.181G > T/p.Val61Leu) is located near one of the 10 conserved cysteine residues and may affect channel function [35]. The c.417G > A/p.Trp139* variant affects the ATP-binding pocket and may impact its accessibility. Additionally, it affects a bisulfide bond involved in covalent linkage and protein structure, thus potentially affecting receptor stability. The ten remaining rare variants include missense variants (7/11, 64%) with unknown effects, early stop codons (2/11, 18%) and loss of the start codon (1/11, 9%). All remaining CNO patients in this cohort carried a combination of more common *P2RX7* SNPs, including the here functionally tested rs208294 (c.489C > T) GOF variant. Rs208294 results in p.His155Tyr, affecting the P2X7 ectodomain involved in ATP binding. It has previously been reported as a

"weak" *P2RX7* GOF polymorphism that is associated with accelerated release of pro-inflammatory cytokines in lupus [36], and female infertility [37]. The current study supports these pro-inflammatory effects.

Because rare likely damaging variants only affected 5.8% of CNO patients, genomic variability among all patients was tested. A markedly higher number of *P2RX7* SNPs unique to one sample was identified in CNO patients compared to 1873 controls (42 versus 3.7), highlighting a possible role of *P2RX7* as a risk locus. In addition to the index family carrying the ultra-rare rs28360445 (c.349C > T/p.Arg117Trp) variant, the CNO cohort included two brothers who developed CNO in adolescence. These patients did not exhibit rare (*in silico* predicted) "high impact" variants but a combination of "moderate impact" variants (monoallelic: rs7958311/c.809G > A/p.Arg270His, rs1718119/c.1042G > A/p.Ala348Thr; bi-allelic: rs208294/c.463T > C/p.Tyr155His), which furthermore suggests combined impact of *P2RX7* variants as a factor in CNO. Previous studies investigating one of these variants, rs208294, suggested LOF [38], and linked with multiple sclerosis [39], chronic pain [40], and severe sepsis. Notably, a proportion of CNO patients will also develop chronic pain [1]. Both mono-allelic variants, rs7958311 and rs1718119, were predicted to cause GOF [41,42]. Rs7958311 has previously been associated with ankylosing spondylitis [43], a condition clinically related to CNO [44]. Also considering reduced pyroptosis associated with functionally tested GOF and LOF variants in the current study, it is tempting to speculate that the combination of LOF and GOF variants in both siblings may result in increased inflammasome assembly and prolonged cell survival. One of the brothers carried an additional "moderate impact" LOF variant rs7958316 (c.827G > A, p.Arg276His) that had been associated with gout, another inflammasome-related condition [45].

Though limited by the number of CNO patients included, this study suggests *P2RX7* variant-associated phenotypic features and disease outcomes. Patients with rare likely damaging variants more frequently displayed lymphadenopathy, abdominal and mandibular involvement. The remaining cohort more frequently exhibited joint and vertebral involvement. Lastly, while not reaching statistical significance level, patients with rare damaging *P2RX7* variants more frequently required 2nd line treatments, including c/bDMARDs or bisphosphonates (63.3% versus 50.8%; $p = 0.08$). While these results need to be confirmed in larger independent cohorts, they may be applied in the future as tools for patient stratification and risk assessment.

Results from this study support NLRP3 inflammasomes and/or pro-inflammatory cytokines as therapeutic targets. NLRP3 inflammasome assembly can be modified (with variable efficacy) by non-steroidal anti-inflammatory drugs (e.g., naproxen) that are routinely used as 1st-line treatment in CNO. Already available IL-1 blocking strategies (e.g., anakinra, canakinumab) may be used to control inflammation [27], while inflammasome assembly may be blocked in the future by small molecules (e.g., MCC950) [13]. As inflammation is promoted by altered P2X7 function, therapeutic targeting of P2X7 appears a promising target. This may, however, be complicated by the fact that both LOF and GOF variants appear to promote inflammation. While P2X7 activation related pyroptosis not only contributes to the inflammation "spreading" through cytokine release and assembled inflammasomes (ASC specks) [19], it also terminates inflammation and tissue damage as pyroptotic cells cannot produce additional pro-inflammatory cytokines [46].

This study has significant strengths, including the comparably large national cohort of CNO patients with associated clinical/demographic datasets, ethnically matched healthy controls, and functional data from primary human and genetically modified cells. Although large for a rare disease, the patient cohort size is not sufficient to deliver statistically significant findings for several rare variants (though not present in control cohort). Primary human cells were only available for the index patient's family. Additional molecular mechanisms associated with P2X7 activation, including Ca^{2+} flux affecting gene expression, protein kinase, transcription factor activation, and oxidative stress were beyond the scope of this report and are the focus of ongoing projects. Lastly, investigation of additional gene variants and/or their combination was beyond the scope of this manuscript and will be the focus of future work.

6. Conclusions

This study links rare variants in *P2RX7* with CNO. All tested variants associate with reduced pyroptosis and prolonged cell survival. This may explain pro-inflammatory monocyte phenotypes and argues for the use of already available cytokine blocking agents that are currently not licensed for use in CNO. Stratification of patients based on *P2RX7* variants may offer an opportunity for individualized care but requires validation in large international cohorts.

Declaration of competing interest

In the past, CMH received research funding from Novartis for research in psoriasis (secukinumab programme, 2017–2019). CMH, PJF and HJG participated in advisory boards on the use of canakinumab in inflammatory bone disease hosted by Novartis.

CRediT authorship contribution statement

Amandine Charras: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Sigrun R. Hofmann:** Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Writing – review & editing. **Allison Cox:** Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. **Felix Schulze:** Data curation, Investigation, Methodology, Resources, Writing – review & editing. **Susanne Russ:** Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. **Sarah Northey:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Xuan Liu:** Data curation, Formal analysis, Writing – review & editing. **Yongxiang Fang:** Data curation, Formal analysis, Writing – review & editing. **Sam Haldenby:** Data curation, Formal analysis. **Hella Hartmann:** Data curation, Formal analysis, Methodology, Resources, Writing – review & editing. **Alexander G. Bassuk:** Investigation, Methodology, Resources, Writing – review & editing. **Ana Carvalho:** Data curation, Formal analysis, Writing – review & editing. **Francesca Sposito:** Data curation, Formal analysis, Writing – review & editing. **Lev Grinstein:** Data curation, Formal analysis, Writing – review & editing. **Angela Rösen-Wolff:** Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. **Almut Meyer-Bahlburg:** Data curation, Resources, Writing – review & editing. **Michael W. Beresford:** Resources, Writing – review & editing. **Elke Lainka:** Resources, Writing – review & editing. **Dirk Föll:** Resources, Writing – review & editing. **Helmut Wittkowski:** Resources, Writing – review & editing. **Hermann J. Girschick:** Data curation, Resources, Writing – review & editing. **Henner Morbach:** Investigation, Resources, Writing – review & editing. **Steffen Uebe:** Formal analysis, Resources, Writing – review & editing. **Ulrike Hüffmeier:** Formal analysis, Investigation, Resources, Writing – review & editing. **Polly J. Ferguson:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing. **Christian M. Hedrich:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2024.103183.

References

[1] S.R. Hofmann, F. Kapplusch, H.J. Girschick, H. Morbach, J. Pablik, P.J. Ferguson, et al., Chronic recurrent multifocal osteomyelitis (CRMO): presentation, pathogenesis, and treatment, Curr. Osteoporos. Rep. 15 (2017) 542–554.
[2] P.J. Ferguson, M. Sandu, Current understanding of the pathogenesis and management of chronic recurrent multifocal osteomyelitis, Curr. Rheumatol. Rep. 14 (2012) 130–141.
[3] C.C.G. Silier, J. Greschik, S. Gesell, V. Grote, A.F. Jansson, Chronic non-bacterial osteitis from the patient perspective: a health services research through data collected from patient conferences, BMJ Open 7 (2017) e017599.
[4] A.M. Huber, P.-Y. Lam, C.M. Duffy, R.S.M. Yeung, M. Ditchfield, D. Laxer, et al., Chronic recurrent multifocal osteomyelitis: clinical outcomes after more than five years of follow-up, J. Pediatr. 141 (2002) 198–203.
[5] A. Jansson, E.D. Renner, J. Ramser, A. Mayer, M. Haban, A. Meindl, et al., Classification of non-bacterial osteitis: retrospective study of clinical, immunological and genetic aspects in 89 patients, Rheumatology 46 (2007) 154–160.
[6] C.M. Hedrich, H. Morbach, C. Reiser, H.J. Girschick, New insights into adult and paediatric chronic non-bacterial osteomyelitis CNO, Curr. Rheumatol. Rep. 22 (2020) 52.
[7] D. Brandt, E. Sohr, J. Pablik, A. Schnabel, F. Kapplusch, K. Mäbert, et al., CD14+ monocytes contribute to inflammation in chronic nonbacterial osteomyelitis (CNO) through increased NLRP3 inflammasome expression, Clin. Immunol. 196 (2018) 77–84.
[8] P. Pelegrin, P2X7 receptor and the NLRP3 inflammasome: partners in crime, Biochem. Pharmacol. 187 (2021) 114385.
[9] M. Idzko, D. Ferrari, H.K. Eltzschig, Nucleotide signalling during inflammation, Nature 509 (2014) 310–317.
[10] D. Zeng, P. Yao, H. Zhao, P2X7, a critical regulator and potential target for bone and joint diseases, J. Cell. Physiol. 234 (2019) 2095–2103.
[11] Z. Li, Z. Huang, L. Bai, The P2X7 receptor in osteoarthritis, Front. Cell Dev. Biol. 9 (2021).
[12] E. Lainka, M. Bielak, V. Hilger, O. Basu, U. Neudorf, H. Wittkowski, et al., Translational research network and patient registry for auto-inflammatory diseases, Rheumatology 50 (2011) 237–242.
[13] L. Grinstein, K. Endter, C.M. Hedrich, S. Reinke, H. Luksch, F. Schulze, et al., An optimized whole blood assay measuring expression and activity of NLRP3, NLRC4 and AIM2 inflammasomes, Clin. Immunol. 191 (2018) 100–109.
[14] D.M. Rowczenio, S. Pathak, J.I. Arostegui, A. Mensa-Vilaro, E. Omoyinmi, P. Brogan, et al., Molecular genetic investigation, clinical features, and response to treatment in 21 patients with Schnitzler syndrome, Blood 131 (2018) 974–981.
[15] F. Ugarte, M. Ryser, S. Thieme, F.A. Fierro, K. Navratiel, M. Bornhäuser, et al., Notch signaling enhances osteogenic differentiation while inhibiting adipogenesis in primary human bone marrow stromal cells, Exp. Hematol. 37 (2009) 867–875. e1.
[16] G. Lordén, I. Sanjuán-García, N. de Pablo, C. Meana, I. Alvarez-Miguel, M.T. Pérez-García, et al., Lipin-2 regulates NLRP3 inflammasome by affecting P2X7 receptor activation, J. Exp. Med. 214 (2017) 511–528.
[17] A. Stutz, G.L. Horvath, B.G. Monks, E. Latz, ASC speck formation as a readout for inflammasome activation, Methods Mol. Biol. 1040 (2013) 91–101.
[18] S.T. Sherry, M.H. Ward, M. Kholodov, J. Baker, L. Phan, E.M. Smigielski, et al., dbSNP: the NCBI database of genetic variation, Nucleic Acids Res. 29 (2001) 308–311.
[19] B.S. Franklin, L. Bossaller, D. De Nardo, J.M. Ratter, A. Stutz, G. Engels, et al., The adaptor ASC has extracellular and "prionoid" activities that propagate inflammation, Nat. Immunol. 15 (2014) 727–737.
[20] B.R. Barker, D.J. Taxman, J.P.-Y. Ting, Cross-regulation between the IL-1β/IL-18 processing inflammasome and other inflammatory cytokines, Curr. Opin. Immunol. 23 (2011) 591–597.
[21] K. Buch, A.C.B. Thuesen, C. Brøns, P. Schwarz, Chronic non-bacterial osteomyelitis: a review, Calcif. Tissue Int. 104 (2019) 544–553.
[22] I. Aksentijevich, S.L. Masters, P.J. Ferguson, P. Dancey, J. Frenkel, A. van Royen-Kerkhoff, et al., An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist, N. Engl. J. Med. 360 (2009) 2426–2437.
[23] S. Reddy, S. Jia, R. Geoffrey, R. Lorier, M. Suchi, U. Broeckel, et al., An autoinflammatory disease due to homozygous deletion of the IL1RN locus, N. Engl. J. Med. 360 (2009) 2438–2444.
[24] C.A. Wise, J.D. Gillum, C.E. Seidman, N.M. Lindor, R. Veile, S. Bashiardes, et al., Mutations in CD2BP1 disrupt binding to PTP PEST and are responsible for PAPA syndrome, an autoinflammatory disorder, Hum. Mol. Genet. 11 (2002) 961–969.
[25] P.J. Ferguson, S. Chen, M.K. Tayeh, L. Ochoa, S.M. Leal, A. Pelet, 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. 42 (2005) 551–557.
[26] M. Colina, C. Pizzirani, M. Khodeir, S. Falzoni, M. Bruschi, F. Trotta, et al., Dysregulation of P2X7 receptor-inflammasome axis in SAPHO syndrome: successful treatment with anakinra, Rheumatology 49 (2010) 1416–1418.
[27] M. Pardeo, D.P. Marafon, V. Messia, M.C. Garganese, F.D. Benedetti, A. Insalaco, Anakinra in a cohort of children with chronic nonbacterial osteomyelitis, J. Rheumatol. 44 (2017) 1231–1238.
[28] R. Lara, E. Adinolfi, C.A. Harwood, M. Philpott, J.A. Barden, F. Di Virgilio, et al., P2X7 in cancer: from molecular mechanisms to therapeutics, Front. Pharmacol. 11 (2020) 793.
[29] R.A. North, Molecular physiology of P2X receptors, Physiol. Rev. 82 (2002) 1013–1067.
[30] A. Gartland, K.K. Skarratt, L.J. Hocking, C. Parsons, L. Stokes, N.R. Jørgensen, et al., Polymorphisms in the P2X7 receptor gene are associated with low lumbar spine bone mineral density and accelerated bone loss in post-menopausal women, Eur. J. Hum. Genet. 20 (2012) 559–564.
[31] J.S. Wiley, R. Sluyter, B.J. Gu, L. Stokes, S.J. Fuller, The human P2X7 receptor and its role in innate immunity, Tissue Antigens 78 (2011) 321–332.
[32] S. Duan, J. Yu, Z. Han, Z. Cheng, P. Liang, Association between P2RX7 gene and hepatocellular carcinoma susceptibility: a case-control study in a Chinese han population, Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. 22 (2016) 1916–1923.
[33] K. Zorina-Lichtenwalter, A.R. Ase, V. Verma, A.I.M. Parra, S. Komarova, A. Khadra, et al., Characterization of common genetic variants in P2RX7 and their contribution to chronic pain conditions, J. Pain (2023), https://doi.org/10.1016/j.jpain.2023.09.011.
[34] D.Y. Zhao, L. McCann, G. Hahn, C.M. Hedrich, Chronic nonbacterial osteomyelitis (CNO) and chronic recurrent multifocal osteomyelitis (CRMO), Journal of Translational Autoimmunity 4 (2021) 100095.
[35] M.-L. He, H. Zemkova, S.S. Stojilkovic, Dependence of purinergic P2X receptor activity on ectodomain structure, J. Biol. Chem. 278 (2003) 10182–10188.
[36] S. Hu, F. Yu, C. Ye, X. Huang, X. Lei, Y. Dai, et al., The presence of P2RX7 single nuclear polymorphism is associated with a gain of function in P2X7 receptor and inflammasome activation in SLE complicated with pericarditis, Clin. Exp. Rheumatol. 38 (2020) 442–449.
[37] A. Pegoraro, D. Bortolotti, R. Marci, E. Caselli, S. Falzoni, E. De Marchi, et al., The P2X7 receptor 489C>T gain of function polymorphism favors HHV-6A infection and associates with female idiopathic infertility, Front. Pharmacol. 11 (2020) 96.
[38] S. Ide, D. Nishizawa, K. Fukuda, S. Kasai, J. Hasegawa, M. Hayashida, et al., Haplotypes of P2RX7 gene polymorphisms are associated with both cold pain sensitivity and analgesic effect of fentanyl, Mol. Pain 10 (2014) 75.
[39] O. Oyanguren-Desez, A. Rodríguez-Antigüedad, P. Villoslada, M. Domercq, E. Alberdi, C. Matute, Gain-of-function of P2X7 receptor gene variants in multiple sclerosis, Cell Calcium 50 (2011) 468–472.
[40] R.E. Sorge, T. Trang, R. Dorfman, S.B. Smith, S. Beggs, J. Ritchie, et al., Genetically determined P2X7 receptor pore formation regulates variability in chronic pain sensitivity, Nat. Med. 18 (2012) 595–599.
[41] L. Stokes, S.J. Fuller, R. Sluyter, K.K. Skarratt, B.J. Gu, J.S. Wiley, Two haplotypes of the P2X(7) receptor containing the Ala-348 to Thr polymorphism exhibit a gain-of-function effect and enhanced interleukin-1beta secretion, Faseb. J. 24 (2010) 2916–2927.
[42] O. Kambur, M.A. Kaunisto, B.S. Winsvold, T. Wilsgaard, A. Stubhaug, J.A. Zwart, et al., Genetic variation in P2RX7 and pain tolerance, Pain 159 (2018) 1064–1073.
[43] Z. Pan, X. Zhang, Y. Ma, S. Xu, Z. Shuai, F. Pan, et al., Genetic variation of rs7958311 in P2X7R gene is associated with the susceptibility and disease activity of ankylosing spondylitis, Postgrad. Med. 95 (2019) 251–257.
[44] O. Vittecoq, L.A. Said, C. Michot, O. Mejjad, J.M. Thomine, P. Mitrofanoff, et al., Evolution of chronic recurrent multifocal osteitis toward spondylarthropathy over the long term, Arthritis Rheum. 43 (2000) 109–119.
[45] J.-H. Tao, M. Cheng, J.-P. Tang, X.-J. Dai, Y. Zhang, X.-P. Li, et al., Single nucleotide polymorphisms associated with P2X7R function regulate the onset of gouty arthritis, PLoS One 12 (2017) e0181685.
[46] D. Zheng, T. Liwinski, E. Elinav, Inflammasome activation and regulation: toward a better understanding of complex mechanisms, Cell Discov 6 (2020) 36.