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Clinical science

Majeed syndrome: first description in a patient of central-European ancestry

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Abstract

Objectives: We present the first case of a Majeed syndrome in a girl of central-European ancestry.

Methods: Patient's medical records were reviewed. A next-generation sequencing (NGS) panel for autoinflammatory diseases was performed and the mutation was confirmed by Sanger analysis. Freshly isolated monocytes were activated with lipopolysaccharide ± ATP. The concentration of inflammatory cytokines was assessed in monocyte supernatants.

Results: A 2-year-old girl presented with pain in the lower limbs, increase of acute phase reactants and persistent microcytic anaemia. The MRI showed bilateral short time inversion recovery (STIR) hyper-intensity of the spongy osseous tissue of the femur, tibia, radius, ulna and astragalus. Bone marrow analysis revealed increased trilinear cellularity with signs of dyserythropoietic anaemia. The NGS panel detected the presence of two novel compound heterozygous mutations in the *LPIN2* gene, confirmed by Sanger analysis. Treatment with anakinra was started with a prompt resolution of the clinical picture. Increased kinetics and concentration of IL-1β were observed in the patient's monocytes compared with healthy controls, with a marked drop following the start of therapy. About 6 months after the start of the therapy, resolution of MRI findings, microcytic anaemia and dyserythropoiesis at bone marrow aspirate were observed.

Conclusion: We describe the first case of Majeed syndrome in a patient of central-European ancestry. The functional test on circulating monocytes before and after therapy with anakinra confirmed pathogenicity of the mutation and the role of LPIN2 in the NLRP3 inflammasome activation. Anti-IL1 agents were effective, leading not only to the resolution of bone lesions but also to an improvement of dyserythropoiesis.

Keywords: Majeed syndrome, osteolytic lesions, periostitis, dyserytropoiesis, neutropenia, IL-1β, anakinra, canakinumab

Rheumatology key messages:

- Exclude Majeed syndrome in children with early-onset CRMO, dyserythropoiesis or neutropenia, regardless of skin involvement.
- Majeed syndrome can occur in the Caucasian population.
- Anti-IL1 agents are effective in Majeed syndrome on inflammatory manifestations and dyserythropoiesis.

Introduction

Majeed syndrome is an autosomal recessive monogenic autoinflammatory disease caused by loss-of-function mutations in the *LPIN2* gene. Clinically, it manifests during the first 2 years of life with triad of early onset chronic recurrent multifocal osteomyelitis (CRMO), congenital dyserythropoietic anaemia (CDA) and Sweet syndrome, characterized by a neutrophilic dermatosis [1, 2]. While most of the patients typically present bone and haematological involvement, skin lesions may be transient and not present at the time of diagnosis [2]. The presence of chronic inflammation, with persistent elevation of blood inflammatory markers, contributes to recurrent fevers, failure to thrive and hepatosplenomegaly [2, 3]. Although there are no shared guidelines for therapy, IL1-blocking agents have been proven effective in controlling febrile episodes, inflammatory bone disease and anaemia [2, 4]. The *LPIN2* gene encodes lipin-2, a protein with phosphatidic acid phosphohydrolase (peroxidase–antiperoxidase) activity involved in glycerolipid metabolism. It converts

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phosphatidic acid to diacylglycerol, which represents a regulatory nodal point that can impact synthesis of phosphatidylcholine, phosphatidylethanolamine and other membrane lipids [2, 5]. *In vitro* studies in human and mouse macrophages reveal that lipin-2 is an important innate immune regulator, controlling both the priming and activation phase of the NLRP3 inflammasome [6]. So far, fewer than 25 patients with the Majeed syndrome have been described in the literature, all of them from Middle Eastern countries, except one patient of Puerto Rican and African American descent [2, 7, 8]. To date, <10 mutations have been reported in the absence of a clear genotype–phenotype correlation [2, 9]. Here, we present the first case of Majeed syndrome in a girl of central-European ancestry.

Methods and case description

Clinical course

The girl, first child of non-consanguineous Caucasian Italian parents, at the age of 6 months presented swelling of the right elbow associated with low-grade fever. The ultrasound highlighted the presence of joint effusion; blood tests revealed elevation of acute-phase reactants (CRP 15 mg/dl). In the suspicion of septic arthritis, broad-spectrum antibiotic treatment was started, associated with NSAIDs, with partial clinical improvement. During the hospitalization, microcytic anaemia was detected, with partial improvement after martial therapy. At the age of 23 months, due to recurrent pain in the lower limbs, the girl was admitted in our hospital. At the clinical evaluation, no sign of arthritis was detected. The blood tests confirmed microcytic anaemia (Hb 10 g/dl, mean corpuscolar volume (MCV) 65 fl) with leucocytes and platelets within the normal range, increased inflammatory markers (CRP 8.59 mg/dl, ESR 43 mm/1 h, serum amyloid A 702 mg/l) and hypergammaglobulinemia. MRI revealed STIR hyperintensity and contrast enhancement of the spongy osseous tissue of femur distal diaphysis and distal metaphysis, tibia proximal epiphysis and part of the diaphysis. Periosteal reaction was present in distal femoral diaphysis, together with STIR hyper-intensity and contrast enhancement of the adjacent soft tissues. The whole-body MRI detected similar bilateral findings in the radius, ulna, astragalus and metatarsus bones, with involvement of the surrounding soft tissue (Fig. 1A and B). Multiple lymphadenopathies and splenomegaly were also detected. A bone marrow aspirate showed increased trilinear cellularity with ineffective erythropoiesis and signs of dyserythropoiesis: binuclear/trinuclear erythroblasts and inter-nuclear bridging (Fig. 1D). A bone biopsy of the proximal right tibial metaphysis showed irregular thickening of the trabeculae, with numerous active osteoblasts and osteoclasts at the surface. Medullary spaces showed diffuse fibrosclerosis, oedema and moderate chronic inflammatory infiltrate. Microbiological investigations were negative.

The study was approved by the Ethical Committee of IRCCS Istituto Giannina Gaslini, and informed consent was provided by participants or legal guardians.

Results

Immunological and genetic assessment

In light of the clinical picture, a large next-generation sequencing (NGS) diagnostic panel for autoinflammatory diseases and immunodeficiencies was performed. The panel detected the presence of two novel compound heterozygous mutations in the *LPIN2* gene: I716Vfs*2 (c.2144_2145dupGT) and p. E565Kfs*15 (c.1693_1694delGA) frameshifts. The first mutation, at exon 16, was inherited from the mother, while the second, at exon 12, was inherited from the father. The mutations were confirmed by Sanger analysis. To verify aberrant inflammasome activation in our patient, we assessed IL-1β, IL-1α, IL-1RA and TNF-α production in stimulated monocytes (Fig. 2A–C). Cells were stimulated with lipopolysaccharide (LPS), a toll-like receptor 4 (TLR4) agonist; secreted IL-1β was quantified at different time points (3, 6 and 18 h). As shown in Fig. 2A, the kinetics of the IL-1β secretion is much faster in the patient (black square) than in healthy monocytes [health donors (HD)] (white circle). Similarly, secretion of IL-1α, TNF-α and IL-1RA was increased in LPS-stimulated patient monocytes collected before treatment. The results were consistent with those observed in other patients with Majeed syndrome [6].

Treatment and follow-up

In view of the clinical picture, the genetic and functional data, the diagnosis of Majeed syndrome was pointed out. At the age of 2 years, treatment with anakinra, the recombinant IL-1 receptor antagonist, at a dosage of 2 mg/kg/day was started, with quick improvement of the clinical picture: pain resolved, no more febrile episodes were reported and acute phase reactants normalized. A whole-body MRI, performed 18 months after the beginning of treatment with anakinra, revealed a decrease both in hyper-intensity in STIR and contrast enhancement in the distal femoral diaphysis bilaterally (Fig. 1C). Moreover, radiological changes previously observed in other bone segments (ulna, radius, talus, etc.) resolved and spleen enlargement reduced. About 6 months after the beginning of the therapy, a complete remission of the microcytic anaemia was observed. A bone marrow aspirate was performed with evidence of a marked reduction of the signs of dyserythropoiesis, with only rare bi/trinucleated cells and rare extracellular chromatin elements (Fig. 1E).

After 3 months of treatment with anakinra, patient monocytes secreted less IL-1β (Fig. 2A). Similarly, the secretion of IL-1α, TNF-α and IL-1RA (Fig. 2B–D), increased before therapy, was strongly reduced after therapy.

Treatment with anakinra was maintained for 18 months, with complete control of the clinical picture; however, mild neutropenia (915 cells/mmc) was observed. In the hypothesis of a side effect related to anakinra and in light of the poor compliance to daily injections, at the age of 4 years, treatment with anakinra was withdrawn and canakinumab, a monoclonal anti-IL1β antibody, was started at the dosage of 4 mg/kg every 4 weeks. The girl is currently 6 years old and is being treated with canakinumab 4 mg/kg every 5 weeks with good control of the inflammatory symptoms. However, mild-to-moderate neutropenia persists.

Discussion

Majeed syndrome is a rare autoinflammatory disorder caused by loss-of-function mutations in the *LPIN2* gene. It is considered, together with the deficiency of the IL-1 receptor antagonist (DIRA), a monogenic syndrome mainly characterized by CRMO. Unlike DIRA, in which cutaneous pustulosis is almost always present and can anticipate systemic complications [10–14], skin lesions of Majeed syndrome, usually represented by Sweet's syndrome, are present in <10% of cases and can appear years after other clinical manifestations [1, 2]. Our case, in

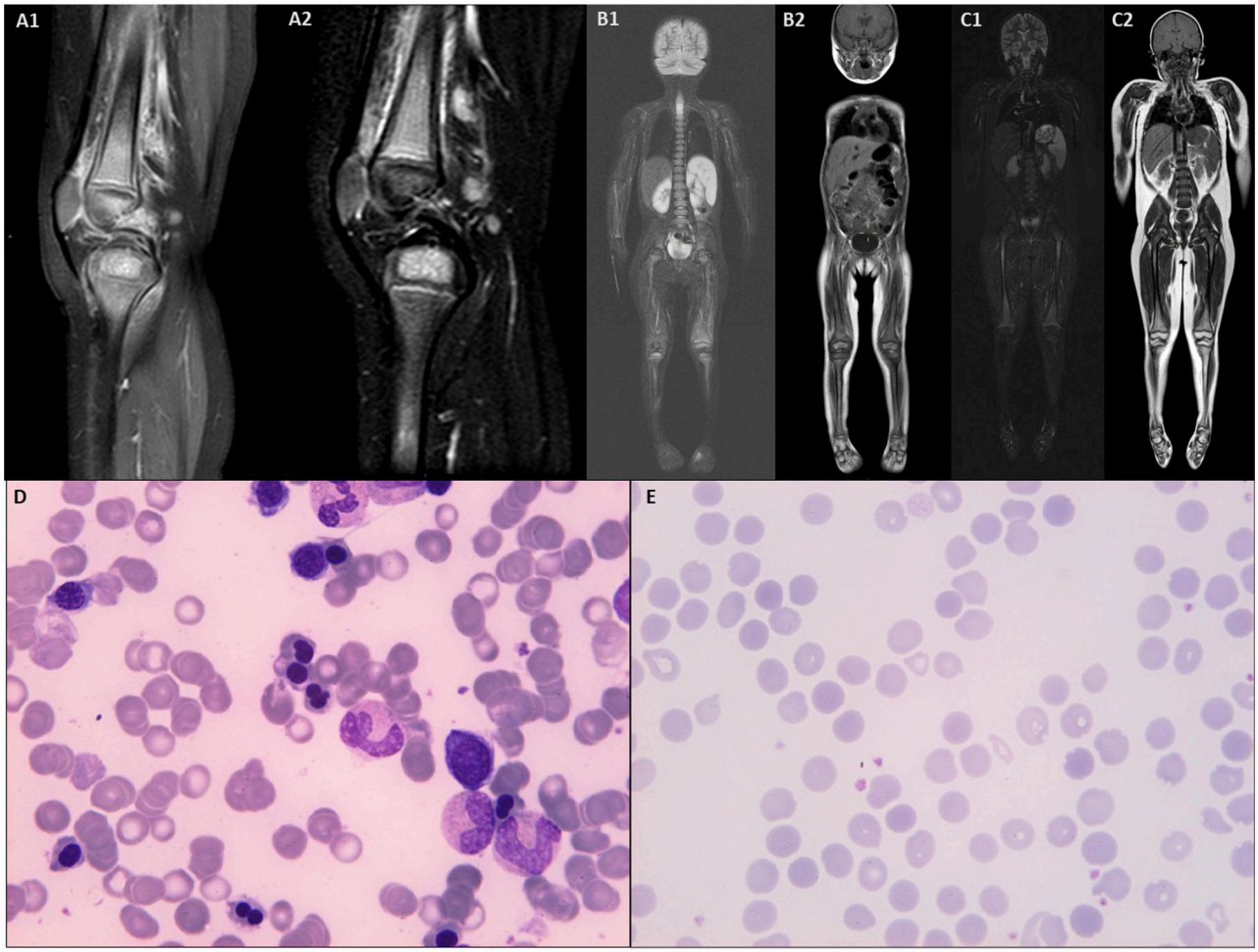

Figure 1. MRI images and bone marrow smears of the described patient before (A1, A2, B1, B2 and D) and after (C1, C2 and E) the beginning of treatment with IL1-blockers. (**A**) Coronal Mobiview reconstruction of turbo-spin-echo (TSE) short time inversion recovery (STIR) and TSE T1 multistack acquisition. Note bilateral meta diaphyseal distal femoral oedema as medullary diffuse hyper–hypointensity in STIR/T1. (**B**) Sagittal TSE t1 fat sat post contrast medium (CM) and TSE T2 fat sat sequence confirms oedema of the distal femoral metaphysis with periosteal and articular contrast enhancement. (**C**) Coronal Mobiview reconstruction of TSE STIR and TSE T1 multistack acquisition. Note the complete resolution of the meta diaphyseal distal femoral oedema. (**D**) Bone marrow dyserythropoiesis, with typical morphological abnormalities represented by numerous bi-plurinuclear erythroblasts, intercytoplasmic bridges, extranuclear chromatin. Myeloid series and megakaryocytes normorepresented. (**E**) Follow-up 6 months after starting anti-IL-1RI therapy: marked improvement in the picture with only a few elements compatible with dyserythropoiesis

which no skin involvement was observed, confirms that Majeed syndrome should be suspected not only in the presence of the 'triad' typical of the disease but rather in presence of a systemic inflammatory disease, with prevalent bone involvement, especially in case of presence of signs of dyserythropoietic anaemia.

To the best of our knowledge, no other reports of a genetically confirmed Majeed syndrome from central-Europe and with uniquely Italian descent are present so far in the literature. Together with the case recently described with Puerto Rican and African American descent [7, 8], the present one broadens the boundaries of the disease, considered as typical only of the Middle East. The NGS panel performed revealed two novel frameshift mutations in both alleles, inherited from parents and therefore compatible with compound heterozygosity, already described in the literature as a possible transmission mechanism causing the disease [7, 15]. Respectively, c.2144_2145dupGT leads to a premature stop codon at position 2 amino acids after the mutation, while c.1693_1694delGA creates a new reading frame that ends with a stop at position 15 after the mutation. Both variants are expected to produce a truncated protein or, more likely, to lead to early degradation of mRNA through the mechanism of nonsense-mediated decay. The mutations detected in our patient are not reported in the INFEVERS database [16] or in other human sequence databases (gnomAD, 1000 Genomes Project, dbSNP). Both variants were classified as pathogenic variants (PVS1-PM2-PP4) according to the ACMG guidelines [17].

Recently, lipin-2 has emerged as a key participant in the regulation of proinflammatory genes' expression by saturated fatty acids in macrophages [18]. LPIN2−/− animals had increased phosphorylation levels for ERK/JNK/p38, a pathway downstream of TLR4 that impacts NLPR3 inflammasome proteins, favouring the second step of inflammasome activation [6]. In addition, lipin-2 maintains a proper lipid environment for the purinergic receptor $P2X_7R$, an extracellular ATP-gated ion channel that plays a pivotal role in activation

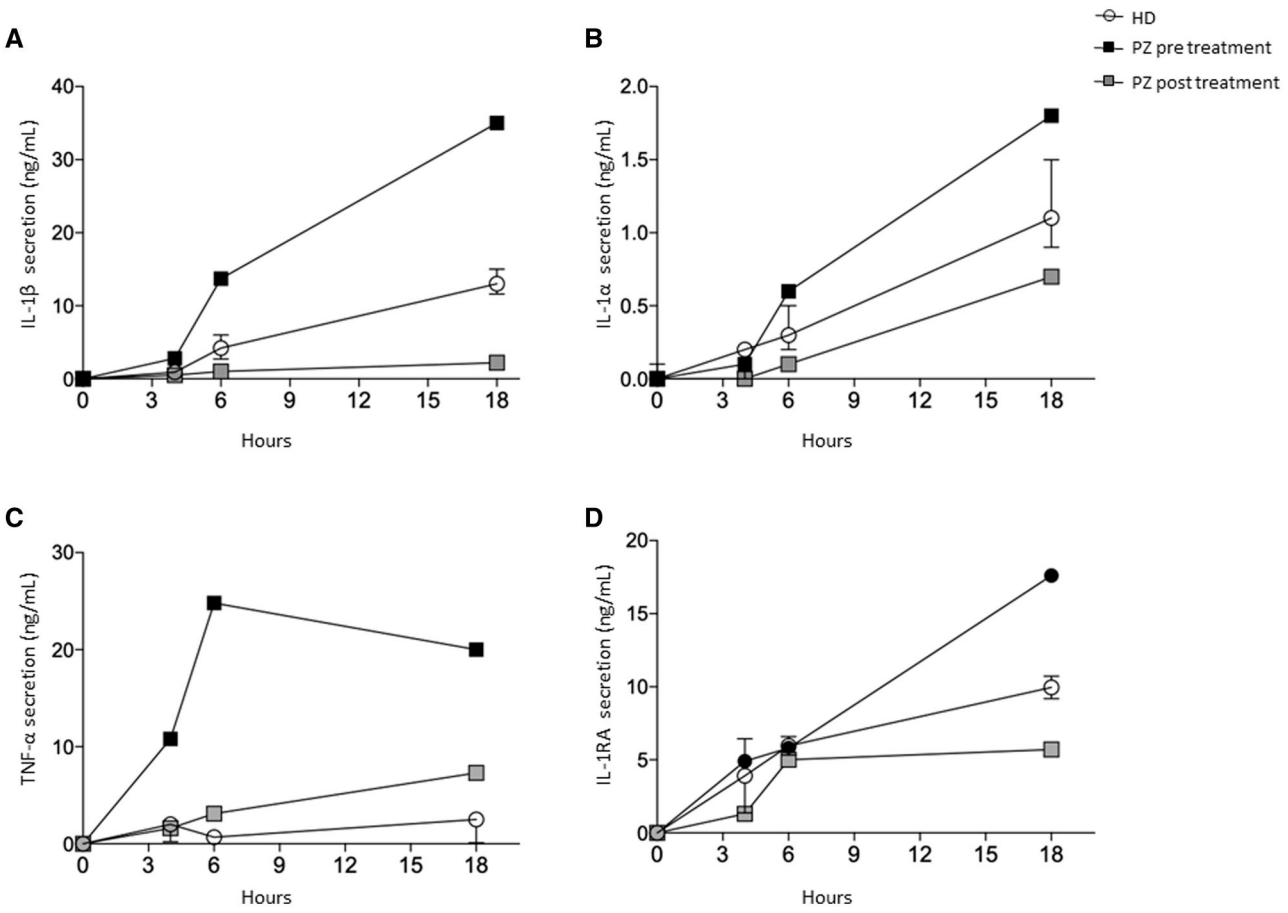

Figure 2. Cytokine secretion by peripheral blood freshly isolated monocytes from patient and health donors (HD). Kinetics of IL-1β (**A**), IL-1α (**B**), TNF-α (**C**) and IL-1RA (**D**) after LPS stimulation (200 ng/ml) from peripheral blood monocytes isolated from the patient before and after therapy and from healthy donors (HD) ($n = 3$). HD data are expressed as medians ± interquartile ranges. The concentration of cytokines was assessed in monocytes supernatants by using R&D Systems kits specific for IL-1β, IL-1α, TNF-α and IL-1RA

of NLRP3 inflammasome [6]. In fact, low cholesterol levels shown in lipin-2-deficient macrophages affect the activation and sensitization of $P2X_7R$ [6].

Thus, mutations in *LPIN2* could stimulate a constitutive NLRP3 activation leading to increased production of IL-1β and other inflammatory cytokines. Functional analysis of peripheral blood monocytes isolated from the patient was performed before and after the treatment with anakinra. As observed in other conditions secondary to a hyper-activation of NLRP3 inflammasome, the kinetics of IL-1β secretion in patient's peripheral blood monocytes was faster than in healthy monocytes (HD). Similarly, the secretion of IL-1α, TNF-α and IL-1RA (Fig. 2) was increased in LPS-stimulated monocytes collected before therapy, while it was strongly reduced in LPS-stimulated monocytes collected after therapy. Taken together, these data confirm that *LPIN2* mutations have important implications for the NLRP3 inflammasome activation, which is mainly responsible for the clinical manifestations of the disease, with a quick improvement following treatment with anti-IL1 agents.

LPIN2–/– mice, although do not display CRMO, develop microcytic anaemia, with features consistent with CDA [19]. However, it is currently unknown whether the inefficient erythropoiesis found in patients with Majeed syndrome is directly caused by a defect in peroxidase–antiperoxidase activity or is secondary to the previously described lack of control of pro-inflammatory pathways, namely NLRP3 inflammasome activation. Our report is the first case in the literature in which a bone marrow aspiration was performed following the beginning of therapy with anti-IL1 agents. The marked improvement of the signs of dyserythropoiesis detected in the bone marrow after the beginning of treatment suggests that the inflammatory dysregulation with increased secretion of IL-1β may play a key role in the development of dyserythropoietic anaemia. A resolution of microcytic anaemia was previously described in patients with Majeed syndrome after treatment with IL-1 blockers, mostly attributed to a possible improvement of the sideroachrestic component mediated by systemic inflammation [2]. The possible role of IL-1β in the pathogenesis of dyserythropoiesis is also supported by the description of such condition in a patient with mevalonate kinase deficiency, another congenital error of lipid metabolism resulting in an autoinflammatory disease with overproduction of IL-1β [20].

While anaemia responded well to treatment with IL-1 blockers, neutropenia appeared during treatment and was not affected by the switch between IL-1 inhibitors. Recently, neutropenia has been described as a possible feature of the disease, being detected in two Turkish siblings with Majeed syndrome [8]. As in our patient, neutropenia did not improve

after the beginning of treatment with anakinra. In light of that, it is conceivable that, while anaemia is related to the increased secretion of IL-1β, the mechanisms leading to neutropenia in Majeed syndrome are different and have not been investigated yet. Further *in vitro* and *in vivo* studies are necessary to better understand the pathogenesis of bone marrow involvement in patients with Majeed syndrome.

In summary, we described the first case of Majeed syndrome of a patient with central-European ancestry, caused by a compound heterozygous *LPIN2* mutation. In light of the presence of this disease in patients with ancestry different from the Middle East, we suggest to rule out Majeed syndrome in all children with an early onset inflammatory phenotype with severe bone involvement, resembling CRMO, especially in the presence of signs of dyserythropoiesis or neutropenia, regardless of the presence of skin involvement. Anti-IL1 agents should be considered the drugs of choice in these patients, allowing control not only of the inflammatory manifestations but also of the anaemia.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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