Mutation screening of the IL-1 receptor antagonist gene in chronic non-bacterial osteomyelitis of childhood and adolescence

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Abstract

Objective

Chronic non-bacterial osteomyelitis CNO is an inflammatory disorder of the musculoskeletal system with unknown etiology. In addition to bone inflammation, patients may present with inflammatory involvement of other tissues including, e.g., skin. Recently, a novel syndrome due to deficiency of interleukin-1 receptor antagonist (IL1RN), DIRA has been identified. Clinically the syndrome is characterized by neonatal onset of pustular dermatosis, periostitis and chronic sterile multifocal osteomyelitis, strongly resembling CNO. Homozygous mutations of IL1RN have been identified and resulted in a truncated protein that is not secreted, hence leaving the action of interleukin-1 unopposed.

Methods

Because of similar clinical, radiological and histological features of CNO and DIRA, we hypothesized that both disorders might share a common autoinflammatory process. Thus, we searched for the presence of mutations in the interleukin-1 receptor antagonist gene in 60 patients diagnosed with CNO.

Results

In one patient with chronic multifocal osteomyelitis a heterozygous missense variant: c.281G>T (p.Cys94Phe) was detected. In the other patients only frequent polymorphisms were found.

Conclusion

Our findings were not able to confirm mutations in IL1RN being an important contributing factor to the pathogenesis of CNO.

Key words

chronic non-bacterial osteomyelitis, DIRA, interleukin-1 receptor antagonist

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Introduction

Chronic non-bacterial osteomyelitis CNO is a rare, inflammatory, non-infectious disorder of the musculoskeletal system of unknown etiology. In children and adolescents, CNO predominantly affects the metaphyses of the long bones, but lesions can occur at any site of the skeleton (3). Both single and multiple lesions have been described and disease course may be recurrent. Chronic recurrent multifocal osteomyelitis is considered the paediatric form of SAPHO syndrome and is the most severe form of CNO. The inflammatory lesions are histologically characterised by lymphocytic cell infiltration, osteolysis, sclerosis or hyperostosis (4).

CNO may manifest with inflammatory bone lesions alone. However, other organs including the skin, gastrointestinal tract and rarely the lungs can also be affected by inflammation (5, 6). The occurrence of palmo-plantar pustulosis, psoriasis, arthritis, sacroiliitis and inflammatory bowel disease in patients with CNO suggests that chronic sterile inflammation is the common pathology in different affected tissues in CNO patients (5, 7).

Pathophysiology of CNO

Although CNO has been recognised for more than 30 years, its origin and pathogenesis is still unclear. A genetic predisposition in CNO is supported by familial clustering of CNO and by the observation of genetic linkage in a CNO cohort as suggested by Golla *et al*. (8). However, consistent evidence for disease associated gene variants is still lacking. Genetic studies revealed no role of different MHC molecules like HLA-B27 or HLA-DR (9), supporting innate immune activation, rather than T-cell mediated autoimmune pathogenesis. Based on the phenotypic similarities with Majeed syndrome in which both copies of the lipin 2 (*LPIN2*) gene are mutated, this gene has been studied in CNO, but appeared unaffected (9). Similarly, in a murine model disruption of proline serine threonine phosphatase interacting protein 2 gene (*pstpip2*) induced a congenital disorder indistinguishable from CNO, but the human ortholog did not show any abnormality in CNO patients (10).

In patients with familial Crohn's disease mutations in the caspase activation and recruitment domain 15 gene/nucleotide-binding oligomerisition domain protein 2 (*CARD15/NOD2*) have been described. CNO without intestinal inflammation is not associated with common CARD15/NOD2 gene variants (5, 9). Polymorphism in the tumour necrosis factor (*TNF*) *promotor* have also been associated with a severe form of Crohn's disease (11). Similar mutations have not yet been described in CNO. In parallel, chronicity, the recurrence of inflammation and effective immunosuppresive or immunmodulative therapies suggest an autoinflammatory and/or rheumatologic cause of CNO (3, 12-14). Therefore, variants of genes coding for proteins involved in innate immunity and inflammation might predispose for the occurrence of CNO.

Deficiency of interleukin-1 receptor antagonist (DIRA)

Recently, we and others identified a novel syndrome due to the deficiency of interleukin-1 receptor antagonist (ILRA), DIRA (1, 2). In this autosomal recessive disorder both copies of IL1RN are incapable of producing any interleukin-1 receptor antagonist protein, hence leaving the action of interleukin-1 (IL-1) α and -β unopposed. Clinically the syndrome is characterised by a pustular dermatosis, periostitis and by chronic progressive multifocal non-bacterial osteomyelitis, strongly resembling CNO.

Although null mutations, as observed in the DIRA patients, give rise to severe neonatal disease, it is conceivable that less disruptive missense mutations could cause milder phenotypes with later disease onset. Based on similar clinical features of CNO and DIRA we hypothesized that CNO and DIRA might share a common autoinflammatory process. The aim of the study was to assess if mutations located in the ILRN gene were associated with CNO. Hence, we genetically analysed stored DNA samples obtained from 60 CNO patients for the presence of mutations in this gene.

Table I. Clinical description of patients with CNO included in the study.

	n.	%
Patients	60	100.0
Female	34	56.7
Multifocal	40	66.7
Unifocal	20	33.3
Inflammatory bowel disease	3	5.0
Palmo-plantar pustulosis	4	6.7
	median	range
Age at diagnosis	12.6 years	2.1-18.3 years
Number of lesions	3.0	1-19

Material and methods

Patients and phenotyping

We studied DNA samples of 60 children diagnosed with CNO at the children's hospital, University of Würzburg (October 2002 to January 2009). Patients were diagnosed using a standardised protocol in diagnosis as described recently (7). The disease was assessed using initial diagnostic biopsy, laboratory tests and magnetic resonance imaging. Inclusion criteria were: age under 18 years at onset of symptoms and presence of CNO. The diagnosis of CNO comprised: typical clinical symptoms (pain and/or swelling), histology (exclusion of malignant process), microbiology (negative culture and eubacterial PCR) and imaging (increased signal intensity in TIRM sequences and post contrast T1). Specific treatment included naproxen and sulfasalazine plus short term oral glucocorticoids in more severe and relapsing cases. No patient was receiving biological therapies. The study was approved by the ethics committee of the University of Würzburg. Signed informed consent was obtained from the patients' parents and from adolescent patients.

Genetic analysis

Genomic DNA of 60 CNO patients was isolated from peripheral blood mononuclear cells by standard procedure (DNeasy Blood and tissue kit, Quiagen, Hilden Germany). Samples were analyzed for mutations in the IL1RN gene (NM_173841). Primers were designed for coding exons, including the intron–exon boundaries: (sequences available upon request). The fragments were amplified and sequenced on an ABI 3100 automated sequencer (PE Applied Biosystems, Foster City, CA, USA) using BigDye® Terminator Cycle Sequencing Kits (primer sequences and PCR conditions are available on request). Data were analysed with Sequencing Analysis (v.3.7) and Mutation Surveyor software (Softgenetics, LLC, State College, PA, USA).

Results

Clinical description of patients with CNO

We investigated 60 patients (34 female, 26 male) with CNO. The patients' characteristic are shown in Table I. Median age at diagnosis was 12.6 years (range 2–18). Osteomyelitis was unifocal in 20 patients (33.3%) and multifocal in 40 (66.7%). The median number of bone lesions was 3.0 (range 1–19). Skin lesions were present in 4 patients (6.7%). 3 patients (5.0%) had Crohn's disease.

Genetic analysis

In one patient with chronic multifocal osteomyelitis a heterozygous missense variant: c.281G>T (p.Cys94Phe) was detected, not described before (Figure 1). The functional consequence of this variant is not known. Moreover, it was not present in 192 control chromosomes, indicating it is not a frequent polymorphism and it might play a potential pathogenic role in CNO. The affected amino-acid is a moderately conserved amino-acid concerning 11 species. The biochemical difference between Cys and Phe, however is large. No other potential pathogenic variants were detected in this patient. In the other patients only common polymorphisms were detected.

Clinical case

This 12-year-old boy with the mutation mentioned above presented with thorax dysplasia and a 3 month history of pain and local bone/tissue swelling in the ribs. MRI showed inflammatory lesions in the left 2nd and 5th rib and bone biopsy confirmed the clinical diagnosis of sterile chronic bone inflammation. The patient did not show any associated diseases. Treatment with naproxen and sulfasalazine was highly effective, with significant amelioration of pain (from 5 to 0.2 on a visual analogue scale VAS from 0 to 10). The CNO overall disease

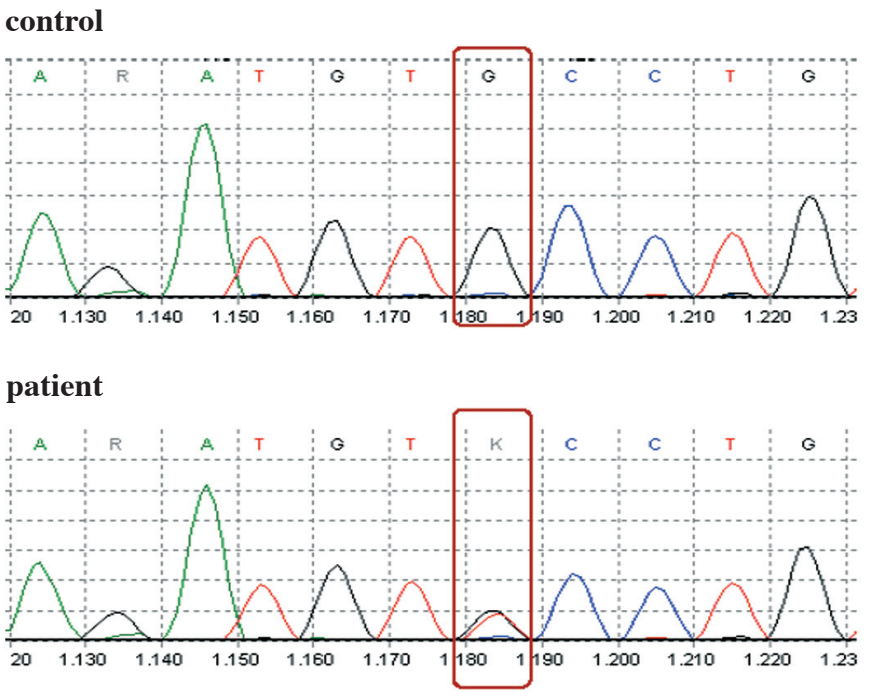

Fig. 1. Genetic analysis, control and patient. Control has a guanine (G) in position 283 – patient shows a heterozygous missense mutation G>T resulting in an amino acid exchange from Cys to Phe.

activity estimated by the patient (from 2 to 0 on the VAS) and by the physician (from 3 to 0.5 on a VAS) showed a significant improvement after 5 years of therapy. Whole body MRI still showed a minor inflammatory swelling of the 5th rib on the left side. There were no new radiological lesions. Of note, after a traumatic ulnar fracture delayed union and pseudarthrosis required surgical correction.

Discussion

CNO is an inflammatory disorder of unknown origin. Susceptibility genes may be involved, as rare familial clustering has been reported. However, previous studies failed to show involvement of HLA genes, PSTPIP2, LIPIN2 and CARD15/NOD2 (5, 8, 9). Because of similar clinical features of CNO and DIRA, a recently described autosomal recessive inflammatory disease with skin and bone involvement, we hypothesized that both disorders might share a common autoinflammatory process. DIRA is due to mutations in the IL1RN that lead to non-expression of the encoded protein IL1RA resulting in an unopposed interleukin-1 receptor activation and an increased response to the proinflammatory cytokines IL-1α and IL-1β (1, 15). *In vitro* studies of leukocytes from DIRA patients with unopposed IL-1 signalling showed that IL-1 drives overproduction of pro-inflammatory cytokines and chemokines. The dramatic clinical phenotype in DIRA patients with life-threatening systemic inflammation underscores the importance of tight regulation of IL-1 in skin and bone (1).

Despite sequencing the coding regions and the intronic areas flanking exon boundaries, we failed to detect homozygous or compound heterozygous mutations in IL1RN in any CNO patient. One patient with multifocal CNO carried a missense variant, of which the functional relevance remains to be determined. He did not show any pustulosis or other associated diseases. Family history revealed no other affected individuals with symptoms suspicious for the presence of CNO. Unfortunatelly no information is available if the mutation is a *de novo* or a transmitted mutation because it was not possible to test the asymptomatic parents. A potential causative role of the detected mutation cannot be totally excluded as this mutation has not been found in 192 control chromosomes. According to recent publications heterozygous carriers of the mutations involved in often lethal diagnosis of DIRA, have been found to be clinically and immunologically normal so far (1). However this does not exclude the potential role of heterozygous mutations in a clinically milder disease like CNO.

Our findings argue against mutations in IL1RN being an important contributing factor to the pathogenesis of CNO. Since this study was limited to genomic DNA samples, we cannot exclude the possibility that IL-1RA function is impaired downstream of its coding gene, *e.g.* due to reduced transcription, reduced translation or due to rapid disappearance of either mRNA or protein. The clinical, radiological and histological similarities do suggest the involvement of some components of the IL-1 signalling pathway, but as long as this hypothesis is not supported by new genetic and functional data, it remains conjectural.

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