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BRIEF COMMUNICATION

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The IL-1 gene family and bone involvement in celiac disease

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Abstract Celiac disease (CD) is associated with decreased bone mineral mass. Its pathogenesis is multifactorial since both systemic and local mechanisms may play a role. Our objective was to determine whether single-nucleotide polymorphisms in genes encoding members of the interleukin-1 family are associated with bone damage measured by densitometry in a series of 71 adult CD patients assessed at diagnosis. When compared with non-carrier CD patients, carriers of allele T of the interleukin-1β gene (*IL1B*-511T) had a significantly lower bone mass at the total skeleton level (p=0.0484) and a greater prevalence of osteopenia/osteoporosis (p=0.0102). To our knowledge, this is the first evidence on the association between a genetic predisposition and low bone mass in CD patients. This finding supports the postulated inflammation-associated bone loss pathogenesis as one of the causes of bone weakness in CD.

Keywords Celiac disease · IL-1 · Genetic polymorphism · Osteoporosis

Introduction

In the past 15 years, a strong body of evidence has shown that celiac disease (CD) is often associated with bone damage with more than 50% of adult patients presenting with osteopenia or osteoporosis at the time of diagnosis (Corazza et al. 2005). Interestingly, although bone involvement in CD was first reported several years ago, the true clinical magnitude of the problem, based on the evidence of fractures, was ignored for a long time, and epidemiological information on fractures in CD has only recently been acquired (Vazquez et al. 2000). In a case-control, cross-sectional study, the occurrence of fractures in CD patients analysed in a referral centre has shown an increased risk of fractures in the peripheral skeleton that seems to be limited to those patients with classical symptoms but not in cases with a subclinical or silent course (Moreno et al. 2004).

The interleukin-1 (IL-1) family (IL-1α, IL-1β and IL-1ra) has been implicated in both the normal bone metabolism and the intestinal lesion of CD. In bones, IL-1 cytokines play a key role in remodelling by regulating the differentiation and activation of osteoblasts and osteoclasts. In this context, abnormal production of IL-1β was formerly identified as a potential factor for increased bone loss in CD (Fornari et al. 1998). Several observations suggest that genetic factors may play an important role in determining both the peak bone mass and the rate of bone loss. However, this genetic predisposition has not been explored in CD. Moreover, the identification of potential candidate-genes predisposing to abnormalities in bones is dampened by at least two factors, i.e. the multifactorial nature of bone involvement in CD and the allelic variability of the genes involved. We hypothesize that a genetically determined variation in the production of cytokines may play a key role in regulating disease-related inflammatory responses and in the inflammation-associated bone loss. Therefore, our objective was to determine the effect of single-nucleotide polymorphisms (SNPs) in genes belonging to the IL-1 family on the bone mass of CD patients because prior studies demonstrated that the production of both IL-1 and IL-1ra protein is related to the *IL1B*-511 C>T SNP or the *IL1B*-31T>C SNP in the TATA box in very strong linkage disequilibrium that influences IL-1β mRNA expression and to the variable number of tandem repeats (VNTR) polymorphism in the *IL1RN* gene

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Table 1 Epidemiology, clinical characteristics and densitometric values at diagnosis of CD patients grouped according to the absence (non-carriers), presence (carriers) or two copies (homozygotes) of allele *IL1B*-511T

	Non-carriers	Carriers	Homozygous
No. of patients	29	42	19
Mean age, years (range)	44 (20–75)	43 (21–75)	44 (21–75)
Gender (male/female)	8:21	7:35	2:17
Clinical features (no. of patients)			
Classical CD	23	29	13
Subclinical CD	6	9	5
Silent CD	0	4	1
Mean BMI (kg/cm^2), Mean±SEM	20.3±0.5	21.6±0.6	21.3±0.8
No. of patients with bone fractures (%)	6 (21)	10 (24)	4 (18)
Bone density values (DEXA) *Z* score, Mean±SEM			
Total skeleton	−0.50±0.46	−1.71±0.32*	−2.24±0.49**
Lumbar spine (L2–L4)	−0.95±0.35	−1.05±0.22	−1.11±0.31
Percentage of patients with osteopenia/ osteoporosis in the peripheral skeleton	23	56***	47

DEXA dual-energy x-ray absorptiometry
*vs non-carriers p=0.0484 (Student's t test)
**vs non-carriers p=0.0394 (Student's t test)
***vs non-carriers p=0.0102 (Chi-square)

in very strong linkage disequilibrium with the *IL1RN*+2018 T>C SNP (Danis et al. 1995; El-Omar et al. 2000; Hurme and Santtila 1998; Santtila et al. 1998, Kimura et al. 2004).

Patients

We collected blood samples from 71 consecutive adult unrelated patients diagnosed with CD (58 female; mean age 36 years, range 20–75) attending the Small Intestinal Disorders Clinic of the "Dr. Carlos Bonorino Udaondo" Gastroenterology Hospital in Buenos Aires, Argentina. The most important inclusion criterion was that patients had a bone mineral density (BMD) determination performed at the time of CD diagnosis by using dual-energy x-ray absorptiometry. While 63 patients had BMD measurements at the lumbar spine (L2–L4), 41 had determinations at the total skeleton level. At the time of blood extraction, patients were informed of the aim of the study and gave their consent to be included.

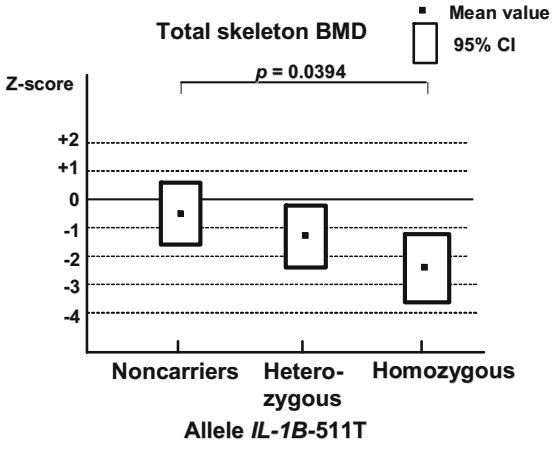

Fig. 1 Mean *Z* scores and 95% confidence interval (CI) for bone mineral densities of CD assessed at diagnosis. Patients are categorized as non-carriers, heterozygous carriers and homozygous of allele *IL1B*-511T

Methods

The *IL1B*-511 C>T SNP (dbSNP ID rs16944), the *IL1A*−*889* T>C SNP (dbSNP ID rs1800587) and the *IL1RN*+2018 T>C SNP (dbSNP ID rs419598) were typed by TaqMan technology in the Laboratory of Immunogenetics, Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands.

Results

At diagnosis, 42 (59%) patients were carriers of allele T of the *IL1B*-511 SNP, 19 (27%) were homozygous and 29 (41%) were non-carriers. When compared with non-carriers CD patients, carriers of allele *IL1B*-511T had significantly lower BMD (*Z* score) at the total skeleton level (p=0.0484) and a greater proportion of patients with osteopenia/osteoporosis (Odds ratio 3.8; 95% CI 1.3–10.8; p=0.0102) (Table 1; Fig. 1) but not in the lumbar spine. This positive association was mainly determined by homozygous carriers of the allele (vs non-carriers p=0.0394). No association was found between bone mass and other *IL1* family SNPs analysed in the study population (data not shown). Furthermore, no statistical differences were detected for BMD measured at other sites. No differences were found for associations with clinical parameters at diagnosis comparing non-carriers with carrier of the minor alleles of either SNP. Thus, no differences were detected in terms of age, gender, clinical characteristics at presentation (classical, subclinical and latent forms) and body mass index and number of fractured patients comparing non-carriers with carriers of *IL1B*-511T or with patients expressing two copies of the allele (homozygous) (Table 1). Multivariate analyses (Cox regression) of potential confounders including age, gender and body mass index determined that the allele *IL1B*-511T was the only variable independently associated with low bone density (Odds ratio 7.50, CI 1.02–54.97). When clinical characteristics of patients were assessed according to the presence of no copies, one copy and two copies of the minor alleles of the

IL1A-889 SNP and the *IL1RN*+2018 SNP, patients did not show significant differences (data not shown).

Discussion

To our knowledge, this is the first study suggesting that low BMD in the total skeleton of CD patients is associated with an allelic variant of the *IL1B* gene. Thus, carriage of *IL1B*-511T is associated with bone loss in the peripheral skeleton of adult CD patients, the site where most bone fractures occur in CD patients. This association was mainly determined by the presence of two copies of the allele. However, our findings do not highlight any differences in the parameters used to evaluate the clinical severity of CD according to the presence of the genetic variables studied. In this context, the present evidence suggests that the *IL1B*-511 SNP may be a marker of bone disease without any other functional expression. At present, it is not clear whether allele *IL1B*-511T results in the loss of a putative AP-2 binding site or the linkage with *IL1B*-31 located in a TATA box. In contrast, no association of bone impairment with other SNPs in genes of the *IL1* gene family (*IL1A* and *IL1RN*) was found. Moreover, we did not find any other genetic association with other areas of the body explored (lumbar spine). Finally, our findings seem to contribute to the postulated inflammation-associated bone loss pathogenesis as one of the causes of bone weakness in CD, as previously suggested in other autoimmune disorders (Schulte et al. 2000; Nemetz et al. 2001; Strand and Kavanaugh 2004). In conclusion, according to our findings, a genetic factor participating in the regulation of the immune response and bone metabolism contributes to CD osteopathy which likely a result of a combination with other factors such as local and systemic immunological disorders, malabsorption and malnutrition. Given the limited number of patients included in this study, we recommend the performance of large-scale prospective studies focusing on the association with SNPs in these and other genes controlling the inflammatory response.

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