GENE POLYMORPHISM FOR A-RECEPTOR OF OESTROGENES AND ALTERATIONS IN BONE MINERAL DENSITY FOR ADULT CELIAC DISEASE PATIENTS

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Abstract

It is well known that osteopenia and osteoporosis are frequently found celiac disease patients presenting classical symptoms of malabsorption[1]. Osteomalacia cases have also been diagnosed in celiac patients who do not present clinical signs of malabsorption , in patients with latent celiac disease, as well as in first degree relatives of patients with celiac disease who do not suffer from celiac disease themselves. This suggests the presence of different pathogenic mechanisms[2]. The analysis of genetic polymorphism represents an effective approach for an in-depth screening of genes potentially implicated in the development of osteoporosis.

Because of the central role that estrogen plays in bone metabolism, ER genes play an important role in the determination of bone mineral density and the risk of osteoporosis. The fact that osteoporotic phenotypes are observed in patients with a destructive mutation of the α receptor gene for estrogen together with the signs of reduced bone mineral density that are found in mice presenting a functional insufficiency of ER α, but not in mice showing reduced ER β function, demonstrates that ER α is one of the principal genes involved in the genesis of osteoporosis[3].

Previously , two intronic polymorphisms of the α ER gene, identified by restriction endonucleases PvuII and TA Xba and repetitive polymorphism sequences, have been linked to bone mass density in the Japanese population and in menopausal Italian women[4].

Keywords: Bone mineral density (BMD), Estrogen receptor(ER), Celiac disease (CD), Standard deviation (SD), Dual Energy X-ray Absorptiometry (DEXA), Polymerase Chain Reaction (PCR), World Health Organization (WHO)

Introduction

It is well known that osteopenia and osteoporosis are frequently found celiac disease patients presenting classical symptoms of malabsorption[1]. Osteomalacia cases have also been diagnosed in celiac patients who do not present clinical signs of malabsorption, in patients with latent celiac disease, as well as in first degree relatives of patients with celiac disease who do not suffer from celiac disease themselves. This suggests the presence of different pathogenic mechanisms[2]. The analysis of genetic polymorphism represents an effective approach for an in-depth screening of genes potentially implicated in the development of osteoporosis.

Because of the central role that estrogen plays in bone metabolism, ER genes play an important role in the determination of bone mineral density and the risk of osteoporosis.

Objective

Evaluate the genotypic distribution of two intronic polymorphisms, PvuII and Xba1, of the α ER receptor gene in a group of celiac disease patients. Study the correlation between particular bone structure and the corresponding genotype in celiac patients, divided into three groups based on their BMD (normal, osteopenia, osteoporosis).

Case Studies

Patients: 80 celiac disease patients between 23 and 74 years of age (mean ± SD = 44.7 ± 1.5). Control: 156 healthy patients (no celiac disease), post-menopausal women between 43 and 75 years of age (mean ± SD = 63.6 ± 0.6).

Methods

Celiac Disease Diagnosis: Dosage of AGA IgA, IgG, TTGA (Eurospital, Trieste), EMA (Euroimmun, Poyesis, Padoue), quantitative immunoglobulin.
Histological exams of biopsies of the second and third part of the duodenum taken during endoscopy.

Laboratory Tests: Parameters of intestinal absorptions of antibodies, some indicators of bone remodeling, and BMD of the entire body.

Table1.Turnover Markers and Bone Resorption Parameters of Celiac Disease Patients.

Parameters	***Normal Values***
Serum Calcium	*8.8-10.7mg/dl*
24 hours Urine Calcium	*100-300mg/24h*
Phosphorus	*2.5-5.0mg/dl*
Phosphaturia	*400-1000/24h*
Serum Magnesium	*1.8-2.6mg/dl*
Parathyroid PTH	*10-70p g/ml*
Osteocalcin	*U 4.4-12.8; D 3.2-16.8*
Total Alkaline Phosphatase	*60-270 U/I*
Bone Alkaline Phosphatase	*U 3.7-22; D 3.4-20 ng/ml*
Urinary D-pyridinoline	*2.0-9 nM/Mm Creat.*
Vitamin D 25-OH	*5.51 Winter; 56.8 Summer*
Vitamin D 1-25 $(OH)_2$	*20.6-70.8 pg/ml*

Evaluation of Bone Mineral Density: Bone mineral density (BMD) was measured in all patients by computerized bone mineralometry (OMC) with a total body DEXA densitometer (Dual Energy X-ray absorptiometry) Hologic QDR-1000.
BMD absolute value was established with a Z-score and a T-score in accordance to WHO classification system.

Table2. WHO T-Score Values

Normal	osteopenia	Osteoporosis
0<T-score<-1 SD	-1<T-score<-2.5 SD	T-score<-2.5 SD

Genotyping

DNA was extracted by the standard procedure with phenol/chloroform and then amplified in 50 µL buffer solution using 1 unit of Taq Polymerase (Promega, Madison WI) and 0,4 µM of oligonucleotidic primers direction 5'-3‘ and opposite direction 5‘-3' using 30 cycles of polymerase chain reaction (PCR) to obtain a part of the intron 1 and exon 2 of the α ER gene. The product of amplification was digested by 10 units of restriction enzyme XbaI or PvuII and subjected to electrophoresis on 2% Agarose Gel.

The presence of the restriction site for each endonuclease was conventionally indicated with lowercase letters p and x, whereas the absence of the restriction site was indicated with capital letters P and X respectively for PvuII and Xba1. Based on the model of digestion of the PCR product, patients were identified as homozygous pp, xx, PP or XX or heterozygous Pp or Xx.

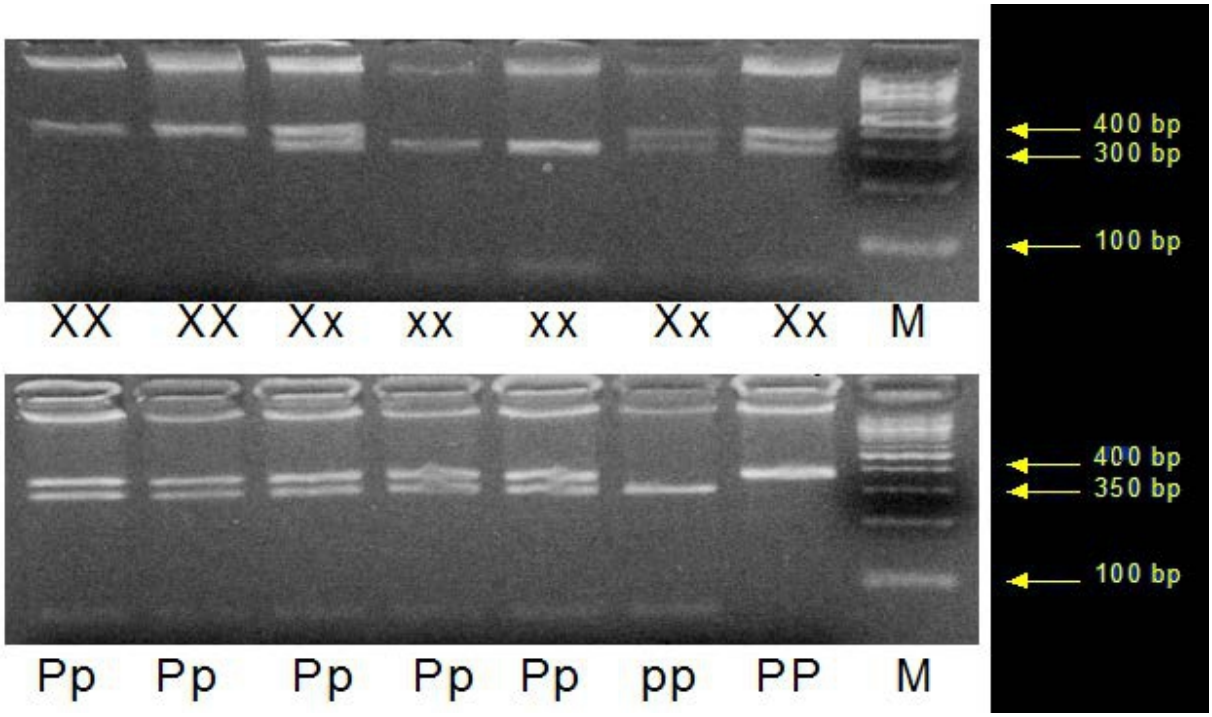
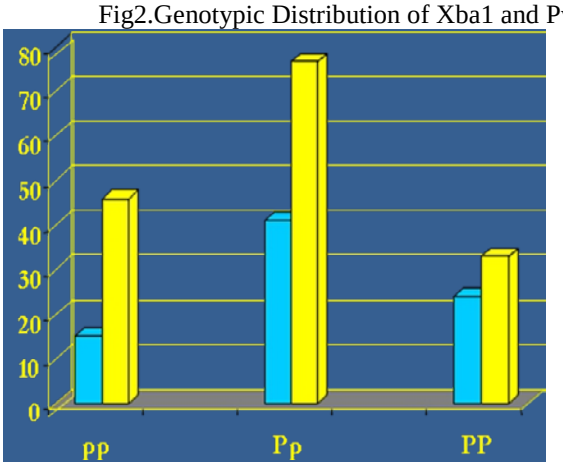


Fig1.Electrophoresis on 2% Agarose Gel of the Product of Digestion for the α ER Gene Xba1 & PvuII

Statistical Analysis

The test χ squared was used to compare the polymorphism distribution of the α ER gene in CD patients and controls. The Fisher test was used to evaluate the difference between the frequency of both polymorphisms in the three groups: normal, osteopenia, osteoporosis.

Fig2.Genotypic Distribution of Xba1 and PvuII Analyzed Separately for Patients and Controls



P>0.05

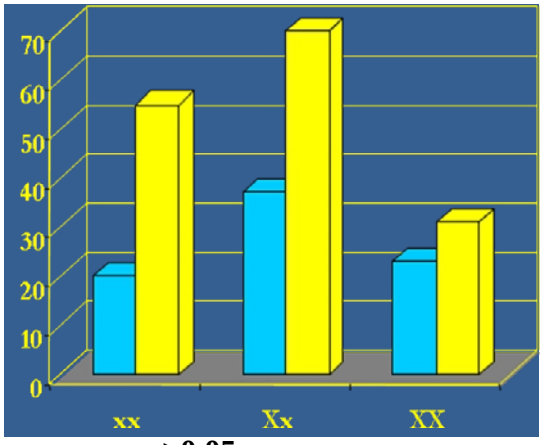


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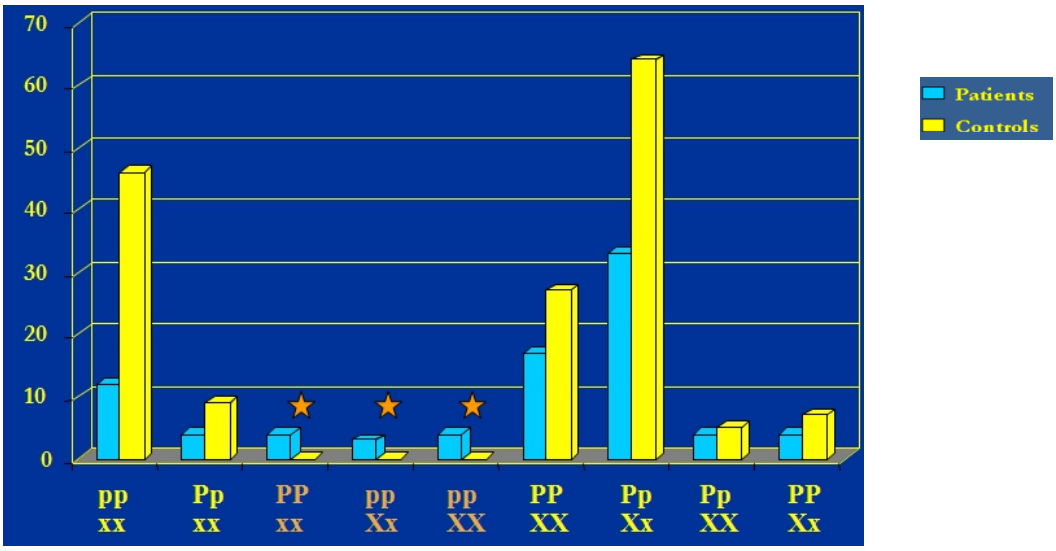
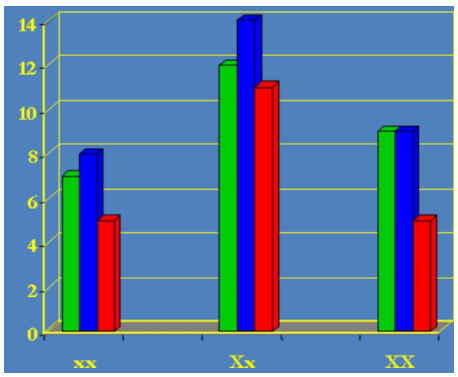
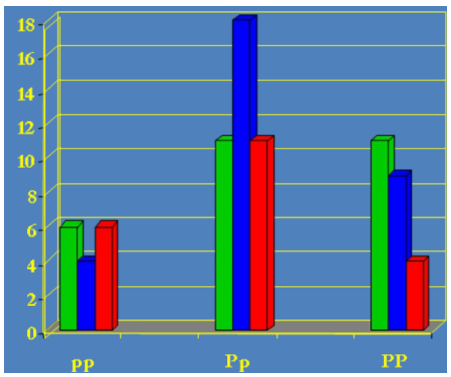
Fig3.Genotypic Distribution of Xba1 and PvuII in Combined Analysis for CD Patients and Controls

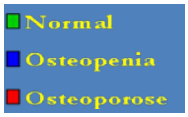


Results

Fig4.Genotypic Distribution of Xba1 and PvuII analyzed Separately for the Three Groups of CD Patients

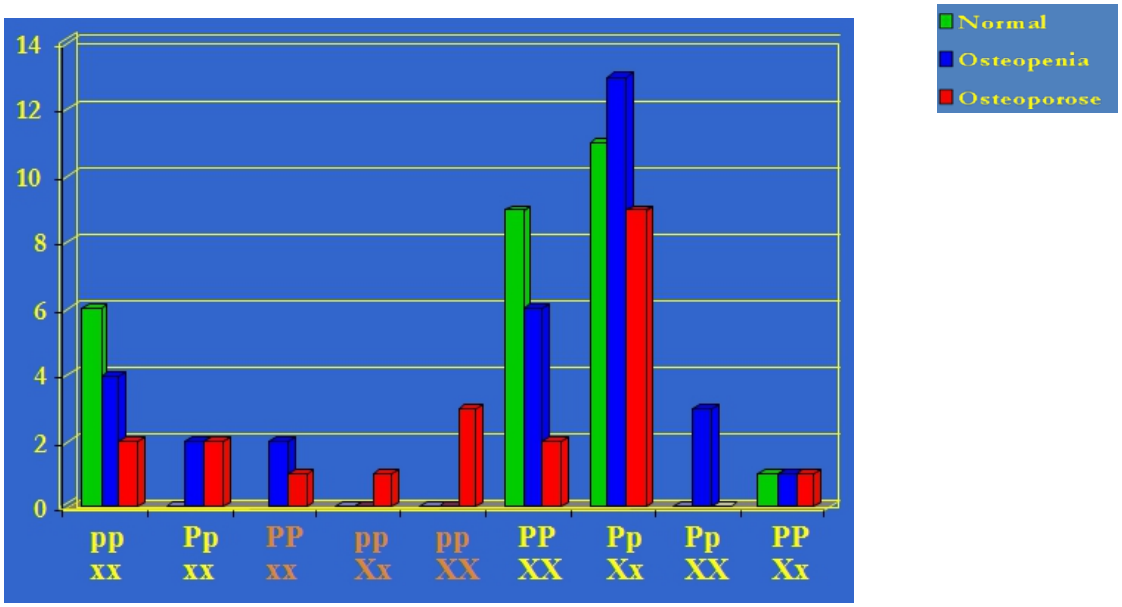






P>0.05

Fig5.Genotypic Distribution of Xba1 and PvuII Analyzed in Combination for the Three Groups of CD Patients
P>0.05



52/80 patients (65%) were diagnosed with osteoporosis or osteopenia.
31/80 osteopenia
21/80 osteoporosis
28/80 normal

No significant differences were found between the three different genotypes in either analysis of the three CD groups (normal, osteopenia, osteoporosis) in both analysis: Separately and in combination for restriction sites.

Conclusion

There is a high prevalence of alterations in bone mineral density in celiac disease. There are significant differences between the genotypic distributions of the two polymorphisms for the CD patient group versus the control group. No significant difference was observed in the two genotypic distributions for the three groups of CD patients. The three genotypes PPxx, ppXx and ppXX are found ONLY in CD patients suffering from osteopenia or osteoporosis.

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