Itai-itai disease is not associated with polymorphisms of the estrogen receptor $\alpha$ gene

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Abstract Itai-itai (or ouch-ouch) disease is a syndrome accompanied by bone mineral disorders, and which may be related to oral cadmium exposure. Itai-itai predominantly affects postmenopausal women with a history of multiple childbirths. Recently, it has been reported that polymorphisms of the estrogen receptor $\alpha$ (ER$\alpha$) gene are associated with postmenopausal reduction of bone mineral density in Japanese women. However, estrogen receptors have never been studied in itai-itai disease. In this study, we examined the genotypic distributions of $Pvu$II and $Xba$I restriction fragment length polymorphisms (RFLPs) of the ER$\alpha$ gene in patients with itai-itai disease and compared them with those of control subjects. The RFLPs are represented here as Pp ($Pvu$II) and Xx ($Xba$I); the capital and small letters signify the absence and presence of restriction sites, respectively. The genotypic distributions of the patient group were: PP, 14.8%; Pp, 55.6%; pp, 29.6%; XX, 7.4%; Xx, 29.6%; and xx, 63.0%. These distributions were similar to those observed for the control groups, hence no pattern of genotypic distribution was observed that could be related to itai-itai disease. We conclude that RFLPs of the ER$\alpha$ gene may not be associated with itai-itai disease.

Key words Itai-itai disease · Cadmium · Estrogen receptor · Restriction fragment length polymorphism

Introduction

Itai-itai (ouch-ouch) disease is a syndrome that is accompanied by bone mineral disorders, osteoporosis and osteomalacia, which may be related to oral cadmium exposure (Tsuchiya 1969; Freiberg et al. 1974). The disease was first seen in the Jinzu River basin of Toyama prefecture, Japan, where heavy metals including cadmium were discharged from the Kamioka mine or refinery (Freiberg et al. 1974). Itai-itai disease predominantly affects postmenopausal women with a history of multiple childbirths, thus disruption of the female endocrine system has been thought to be associated with the disease, perhaps as a predisposing factor (Murata and Nakagawa 1958). However, there are no significant differences in the urinary levels of estrogen and its metabolite between affected and healthy women (The Study Group of Kanazawa University School of Medicine 1967).

Estrogen receptor $\alpha$ (ER$\alpha$) is a critical factor for bone metabolism. Osteoblasts and osteoclasts express ER$\alpha$, which suggests that the receptor regulates bone formation and resorption (Eriksen et al. 1988; Ikegami et al. 1994; Kameda et al. 1997; Komm et al. 1988). It has been proved that mutations of the ER$\alpha$ gene cause bone resorption in affected individuals (Smith et al. 1994). Recently, Kobayashi et al. (1996) reported that $Pvu$II and $Xba$I restriction fragment length polymorphisms (RFLPs) of the ER$\alpha$ gene are associated with postmenopausal reduction of bone mineral density in Japanese women. On the other hand, cadmium has been shown to inhibit transcription of the ER$\alpha$ gene (Garcia-Morales et al. 1994). These findings led us to consider the role of ER$\alpha$ in the pathogenesis of itai-itai disease. However, estrogen receptors have never previously been studied in Itai-Itai disease.

We hypothesized that a polymorphism of the ER$\alpha$ gene is associated with itai-itai disease, as well as with postmenopausal reduction of bone mineral density. A polymorphism of the ER$\alpha$ gene, if associated with itai-itai disease, will be linked to a gene mutation that is a pre-
disposing factor for itai-itai disease. To test this hypothesis, we examined the genotypic distributions of PvuII and XbaI RFLPs of the ERα gene in a group of patients with itai-itai disease and compared them with those of control non-itai-itai patients and healthy individuals.

**Subjects and methods**

**Sample collection**

We performed DNA analysis using autopsy specimens (liver or muscle) from 54 patients with itai-itai disease (1 male and 53 females) who lived in the cadmium-polluted area of Toyama prefecture. Two sets of control samples were examined; autopsy specimens (liver or muscle) from 37 non-itai-itai patients who lived in the unpolluted area of Toyama prefecture (control non-itai-itai patients; 16 males and 21 females) and blood samples from 99 healthy volunteers who lived in Hyogo prefecture (healthy subjects; 36 males and 63 females). Toyama and Hyogo prefectures are on Honshu island, Japan. The autopsy specimens had been stored at −20°C before DNA extraction. DNA was extracted from blood samples within 4 h of the sample being collected.

**DNA analysis**

DNA was extracted from the autopsy specimens using a QIAamp tissue kit (Qiagen GmbH, Hilden, Germany), and from blood using a SepaGene Kit (Sanko Junyaku Co., Ltd. Tokyo, Japan) according to the manufacturers’ instructions. PvuII and XbaI RFLPs of the ERα gene were detected by the method of Kobayashi et al. (1996). Polymerase chain reaction (PCR) was carried out using a PCR thermal cycler (TP2000; Takara Shuzo, Kyoto, Japan) in a reaction volume of 30 μl containing 300 ng of genomic DNA in ×1 Expand HF buffer (Roche Diagnostics GmbH, Roche Molecular Biochemicals, Mannheim, Germany), 1.5 mM MgCl2, 250 μM dNTPs, each of two primers at 0.5 μM, and 0.7 Units of Expand high fidelity PCR system enzyme mix (Roche Diagnostics). The sequences of the primers were 5'-CTGCCACCACTATCTGATTTTCATCC-3' and 5'-TCTTTCTGTCACCCCATGCGCCTGATATCTGAA-3'. The PCR temperature conditions were initial denaturation at 94°C for 7 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 2 min and extension at 72°C for 3 min. The final extension was at 72°C for 7 min. After amplification, the PCR products were digested with restriction endonucleases (PvuII or XbaI; Takara Biomedicals). The digested fragments were separated by electrophoresis in 2% agarose gel and visualized by ethidium bromide staining.

**Results and discussion**

The RFLPs are represented here as Pp (PvuII) and Xx (XbaI); capital and small letters signify the absence and the presence of restriction sites, respectively (Table 1). The genotypic distributions in the group of itai-itai patients (*n* = 54) were: PP, 14.8%; Pp, 55.6%; pp, 29.6%; XX, 7.4%; Xx, 29.6%; and xx, 63.0%. Similar results were obtained from two control groups (non-itai-itai patients in Toyama prefecture and healthy individuals in Hyogo prefecture). No significant differences were shown between the genotypic distributions of the itai-itai patient group and those of control groups.

Since the itai-itai patient group were almost all females whereas the two control groups consisted of a larger proportion of males, one could argue that the group comparisons were possibly affected by this factor. To address the possibility, we evaluated the genotypic distributions in females in each group (Table 2). No significant differences were detected between the genotypic distributions of the female itai-itai patients and those of females in the control groups. In addition, there were no significant differences between the genotypic distributions of the patient group and those of Japanese postmenopausal women in Nagano prefecture on Honshu island in Japan (Kobayashi et al. 1996). Hence, we...