Objective: Osteoarthritis (OA) which is a disease characterized by the destruction of joint cartilage is one of the most common joint diseases that affect human beings. Smad3 is the mediator molecule of transforming growth factor-beta (TGF-β) signal pathway and it generates inhibitory effects in the maturation process of chondrocytes. In our study, the relationship between primary knee osteoarthritis and nucleotide changes in Smad3 gene was investigated. Material and Methods: Smad3 gene was scanned in the patients with primary knee osteoarthritis (n=92) and in controls (n=87) with single strand conformation polymorphism analysis of polymerase chain reaction (PCR-SSCP) in terms of mutations. DNA sequence analysis was performed for the patients with distinct band pattern in SSCP. G→C transversion in the 59th position of intron 2 was scanned with PCR/RFLP method. Results: Three distinct single nucleotide polymorphisms (SNP) were detected including G→C transversion in the 59th position of intron 2, silent A→G transition on the codon 103 and A→G transition on the second position of codon 170. While statistically significant differences were found between OA patients and control group (P= 0.030) and severe OA patients and control group (P= 0.037) when the G/C allele frequencies in the 59th position of intron 2 were evaluated, no difference was found in terms of genotype frequencies. The difference of C allele frequency in radiologically severe OA patients suggested that C allele could be related to the severity of the disease. Conclusion: We detected a region on Smad3 gene associated with OA for the first time in our study and developed Bme 13901 enzyme cut method for a rapid investigation of the frequencies of this region; we believe that our study will enlighten the studies about osteoarthritis.