Defense Health Service Review Committee, Athens, Greece. A control group of 12 Greek army recruits (mean age 24 years), without known history of LDD or complaints of lower back pain, was also studied. All subjects signed an informed consent form permitting the blood sampling. All patients and all controls belonged to the same draft group. An additional group of 54 gender- (males) and age-matched control subjects who, however, were not army recruits but selected from the general population, were genotyped to increase statistical strength to a total of 66 controls. Blood was collected in standard EDTA-coated tubes.

**DNA studies**

Genomic DNA was extracted from the blood samples by standard methods. To detect the A1 and A2 alleles, we used a polymerase chain reaction-based method; 4 positive samples were sequenced for confirmation of the sequence change. Primers used to amplify the 4bp insertion polymorphism were: 5’-CCTTTCTGCTCCTTTCTCCA-3’ (forward primer) and 5’-GCAACACAGTTACACAAGG-3’ (reverse primer). The reverse primer was end-labelled with γ-³²P using polynucleotide kinase (New England Biolabs, Beverly, MA). A fragment of 430bp (allele A1) and/or 434bp (allele A2) was obtained using a 6% acrylamide gel (Promega, Madison, WI) according to a previously described protocol.⁸,¹⁰

**Statistics**

Results are expressed as percentages and χ² analysis was instituted (with Fisher’s correction, where appropriate): the distribution of the A1 and A2 alleles (A1A1, A1A2, and A2A2) was compared in the two groups (patients with LDD and controls). The data were also tested against the Hardy-Weinberg equilibrium by χ² analysis. P-Values<0.05 were considered significant.

**RESULTS**

PCR analysis with end-labeling of the primer produced easily readable results (Figure 1). Sequencing confirmed the genotyping (data not shown).

Fourteen patients (58.3%) were homozygous for A2A2 versus 35 controls (53%), while 3 patients (12.5%) were A1A1, and 8 of the control subjects (12%) had this genotype (Table 1). There were no statistically significant differences in the presence of the two alleles of this polymorphism between patients with LDD and control subjects and there was no deviation from the Hardy-Weinberg equilibrium for both groups (patients and controls).

**DISCUSSION**

We recently reported an association of the Sp1 site COL1A1 polymorphism with LDD⁸ in the patients described in this report. The present study, although negative, strengthens our previous observations: the association of LDD with the functional Sp1 polymorphism of the COL1A1 gene is most likely specific and not in linkage disequilibrium with another locus, at least not as regards the 3’ UTR site of the gene that was investigated in the present study.

Our previous report was, to our knowledge, the first of a positive association of a collagen I polymorphism with LDD. We demonstrated that patients with known LDD were much more likely to be homozygous for the T/T Sp1 polymorphism than asymptomatic controls. Collagen I contributes a significant portion of the annulus fibrosus.⁴,⁵ The effect of the Sp1 polymorphism on collagen I appears to relate to an abnormal ratio of the collagen alpha-1 chain production compared with alpha-2 chain production; this may account for structural alterations, which in turn lead to a suboptimal annulus fibrosus quality, as is the case in bone. LDD may be a disease of more than the intervertebral disks alone. Although the literature