INTRODUCTION
Osteoarthritis is the most common joint disorder in all ethnic groups with a strong genetic basis, although the genes involved are largely unknown. The identification of genetic markers associated with the severity of joint diseases could be used for an early diagnosis and for monitoring of the course of the disease. Short DNA sequences that differ between normal individuals without having a detectable effect on the organism may be used as genetic markers. These ‘Restriction Fragment Length Polymorphisms’ (RFLPs) reflect sequence variations that result in fragments of different sizes: a single base-pair difference may eliminate, or introduce, a restriction-enzyme cutting site. We evaluated the RFLP frequency distribution of two candidate genes, namely the PvuII for the alpha-1-chain of type II collagen (COL2A1) and the BsmI for the vitamin D receptor (VDR), in patients with severe hip osteoarthritis, all candidates to total joint replacement.

MATERIALS AND METHODS
A consecutive series of 143 caucasian individuals undergoing total joint replacement for severe osteoarthritis of the hip was enrolled in this study. We collected a written informed consent; a detailed clinical history; and information on height and weight, menopausal status, and familiarity of the disease. As a control group, 50 donors were selected featuring the same gender distribution (males, 30%; females, 70%), a more than 50 years of age (range 50–70), and the absence of pain necessitating anti-inflammatory drugs. A small amount of peripheral blood was collected from each patient, and the genomic DNA was isolated by a spin column procedure. The COL2A1/PvuII polymorphic site was amplified by the method of Vikkula et al. (1). The VDR/BsmI polymorphic site was amplified by the McClure method (2). For both amplification products, a ‘capital letter’ indicates the absence of the restriction site on each of the alleles, and a ‘lower-case letter’ denotes its presence. The letter ‘p’ was used for the PvuII restriction site, and ‘b’ for the BsmI one. The frequency of both genotypes was calculated, and the chi-square test was used to test differences due to clinical variables. The relative risk and the 95% confidence limits were calculated to analyze the probabilities for each couple of alleles to be associated with clinical characteristics. The effects of different genotypes on the measurable parameters were performed by the analysis of variance.

RESULTS
In the healthy donors the frequency distribution of the genotypes was similar to that seen in larger studies (3). We found no differences due to clinical features, including sex, age, height and weight, menopausal status, and a family history of osteoarthritis. Significant results were found with regard to the underlying disease, i.e. idiopathic osteoarthritis versus osteoarthritis secondary to developmental hip dysplasia (DDH): a significantly higher frequency of ‘PP’ and decrease in ‘pp’ (P=0.02), as well as a higher frequency of ‘bb’ (P=0.05), was observed in patients with DDH. The relationship allele/disease was analyzed by separately comparing individuals carrying one or two alleles with the group carrying out no tested alleles. In our series, the probability to have at least one P allele was 3-fold greater in patients with osteoarthritis secondary to DDH (95%CI: 1.120–8.730), while the probability to have on p allele was significantly lower (OR 0.439 and 95%CI 0.205–0.938). The link between the two polymorphisms and the association of all the possible haplotypes were considered. In the Pp-BB subgroup, no patients with DDH were found (P= 0.005 and OR 0.088). The wide range observed in the 95%CI (0.005 – 1.531) are probably explained by the limited number of subjects in each group.

DISCUSSION
Both COL2A1 and VDR have been considered as candidate genes to be related to osteoarthritis for the following reasons: a) type II collagen is the most abundant structural protein of cartilage, and mutations of alpha-1-chain have been related to the pathogenesis of rare familial variant of osteoarthritis (3); b) the VDR genotype has been associated with bone mineral density, a high bone density being generally present in patients with degenerative joint disease (4), and c) the close proximity of these two genes, which are separated by less than 740 kb on the 12q chromosome (5).

Our findings may help defining the natural history of DDH, a well-known polygenic disease. The severity of DDH ranges from the simple unstable neonatal hip due to a slight capsular laxity, through moderate lateral displacement of the femoral head, up to complete dislocation of the femoral head from the acetabulum. Due to the wide range of joint abnormalities, a definition of DDH may therefore include different clinical conditions. Although ultrasound represents an effective method for an early diagnosis, the cost-effectiveness of universal screening is heavily debated, because of the potential overtreatment of infants with non-pathologic hip joint immaturity. The risk of complications, such as treatment-related osteonecrosis, and the possible occurrence of the disease after ultrasound examination. Detection of reliable genetic markers may contribute to improve of the effectiveness of current methods of screening. Since this series of patients was limited to candidates to total hip replacement, we could not establish if their genotypes were directly related to DDH or if they represented a subset with particularly poor outcome among the broader group of patients with DDH.

REFERENCES