INTRODUCTION
Bone regeneration is a complex process that involves several interacting biological mechanisms and is a critical component of many aspects of musculoskeletal care including fracture healing, spinal fusion, and osteo-integration of implants. It is estimated that of the approximately 7.9 million fractures that occur in the United States each year, nearly 10% are complicated by impaired healing. The cost for treating delayed or non-unions can be as high as $7500 per patient and lost productivity can cost as much as $17,000 per patient. Therefore, identifying patients at risk for delayed fracture healing and aggressively treating them on initial presentation will have an important health and economic impact on the nation.

We propose that an individual patient has a fracture healing potential based, in part, on their genome. This potential is then phenotypically expressed at the time of injury. Knowledge of this genetic potential at the time of initiation of fracture care will allow optimization of treatment with modalities such as bone grafting, BMP’s, parathyroid hormone (rhPTH1-34), or the immediate use of physical modalities such as ultrasound or electric field therapy. Identification of the genetics associated with impaired fracture healing may also provide further insight into the molecular genetics of bone healing and may yield additional gene-based treatments for these patients.

Our hypothesis is that patients who experience defective fracture healing have a unique genomic profile of one or more single nucleotide polymorphisms (SNPs) at a series of bone-related genes. These SNP’s may affect fracture healing directly, or may interact with other host factors to result in delayed fracture union. There is only one published study which has investigated a genetic predisposition to the development of a nonunion. [1] Dimitriou et al. examined a total of 15 SNPs within 4 genes (BMP-2, BMP-7, Noggin, and SMAD6) in 109 patients (62 nonunion and 47 healers). They found two SNPs (rs1372857 SNP, located on NOGGIN and rs2053423 SNP, located on SMAD6) which had a potential association with the development of an atrophic nonunion. In their study, the authors looked at a small number of SNPs and genes. Additionally, they did not take into account Hardy-Weinberg disequilibrium.

METHODS
A total of 144 SNPs within 30 genes were examined, in 78 selected adult patients with long bone fractures. There were 39 patients with atrophic non-unions and 39 patients with eventful fracture union. An atrophic nonunion was defined as a patient requiring surgical fracture stabilization with bone grafting to obtain union. SNP genotyping was performed with the Illumina GoldenGate genotyping bead array technology. Overall SNP frequencies were computed with respect to patient’s age, gender, smoking habits, and diabetes, and tested for their association to the development of a fracture nonunion, using binary logistic regression [3].

RESULTS
A total of 144 SNPs were genotyped from 30 different genes. SNP genotyping quality control involved retaining only the genotypes with a GenCall score larger than 0.25 and retaining the SNPs having a GenTrain score larger than 0.25. GenTrain scores measure the reliability of SNP detection based on the distribution of genotypic classes [2]. Three SNPs (rs3758853, rs1143641, rs2075554) did not segregate in the population and were therefore excluded from the analysis. Finally, SNPs were tested for Hardy-Weinberg disequilibrium and are noted if the Hardy-Weinberg p-value is smaller than the Bonferonni corrected level of 0.05/144 = 0.00035. A total of 8 SNPs were noted to have rejected Hardy-Weinberg.

Table 1: SNPs and associated genes using the additive genotyping model. Odds ratios of developing a nonunion if the genome contains a particular SNP are included

A total of 8 SNPs genotypes were found to have a p-value of less than 0.05 (Table 1). Of these 8 SNPs, 3 (rs1998190, rs2301914, and rs6070034) were located in the BMP-7 gene, 2 (rs2297514 and rs2248814) were located in the iNOS gene, and rs1793937, rs1672195, and rs13199729 were located in the Col2A1, ANG1 and BMP-6 genes respectively. There was no association with age, gender, smoking, or diabetes in the development of an atrophic nonunion.

DISCUSSION
In this pilot study, we sought to determine a genetic predisposition to developing an atrophic nonunion. Our results indicate that there is a potential genetic impairment in the BMP-7 and iNOS genes that could predispose a patient to the development of an atrophic nonunion. Further studies with larger patient samples, and targeted genotype testing of BMP-7 and the NitrA oxide pathways need to be performed to confirm that a genetic impairment in the aforementioned pathways can lead to an atrophic nonunion. However, these results illustrate the potential of genomic profiling in identifying a predisposition to dysfunctional fracture healing.

SIGNIFICANCE
Single nucleotide polymorphisms (SNP’s) in selected osteogenic genes may predict impaired fracture healing. Knowledge of these SNP’s may minimize fracture morbidity by altering the initial fracture treatment and yield further insight into the molecular genetics of fracture healing.

REFERENCES