INTRODUCTION:
Degeneration of the intervertebral disc (IVD) has been identified as a leading cause of both neck pain and low back pain (LBP), which are major causes of morbidity in developed societies. Changes to the structure, composition and function of the central nucleus pulposus (NP) matrix has been linked to the underlying aetiology of IVD degeneration. As matrix homeostasis is primarily a cellular-controlled process within the NP, determining the phenotypic profile of such cells is required in order that the underlying pathological process of disc degeneration is understood, and so that effective regenerative therapies against the disease may be developed.

Currently, much of the research undertaken regarding the IVD has been performed using samples obtained from the lumbar spine, whilst relatively little is known about the molecular biology of NP cells from the cervical spine region. It is well documented however, that differences in structure exist between discs of the two regions, and that they are exposed to distinct loading patterns, and thus it is thought that cells in these discs will also be phenotypically distinct. Recently, a range of phenotypic markers for cells of the NP have been identified, but their expression levels in lumbar and cervical NP cells has yet to be assessed, and was thus the focus of this study.

METHODS:
Human NP samples were obtained with informed consent during discectomy in accordance with university and ethical committee policies and HTA legislation. A cohort of 27 lumbar (aged 25-75 years) and 27 cervical samples (aged 33-72 years) was used for this study. Samples were histologically graded for their level of degeneration according to a previously published method1. RNA was extracted, DNase treated and reverse transcribed to cDNA. Following this, QPCR analysis was performed for a range of markers, including classic chondrogenic markers (SOX9, COL2A1, ACAN, VCAN), novel NP markers (FOXF1, PAX-1, KRT8, KRT18, KRT19, CAXII) and novel NP negative markers (IBSP, FBLN1). Moderately degenerate samples were analysed, whilst data was assessed according to the Δct method and all expression was normalised to that of the housekeeping genes EIF2B1 and MRP19.

RESULTS:
No significant differences in the expression of SOX9, COL2A1 and ACAN between lumbar and cervical samples were noted (Figure 1). Interestingly, VCAN expression was significantly upregulated in lumbar NP specimens. Expression of novel NP markers (Figure 2) FOXF1, PAX-1 and KRT19 did not differ between lumbar and cervical samples, although KRT8 and KRT18 were significantly upregulated in cervical NP cells. Additionally, significantly increased CAXII expression was demonstrated in lumbar spine samples. Many of the cervical and lumbar specimens did not express the NP negative marker FBLN1, although when expressed was significantly increased in cervical NP cells. IBSP was significantly more abundant in the cervical NP, but was notably lower than that of other genes analysed.

DISCUSSION:
The findings presented here corroborate recent studies investigating novel NP phenotypic markers in that NP positive markers were highly expressed, and downregulation in NP negative markers was observed. Upregulated expression of CAXII in lumbar specimens may be indicative of the increased impact of alterations in disc nutrition associated with degeneration and therefore a more harsh in vivo environment, as CAXII is associated with pH buffering.

SIGNIFICANCE:
This study indicates that whilst not all genes differ in expression according to location within the spine, some markers are altered, raising the question of whether the degenerative process as extensively described in the lumbar spine, is in fact different in the cervical region.

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