INTRODUCTION:
Tissue engineering and cell-based regenerative therapy is increasingly considered for treatment of intervertebral disc (IVD) diseases. Nevertheless the optimal cell phenotype suitable to regenerate IVD and in particular nucleus pulposus (NP) tissue is not still well defined. Mesenchymal stem cells (MSC) from different sources have been shown to induce regenerative effects and to be able to differentiate towards IVD-like cells. However, due to the phenotypical similarities between NP cells and articular chondrocytes (AC), identical markers have generally been used to monitor chondrogenic and IVD-like differentiation. Recently efforts to define the phenotype of IVD cells and to identify molecular markers that may distinguish IVD (NP and annulus fibrosus (AF)) cells from AC have been undertaken [1-3]. While results from these animal studies are of interest for investigations on animal models, there is increasing evidence that data from animal studies cannot be translated to human IVD cells [3,4]. In this study we used microarray and quantitative RT-PCR to compare gene expression profiles of human IVD cells and AC for the purpose of identifying markers that may define disc cells and separate them from chondrocytes found in articular cartilage.

METHODS:
The study was conducted in accordance to the ethical committee guidelines of the University Medical Center Utrecht, University of Basel, and University of Munich. Patients with no known history of IVD disease were included. IVD tissue was harvested from segments between L1 and L5 and was separated into NP and AF tissue. Articular cartilage was harvested from the patellae of the same patients. Cells were enzymatically isolated and total RNA was extracted. RNA from NP, AF, and AC of 5 patients (age range 2y-46y, average 27y) was sent to SCIENION AG (Berlin, Germany) for microarray analysis using Code Link Human Whole Genome Bioarray (Amersham GE Healthcare). This study focused on molecules with high NP/AC signal intensity ratio as the differentiation between NP cells and AC is of particular interest. Therefore, 27 genes with a NP/AC ratio of >10 in at least 4 out of 5 human donors and with functions potentially relevant to the NP were selected for quantification of expression levels in NP, AF, and AC cells of 7 additional patients (age range 25y-81y, average 52.6y; average Thompson disc degeneration grade 2.5 [5]). Real time RT-PCR was carried out using gene expression assays (Applied Biosystems™). Gene expression levels were normalized to the average of 4 housekeeping genes (18S rRNA, gaddph, hprt1, gusb) and expressed as log(2) transformed data. ANOVA with Tukey HSD post hoc test was performed using SPSS, and p<0.05 considered significant. Pearson correlation was determined to identify potential associations between gene expression pattern and age or degree of disc degeneration (DD).

RESULTS:
Microarray comparison of NP versus AC samples revealed the following genes elevated in NP that were further analyzed by RT-PCR: agt, sgcg, lpl, orml1, lendl1, csrp2, foxf1, foxf2, uch11, clic3, cldn11, sfn, scgb1d2, spon2, tppp3, jph3, tmem27, cdh19, bcan, lrc2, syn1c, cpvl, slamf9, krt6c, ca12, rgs1, slca2a. Nineteen of these genes, specifically sgcg, lpl, orml1, lendl1, csrp2, foxf1, foxf2, uch11, clic3, cldn11, sfn, scgb1d2, spon2, tppp3, jph3, tmem27, cdh19, bcan, lrc2, ca12, and rgs1 were found to be expressed significantly more highly in NP cells compared to AC as assessed by real time RT-PCR. Sgcg, cdh19, ca12, and rgs1 were also enhanced in NP compared to AF cells. Fig. 1 shows the expression pattern in NP, AF, and AC samples of the 10 genes with an average mRNA level at least 10-fold higher in NP compared to AC. None of the genes showed more than 100 times enhanced mRNA levels in the NP. In the NP cells, the expression of orml1 was positively correlated with DD. On the other hand cldn11 and ca12 were negatively correlated with age and DD, while tmem27 was negatively correlated with age. In the AF cells, a positive correlation of orml1 and negative correlations of spon2 and tppp3 with age and DD were noted. The most significant association was a decrease in ca12 expression with age (p<0.003, correlation -0.92) and DD (p<0.001, correlation -0.98). As expected a strong association of age and DD was observed (p=0.006, correlation 0.90).

DISCUSSION:
The aim of this study was to identify molecular markers that may characterize the human NP cell phenotype and are not detected or expressed at negligible levels in AC. While no clear on/off marker was identified, ten genes were found with at least 10-fold higher mRNA levels in NP cells as compared to AC. The finding that none of the genes analyzed by RT-PCR showed more than 100 times enhanced mRNA levels in the NP corroborates the similarities between disc and cartilage cell phenotypes. Although these genes with elevated expression may be considered to distinguish between NP and AC, some of them, e.g. lendl1, show a relatively low expression even in the NP and are therefore less suitable than genes with a comparably high expression in the NP, such as foxf1. Interestingly, none of the ten genes showing largest NP versus AC expression differences were found to be associated with age or degree of disc degeneration. This indicates that these genes are stably expressed, which is an advantage for a marker gene. Correlations with age and/or DD were found for certain other genes. Although for this study discs with no history of disease were taken, aging is generally associated with some degree of degeneration. Hence, the molecules found to decrease with age may be associated with young healthy discs, whereas the markers that increase with age/DD may indicate a degenerative disc. Taken together, our data present a set of potential new human NP marker genes. To more comprehensively characterize the normal NP and AC cell phenotype, further young and healthy IVD samples need to be analyzed and the protein expression pattern investigated. Finally, further studies will evaluate the suitability of the identified NP marker genes to monitor MSC differentiation towards NP cells.

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REFERENCES: