OA candidate genes including 50 kb of flanking sequence to either side were obtained from Ensembl. Autosomal SNPs with minor allele frequency (MAF) in Europeans >5% and mapping to these sequences were extracted from HapMap. Fixed effect meta-analysis across 8 OA GWAS was performed for these SNPs. Significance threshold was set after Bonferroni correction for the effective number of independent test determined with the simpleM method.

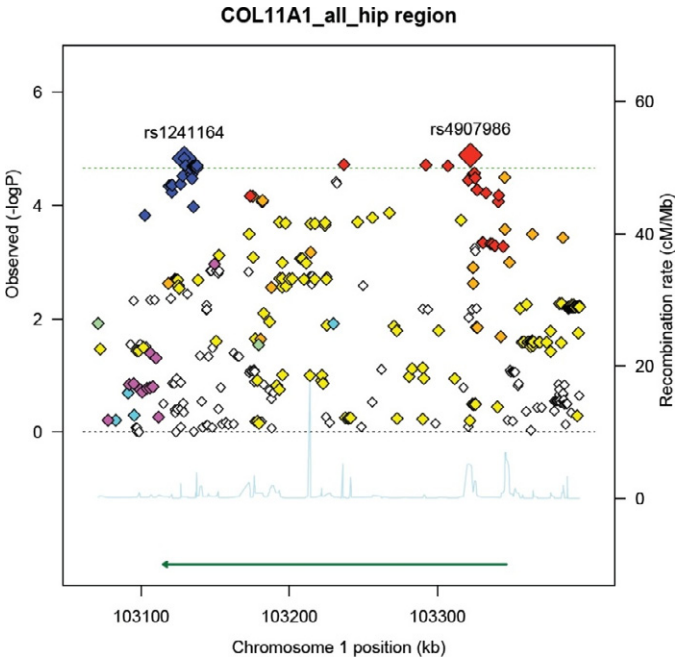

Fig. 1.

Results: A total of 186 OA candidate genes including 24298 SNPs were analyzed in GWAS results from 5630 subjects with knee OA, 5180 with hip OA and 31272 controls. This allowed 0.8 power to detect association of a SNP with O.R. = 1.14 and 0.2 MAF at the $P < 2.2 \times 10^{-5}$ significance threshold calculated for this study. Nine SNPs in the 7q22 OA locus were significantly associated with knee OA. The most highly associated SNP in this locus, rs4730250, has already shown GWAS level significant association in a previous study. There were also seven *COL11A1* SNPs associated with hip OA. They represent, at least, two independent association signals suggesting the presence of multiple causal polymorphisms in this gene (labelled in blue and in red in Figure 1).

Gender stratified analyses showed association of 17 SNPs in *GDF5* (top SNP *rs224329*, $P = 1.2 \times 10^{-5}$) with knee OA in females and of *rs833058* in *VEGF* with hip OA in males ($P = 1.3 \times 10^{-5}$).

Conclusions: We have found that only a small fraction of OA candidate genes were confirmed in our meta-analysis, which is the largest done to date. This is a common finding in complex diseases. Two of the loci, *7q22* and *GDF5*, were already widely recognized as truthfully involved in OA and our analysis did not identify new signals in them. The other two loci were not renowned in genetics. However, they are particularly interesting because of the functional implications of *VEGF*, which is well known for promoting angiogenesis, and for the possibility of multiple causal polymorphisms in *COL11A1*, which codes for one of the chains of type XI collagen that is involved in maintaining cartilage integrity and cohesion.

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IDENTIFICATION OF INTERLEUKIN-16 AS A NOVEL EPIGENETICALLY-REGULATED FACTOR IN CHONDROGENIC DIFFERENTIATION OF ADULT STEM CELLS

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Purpose: As mesenchymal stem cells (MSCs) differentiate they undergo epigenetic changes. We hypothesized that elucidation of changes such as alterations in DNA methylation would lead to increased understanding of differentiation along specific lineages. DNA methylation is an epigenetic process whereby methyl groups are added to cytosine residues (CpG) typically resulting in reduced gene expression. The aim of this project was to investigate global changes in gene promoter methylation patterns as MSCs differentiated down various lineages and thereby identify differentially methylated genes associated with specific differentiation pathways.

Methods: Genomic DNA was extracted from undifferentiated, chondrogenic, osteogenic and adipogenic MSCs at 14 days and analysed for changes in promoter methylation patterns using a high throughput epigenomic discovery platform with custom CpG island arrays (Infinium Human Methylation 27BeadChip Assay). 28K CpG probes were used to analyze gene promoter regions of 14K genes yielding beta values ranging from 0–1 depending on level of promoter methylation (0=unmethylated, 1=methylated). Stringent selection criteria were applied to the data generated to select genes for further study. RNA was extracted from undifferentiated and differentiated MSCs at 0, 2, 4, 7 and 14 days and quantitative RT-PCR performed to validate array results and investigate temporal gene expression levels of selected targets. Protein levels were determined by Western Blotting or ELISA.

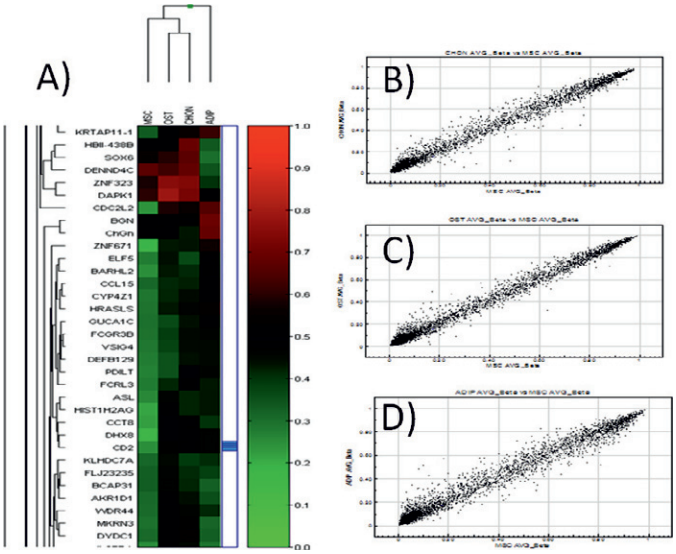

Fig. 1. (A) Heat map of representative genes illustrating relative hypomethylation of undifferentiated MSC promoter regions in comparison to differentiated MSCs. Scatter plots showing clustering of undifferentiated MSC methylation patterns to those of (B) chondrocytes, (C) osteocytes and (D) adipocytes.

Results: Analysis of the methylation patterns indicated that promoters of undifferentiated MSCs were generally hypomethylated in agreement with the comparatively unrestricted gene expression of these cells (Figure 1A). Chondro- and osteo-differentiated MSC clustered closer than to undifferentiated MSC or to adipogenic progeny, with the latter two groups showing the least similarity (Figure 1B-D). Lineage-specific, differentially methylated genes were selected to create differentiation-specific methylation signatures for validation by qRT-PCR and bisulphite sequencing. This analysis showed that DNA methylation contributed to the rapid down regulation of superfluous genes on commitment to a specific lineage whereas hypomethylation of specific CpGs was associated with upregulation of genes associated with the differentiation process. Based the stringent selection criteria, 15 genes (9 chondro-, 4 adipo- and 2 osteo-specific) were selected for array validation and further study. Of the chondrogenic genes chosen, 8 were hypomethylated following differentiation and one hypermethylated. Five of the genes showed changes in gene expression corresponding to their methylation signature. Of the four validated hypomethylated genes, MIA/CD-RAP and SCRG1 have been previously associated with chondrogenesis whereas Interleukin 16 (IL16) was identified as a novel hypomethylated factor in chondrogenesis of MSCs. Expression of this cytokine mimicked that of CD-RAP increasing expression over the first 7 days of chondrogenesis and thereafter decreasing; gene expression was rapidly downregulated on differentiation to the adipo- and osteo- lineages. IL16 protein was

detected in chondrogenic pellets and secreted by the differentiating MSCs. Finally, high levels of IL16 were detected in osteoarthritic cartilage.

Conclusions: We hypothesise that differentiation of MSCs initially involves hypermethylation to facilitate the rapid down regulatation of genes involved in self-renewal or alternative differentiation pathways. Epigenetic mechanisms also represent an important aspect of control to ensure the sequential and temporal expression of critical genes throughout the differentiation process. IL16, previously associated with synovial fibroblasts from rheumatoid joints was identified as a novel factor in chondrogenesis.

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NEUROMUSCULAR EXERCISE IMPROVES FUNCTIONAL PERFORMANCE IN PATIENTS WITH SEVERE HIP OSTEOARTHRITIS

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Purpose: Exercise is regarded a cornerstone in the treatment of mild to moderate osteoarthritis (OA). However, little is known of the effects in patients with advanced and end-stage OA. The purpose was to evaluate the effect of neuromuscular exercise in patients with severe hip OA.

Methods: *Design*. Randomized controlled trial (Clinicaltrials.gov identifier: NCT01003756). 84 patients, 51% female, mean age 68.6±7.8 years, BMI 28.7±4.7 scheduled for total hip replacement at Svendborg Community Hospital, Odense University Hospital, Denmark were included. *Intervention*. Participants were randomized to an eight-week neuromuscular exercise (NEMEX-TJR) intervention or care-as-usual (verbal and written preoperative information). Intervention was supervised and offered twice a week with each session lasting one hour. The program is considered feasible and safe in this patient group and previously described in detail. Assessments were carried out at baseline and within one week after the intervention. *Outcomes*. Functional performance: 20-m walk at maximal pace and 5 repeated chair stands timed. Muscle power: Unilateral multi-joint leg extension power and unilateral single-joint knee extension power evaluated with a leg extension press (Nottingham Power Rig, Nottingham University, Nottingham, UK) and a seated knee extension machine (Oemmebi, Moglia, Italy) adapted with a linear encoder (MuscleLab Power, Ergotest Technology, Langesund, Norway), respectively.

Results: On average the intervention group attended 13±4 sessions (Table 1). In favor of the intervention group, the between-group difference was significant for 20-m walk (2.2 seconds, p = 0.009), chair stands (1.7 seconds, p = 0.022) and leg extension for the non operated leg (.17 W/kg, p = 0.049) (Table 2).

Table 1. Baseline characteristics of study participants

	Exercise intervention	Care-as-usual
Scheduled for operation – no.	43	41
Female sex – no. (%)	22 (51)	21 (51)
Age – yr	68.7 [66.1;71.3]	68.6 [66.3;70.8]
BMI – kg m^{-2}	28.5 [27.3;29.7]	28.8 [27.1;30.5]
Exercise sessions – no.	13 [12.2;14.7]	–

Table 2. Baseline values and change over time (mean ± SD)

Outcome measure	Baseline		Change			
	Exercise intervention	Care-as-usual	Exercise intervention	Care-as-usual	Between-group mean difference	p-value
Muscle power (W kg^{-1})						
Multi-joint leg extension						
Non operated	1.28 [1.11;1.45]	1.33 [1.12;1.54]	0.22 [0.09;0.35]	0.05 [−.05;0.16]	0.17	0.049
Operated	1.02 [0.85;1.20]	1.09 [0.9;1.3]	0.07 [−0.07;0.20]	−0.05 [−0.18;0.08]	0.12	0.200
Single-joint knee extension						
Non operated	0.82 [0.67;0.96]	0.81 [0.65;0.97]	0.10 [0.05;0.16]	−0.01 [−0.14;0.11]	0.12	0.091
Operated	0.59 [0.47;0.72	0.58 [0.42;0.74	0.08 [0.01;0.14]	0.10 [−0.07;0.27]	0.03	0.761
Functional performance (s)						
Chair stands	13.1 [11.5;14.7]	13.4 [11.9;14.9]	−2.7 [−3.9;-1.4]	−0.5 [−1.6;0.7]	2.2	0.009
20-m max pace	15.2 [13.6;16.8]	15.6 [14.1;17.1]	−1.0 [−1.8;-.1]	0.7 [−0.5;1.9]	1.7	0.022

Conclusion: Eight weeks neuromuscular exercise according to the NEMEX-TJR program improves functional performance and leg extension power in patients with severe OA of the hip joint.

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TIME TO TOTAL HIP REPLACEMENT SURGERY AFTER SUPERVISED EXERCISE AND PATIENT EDUCATION IN PATIENTS WITH HIP OSTEOARTHRITIS. A RANDOMIZED INTERVENTION STUDY WITH BETWEEN 3.5 AND 6 YEARS FOLLOW UP

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Purpose: The purpose of the study was to evaluate time to total hip replacement (THR) surgery in patients with hip osteoarthritis going through both a supervised exercise program and patient education (SE+PE) compared to patients going through patient education only (PE).

Methods: One hundred and nine patients were included in the study between April 2005 and October 2007. Inclusion criteria were age 40–80 years, hip pain for three months or more, radiographically verified hip osteoarthritis (Danielson's criteria), and Harris Hip Score between 60–95 points, i.e. their impairments were not severe enough for considering THR at time of inclusion. All patients initially had three sessions of patient education. After completing the education program baseline assessments were conducted, and the patients were then randomized to 1) a 12 week supervised exercise program (SE+PE, n = 55), or 2) no further treatment (PE, n = 54). Both groups were recommended to follow the information giving during the patient education. The SE+PE group performed exercises 2–3 times weekly supervised by a physical therapist. The exercises consisted of strength training, functional exercises, and flexibility exercises.

Between April 12th and May 3rd 2011, 3.5 to 6 years after inclusion, all patients were contacted by telephone and information on whether and when THR surgery had been performed were collected. Survival analysis (Kaplan-Maier) were used to assess time to THR surgery in both groups. Group differences were tested by the Log Rank test.

Results: Twelve patients (11%) did not respond at latest follow-up (April/May 2011). Six of these patients had previously informed us that they had gone through THR surgery and at what time, and the remaining six patients were censored.

In total, 53 (48.6%) patients went through THR surgery within the follow-up time, 22 (40.0%) in the SE+PE group and 31 (57.4%) in the PE group. Median time to THR surgery was 1953 days (95% confidence interval (CI): 1634, 2272) and 1260 days (95% CI: 850, 1670) in the PE group. Cumulative survival without THR surgery after 6 years was 41.4% in the SE+PE group and 25.4% in the SE group (Figure 1, p = 0.034).

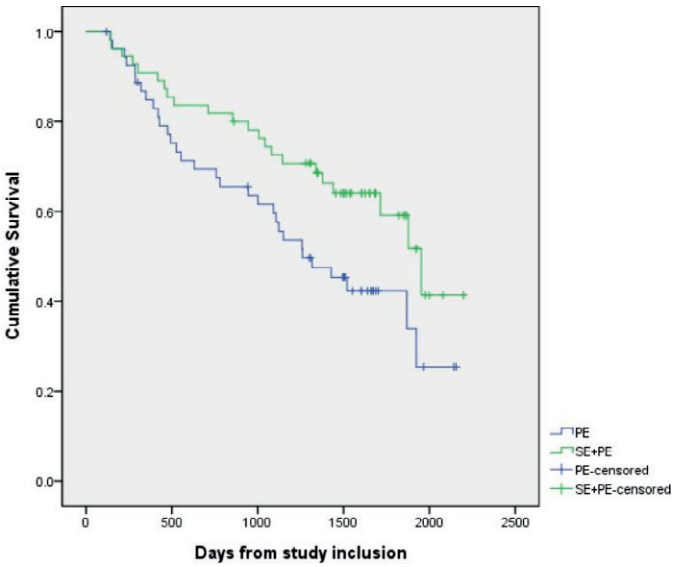

Fig. 1. Kaplan–Meier plot. Cumulative survival (without THR surgery) in the SE+PE group (green line) and the PE group (blue line). Censored data is marked at each line.