management of CPSP as difficult, and there was a tension between their desire to help patients and the concern that keeping patients 'in the system' of secondary care might not always be helpful. Identifying ways to improve services, participants described specialist services, specifically multidisciplinary pain clinics and pain management programmes, as having a vital and specific role in helping patients with CPSP.
Conclusion: Despite healthcare professionals' commitment to helping patients with CPSP, coherent referral pathways and services to manage CPSP were described as lacking, resulting in an unsatisfactory approach. By corollary, better services would provide a joined up pathway that would assist patients and healthcare professionals involved in their care. This small-scale study complements ongoing work that highlights the diversity of services for CPSP after knee replacement across the UK, in which some examples of clear pathways are apparent. Future research is needed to assess the impact of clear referral pathways and integrated service provision for patients with CPSP after total knee replacement.

402
QUADRICEPS MUSCLE STRENGTH AND ITS RELATIONSHIP TO RADIOGRAPHIC KNEE OSTEOARTHRITIS

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Purpose: Knee osteoarthritis (OA) is a multifactorial disease and strongly affected by mechanical factors.
The aim of the present study was to evaluate the relationship between quadriceps strength measured by the Quadriceps Training Machine (QTM) (QTM-05F, Alcare Co., Ltd. Tokyo, Japan) and radiographic knee OA by epidemiological survey.
Methods: The relationship between radiographic knee OA and quadriceps strength was investigated with 1,112 knees in 556 subjects by epidemiological survey. They were inhabitants in the Matsudai district in Niigata prefecture and they all participated in an extensive survey of knee OA in 2013 (Matsudai knee Osteoarthritis Survey). Bilateral quadriceps strength of each subject was measured by the QTM. Measured value was divided by body weight and we evaluated this data as muscle strength per body weight ratio (M/P ratio). Furthermore, a weight-bearing standing knee radiograph was obtained and graded according to the Kellgren–Lawrence classification. Radiographic knee OA was defined if a Kellgren–Lawrence grade of II or higher was detected. From these results, the change in quadriceps muscle strength level by gender and by age, comparison of the quadriceps muscle strength level between the non-OA group and OA group, and the change in quadriceps muscle strength level by gender and by knee OA grades, were investigated.
Results: In the Matsudai Knee Osteoarthritis Survey, the prevalence of radiographic OA (grade II or higher upon Kellgren-Lawrence classification) was: 10.9, 39.6, 64.6, and 84.1%, regarding women in their fifties, sixties, seventies, and eighties, and was: 9.5, 13.8, 37.3, and 53.8% regarding men, respectively. Quadriceps muscle strength declined following 60 years of age in women, and significant decline was observed in their seventies and eighties. Quadriceps muscle strength of the OA group (grades II, III and IV) was significantly declined compared with that of the Non-OA group (grade 0 and I). Furthermore, the tendency of the muscle strength level to decline with the progression of knee OA grade was particularly observed between grade I and grade II in both men and women.
Conclusions: The relationship between radiographic knee OA and quadriceps strength was quantitatively evaluated by an epidemiological survey (Matsudai knee Osteoarthritis Survey in 2013), and we found a correlation between knee OA and the decline in quadriceps strength. Furthermore, it was suggested that the decline in quadriceps muscle strength may be more strongly related to the incidence of the knee OA than to its progression. We have conducted the extensive survey of knee OA (Matsudai knee Osteoarthritis Survey) since 1979, and quadriceps muscle strength was measured by the QTM every three years since 2010. We believe that longitudinal analyzing the same cohorts and investigating whether or not a decrease in quadriceps muscle strength is the cause of incidence or progression of knee OA in addition to conducting the same investigation regarding symptomatic knee OA are necessary.

Genetics and Genomics and Epigenetics

403
A GAIN OF FUNCTION MUTATION IN *TNFRSF11B* CAUSES OSTEOARTHRITIS WITH CHONDROCALCINOSIS

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Purpose: Osteoarthritis (OA) is the most common arthritic disease and a leading cause of disability among elderly. Developing new therapies to slow down or cure OA requires deeper insight into underlying biological mechanisms driving OA. We hypothesized that next generation sequencing using OA patients with a familial history of OA can contribute to the identification of causal pathogenic mutations leading to more general pathways in which other genetic variations may affect susceptibility to common OA.
Methods: Exome sequencing was applied to two distant family members with dominantly inherited early onset primary OA at multiple joint sites characterized by chondrocalcinosis. By applying an eligible prioritization scheme five candidates were selected for *de novo* genotyping and linkage analysis across the extended family. The effect of the mutation was investigated by means of a cell-based assay and to investigate generalizability of the genes that act in the identified pathway, expression analyses was performed in preserved and osteoarthritic cartilage of 33 independent patients.
Results: Exome sequencing of the 2 individuals with familial early onset OA (FOA) identified 57,018 and 60,652 variants, respectively. The heterozygous, read-through mutation (c.1205A=>T;p.Stop402Leu) in *TNFRSF11B* encoding osteoprotegerin (OPG) showed complete co-segregation with the OA phenotype (LOD-score 3.48) and is, therefore, likely causal to the disease in this family. The mutation was absent in 1,467 independent OA affected subjects and 744 random controls indicating that it may be private for this family. OPG is a soluble decoy receptor which inhibits osteoclastogenesis by competing with the receptor activator of the nuclear factor-KB (RANK, encoded by *TNFRSF11A*), expressed on the membrane of pre-osteoclasts, for the binding of the nuclear factor-KB ligand (RANKL, encoded by *TNFSF11*). The mutant OPG protein showed enhanced capacity to inhibit osteoclastogenesis and concurrent bone resorption (P-value <0.05), indicating a gain of function mutation. Expression analyses in preserved and osteoarthritic cartilage indicated that up-regulation of the *TNFRSF11B/TNFSF11* ratio (1.1-fold, $P = 2.0 \times 10^{-4}$) is a general phenomenon in the pathophysiological OA process.
Conclusions: We are the first to demonstrate that enhanced OPG function towards matrix mineralization could be directly causal to the onset of OA. Even more, the gene expression pattern indicated that enhanced OPG-mediated antagonism could be a more general phenomenon in the pathophysiological process of OA. These findings and extensive literature showing high systemic bone mass as a consistent OA risk factor are in contrast to recent studies reporting on beneficial effects of anti-osteoporotic drugs in OA patients. We strongly recommend investigation of long term effects of these bone forming therapies in OA and advocate that agents counteracting the function of OPG could comply with development of new disease modifying treatments in OA.

404
DISCOVERY AND ANALYSIS OF RARE CODING VARIANTS FOR HIPOA BY EXOME-SEQUENCING

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Purpose: It is well established that Osteoarthritis (OA) is to a large extend genetically determined. Over the last years several GWAS studies have identified multiple genetic loci associated with OA. However, only a small proportion of the heritability is explained by these loci. It is anticipated that a part of the unexplained variance must be due to rare variants, possibly unique to the disease under study. These rare variants are missed in a common GWAS study, where only common variants are examined. We here present the first exome-sequencing

results done for OA. We focus our analysis on the exome, since variants in the protein-coding part of the genome are more likely to have a functional consequence.

Methods: The Rotterdam study is a large prospective study population of over 15.000 individuals ages 45 years and older all living in the Ommoord district of Rotterdam in the Netherlands. The first participants were recruited in 1990, and 4 follow-up visits have been done so far, while continuous monitoring through general practitioners and pharmacies is also in place. All of the participants were deeply phenotyped for osteoarthritis by radiographs and questionnaires. For 1,524 participants, whole exome-sequencing has been performed at a mean coverage of 40x per base. This allows to find novel rare variants, not identified previously. We aim to identify functional variants associated with hipOA and its endophenotype, minimal joint space width (mJSW) a proxy for cartilage thickness in the hip joint. Our approach included a candidate gene approach for all genes previously found associated with hipOA. In addition, we conducted exome-wide association analysis on all variants found (199 cases, Kellgren-Lawrence grading $\geq$2; 1337 controls) and mJSW (minimal cartilage thickness in mm) as the phenotypes of interest.

Results: We analyzed in total 199 hipOA cases and 1337 controls, while we had in total 1242 individuals available with data for mJSW. To investigate if other coding variants associated to hipOA and mJSW might be located in the previously identified hipOA genes, we analyzed a group of 13 candidate genes in our exome sequencing data (see table 1). This list includes also 3 genes we identified to be associated to mJSW with a GWAS meta-analysis (FGFR3, RUNX2, and TGFα). We identified 761 variants in these genes, from which the majority was found only once (576 singletons). Of the remaining variants, 70 were non-synonymous, 86 synonymous and 52 intronic; these variants (excluding the singletons) were analyzed further. We next performed gene-based rare-variant tests on these hipOA genes, by comparing the burden, i.e. amount of rare variants, in OA cases compared to controls. In 3 candidate genes a nominal significant hit was found (FGFR3, TGFα and NCOA3), after Bonferroni correction only FGFR3($p = 8.68E-05$) remained highly significant. The single-variant analysis yielded 3 signals with a strong association to the mJSW or hipOA in the exome sequencing data set ($p < 10E-2$, see table 2A). The strongest signal for mJSW was a rare variant in the FGF3 gene, the same gene also identified in the gene specific burden test. This rare variant was not associated to the GWAS signal identified before by us ($r^2 < 0.1$). FGFR3 has previously been shown to be involved in endochondral bone formation. In addition, mutations in FGFR3 result in achondroplasia.

Conclusions: We identified rare variants in the FGF3 gene to be associated with extreme values of mJSW. Currently we are working on replication of these findings. Future analysis will consist of further functional assessment of the identified variants.

Table 1
Variants found in OA candidate genes in the Rotterdam Study – 1 cohort

Candidate OA gene	Variants	Singletons	Synonymous	Nonsynonymous	Intronic
ASTN2	77	49	11	12	5
DIO2	18	13	4	1	0
DOT1L	117	89	7	8	13
FGFR3	72	47	11	4	10
FILIP1	41	27	6	7	1
GLT8D1	16	14	1	0	1
GNL3	25	18	22	3	2
MCF2L	126	86	16	14	10
NCOA3	122	107	4	8	3
PIK3R1	2	1	0	1	0
PTHLH	13	12	0	0	1
RUNX2	14	12	1	3	1
SENP6	118	101	3	9	5

Table 2
Variants associated to mJSW and hipOA in the Rotterdam Study – I cohort

A. Single-variant analysis

										Discovery	
Variant	Chr	Position	Reference allele	Variant allele	Gene	Type	Phenotype	Frequency	SE	P	N
rs3U5898†‡	***4***	***1807922***	**G**	**A**	**FGFR3**	**Intronic**	**mJSW**	**0.0099**	**0.1896**	**4.92E-06**	**1006**
rs138836456	*9*	*119568036*	G	A	ASTN2	Synonymous	**mJSW**	0.0027	0.3435	5.01E-03	1097
rs114754024†‡	*4*	*1806044*	C	T	FGFR3	Intronic	**hipOA***	0.0083	0.5006	7.90E-03	1082

*Kellgren-Lawrence Grading $\geq$2
†Some intronic/intergenic regions are present in exome-sequencing data
‡Variants not in linkage disequilibrium $r^2 < 0.1$

B. Candidate gene Burden test

Gene	Chr	Position	Variants	Phenotype	P
FGFR3	***4***	***1801219 – 1807922***	**17**	**mJSW/HipOA***	**8.68E-05**
TFGα	*2*	70677994 – *70677994*	1	mJSW	0.00786678
NCOA3	*20*	*46256424 - 46280031*	9	HipOA	0.0163782

*Kellgren-Lawrence Grading $\geq$2

405

EXPRESSION PROFILING OF SUBCHONDRAL BONE IN OSTEOARTHRITIS KNEE JOINT TISSUE

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Purpose: Osteoarthritis (OA) is a disease of the whole joint organ involving pathological changes of articular cartilage (AC), structural changes of underlying subchondral bone (SB) and synovitis. Many studies utilizing expression array chip technology have been performed on AC, however, very few has been performed on the SB. We have