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countries, range from $3,200–$18,000 per OA patient (excluding surgeries). In addition, costs associated with side effects of currently available drugs (e.g. NSAID-related serious GI event) were also found to be significant. **Conclusions:** Based on the findings of this literature review, the burden of OA pain is significant. There appears to be a great unmet need for new innovative treatments that can significantly reduce pain without the sides effects associated with currently available treatment options and/or drugs which may delay progression of disease.

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**DEEP SEQUENCING OF GDF5 IN OVER 1900 OSTEOARTHRITIS CASES AND CONTROLS REVEALS NOVEL AND POTENTIALLY FUNCTIONAL RARE VARIANTS IN THE PROTEIN CODING AND PROMOTER REGIONS OF THE GENE**

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**Purpose:** We have assessed whether rare DNA variants in a gene already known to be associated with knee and hip osteoarthritis (OA) contribute to OA susceptibility. The selected gene is GDF5 (growth and differentiation factor 5), which harbours a single nucleotide polymorphism, rs143383, in its 5’ untranslated region that shows very robust association to OA in European and Asian populations. The risk allele of this SNP mediates a small decrease in GDF5 expression. These results indicate that polymorphism in this gene of small singular impact are important for OA development and suggest that rarer but more disruptive variants may have greater effects on gene function and therefore OA susceptibility.

**Methods:** Three groups of patients and controls were studied from the UK, Spain and Greece. There were 992 patients (502 British, 264 Spanish and 226 Greek) that had each undergone a total knee or a total hip joint replacement due to severe primary OA and 944 controls (460 British, 294 Spanish and 226 Greek). In the Spanish and Greek cohorts the search for rare variants focussed on the two exons of GDF5 and was performed by Sanger sequencing after PCR amplification. The Polypyhred software was then used to align sequences and to identify DNA changes. The UK search encompassed the two exons as well as both untranslated regions and 100bp of the proximal promoter of the gene. Sanger sequencing after PCR amplification was also performed, with the SeqScape software used to identify DNA changes.

**Results:** In the UK cohort we discovered five novel rare variants in GDF5, four in control individuals and one in a case. One of the variants, identified in a control, is located 40 base pairs upstream of the transcriptional start site and is predicted to interfere with a highly conserved SOX9 transcription factor binding site. Three of the remaining variants were found in exon 2, all in control individuals, one of which is predicted to cause a non-conservative substitution of a highly conserved threonine to an arginine. The final variant was a synonymous substitution in exon 1 detected in a female patient with knee OA. Only a synonymous substitution was discovered in the Spanish or Greek samples, in a control individual.

**Conclusions:** Our deep-sequencing analysis of over 1900 OA cases and controls from northern and southern Europe has identified several novel rare variants in the OA-associated gene GDF5. The effects of these variants upon GDF5 expression and protein structure will now be investigated using luciferase assays, in-vitro splicing studies and protein modeling to discover whether these alleles are potentially protective, harmful or neutral to OA susceptibility.

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**A MULTICENTER STUDY OF THE ASSOCIATION OF AROMATASE AND ESTROGEN RECEPTOR GENES WITH HIP AND KNEE OSTEOARTHRITIS**

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**Purpose:** Several lines of evidence suggest that estrogens influence the development of osteoarthritis (OA). The aim of this study was to explore the genetic and functional association of two common polymorphisms from within the aromatase (CYP19A1) and estrogen receptor α (ESR1) genes with severe OA of the lower limbs.

**Methods:** The rs1062033 (CYP19A1) and rs2234693 (ESR1) single nucleotide polymorphisms (SNPs) were genotyped in 5,479 individuals (3,098 patients with hip or knee OA ascertained by the need for joint replacement surgery due to severe primary OA, and 2,381 controls) from three centres in Spain and one centre in the UK. Expression of CYP19A1 and ESR1 was measured in femoral bone RNA samples from a group of patients by real-time quantitative PCR and was subsequently stratified by donor genotype at rs1062033 and rs2234693.

**Results:** In the global analysis, both polymorphisms were associated with OA, but there was a significant sex interaction. The G allele at rs1062033 (a C/G transversion SNP) was associated with an increased risk of knee OA in women (OR 1.23; p=0.04). The CC genotype at rs2234693 (a C/T transition SNP) tended to be associated with reduced OA risk in women (OR 0.76, p=0.028, for knee OA; OR=0.84, p=0.076 for hip OA), but with increased risk of hip OA in men (OR 1.28; p=0.029). Women carrying two copies of the rs1062033 G-allele and no copies of the rs2234693 C-allele were at particular risk for knee OA, with an OR of 1.61 (p=0.006).

The rs1062033 GG genotype associated with increased OA risk was also associated with reduced expression of CYP19A1 in bone (p=0.036).

**Conclusions:** Common genetic variations of the aromatase and estrogen receptor α genes CYP19A1 and ESR1 are associated with the risk of severe OA of the large joints of the lower limb in a sex-specific manner. These results are consistent with the hypothesis that estrogen activity influences the development of large-joint OA. The CYP19A1 SNP rs1062033, or a SNP in linkage disequilibrium with it, may mediate its effect on OA susceptibility by regulating the expression of CYP19A1.

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**META-ANALYSIS OF GENOME-WIDE ASSOCIATION DATA IMPLICATES THE 4P15.3 REGION TO INFLUENCE CHRONIC WIDESPREAD PAIN IN WOMEN**

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**Purpose:** Chronic Widespread Pain (CWP) is prevalent in approximately 10% of the general population and may result from inadequately treated chronic focal pain problems such as osteoarthritis (OA) and influences human well-being. The prognosis of CWP patients appears to be poor and it is associated with increased consumption of health care and working disability. CWP is regarded to be a complex trait, meaning that both environmental and genetic factors play a role in the etiology. Twins studies have estimated a heritability of 48-54% for CWP. So far candidate gene studies have not been able to discover genes involved in the pathogenesis of CWP, possibly due to small sample sizes. Meta-analysis of Genome Wide Association Studies (GWAS) from several population based cohort studies is a hypothesis-free and more robust state-of-the-art approach to identify genetic risk factors. The objective of this study was to identify genes involved in CWP by means of a large-scale GWAS meta-analysis.

**Methods:** CWP is defined as pain present in at least both sides of the body, pain above and below the waist, and axial skeletal pain (the FMS criteria of the American College of Rheumatology (ACR)). We conducted