Functional analysis of the osteoarthritis susceptibility mapping to the gene *NCOA3*.

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Introduction

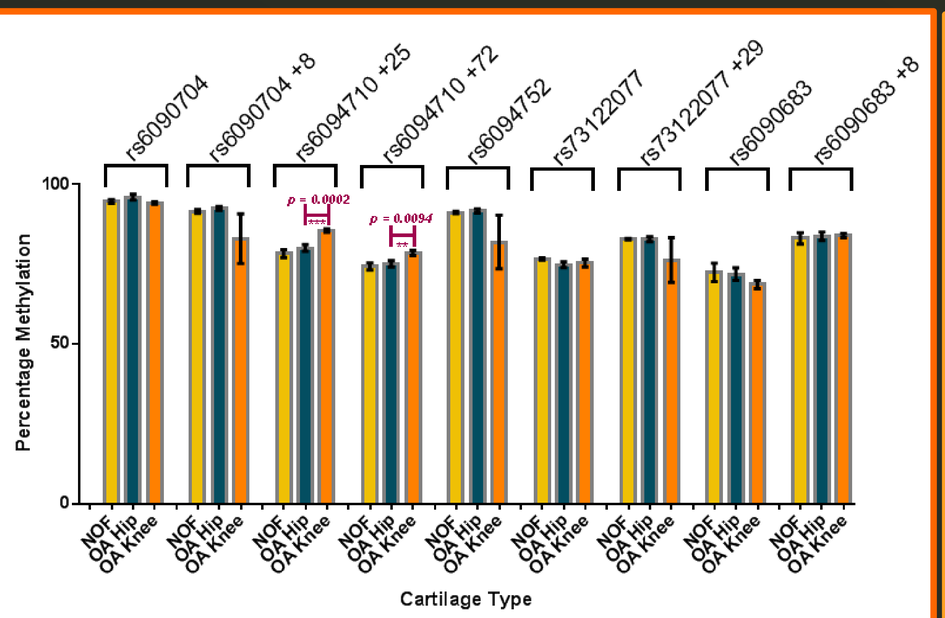
Osteoarthritis is a debilitating disease affecting cartilage tissue which is increasing in prevalence as lifespan and obesity levels rise. *NCOA3* is an osteoarthritis susceptibility gene which has lower expression in osteoarthritic cartilage than healthy cartilage. A number of mutations, (SNPs), enhance it's expression. However, short sequences of DNA known as CpG sites can go through further modification in vivo known as methylation, and this may alter the SNPs effects on *NCOA3* expression. If this is the case, it may be the methylation of a particular SNP causing decreased expression in osteoarthritic tissue.

Objectives and Methods

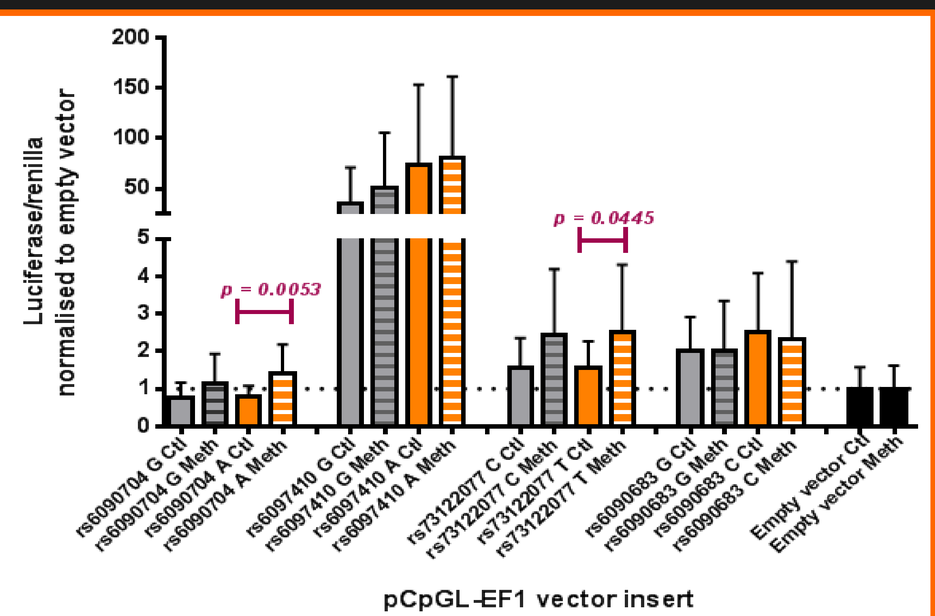
1. Discover the percentage methylation of each of the CpG sites of interest in healthy hip cartilage as well as osteoarthritic hip and knee cartilage. This was done by bisulphite converting DNA, amplifying it using PCR and then analysing it on a CpG pyrosequencer programme to give a percentage methylation reading. It was important to analyse different cartilage types as methylation levels can differ between different tissues.

2. For each site with at least ten percent methylation, ascertain whether the methylation alters the SNPs' effect on *NCOA3* expression by inserting each major and minor variation of each SNP into a pCpGL-EF1 plasmid vector and then transfecting these into SW1353 chondrosarcoma cells. Luciferase assays were then carried out and the results normalised against renilla and empty vector readings.

Results



1. Every CpG site analysed is methylated by at least 60% in each cartilage type. The purple bars show that the osteoarthritic knee samples are more methylated than the osteoarthritic hip cartilage for two of the CpG sites with statistical significance.



2. The dotted horizontal line represents the baseline expression level of *NCOA3*. It appears that methylation of each SNP variant enhances their effect on *NCOA3* expression, rather than diminishing it.

Conclusions

- Previous work on this gene has only looked at non-modified DNA, but as DNA can be modified in vivo, it is important to verify that any findings in the lab are consistent with what happens in the body. My results show that these SNPs are all heavily methylated in vivo, and this methylation increases their enhancement of *NCOA3*.
- If we can understand the function of osteoarthritis susceptibility genes such as *NCOA3*, we may one day be able to develop novel treatments which target the effect of each of these genetic components, allowing effective treatment tailored to a patient's genotype.

Acknowledgements

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