Bioinformatic analysis of an osteoarthritis susceptibility gene.

Joe Crutwell* Supervisors: Louise Reynard & John Loughlin

Dept. of musculoskeletal research

j.crutwell@ncl.ac.uk

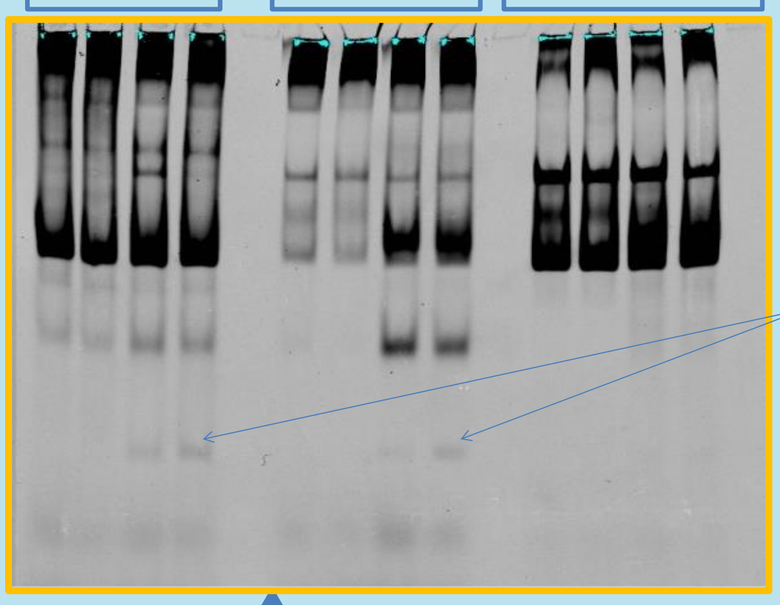
Osteoarthritis is a condition categorised as a loss of articular cartilage over time, a genome wide association study identified the gene ***CHST11*** as being associated with osteoarthritis, specifically the single nucleotide polymorphisms **rs835487** and **rs835488** (rs87/88 for short). As there was no amino acid substitution caused by these SNPs, It was decided they must affect the binding of regulatory proteins. The area which these SNP's are found is known to be an **enhancer region**.

My objective was to assay the genetic basis of this association, I did this by:

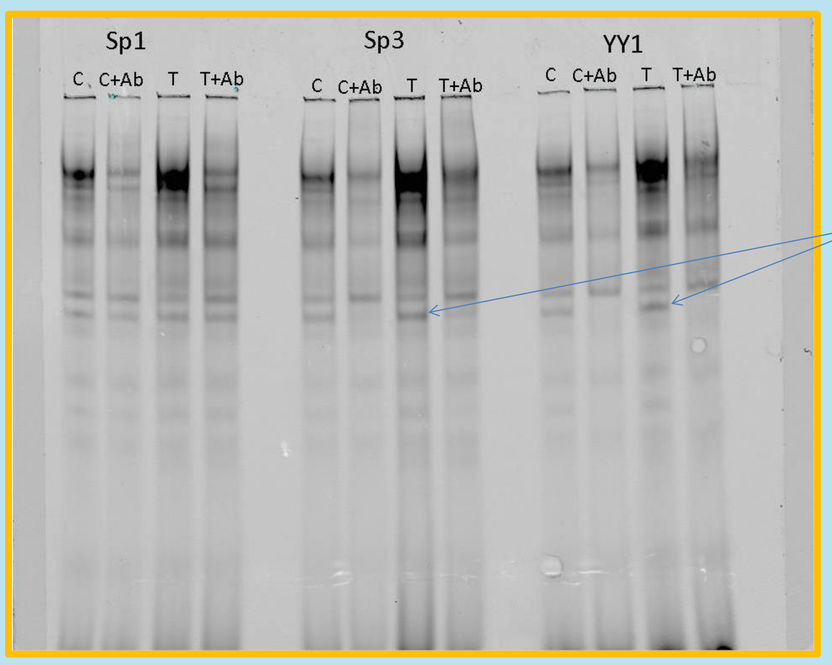
1. Assessing enhancer activity of the different SNPs through luciferase assays
2. Checking for Transcription Factors binding to the SNPs rs835487 and rs835488 using electro-mobility shift assays

rs835487/rs835488 EMSA's

Every 4 lanes are:
A allele / A allele with Pax 9 Antibody / G allele / G allele with pax9 antibody



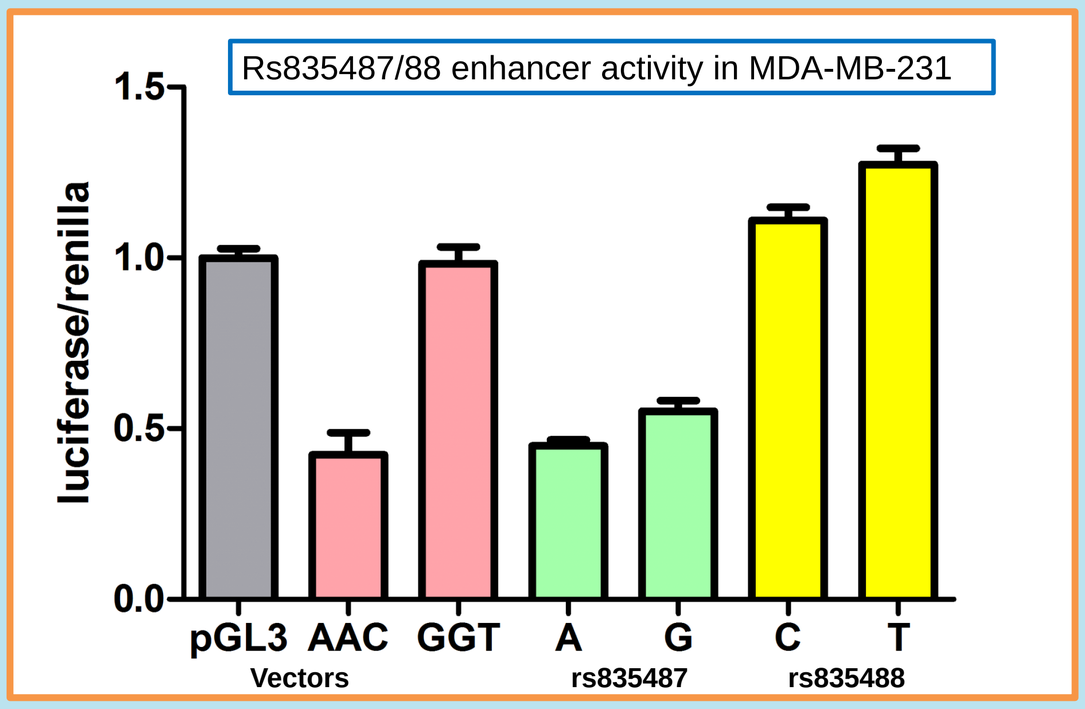
Pax9, a transcription factor with a consensus sequence in rs835487 was identified as binding to the SNP in the cell line sw1353 in an earlier EMSA, this image shows the repeated experiment in multiple cell lines. It shows that a band **specifically binding to the G allele** is apparent in sw1353 and sw872 cell lines but not MDA.

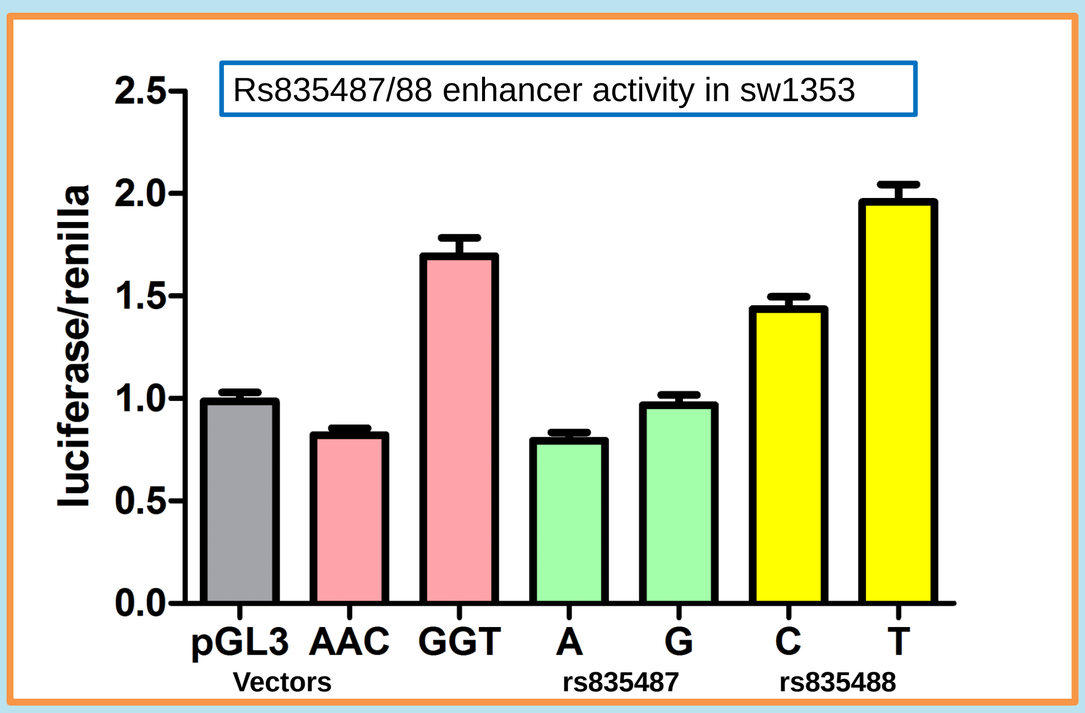


In rs835488, Sp1, Sp3 and YY1 transcription factors were found to bind. More interestingly, Sp3 and YY1 antibodies were found to shift exactly the same bands, this suggests that **YY1 and Sp3 form a complex**.

Luciferase Assays

By inserting the enhancer region with both variations of the rs87/88 SNPS into a pGL3 vector and expressing this plasmid in cells, you can test how each SNPs variant influences enhancer expression. This was done in both sw1353 and MDA-MB-231 cell lines.





These results show that that **G allele in rs835487 and T allele in rs835488 result in increased enhancer activity.** These are both the forms that are shown to be more associated with developing OA. The results also suggest that **there's a cumulative effect of the two SNPs on OA likelihood rather than a single causal SNP.**

These results lead to some interesting conclusions:

1. Pax9 Binds preferentially to the G allele in rs835487, meaning it may play a part in the differential allelic expression
2. Rs835488 is bound by a potential complex between Sp1 and YY1, further research will yield more about this interaction
3. Differences in the base at both SNP's studies results in greater enhancer activity, meaning higher and not lower enhancer activity results in the diseased state