Gene Correlation with Osteoporosis in Mice

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INTRODUCTION

Osteoporosis is a bone disease affecting 70% of white people over 80 years old. It is characterized by a loss in bone mass and thinning bone structures, and is defined as having a bone density of 2.5 standard deviations below that of a normal adult. Normally, white and Asian people are at greater risk, and women have far higher risk of osteoporosis than men.

A loss in bone mass causes many complications. It is the most common reason for broken bones among the elderly. It can cause microfractures that lead to acute or chronic pain that may interfere with daily activities. These fractures can lead to a stooped posture and loss of height. Blood clots, particularly deep vein thrombosis, are associated with osteoporosis. It also leads to falls, as bones are too weak to support the body weight, which in turn leads to more injury and further exacerbates the symptoms of the disease.

It has been shown that osteoporosis tends to run in families. This, combined with the fact that certain races are more susceptible to the disease than others, leads scientists to suspect a genetic factor behind the disease. In our research, we are tried to identify the genes that may be associated with the disease. Many genes of interest have been identified by previous research, and the goal of our research is to use scientific techniques to reexamine and confirm these results, as well as to measure the strength of the correlation. If we have a specific gene or number of genes that are specifically related to the disease, we can target these genes for future research into developing a cure or treatment.

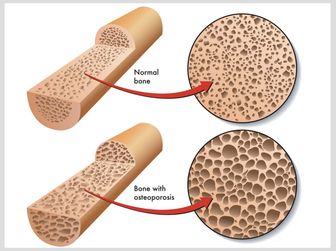


Fig. 1 Diagram showing the difference between normal and osteoporotic bones (image from www.painmanagementworks.com)

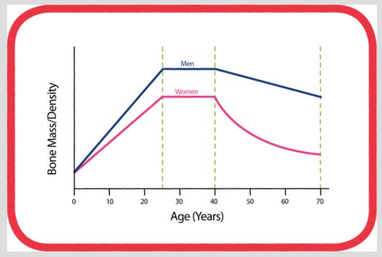


Fig. 2 Graph showing the difference in risk of osteoporosis between men and women (image from Reddit)

MATERIALS & METHODS

The first step of research was to identify all genes that are possibly associated with the disease. This step has already been done for us. Researchers at Dr. Wu's lab have identified around 20 genes that may be related with the loss of bone mass in osteoporotic lab rats, which have been shown already to have a link to bones and are expressed in blood samples taken from the bone.

The next step was to identify primer sequences for these genes. Our eventual goal was to isolate these sequences and measure their prevalence, so it is necessary to locate these genes. The idea of the primer is to provide two segments of around 20 nucleotides, which represent the beginning and end of the target gene. We first went through scientific literature to see which primers we could find from past research, and then checked the primers for validity by using the BLAST program to backwards-derive the gene. If we could not locate the primer in past research, our next step was to design primers using programs and algorithms available to us. Figure 3 shows the list of primers that we found and ordered.

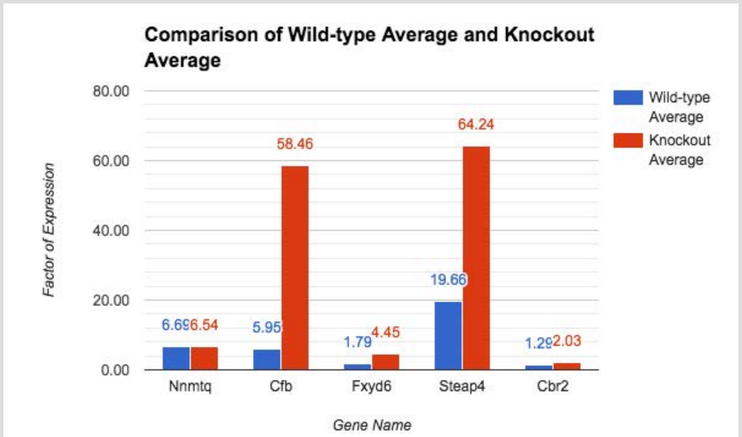
Once we had the primer sequences, we used Quantitative Real-Time PCR (qPCR) to obtain a count of the relative amounts of each gene there is in a sample from osteoporotic rats. This process first uses enzymes to locate the primer sequences, and then transcribes the target gene, effectively duplicating it. At the end, we had a measurable amount of each gene, and we could then mathematically derive how many of the gene were present in the original sample. The data for these genes can be found on the graphs.

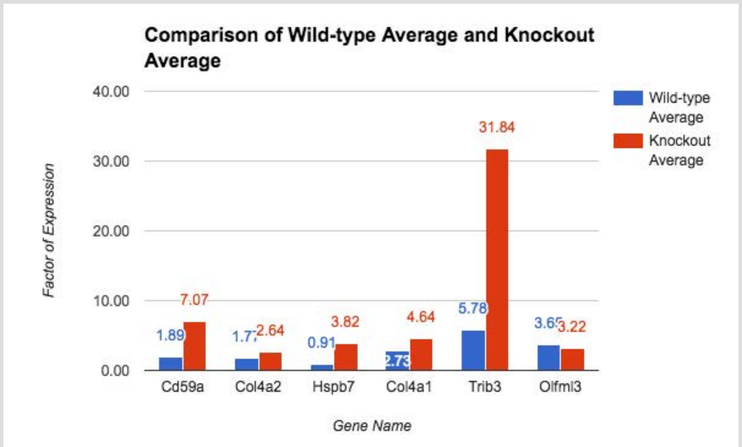
Gene Name	Forward Primer Sequence(5'→3')	Reverse Primer Sequence(3'→5')
Nnmt[2]	GCCCCACCATCTATCAGCTTC	CCCTTCATTCTGTTGCCTTCAAG
Cfb[1]	CTCGAACCTGCAGATCCAC	TCAAAGTCCTGCGGTCGT
Fxyd6[4]	AGCCCCAGAAGGCAGAGAAC	GCACAGGGAACACGCATTCT
Cbr2[6]	AGGAAGTTCGCAGAGGTTGA	GGCAACTGAGCAGACTAGGA
Asb4[5]	TCCTGCTGCACAGCCTGAGAT	TTCGGGCAAGAGTGGCAAGC
Mlh1[8]	TGCTGGCCTTAGACAGTCCT	ATACACTTCGCCTTGGATGG
Col4a2[3]	CGACCGAGTGCGGTTCAAA	ACCGCAGGGCACATCCAACT
Hspb7[11]	TGTCACCACCTTCAACAACCAC	TCATGACTGTGCCATCAGCTG
Col4a1[7]	CTGGAGAAAAGGGCCAGAT	TCCTTAACTTGTGCCTGTCCA
Trib3[10]	TCTTCCGGCAGATGGCTAGT	GGTTCTCCAGCACCAGCTTC
Olfml3[9]	CTGCTGCTCCTCTTCTTTTTG	CTACTCTGATCCTGGCATTGG

Fig. 3 A list of primers that we found in scientific literature that were designed for mouse qPCR (reference numbers in superscript)

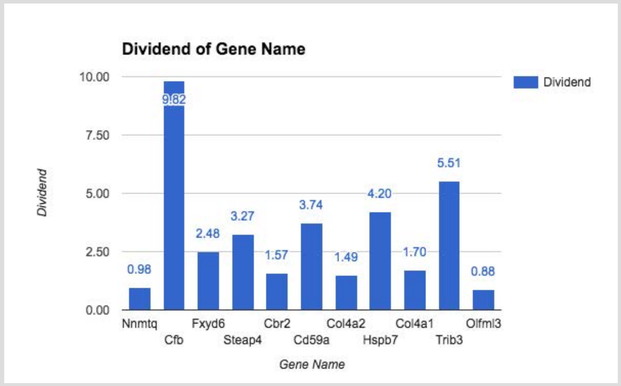
RESULTS

- Graphs of the factor of expression in wild type mice compared with mice with osteoporosis





- Graphs of the dividend of each gene (Knockout Average/Wild-type)



SUMMARY / CONCLUSIONS

Our goal was to identify genes that were significantly correlated with osteoporosis in mice. The strongest correlation we managed to find was with the Cfb gene, which on average was present 9.81 times more often in knockout mice than wild type mice. Other genes of significant interest that we had were Trib3 and Hspb7, which were present 5.51 and 4.20 times more often in knockout mice, respectively. Further research is needed to determine the cause and effect relationship between the presence of these genes and osteoporosis.

Cfb is known to be a complement gene to factor B, which itself complements the abilities of antibodies and phagocytic cells in the immune system. We think that this link between osteoporosis and the immune system is intriguing, but further research is needed to further examine this relationship. Trib3 codes for a protein that is involved in the phosphorylation chain of proteins found in signal transduction. We think that this is very interesting, and it is possible that there is a link between osteoporosis and improper signalling between cells. Finally, Hspb7 is a heat shock protein that is expressed in the heart, and has a protective function against cardiovascular disease. Again, the relationship between this gene and osteoporosis is unclear, prompting future work.

Further research is definitely necessary to confirm and further our findings. One of the limitations that we had was the large amount of human error that occurred in the experiment. We frequently had slightly different results between identical trials, and our final data was based on the average of these results. This likely reduced the statistical significance of our results, indicating that our research could be redone for more accurate results. In addition, we hope that research will be done between the exact relationship between the genes that we identified and the development of osteoporosis.

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