Optimizing a Genomic-Wide Association Study in Ligature-Induced Peri-implantitis

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Abstract: Dental implants are increasing in prevalence as desirable options in replacement of missing teeth. Unfortunately, implants come with complications, and animal models are key to studying the pathophysiology of complications, such as peri-implantitis (PI). PI is characterized by inflammation in the tissues around dental implants with progressive loss of supporting bone. VCF files from the Mouse Genomes Project were merged using bcftools. PLINK was employed to create filtered bed files. This study outlines a genome-wide association study (GWAS) for peri-implantitis in mice. Phenotype files were generated separately for control and ligature-induced peri-implantitis groups, incorporating average linear and volumetric bone loss measurements. The GWAS analysis was performed using FaST-LMM. This approach allows for identifying genetic variants associated with peri-implantitis susceptibility, potentially revealing novel insights into the disease's molecular mechanisms.

Background

Experimental Induction of Peri-Implantitis in Mice

Peri-implantitis (PI) is a significant complication in dental implantology, characterized by inflammation in peri-implant tissues and progressive loss of supporting bone. To study this condition, our team developed an experimental model of ligature-induced peri-implantitis in mice [1]. The process involves:

1. Tooth extraction in 3-week-old male and female mice.
2. Implant placement after a 4-week healing period.
3. Ligature placement around implants to induce peri-implantitis.
4. Evaluation after 2 weeks using clinical, radiographic, and histological methods.

This model successfully recreates the key features of peri-implantitis, including increased soft tissue edema, significant bone loss (linear and volumetric), and enhanced inflammatory infiltrate.

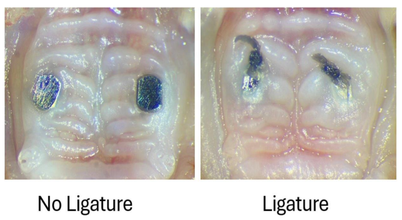

Figure 1A: Non-ligature dental implants and ligature-induced peri-implantitis .

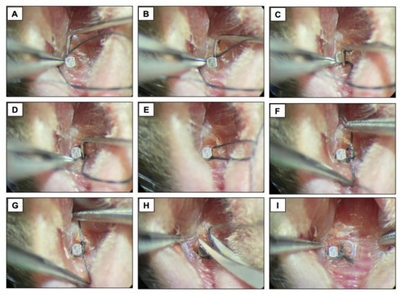

Figure 1B: Ligature-induced peri-implantitis sequence. (**A-D**) Silk ligature (6.0) placed around the implant head. (**E-G**) Knot closure. (**H**) Ligature cut. (**I**) Final appearance. Clinical images obtained from live animals under sedation.

Methodology

Peri-implantitis induction was done using mice from the Jackson Laboratory. Control and treatment mice were divided by those with and without ligature-induced PI, and our phenotype data measured bone loss between the control and treatment groups. Genotype data was extracted from sequences available on the Mouse Genome Project. We then performed quality control on SNPs and samples by filtering out insignificant data. FaSTLMM was then used to associate genotypes [2] with the phenotypes, and to visualize this, we generated Manhattan plots to visualize the association signals seen across the genome (Fig. 3). The Fig. 3 plots the –log10(p-value) on the y axis versus the chromosome position on the x axis for each tested genetic variant.

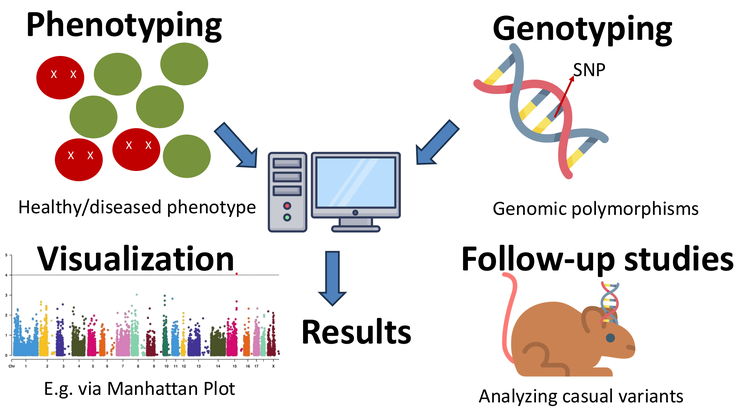

Figure 2. Diagram illustrating the essential steps of the GWAS.

Results

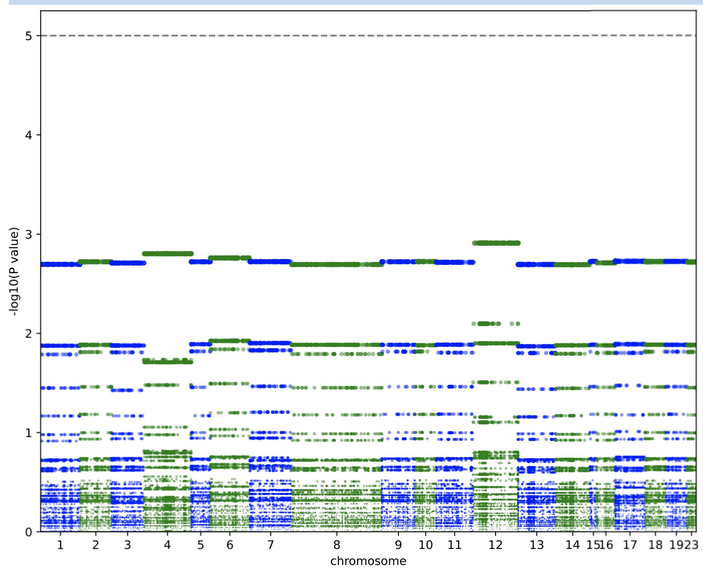

Figure 3. Manhattan plot for ligature-induced PI bone loss for major 5 mice strains (A_J, AKR_J, BALB_cJ, C3H_HeJ, CBA_J). No significant SNPs were observed in the Manhattan plot. The small sample size of 5 strains was insufficient to reach significance, as none of the SNPs surpassed the significance threshold.;

A.

	Linear Bone Loss (mm)		Volumetric Bone Loss (mm3)	
Strain Name	**Control**	**Ligature**	**Control**	**Ligature**
A/J	0.11065542	0.352935	0.1922767	0.3063457
AKR/J	0.14845188	0.4869175	0.350035	0.58632
BALB/cJ	0.11700357	0.37052438	0.23549429	0.9849625
C3H/HeJ	0.16477583	0.420125	0.06347667	0.26967333
CBA/J	0.25022375	0.413412	0.457955	0.376322
FVB/NJ	0.20893417	0.23581938	0.31407	0.520365
NOD/ShiLtJ	0.09741917	0.2879375	1.60045667	0.3126475
NZW/LacJ	0.37860357	0.2783333	0.3714571	0.2102367
Paired T-Test	0.001826387		0.990637882	

B.

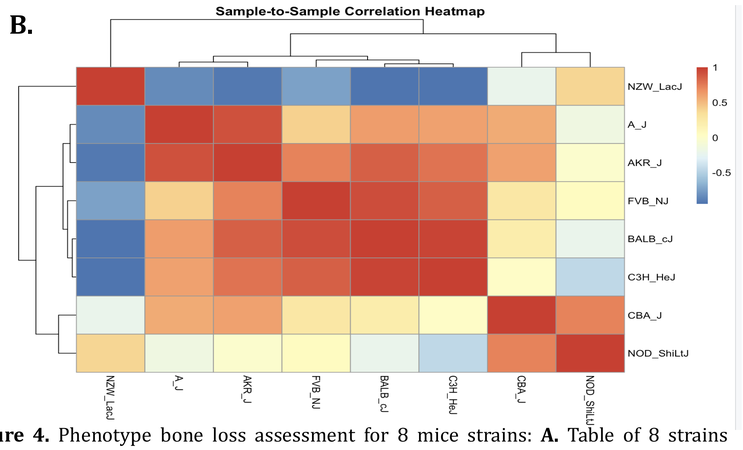

Figure 4. Phenotype bone loss assessment for 8 mice strains: **A.** Table of 8 strains showing linear and volumetric bone loss measurements; **B.** Correlation analysis of male and female phenotypes for eight mice strains.

Discussion

The sample-to-sample correlation heatmap reveals distinct clustering of male and female phenotypes, suggesting sex-specific genetic or environmental influences. The most significant bone loss experienced Balb/cJ ($p<0.05$), while the least significant bone loss was observed for NZW/LacJ ($p<0.05$), thus suggesting that in female mice we observe much higher degree of bone loss compared to male mice. The Manhattan plot, representing SNPs for 5 out of the original 38 strains, does not show any significant peaks that surpass the typical genome-wide significance threshold (usually $p < 5x10^{-8}$). This lack of significant associations could be due to the limited sample size, as we were only able to analyze a subset of the intended strains. It's important to note that the current analysis is constrained by issues in accessing the complete genotype data, preventing a full-scale GWAS on the entire dataset. Despite these limitations, this preliminary analysis provides valuable insights and a foundation for future studies. Once complete genotype data is available, a comprehensive GWAS may uncover significant genetic associations underpinning peri-implantitis not detectable in this limited analysis. This research experience has equipped us with the necessary tools and knowledge for more extensive genetic studies in the future.

Conclusion

This study represents a crucial step towards optimizing a GWAS for ligature-induced peri-implantitis in mice. While our analysis was limited by incomplete genotype data, preventing a full-scale study, the methodological framework established here is strong for future, more comprehensive analyses. This study underscores the potential of GWAS in uncovering the genetic basis of PI susceptibility and sets the stage for more in-depth investigations that may provide novel insights in PI.

References & Acknowledgements

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References:

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2. *Reference Genomes | The Mouse Genomes Project*. (2023). Mousegenomes.org. https://www.mousegenomes.org/reference-genomes/