

Investigating the Effect of NF1 Loss on Spermatogonial Stem Cells in a Mouse Model.

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Lay Summary (200 words)

Some genetic conditions, including several rare developmental disorders, are more common in the children of older fathers and are termed Paternal Age Effect (PAE) disorders. Many are caused by mutations in genes that regulate the RAS/MAPK signalling pathway, which controls how cells grow and divide. In most PAE disorders, the mutations are gain-of-function, meaning they over-activate the pathway. This gives the affected sperm-producing stem cells, spermatogonial stem cells (SSCs), a competitive advantage. These mutated SSCs multiply more than their non-mutated neighbours and gradually take over more space in the testis, a process known as clonal expansion. As a result, the chance of passing on the mutation to children increases with age. Because such mutations benefit SSCs in the father while causing disease in children, they are termed “selfish mutations.”

This project focused on the Neurofibromatosis type 1 (*Nf1*) gene, which is a negative regulator of the RAS/MAPK thus loss of NF1 is expected to cause an increase in RAS/MAPK activity.

Using a mouse model heterozygous for a loss-of-function mutation in *NF1*, I examined whether loss of one copy of *NF1* increased the density of GFR α 1-positive SSCs in the testis.



Aim:

Does heterozygous loss of *Nf1* alter the density of spermatogonial stem in mouse seminiferous tubules?

Methods:

I used a C57BL/6 mouse model carrying a heterozygous loss-of-function mutation in the *Nf1* gene. My hypothesis was that reduced NF1 function would increase RAS/MAPK activity, leading to greater maintenance or proliferation of undifferentiated spermatogonial stem cells (SSCs).

To test this, I dissected seminiferous tubules from testes of *Nf1* heterozygous and wild-type littermates (wild-type

serving as controls). SSCs were identified by immunofluorescent staining for GFR α 1, a well-established surface marker of undifferentiated SSCs. Samples were imaged using widefield and confocal microscopy, and ImageJ software was used to quantify GFR α 1-positive cell density. Consistent protocols for sample preparation, imaging, and analysis were applied across groups to ensure comparability. Additionally, the process was blinded, as in, it was unknown which tubules had the mutation during labelling, mounting, imaging, and counting.

Because all SSCs in heterozygous mice carried the same Nf1 mutation, no competitive advantage was possible. Any observed increase would therefore reflect a cell-intrinsic effect of NF1 loss on SSC behaviour rather than selfish selection. To test whether Nf1 mutations behave as classical selfish mutations, future experiments will need mosaic models where mutant and wild-type SSCs coexist in the same testis, allowing direct assessment of competitive dynamics.

Findings:

My results showed no significant difference in the density of GFR α 1-positive spermatogonial stem cells (SSCs) between testes of Nf1 heterozygous and wild-type tubules. This suggests that heterozygous loss of NF1 alone does not alter the abundance of undifferentiated SSCs.

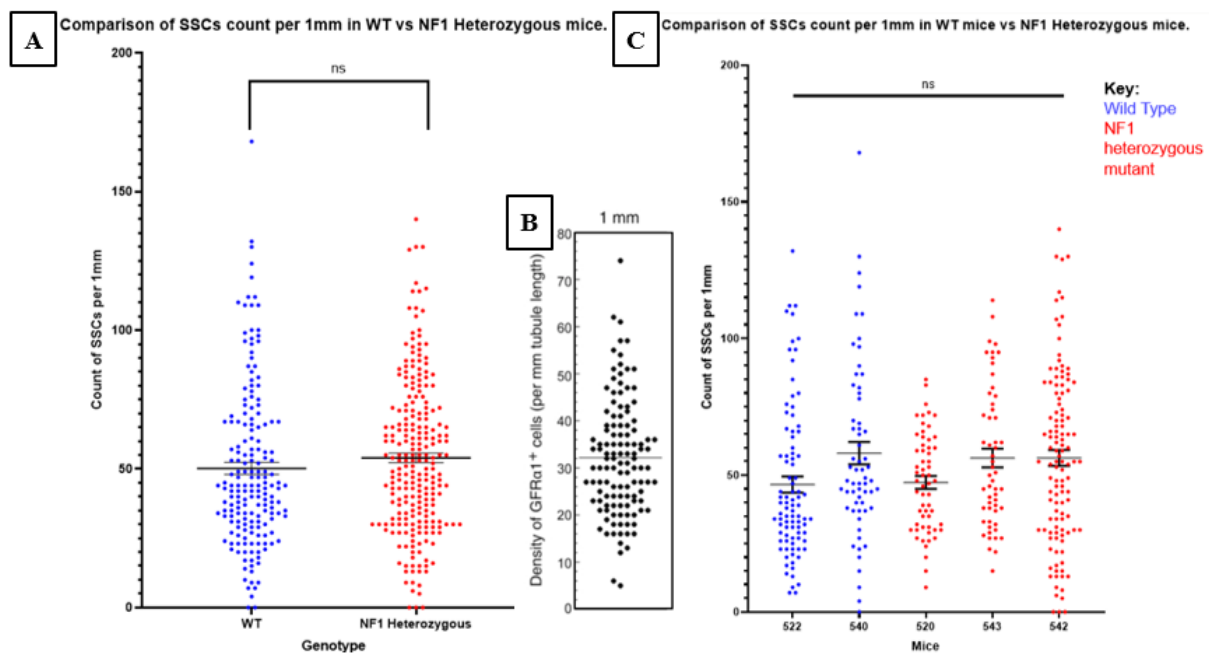


Figure 1. (A) Comparison of spermatogonial stem cell (SSC) density between wild-type (blue) and Nf1 heterozygous (red) mice. Each point represents the number of GFR α 1⁺ SSCs per 1 mm of seminiferous tubule. Horizontal lines indicate mean $\pm$ SEM. An unpaired t-test showed no significant difference (ns) between the two groups. (B) Published reference data illustrating density of GFR α 1⁺ cells per mm of seminiferous tubule, included for comparison with my results. (C) SSC density compared across individual seminiferous tubules from each mouse (genotypes as in A). One-way ANOVA again showed no significant differences between groups.

Interestingly, the wild-type controls in my dataset displayed a higher density of GFR α 1-positive SSCs compared with values reported in the literature and in previous work from my supervisor's group. This discrepancy may reflect biological variability between mouse cohorts, differences in genetic background, or subtle methodological differences in how SSCs were quantified. It is also possible that SSC numbers fluctuate depending on age or physiological state, factors which can vary even within the same strain.

These findings do not exclude a role for NF1 in SSC regulation. Subtle changes in RAS/MAPK signalling may not be captured by GFR α 1 staining alone, as this marker identifies undifferentiated SSCs but does not distinguish whether they are dividing more rapidly or persisting longer. Additional markers, such as Ki67 for proliferation, or lineage-tracing approaches, would provide further resolution of SSC dynamics.

Finally, NF1 loss might influence later stages of spermatogenesis or manifest only in a competitive environment. Future studies using mosaic models, where mutant and wild-type SSCs coexist within the same testis, would provide a more realistic context for testing whether NF1 mutations confer a competitive advantage and drive clonal selection.

What did I learn:

This studentship taught me much more than just lab techniques. I learned how to think like a scientist: to ask precise questions, design experiments to test hypotheses, and critically interpret the results. I also gained experience with troubleshooting and adapting methods when things did not go to plan, which was an essential part of the learning process. One of the highlights was completing the OxMic microscopy training programme, where I gained theoretical knowledge in imaging. This included sessions on microscope design, sample preparation, and advanced techniques such as spinning disc and super-resolution microscopy. In addition to that, I trained on Leica widefield and confocal systems and applied these directly in my research by imaging immunofluorescently stained seminiferous tubules. I also became confident using image analysis software such as ImageJ to quantify my results. Overall, the project deepened my appreciation for microscopy as a tool to investigate cellular and tissue-level phenomena, and taught me the importance of combining good experimental design with rigorous data analysis.

Long Term Goals:

This project confirmed that I want to pursue a career in clinical genetics. I have always been interested in the intersection between research and medicine, and this experience has shown me how fundamental research into cell signalling and genetics can directly inform our understanding of inherited diseases. Working in a lab that studies the origins of de novo mutations and how they arise during spermatogenesis has given me a new appreciation for the complexity of the germline. I enjoyed the intellectual challenge of working with signalling pathways like RAS/MAPK, which are involved in many developmental disorders as well as cancer. I plan to apply for a PhD in the future, ideally in a research setting where I can continue to investigate rare genetic conditions. I also hope to continue using microscopy, as I found it to be both a powerful and satisfying technique. More than anything, this project showed me that I want to be in a field where scientific discovery can be translated into better diagnostics, treatments, and outcomes for patients with genetic disease.