

- The [REDACTED] and [REDACTED] labs started collaborating on a project to generate novel Matrin 3 transgenic mice soon after mutations in Matrin 3 were described in patients with familial amyotrophic lateral sclerosis, with the potential that one specific mutation in the gene may also cause distal myopathy. No transgenic mouse models existed and the function of the mutations that are associated with Matrin 3-linked genes were and still are unknown.
- A very senior and experienced technician ([REDACTED] [REDACTED] in [REDACTED] lab, cloned and sequenced the constructs for WT Matrin 3 and F115C Matrin 3.
- [REDACTED] [REDACTED] of the UF transgenic core received those constructs from the [REDACTED] lab and produced potential founders which were first identified by PCR by the [REDACTED] lab and subsequently by the [REDACTED] lab.
- Once founders had been identified, [REDACTED] transferred the founders into the [REDACTED] / [REDACTED] colony. Founder lines labelled for WT were kept on separate racks (and sides of the room) from the lines labelled as F115C to avoid any accidental switches due to ACS or lab mix up.
- Founder lines that were labelled as expressing WT Matrin 3 were assigned a number with the prefix "MA" (e.g. MA1573) and Founder lines that were labelled as expressing F115C Matrin were assigned a number with the prefix "MF" which eventually became "F" (e.g. F####). Within the MA and F colonies, the origin of parent and founder line that the mice arose from were tracked and were documented in the Lewis lab colony records. Pedigrees of all mice in the Matrin 3 colony are available. As far as we can determine, there was no accidental interbreeding of mice from the "MA" lineages with mice from the "F" lineages.
- Because founders from both the MA and F colonies were mosaic, there was a substantial amount of breeding that was involved to establish lead lines. Additionally, since we had very little funding, our colony was also kept at very low numbers, also slowing the rate at which these lines could be fully analyzed.
- The MA colony was worked out the quickest, both by chance and a focused effort in establishing the "WT" Matrin 3 lines as a baseline to understanding the effect of wild-type human Matrin 3 in mice.
- One of the main ways that the MA colony was established was the observation that mice from line MA1579 developed a robust and early motor phenotype that had high, but incomplete penetrance. We then had a few animals from two other founder lines (MA1573, MA1576) that also developed the same phenotypes (one around 5-7 months, one around 13 months). In both cases, the phenotype had a low, incomplete penetrance.

- Expression of the transgene in these mice was primarily detected in the muscle, with much lower and variable levels in the spinal cord. The MA transgenic mice from the three founder lines that developed motor phenotype abnormalities showed muscle pathology that included vacuoles, centralized nuclei and gross muscle atrophy. The spinal cords of motor impaired mice had gliosis, but the pathology was less striking than in the muscle. We also observed neuromuscular junction defects. Based in part of prior observations that over-expression of WT-TDP43 can produce neuromuscular phenotypes similar to what we observed here, we accepted the results as plausible and moved ahead to publish a paper describing the MA- lineages mice in late 2016 in *Acta Neuropathologica Communications*. Part of the impetus to publish was pressure to support potential grant applications to fund further characterization of this new model. Without funding, additional characterization of the MA lines as well as all characterization of the F lines would not have occurred, in which case, we would have never been able to catch this mistake.
- At the time of publication of the first paper about the MA lines, we were still trying to sort out which F lines we would continue with. A couple of mice from one F line had developed a phenotype so we anticipated that we were again dealing with mosaicism. Additionally, of the mice that we did have, we saw increased random deaths – an initial event that we saw as we were establishing the MA lines. The mice from the highest expressing F lines had similar Matrin 3 expression to MA1573 and MA1576; therefore, we anticipated that a motor phenotype with low penetrance may be observed in these lines, necessitating a large N number in order to detect the low frequency event.
- Jackson labs had tried to create F115C mice (equivalent to what we thought was our F lines) and they also had trouble getting lines with a consistent phenotype. This gave us some reassurance that our variability and the overall lack in a phenotype was consistent with other people's efforts.
- After publishing the paper on MA mice, we only had one person working on the mice ( [REDACTED] ) and thus our scientific hands were limited. We were still focusing much of our attention on further MA characterization, but started collecting some data on the F mice. We saw muscle pathology in a few of the F mice that we had collected, similar to what we saw in the age-matched MA lines. Because of the low N number and the little person power we have, we have not done a direct comparison at multiple timepoints.
- In March 2017, we performed some analysis of neuromuscular junctions in a subset of 3-5M old (non-phenotypic) mice from the F-lineage mice, finding no evidence of abnormality. Since these F-mice were younger than the phenotypic MA mice that we utilized for NMJ studies in the paper, we hypothesized that age and/or phenotype may influence the NMJ findings.

- In August 2017, the student involved in characterizing the mice (██████████) gave a committee meeting indicating that relatively few mice of the F-lineage were developing motor abnormalities despite similar levels of transgene expression in muscle.
- Until August 31, 2017, we had been working under an assumption (based on lab meeting conversations) that the DNA constructs that were injected were prepared separately and provided to the Core at different times. It was rare that the ██████████ lab prepared and injected the WT and mutant construct of the same transgene at the same time.
- On August 31, 2017, ██████████ emailed ██████████ that ██████████ had checked ██████████ notes and discovered that both the F115C construct and the WT construct had been given to ██████████ at the same time. ██████████ is not sure what prompted ██████████ to check ██████████ notes on this date. ██████████ immediately went down to ██████████ and they designed a restriction digestion of tail DNA PCR products used for genotyping that could distinguish mutant and WT Matr3 transgenes. The required enzyme was ordered and it arrived after Labor Day.
- Immediately before the hurricane, ██████████ told ██████████ that the restriction analysis suggested that the mice from the MA-lineage harbored the mutant F115C Matr3 transgene and that the mice from the F-lineage harbored the WT transgene. Importantly, at the same time, ██████████ pulled aliquots of the DNA preparations that had been provided to the Core and determined that the DNA ██████████ had provided to the Core has been mislabeled.
- To be absolutely certain, we confirmed the mistake by sequence analysis of PCR products from tail DNA from 2 MA mice and 2 F mice.
- Because of the hurricane, the sequencing results were not available until Sept. 18, 2017.
- After speaking with ██████████ and informing him of the issue on the 18th, ██████████ contacted the Editor of *Acta Neuropathologica Communications*, on behalf of both ██████████ and ██████████ and the Editor suggested that we retract with a note. The Editor indicated that the journal would like to consider a paper that set the record straight describing data for both WT and F115C data.
- On Sept 19<sup>th</sup>, ██████████ notified the other authors and the funding agency (ALSA). There were two NIH grants that were submitted that described data on these mice, but no experiments directly using the animals. Neither of these grants scored well and there are no pending grants still under consideration at the NIH that reference the *Acta Neuropathologica Communications* paper. There were three NIH grants cited on the paper which covered salary support for ██████████, ██████████, ██████████, and ██████████. None of the NIH grants proposed work on the Matr3 mice. Statement of funding from paper is below.

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### **How do we prevent a recurrence of this event?**

In Matrin 3 transgenic mice, the transgenic protein product of the mutant allele does not have an obvious distinguishing abnormality compared to the wild-type Matrin 3 transgenic protein that allows an observer to be confident of genotype. For many other transgenic mice that we work with, it is possible to detect a mistake in genotyping (e.g. mislabeling of WT and mutant lines) by the biochemical aspects of the protein produced by the transgene (e.g. protein is truncated, or prone to aggregate, etc). We should have confirmed the identity of our founder lines at the outset by sequencing PCR amplified transgene sequences from tail DNA. Our mistake was trusting our staff a little too much, and we should have been more rigorous in verifying. We have learned a painful lesson in that even a very experienced technician can make a mistake. It is possible to verify genotypes by sequencing PCR amplified transgene sequences from tail DNA. This level of validation should have been included in screening the initial founders and will be employed to randomly, and periodically, sample animals from the colony to make sure that no mislabeling has occurred in the future.