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Percent Effort: 20%
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Introduction.

Our research uses forward mouse genetics to identify genes necessary for normal CNS development. To this end, we have been studying the spontaneous mouse mutant, *vacuolated lens* (*vl*). *Vl* homozygotes display three different neurodevelopmental phenotypes that include congenital cataracts, spina bifida and if the neural tube closes, an attenuated dorsal midline.

Original Aims of the Project:

There were two aims of the original project.

1. To positionally clone the *vacuolated lens* locus
2. To characterize the phenotypic effects of the *vacuolated lens* mutation on spinal cord development.

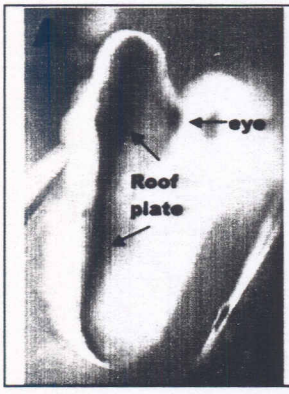

Figure 1. An *in situ* hybridization for *vacuolin* on E9.5 +/+ embryos demonstrate expression in the dorsal spinal cord and eye.

Project successes.

Our greatest success despite great technical difficulty was the positional cloning of the *vacuolated lens* locus, accomplishing Aim 1 of the original project.

We have demonstrated that the *vacuolated lens* locus is encoded by an orphan GPCR that is expressed in the developing eye and spinal cord (see Figure 1). The mutation causes an 8 base pair deletion that results in a frame shift and early termination of the protein.

The following genetic data supports our conclusion that this orphan GPCR encodes the *vl* locus.

1. The 8 base pair deletion segregates with the *vl* phenotype in over 3000 meoises form three separate crosses.
2. The 8 base pair deletion is the only DNA alteration observed in the minimal genetic interval defined by our crosses.
3. The 8 base pair deletion is not observed in 23 other inbred strains indicating that it is not a polymorphism
4. Expression analysis has demonstrated that none of the other genes in our minimal genetic interval are affected by the *vl* mutation.

For these reasons, we conclude that this orphan GPCR is responsible for the *vl* mutation and in accordance with mouse and human nomenclature we are calling this orphan GPCR, *vacuolin*.

The vl mutation truncates the C terminal tail of *vacuolin*. When a similar C terminal tail truncation is generated in other GPCRs, this results in a constitutively active receptor (Gainetdinov et al., 2004). Interestingly, our preliminary phenotypic analysis has demonstrtaed an increase in the number of cells in the lens and spinal cord, consistent with this possibility.

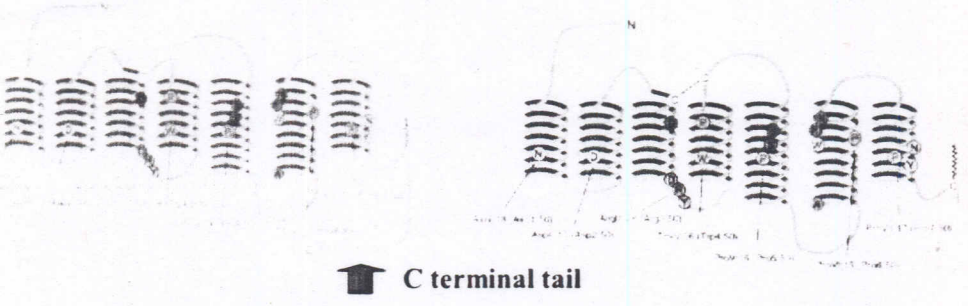

Figure 2. The effect of the 8 base pair deletion of vacuolin protein structure is schematically represented.

In summary, our positional cloning of the *vacuolated lens* locus has identified a new receptor important for spinal cord and lens development. This result suggests that *vacuolin* is part of a novel receptor-ligand pathway that is normally used during lens and spinal cord development.

Project challenges.

The biggest challenge for the phenotypic characterization and genetic mapping of the *vl* locus has been the suppressing effects of the different genetic backgrounds. Initially, our goal was to characterize the *vl* phenotype concomitant with our positional cloning. Because *vl* arose on the *C3H/HeSnJ* background, the plan was to cross the +/*vl C3H* mice to +/+ *C57BL6/J* mice and then intercross the +/*vl C3H/BL6* mice to generate F2 embryos. Because *vl* arose on the *C3H* background, *vl/vl* embryos would have a *C3H/C3H* genotype for polymorphic markers on distal chromosome 1 while +/*vl* and +/+ embryos would have the *C3H/BL6* and *BL6/BL6* genotype respectively. Although this plan was expected to work and had been used successfully by us in the past to characterize another mouse mutant (Millonig et al., 2000), we discovered that unlinked *C57BL6/J* modifier loci suppressed the *vl* phenotypes. This phenomenon was then confirmed by Bev Paigen at The Jackson Laboratories, our now present collaborator, who had previously done a *vl* x *BL6* cross. This suppressing effect of the *BL6* background meant that the phenotypic analysis had to be delayed until the *vl* locus was cloned. Because *vacuolin* has been cloned, we are now in the process of performing these experiments.

Advantages of Project Challenges.

Despite this set back, we have taken advantage of this suppressing effect to map these interacting genetic modifiers. The *vl* mutation has been crossed onto three separate genetic backgrounds, *C57BL6/J*, *Mus castaneus* (*CAST/Ei*) and *Mus. molossinus* (*MOLF/Ei*). In all three crosses, the genetic backgrounds have suppressed the associated *vl* mutant phenotypes. This result means that all three genetic backgrounds have modifier loci that can compensate for the *vl* mutation.

Because the cloning of *vacuolin* has likely identified a novel pathway for spinal cord and lens development, it is important to identify other genes in the pathway. The mapping

and eventual cloning of these modifier genes provides a means of accomplishing this goal.

In collaboration with Bev Paigen, we have mapped the *vl* modifier loci for each of the genetic backgrounds. In total, 5 modifier loci have been mapped, 1 for *BL6*, 1 for *CAST/Ei* and 3 for *MOLF/Ei*. Their respective LOD scores and map positions are shown on Figure 3. The eventual cloning of these modifier loci will provide further insight into how the *vacuolin* pathway controls CNS development.

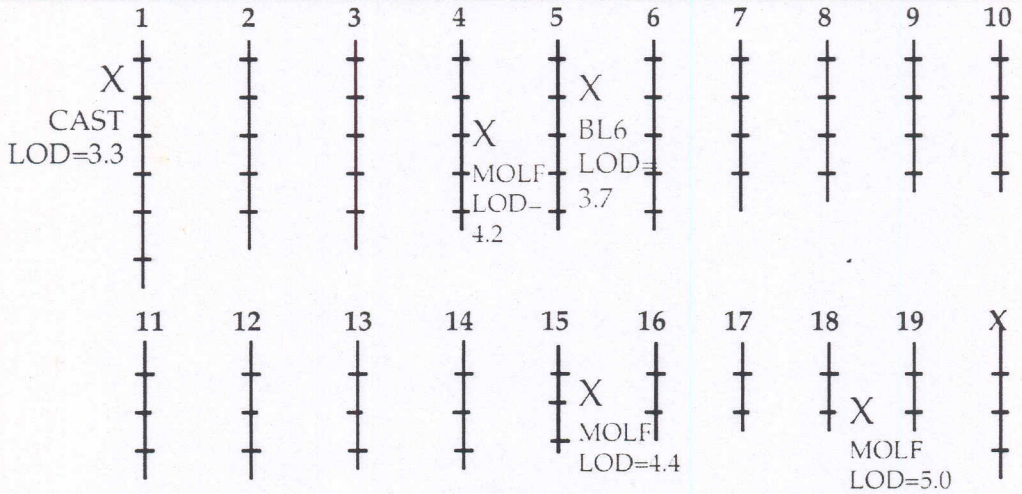

Figure 3. The chromosomal position and LOD scores of the different *vl* modifiers is diagrammatically represented.

Implications for future research/clinical treatment.

Stem cells provide a potential means to treat the neurological disorders including spinal cord injury and disease. Others have demonstrated that exposure of stem cells in culture to different combinations of secreted factors is sufficient to drive stem cells to a particular neuronal cell fate (Barberi et al., 2003). The identification of *vacuolin* as an orphan GPCR indicates that an unidentified ligand exists that is important for spinal cord development. Thus, the identification of this ligand in combination with stem cell therapies could have therapeutic consequences for spinal cord injury and disease in the future.

For these reasons, we will continue to characterize the *vl* phenotype and pathway. In addition, we will perform genetic and molecular experiments to fine map and positionally clone the modifier loci since they could encode the ligand or provide molecular clues to the identity of the ligand.

Future Plans

We have already submitted and been awarded a grant to NJSCR to follow up on these important findings. Part of this research will also be submitted to NIH for review.

Publications

Desai J, Korstanje R, Lazar G, Rollins J, Mancuso V, Paigen B, Millonig JH. *Vacuolin*, an orphan GPCR, is required for normal lens and spinal cord development.

Korstanje R, Desai J, Rollins J, Lazar G, Spurr M, Joseph J, Kadambi S, Cherry A, Paigen B, Millonig JH. Localization of modifier loci of the *vacuolated lens* mutant, a mouse model of spina bifida and congenital cataracts (in preparation).