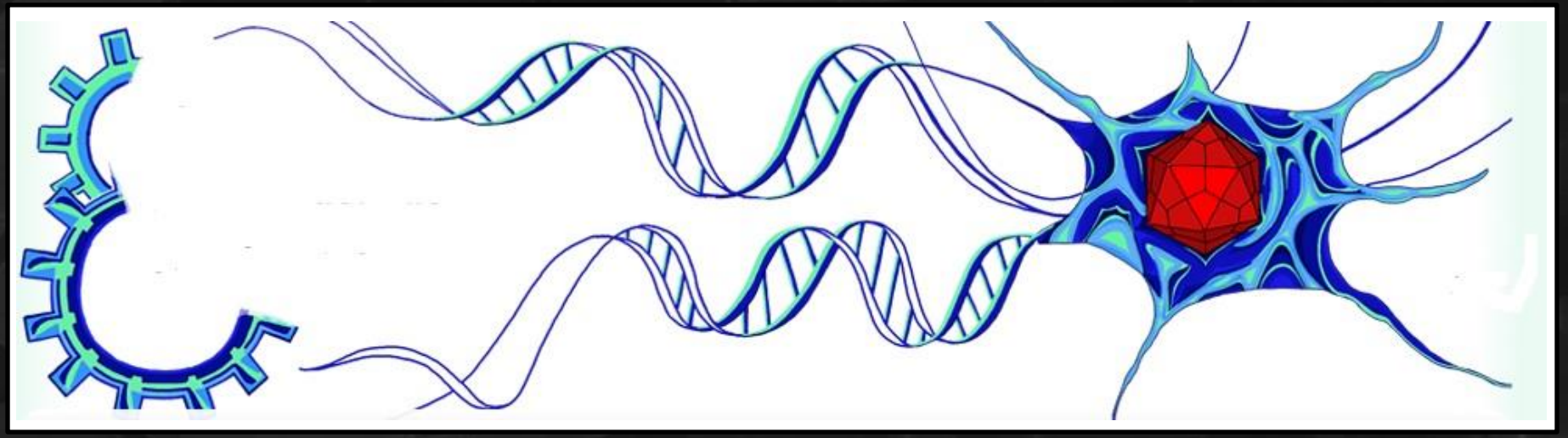
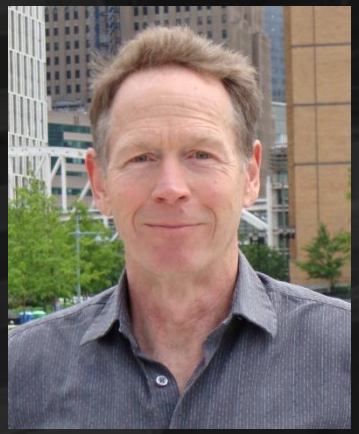
Viral vector mediated Fmr1 gene delivery in Fragile X Knock-out Mice



Dr. Shervin Gholizadeh
Dr. Jason Arsenault
and
Dr. David R. Hampson



David R. Hampson, Ph.D. is a Professor in the Department of Pharmaceutical Sciences at the University of Toronto in Toronto, Ontario, Canada. He is also the Director of the Canadian Institutes for Health Research sponsored Training Program in Biological Therapeutics. In addition to the study of fragile X syndrome, work in Dr. Hampson's laboratory is also directed towards the study the molecular pharmacology and biological roles of G-protein coupled receptors (GPCRs) in the central nervous system. His research on GPCRs has centered on members of subfamily C within the GPCR superfamily; this group includes the metabotropic glutamate receptors, the $GABA_B$ receptor, and the calcium-sensing receptor.

Shervin Gholizadeh is a Ph.D. candidate in Dr. Hampson's laboratory. He obtained his Pharm.D. degree from Tehran university of medical sciences in 2007. Prior to joining Dr Hampson's research group, he completed a postdoctoral fellowship at university of Western Ontario in 2010 under the supervision of Dr Steven Laviolette.

Jason Arsenault is a postdoctoral fellow in Dr. Hampson's laboratory. He obtained his Ph.D. from Université de Sherbrooke in 2010 and completed his first postdoctoral fellowship in Cambridge university under Dr Bazbek Davletov's supervision. He joined Dr Hampson's laboratory in 2013 as a post-doctoral fellow.

Prospects for gene therapy in Fragile X syndrome

- FMRP protein replacement in the brain:
  - Localized vs. global transgene expression in the brain
  - FMRP expression in different cell types
  - Therapeutic window to rescue the behavioral abnormalities
  - Reversal of learning deficits in fragile X syndrome by restoration of FMRP in adult neural stem cells (Guo et al., 2012)

Specific aims

1. To design efficient adeno-associated viral vectors (AAVs) containing Fmr1 gene with widespread strong neuron-specific transduction in neonatal mice.

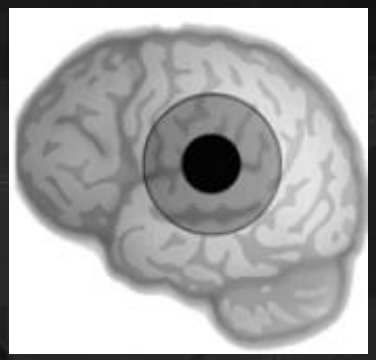
2. To identify the most effective route(s) of administration and age of injection

3. To assess levels and distributions of FMRP in vector-injected Fmr1-knockout mice compared with wild-type mice by immunocytochemistry and quantitative Western blotting.

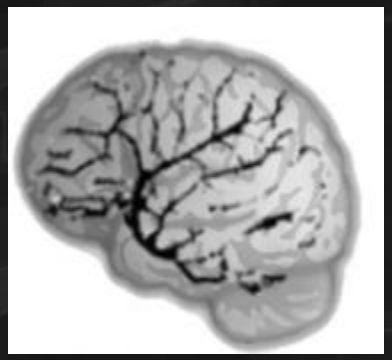
4. To assess a range of behavioral phenotypes associated with fragile X syndrome in AAV-treated Fmr1 KO mice.

Major factors affecting efficiency AAV transduction

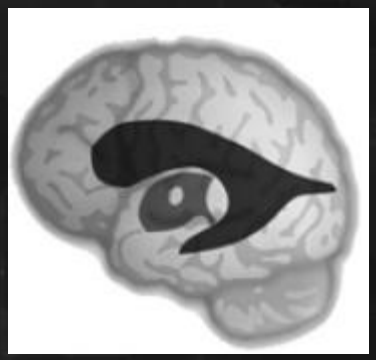
- 1. AAV serotype
- 2. Route and age of administration



intracranial



Intravascular(i.v.)

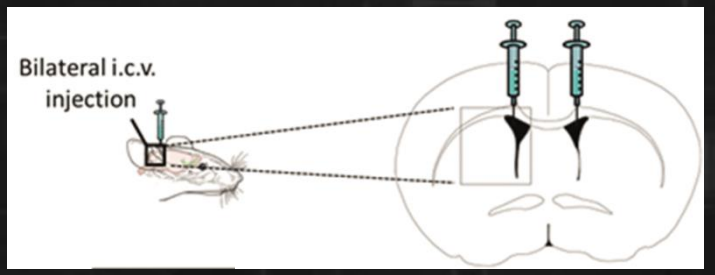


Intracerebroventricular(i.c.v.)

- 3. Promoter selection

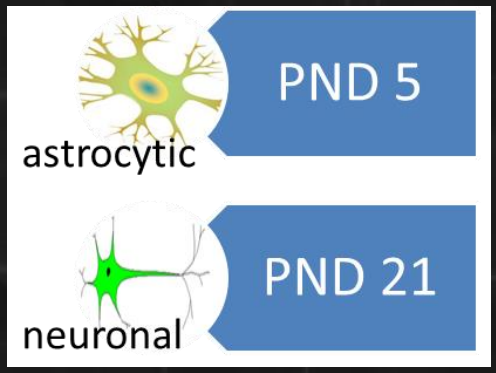
Lessons learned from AAV2/9-eGFP injections

- Higher distribution of AAV2/9 vectors in the brain at postnatal day 5 compared to postnatal day 21 injections



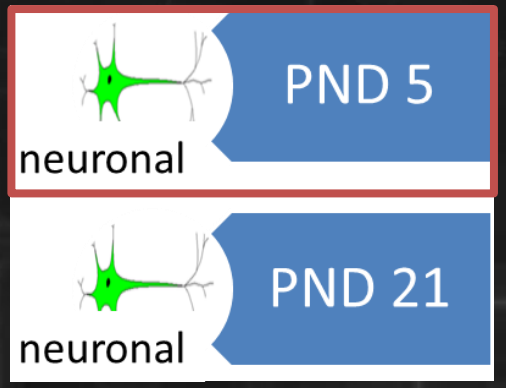
- **AAV2/9-CMV-eGFP**:

Preferential transduction of astrocytes in neonatal mice but neurons in juvenile mice



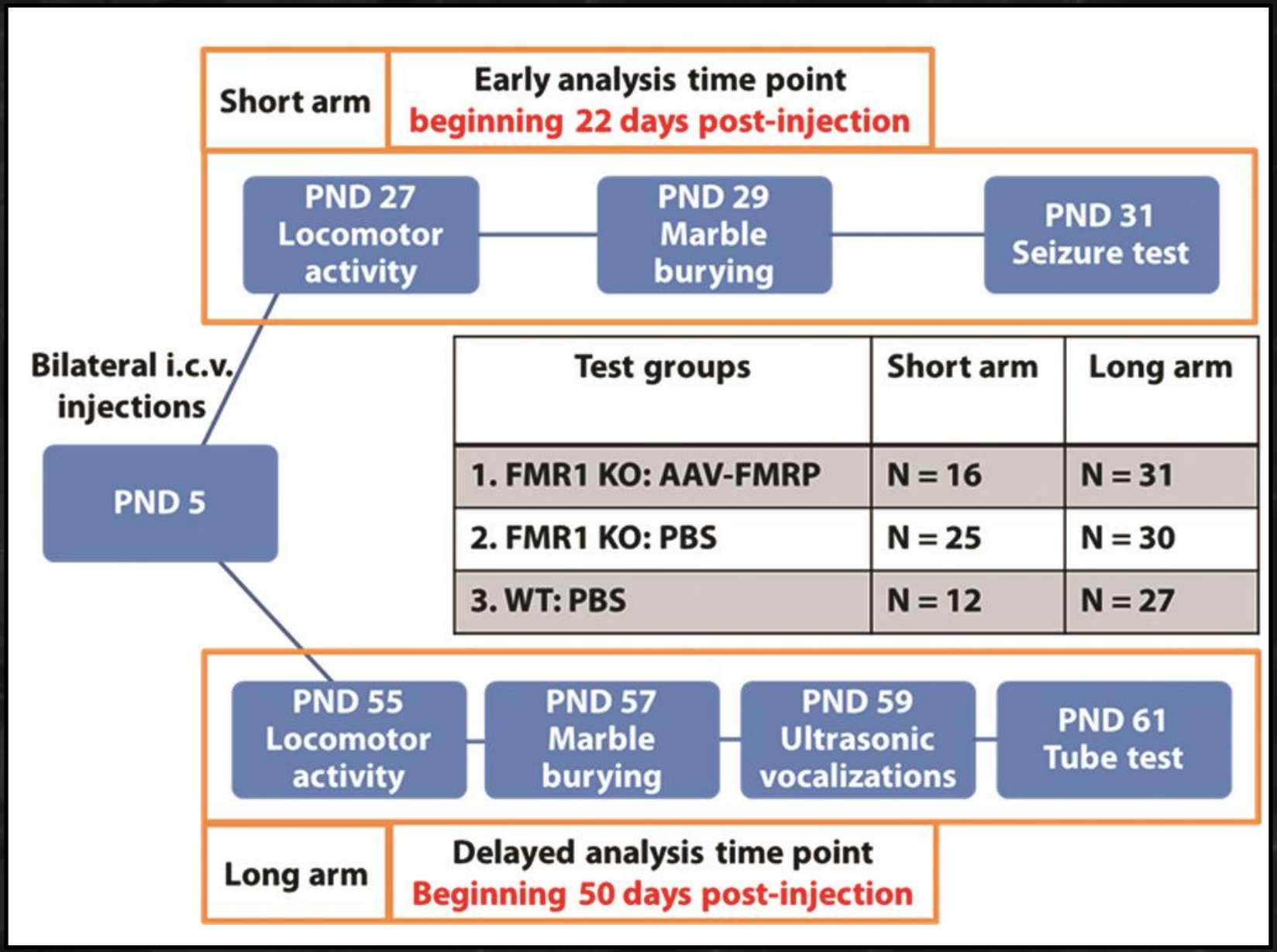
- **AAV2/9-SYN-eGFP** :

Extensive neuron-specific transduction in the brain regardless of the age of injections



Gholizadeh et al., 2013

Study outline



Results & Conclusions

- After a single vector injection in neonatal Fmr1 mice, we observed partial rescue of several behavioral impairments in *adult* AAV-treated Fmr1 KO mice.

- This work represents a proof-of-principle for gene therapy in an animal model of FXS.

- This research also provides us with a better understanding of the role of FMRP in neurons, and its contribution to reversing abnormal behaviors in Fmr1 KO mice.

Perspectives

- Fine tune transgene delivery to have widest possible distribution that approaches 100% of WT expression levels
  - Over expression might lead to pathological effects
- Investigate new behavioral tests
- Alternative injection strategies and vector constructs for improved outcomes

Acknowledgments

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Dr. David R. Hampson

- **Lab members:**
  - **Dr. Jason Arsenault**
  - Dr. Laura Pacey
  - Dr. Sujeenthar Tharmalingam
  - Ingrid Xuan
  - Dr. Chiping Wu
  - David Jiang
  - Dr. Sebok Halder

Vanier Canada Graduate Scholarships

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