



Universiteit
Leiden
The Netherlands

CRB1 gene therapy coming of age: mechanistic insight and rAAV assays on mouse & human retinal organoid models

Buck, T.M.

Citation

Buck, T. M. (2022, September 28). *CRB1 gene therapy coming of age: mechanistic insight and rAAV assays on mouse & human retinal organoid models*. Retrieved from <https://hdl.handle.net/1887/3464695>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3464695>

Note: To cite this publication please use the final published version (if applicable).

ACKNOWLEDGEMENTS

Time flies at the LUMC! It has been a wonderful, sometimes frustrating but exciting PhD time, working side-by-side with all of you over the last six years. I very much enjoyed the comradeship, guidance and all the helping hands! I would not have lasted so long without you (and you made the ride much more enjoyable).

Thank you, Jan Wijnholds (supervisor) and Prof. Gré Luyten (promoter), for the patience and mentoring over the years. You always placed importance on having students around to sharpen our minds & giving back to the next generation, a tradition I will take with me.

To the current and former members of the Jan Wijnholds lab: Rogier, Peter, Jeroen, Lucie, Henrique, Alben, Michaela, Nanda, Andy, Carmen, Charlotte, and Xuefei. Special thanks to Lucie (my MSc student supervisor), Rogier (AAV production / cell culture / subcloning) and Henrique (retina research / co-supervisor) who all helped me transitioning from a MSc student to a PhD student and stoke the fire for the field of gene therapy. It was my privilege to supervise so many students. Thank you for your efforts: Martijn, Sarina, Ayse, Charlotte, Mees, Hind, Ilse, Yacintha, Ariadna, Mariska, and Daniëlle.

Thanks to all people in different departments. OOG: Camiel, Martine, Mieke, Elon, Danial, Annemijn, Mays, Annelies, and Judith. Transgenic animal unit: Jan-Bas, Ben, Fred, Martine, Ewoud, Jos, Nelleke, Marloe; and, the fruitful discussion during the experimental animal coordinator meetings. We could not have done all the mouse behaviour studies (ERG, OKT, cSLO, OCT) and viral injection rescue studies without you. The microscopy team & microscope user meetings: Joop, Annelies, Karien, Aat, Bram, Carolina, Lennard, and Willem. You made my images pop! The Virus / Stem Cell Biology / Human iPSC Hotel groups: Rob, Martijn, Harald, Christian, Christiaan, Marga, Arnaud, Maaïke, Manuel, Diana, Amiet, Anna, Francesca, Hidde, Igno, Iris, Jin, Kim, Marjolein, Sanne, Timo, Tobias, Vera, Xiaoyu, Marcella, Silvana, Qian, Zhen, Merve, Sanne, Selas, Willemijn, Sofia, and Lonneke. Thanks Sanne for organizing game nights! Also, the generation of viral vectors and iPSCs has been essential for my thesis. Special thanks to Steve for his guidance on all molecular techniques & whisky. The CCB: Sjaak, Ilana, Marlieke, Peter, Catalina, Chuannan, Dieuwke, Joke, Tom, Mayke, Felix, Marten, David, Zary, Pauline, Julia, and Marianne. Thank you, Ilana, for bringing the endolysosomal system a bit closer to me.

To my paranympths Nanda and Francesca, thanks for all your support and advice. Additionally, I would like to thank my Alb friends, the CV Parterretrap VC group, and my NOLA friends.

Thanks to my extended family (Böckeler, vd Brand, Broeders/Adams, Buck, Calmbach). A special thanks to my grandpa Theo who inspired me to become a scientist. Finally, I would like to dedicate the Thesis to my wife Claartje and my child Luna. Thanks for staying patient with me during the many stressful hours as a PhD student.

Curriculum Vitae

Thilo Matthias Buck was born on June 22nd, 1988 in Esslingen am Neckar, Germany. After finishing his business & economics high school at the Theodor-Heuss-Gymnasium (Abitur), Reutlingen, Germany, he went on to study psychology and economics in his bachelor studies at the University of Innsbruck, Austria. He transferred to the University of New Orleans, USA, in 2009, finishing his Bachelor's Degree (*magna cum laude*) in psychology with minors in chemistry and biology in 2012. During his bachelor studies he broadened his skills in internships in stress research (SPIT lab; supervisor: Dr. Elizabeth Shirtcliff), brain development (supervisor: Dr. Ben Hall), and Huntington's disease (supervisor: Dr. Gerald J. LaHoste).

He was awarded to the Erasmus Mundus NEURASMUS master's scholarship program in neuroscience in 2012. During 2012-2014, he interned at the labs of Dr. Marc Landry (chronic pain mouse models, INSERM/CNRS, France), Dr. Olaf Strauss (RPE and bestrophin-1, Berlin Charité, Germany), and Dr. Jan Wijnholds (rAAV tropism in mouse models, NIN, Amsterdam). He received his Master's Degree in Neuroscience from the VU Amsterdam (The Netherlands), the Bordeaux University (France), and the Humboldt University (Germany).

After a short stay as an English teacher in Siem Reap, Cambodia, in 2014/2015, he continued his work first as a research analyst (2015/2016) in Dr. Jan Wijnholds laboratory, and then later as a PhD candidate at the Department of Ophthalmology, at the Leiden University Medical Center (LUMC), in Leiden under the supervision of Dr. Jan Wijnholds on rAAV vectors and CRB1/CRB2 protein functions in the retina.

In 2021, he works as a viral vector specialist at the Netherlands Center for the Clinical Advancement of Cell & Gene Therapies (NecstGen), Leiden. NecstGen helps early-stage research organizations and start-ups to enter the clinical stage by bringing together the required expertise from R&D, development, production, QA and regulatory affairs in a non-profit GMP production.

List of Publications

Buck, T.M., Wijnholds, J (2020). *Recombinant Adeno-Associated Viral Vectors(rAAV)-Vector Elements in Ocular Gene Therapy Clinical Trials and Transgene Expression and Bioactivity Assays*. Int. J. Mol. Sci., 21, 4197.

Buck, T.M., Vos, R.M., Alves, C.H. Wijnholds, J. (2021). *AAV-CRB2 protects against vision loss in an inducible CRB1 retinitis pigmentosa mouse model*. Molecular Therapy: Methods & Clinical Development, 20, 423-441.

Buck, T.M., Pellissier, L.P., Vos, R.M., van Dijk, E.H.C., Boon, C.J.F., Wijnholds, J. (2018). *AAV serotype testing on cultured human donor retinal explants*. Methods in Molecular Biology, 1715, 275-288.

*Quinn, P. M., ***Buck, T.M.**, Mulder, A.A., Ohonin, C., Alves, C. H., Vos, R. M., Bialecka, M., Herwaarden, T. v., van Dijk, E. H.C., Talib, M., Freund, C., Mikkers, H. M. M., Hoebe, R. C., Goumans, M.-J., Boon, C. J. F., Koster, A. J., Chuva de Sousa Lopes, S. M., Jost, C. R. and Wijnholds, J. (2019). *Human iPSC-derived retinas recapitulate the fetal CRB1 CRB2 complex formation and demonstrate that photoreceptors and Müller glia are targets of AAV5*. Stem Cell Reports, 12, 906–919. ***Authors contributed equally to this work.**

Buck, T.M., Quinn, P. M., Pellissier, L.P., Mulder, A.A., et al. (2022). *CRB1 is required for the recruitment of NOTCH1 and proper recycling by RAB11A+ vesicles in human retinal organoids*. Manuscript submitted.

Quinn, P. M., **Buck, T.M.**, Ohonin, C., Mikkers, H. M. M., Wijnholds, J. (2018). *Production of iPS-Derived Human Retinal Organoids for Use in Transgene Expression Assays*. Methods Mol. Biol., 1715, 261–273.

Boon, N, Alves, C.H., Mulder, A.A., Andriessen, C.A., **Buck, T.M.**, Quinn, P.M.J., Vos, R.M., Koster, A.J., Jost, C.R., Wijnholds, J. (2021). *Defining Phenotype, Tropism, and Retinal Gene Therapy Using Adeno-Associated Viral Vectors (AAVs) in New-Born Brown Norway Rats with a Spontaneous Mutation in Crb1*. Int. J. Mol. Sci., 22, 3563.

Schoonderwoerd, R.A., **Buck, T.M.**, Andriessen, C.A., Wijnholds, J., Hattar, S., Meijer, J.H., DeBoer, T. (2021). *Sleep deprivation does not change the flash electroretinogram in wildtype and Opn4^{-/-} Gnat1^{-/-} mice*. J. Biol. Rhythms 37, 216-221.