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Functional read-outs and novel interventional targets in a transgenic MSA model
2nd Year Progress Report

Aim 1: Non-motor read-outs in transgenic MSA mice

In collaboration with P10/Singewald, cardiovascular autonomic dysfunction was studied in the PLP mouse model. Decreased heart rate variability and alterations in the circadian rhythmicity were detected in the transgenic MSA group (Kuzdas et al., Exp Neurol. 2013. doi: 10.1016/j.expneurol.2013.02.002. [Epub ahead of print]). In collaboration with Dirk De Ridder, Leuven University, Belgium we showed that MSA mice recapitulated major urological symptoms of human MSA including increased post-void residual volume linked to detrusor contraction inefficiency with indirect evidence of detrusor-sphincter dyssynergia that may be linked to αSyn-related central and peripheral neuropathology and can be further used as a pre-clinical model to decipher pathomechanisms of autonomic dysfunction in MSA (Boudes et al. Mov Disord. 2013 Mar;28(3):347-55) Olfactory function and underlying neuropathological changes were investigated in a transgenic MSA mouse model based on targeted oligodendroglial overexpression of α-synuclein. Despite progressive α-synucleinopathy in the olfactory bulb, preserved olfaction was observed in MSA mice (Krismer et al., PLOS ONE, under review). In addition, sleep EEG recordings were proposed as additional non-motor readout in order to optimize the current drug development protocols. The start of this project had to be postponed due to the fact that our collaborator (T. Fenzl) moved from Munich to Innsbruck requiring the establishment of sleep testing infrastructure in Innsbruck. However, we are confident that in vivo sleep analysis will be completed by year 3. Finally, sudomotor testing will be added to the battery of non-motor functional read-outs. Thus far, we successfully established the mold impression technique in our laboratory. Briefly, the plantar surface of the hindpaw will be cleaned using ethanol and, subsequently, covered by an elastic silicone-like material. As the silicone hardens, it will retain the impressions of emerging sweat droplets representing activated sweat glands. The amount of sweating will be determined by counting the number of sweat droplet impressions appearing in the mold (see figure on the right hand sight).

Aim 2: Contribution of L-Type Calcium channels to the pathogenesis of MSA

Our first goal was to determine the Cav1.2 and Cav1.3 expression in brains of aging PLP-α-syn mice and human MSA brain tissue. To this end, we applied immunohistochemistry for Cav1.2 (validated Cav1.3 antibodies for immunstaining are currently not available) and established protocols for in situ hybridization of both Cav1.2 and Cav1.3 (in collaboration with P02/Striessnig-Koschak and P10/Singewald). Analysis of channel distribution is expected to be finished by month 30. To further define the in vivo role of Cav1.3 in α-syn induced neurodegeneration cross-breeding of PLP-α-syn mice with Cav1.3-/- mice will be performed. In our cross-breeding experiments, we are currently at the CaV1.3-/-,PLP-αSYN+/- stage. These experiments are currently delayed due to unexpected long generation time of the mouse strains and limited space in the animal facility. However, we are expecting to receive a double-homozygote status by month 42.

To address the postulated neuroprotective effects of L-type Calcium channel blockade, isradipine is currently being tested in tg PLP-α-syn mice. The in vivo part of this study will be completed by month 30 and followed by neuropathological work-up. A study flow diagram is given below.

Aim 3. HDAC

To test the neuroprotective capacity of HDAC inhibitors in MSA, as observed in other neurodegenerative disorders, we analyzed the efficacy of sodium phenylbutyrate (NaPB), a pan-HDAC inhibtor in transgenic MSA mice. Our preliminary results show neuroprotection at the level of SNc and locus coeruleus, suggesting a possible role of changes in histone acetylation profile of the brain in the pathogenesis of MSA. In a conformational step of the study these effects will be further analyzed to define the specific mechanisms of neuroprotection achieved by this non-specific pan-HDAC inhibitor. To investigate age dependent chromatin-related differences in the brains of MSA and control mice, we have extracted histones from 4 brain regions (forebrain, midbrain, brain stem, cerebellum) at 2, 6 and 12 months of age and examined their acetylation levels. This part of the project is performed in close collaboration with Alexandra Lusser. Our preliminary results in 6 months old mice suggest changes in H3 and H4 acetylation, which may link to disease pathogenesis. In addition, we have examined the expression of several genes that may have potential role in the disease pathogenesis by RT-qPCR. At 6 months of age, the signaling factor Rab5a was upregulated in midbrain of MSA mice and the transcription factor Sox17 exhibited moderate downregulation in the

brain stem. In ongoing and future experiments we will attempt to identify additional misregulated genes and use ChIP assays to determine, if the misregulation is accompanied or caused by chromatin changes at the gene loci.

Aim 4. Non-coding RNAs

In collaboration with Alexander Hüttenhofer we aim to determine whether ncRNAs may play a role in the pathogenesis of MSA. To achieve this we collect brain tissue (forebrain, midbrain, brain stem and cerebellum) from young (non-symptomatic) and old (symptomatic) transgenic MSA mice and age matched wild type controls and screen the expression of ncRNAs by the application of the ncRNAs chip

List of peer-reviewed publications

- *Wenning GK, Krismer F, Poewe W. New insights into atypical parkinsonism. Curr Opin Neurol. 2011;24(4):331-8. (IF: 4.9)*
- *Krismer F, Seppi K, Tison F, et al. The Unified Multiple System Atrophy Rating Scale: intrarater reliability. Mov Disord. 2012;27(13):1683-5. (IF: 4.5)*
- *Metzler M, Duerr S, Krismer F, et al. Neurogenic orthostatic hypotension: pathophysiology, evaluation, and management. J Neurol. 2012 Nov 20. [Epub ahead of print] (IF: 3.4)*
- *Kuzdas D, Stemberger S, Gaburro S, et al. Oligodendroglial alpha-synucleinopathy and MSA-like cardiovascular autonomic failure: Experimental evidence. Exp Neurol. 2013. doi: 10.1016/j.expneurol.2013.02.002 (IF: 4.4)*
- *Wenning GK, Geser F, Krismer F, et al. The natural history of multiple system atrophy: a prospective European cohort study. Lancet Neurol. 2013;12(3):264-74. (IF: 23.4)*
- *Krismer F, Duerr S, Minnerop M, et al. MSA-QoL: Ein spezifisches Bewertungsinstrument zur Erfassung der Lebensqualität bei Patienten mit Multisystematrophie – Validierung der deutschsprachigen Übersetzung. Der Nervenarzt, in press (IF: 0.7)*
- *Krismer F, Wenning GK, Li Y, et al. Intact olfaction as hallmark feature of Multiple System Atrophy: experimental evidence. Plos ONE, under revision (IF: 4.1)*
- *Krismer F, Jellinger KA, Scholz SW, et al. Multiple System Atrophy: An Emerging Template for Accelerated Drug Discovery in Parkinson's Disease. Neurology, under review (IF: 8.2)*
- *Boudes M, Uvin P, Pinto S, et al. Bladder dysfunction in a transgenic mouse model of multiple system atrophy. Mov Disord. 2013 Mar;28(3):347-55. (IF: 4.505)*
- *Fellner L, Irschick R, Schanda K, et al. Toll-like receptor 4 is required for α-synuclein dependent activation of microglia and astroglia. Glia. 2013 Mar;61(3):349-60. (IF: 4.82)*
- *Fellner L, Stefanova N. The role of glia in alpha-synucleinopathies. Mol Neurobiol. 2013 Apr;47(2):575-86. (IF:5.735)*
- *Fellner L, Jellinger KA, Wenning GK, Stefanova N. Glial dysfunction in the pathogenesis of α-synucleinopathies: emerging concepts. Acta Neuropathol. 2011 Jun;121(6):675-93. (IF: 9.32)Stefanova N, Kaufmann WA, Humpel C, et al. Systemic proteasome inhibition triggers neurodegeneration in a transgenic mouse model expressing human α-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. Acta Neuropathol. 2012 Jul;124(1):51-65. (IF: 9.32)Stefanova N, Fellner L, Reindl M, et al. Toll-like receptor 4 promotes α-synuclein clearance and survival of nigral dopaminergic neurons. Am J Pathol. 2011 Aug;179(2):954-63. (IF: 4.89)*