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Identifiering av nya behandlingsmål kopplade till spridningsmekanismer och symtomutveckling vid Lewykroppsdemens

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Synukleinopatier, så som Parkinsons sjukdom och Lewykroppsdemens, kännetecknas av att proteinet alfa-synuklein ansamlas i hjärnan och bildar Lewykroppar. Lewykroppar bildas genom att alfa-synuklein antar en felaktig struktur och klumpar ihop sig som plack i nervcellerna som då dör. Senare års studier har visat att placken uppstår i vissa delar av hjärnan och att patologin sedan sprider sig. Att felveckat protein kan spridas till olika delar i hjärnan har lett till hypotesen att alfa-synuklein har så kallade ”prion-liknande” egenskaper, d.v.s. att protein som fått en avvikande struktur kan fungera som en felveckningsmall och påverka andra proteiner i närheten att kopiera den felaktiga strukturen och också börja klumpa ihop sig.

För att kunna förhindra nervcellsdöd och utveckla nya läkemedel krävs mer kunskap om flera idag okända mekanismer. I det här projektet studerar vi några centrala mekanismer för hur alfa-synuklein antar prion-liknande egenskaper och sprider sig i hjärnan och även till andra organ. Vidare kommer vi studera kopplingen mellan patologi och utveckling av neurologiska symptom. Sammantaget kan forskningen leda till ny kunskap om viktiga sjukdomsprocesser vid synukleinopatier, sjukdomar där det i dagsläget inte finns någon botande behandling.

Alpha-synucleinopathies, such as Parkinson´s disease (PD) and Lewy body dementia (DLB), are some of the most common neurodegenerative disorders and there is currently no cure. Existing treatments are symptomatic rather than targeting the underlying cause of the diseases. The number of individuals affected is expected to dramatically increase as the population ages (1).

The alpha-synuclein (α-syn) protein is a 140-amino-acid cytoplasmic protein that is found within presynaptic nerve terminals and is involved in the assembly of synaptic vesicle complexes (2). It is believed to play a central pathogenic role in the α-synucleinopathies, since mutations in the gene encoding α-syn cause early-onset PD or DLB (3). In disease, α-syn polymerizes into insoluble β-sheet-rich protein aggregates that become phosphorylated at residue serine 129 and deposit within the central nervous system (CNS) (4, 5). Soluble α-syn oligomers have been shown to be toxic both *in vitro* (6-8) and *in vivo* (9, 10) and are thus believed to play a central pathophysiological role (11-13). As histopathological hallmarks of α-synucleinopathies intraneuronal α-syn deposits as Lewy bodies and Lewy neurites (14).

Interestingly, α-synucleinopathies have pathogenic similarities to prion-protein induced neurodegeneration. Prions are proteins that can self-propagate and cause degenerative brain disorders such as Creutzfeldt–Jakob disease. Misfolded prion protein has been suggested to catalyze the conformational conversion of properly folded prion protein into additional copies of the misfolded form via a template-directed refolding mechanism. There is today growing evidence indicating that α-syn becomes “prion-like” during disease, leading to a progressive cell-to-cell spreading of protein aggregates within the brain (15). Similar to experimental transmission of prion disease, injection of α-syn aggregates into the brain of transgenic α-syn mice induces aggregation and deposition of α-syn and accelerates the onset of behavioral dysfunction in the mice (16-18).

The prion-like behavior of α-syn aggregates provides a potential molecular explanation for the progressive nature of DLB and related α-synucleinopathies. Identifying such mechanisms is of critical importance both for the development of early diagnostic methods and for impeding disease progression. Today little is known regarding which factors that affect this progression and more research is needed in order to develop curative treatments.

Purpose and aims/methods used

The purpose of this project is to increase the knowledge of early pathological events central for α-syn aggregation and spreading in DLB. Further, we aim to elucidate how these pathological alterations correlate with symptom development, in order to identify new targets for therapeutic intervention. We will study mechanisms central for prion-like propagation of the α-syn protein, including phosphorylation of α-syn oligomers. It has been shown that α-syn can aggregate in the brain and spread, both within and outside the CNS. This leads to subsequent symptom development and eventually death. However, the mechanisms for spreading is unknown and spreading to and from the periphery has not been well studied. Further, the spreading of α-syn and how it is related to the development of neurological symptoms is not well understood.

In detail, the following aims will be explored:

Aim 1: We hypothesize that the phosphorylation of α-syn oligomers is *central* for the aggregation process and the prion-like propagation of α-syn. Several studies have reported that ~90 % of the α-syn species within Lewy bodies are phosphorylated at Ser129 (pS129). However, it has been difficult to detect α-syn oligomers on brain tissues using conventional staining techniques.

The first aim for this project is to investigate mechanisms central for α-syn aggregation and spreading by using the sensitive oligomeric α-syn-proximity ligation assay (ASO-PLA) that we developed in our laboratory. We recently evaluated the ASO-PLA method and showed that it is a highly sensitive method for measurement of α-syn oligomers on mouse tissue (Behere et al., *submitted to J of Neurosci. Res.*). Human brain tissue from diseased α-synucleinopathy patients will be used as controls for evaluation of relevant pathology. We will in detail study phosphorylation of α-syn oligomers on tissue sections using the ASO-PLA combining different oligomer-specific antibodies. We will investigate spreading of α-syn pathology into different brain areas and to peripheral tissue including liver, gut, kidneys and also blood. We will further investigate to which cell types that aggregated α-syn species accumulate and spread.

To further study the prion-like spreading of α-syn, the previously established intracranial α-syn injection model will be used (19). We will use recombinant α-syn for the generation of preformed fibrils for injection into the brain. The monomeric α-syn protein will be purchased from Proteos Inc, USA and fibrils will be made according to an established protocol in our laboratory. We have previously tested and evaluated these fibrils (*preliminary data, fig. 1*). We will focus on fibrils since they appear to be more potent inducers of PD-like neuropathology than α-syn oligomers after injection into mice. We will inject recombinant mouse α-syn preformed fibrils into the unilateral or bilateral dorsal striatum in wild type control and α-syn transgenic mice (n=12/group). The acute spreading of misfolded α-syn into the bloodstream and to peripheral organs will be investigated with the ASO-PLA by taking blood samples and tissue for *ex vivo* analysis, at 24h – 30 days, 3 and 6 months after injections. We have already initiated a pilot experiment including thirty wild type mice inoculated with α-syn pre-formed fibrils and collected tissue from early time-points.

Aim 2. The second aim is to characterize subsequent symptom development using sensitive behavioral measurements in order to understand the disease progression and to get better readouts for preclinical testing of new drugs. We will use behavioral models that measure clinically relevant motor and non-motor symptoms, and investigate if such symptoms correlate to brain pathology. The following tests will be used; challenging beam test for fine motor impairments, open field test for general activity and locomotion, multivariate concentric square field (MCSF) test for behavioral profiling and risk taking behaviors, novel object recognition for memory deficits, and wire hanging test for muscle strength and coordination. An ethological approach will be used in the design of the experiments.

We have a long experience of behavioral testing and analysis of behavioral data in our research group. The videos from the behavioral tests will be analyzed using an automatic software (Ethovision XT) in combination with manual scoring of specific behaviors. Data will be analyzed with appropriate statistical methods based on data distribution. Data from the MCSF test will be analyzed using principal component analysis, a complement to conventional statistical methods.

Time-line:

Fall 2021: Analysis of pilot inoculation experiment. Set-up of long-term injection experiments.

Spring 2022: Long-term injection experiments including behavioral analysis.

Fall 2022: Analysis of pathology and behavioral data.

Preliminary results

We carefully characterized pre-formed α-syn fibrils (PFFs) and oligomers, made from mouse recombinant α-syn, using electron microscopy. We showed that a majority of the fibrils were

20-40 nm after sonication and with high stability in room temperature, which is of great importance for the experimental procedures (fig 1). The oligomers showed higher variability in size, between 40-120 nm, but had the same stability as fibrils (not shown). Stereotaxic injections of these α-syn PFFs and oligomers into the dorsal striatum in wild type mice and showed that fibrils, but not oligomers, induced α-syn pathology (pS129) in the ipsilateral and contralateral cortex, striatum and amygdala, at one month after injection. The injections further resulted in a limited inflammatory response (increased GFAP staining) in the same areas. However, further studies are needed to find out earlier pathological events, study the different cell types α-syn is spreading to and if one single injection of α-syn PFFs is enough to induce DLB-like symptoms in normal wild type mice.

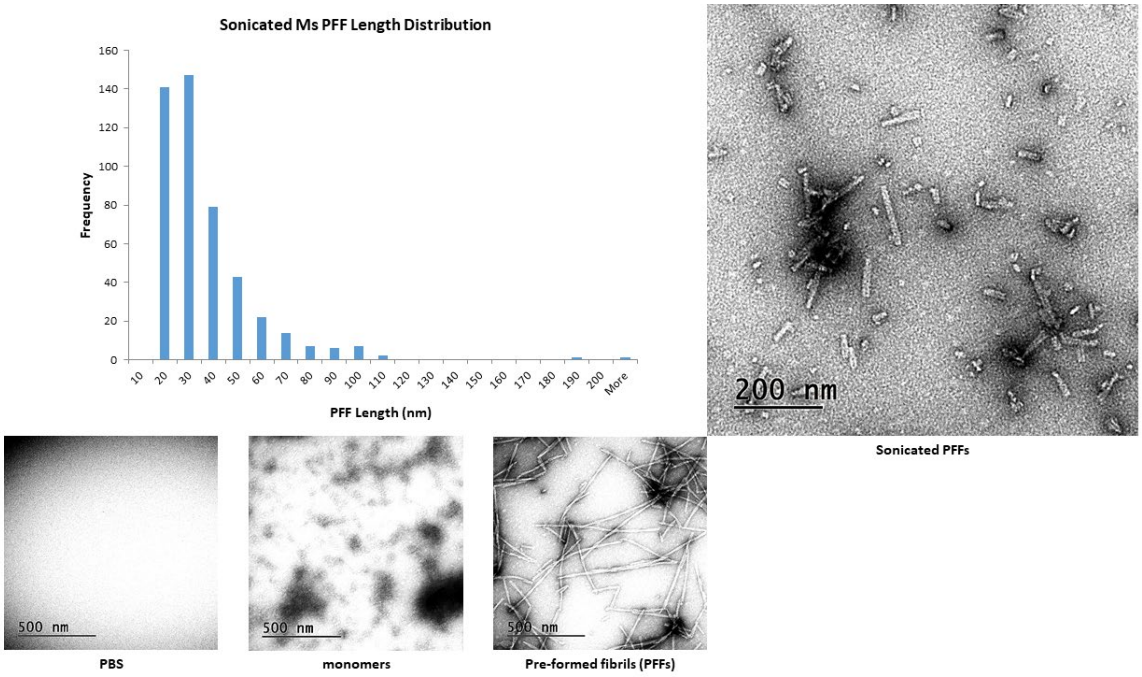

Fig. 1. Electron microscopy picture of α-syn fibrils used for the injections and quantification of the amount of fibrils with a certain length directly after sonication (*upper section*). The electron microscopy pictures show PBS solution only in which the protein is solved, monomeric α-syn and pre-formed fibrils before sonication (*lower section*).

Significance

Therapies for α-synucleinopathies including DLB should not only target α-syn pathology, but also improve symptoms that have an effect on the patients' daily life, in order to be considered for clinical use. This project will increase our knowledge of mechanisms central for α-syn aggregation and propagation, as well as provide us with a better understanding for symptom development during disease progression. In a longer perspective, such insights may lead to the identification of new targets for treatment of these diseases.

References

1. Kalia LV, Lang AE. Parkinson's disease. Lancet. 2015;386(9996):896-912.
2. Burre J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Sudhof TC. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science. 2010;329(5999):1663-7.
3. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science. 1997;276(5321):2045-7.
4. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, et al. alpha-Synuclein is phosphorylated in synucleinopathy lesions. Nat Cell Biol. 2002;4(2):160-4.
5. Lashuel HA, Overk CR, Oueslati A, Masliah E. The many faces of alpha-synuclein: from structure and toxicity to therapeutic target. Nat Rev Neurosci. 2013;14(1):38-48.
6. Danzer KM, Haasen D, Karow AR, Moussaud S, Habeck M, Giese A, et al. Different species of alpha-synuclein oligomers induce calcium influx and seeding. J Neurosci. 2007;27(34):9220-32.
7. Kayed R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, et al. Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science. 2003;300(5618):486-9.
8. van Rooijen BD, Claessens MM, Subramaniam V. Membrane binding of oligomeric alpha-synuclein depends on bilayer charge and packing. FEBS Lett. 2008;582(27):3788-92.
9. Karpinar DP, Balija MB, Kugler S, Opazo F, Rezaei-Ghaleh N, Wender N, et al. Pre-fibrillar alpha-synuclein variants with impaired beta-structure increase neurotoxicity in Parkinson's disease models. EMBO J. 2009;28(20):3256-68.
10. Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. Proc Natl Acad Sci U S A. 2011;108(10):4194-9.
11. Sharon R, Bar-Joseph I, Frosch MP, Walsh DM, Hamilton JA, Selkoe DJ. The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. Neuron. 2003;37(4):583-95.
12. Kramer ML, Schulz-Schaeffer WJ. Presynaptic alpha-synuclein aggregates, not Lewy bodies, cause neurodegeneration in dementia with Lewy bodies. J Neurosci. 2007;27(6):1405-10.
13. Paleologou KE, Kragh CL, Mann DM, Salem SA, Al-Shami R, Allsop D, et al. Detection of elevated levels of soluble alpha-synuclein oligomers in post-mortem brain extracts from patients with dementia with Lewy bodies. Brain. 2009;132(Pt 4):1093-101.
14. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997;388(6645):839-40.
15. Jucker M, Walker LC. Propagation and spread of pathogenic protein assemblies in neurodegenerative diseases. Nat Neurosci. 2018;21(10):1341-9.
16. Mougenot AL, Nicot S, Bencsik A, Morignat E, Verchere J, Lakhdar L, et al. Prion-like acceleration of a synucleinopathy in a transgenic mouse model. Neurobiol Aging. 2012;33(9):2225-8.
17. Luk KC, Kehm VM, Zhang B, O'Brien P, Trojanowski JQ, Lee VM. Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. J Exp Med. 2012;209(5):975-86.
18. Prusiner SB, Woerman AL, Mordes DA, Watts JC, Rampersaud R, Berry DB, et al. Evidence for alpha-synuclein prions causing multiple system atrophy in humans with parkinsonism. Proc Natl Acad Sci U S A. 2015;112(38):E5308-17.
19. Luk KC, Kehm V, Carroll J, Zhang B, O'Brien P, Trojanowski JQ, et al. Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science. 2012;338(6109):949-53.

FÖRDELNING AV KOSTNADER/SÖKT BELOPP

Antikroppar och reagenser	20 000 SEK
Kemikalier	20 000 SEK
Alfa-synuklein för injektioner	15 000 SEK
Förbrukningsmaterial för intrakraniella injektioner	35 000 SEK
Djurhållning	10 000 SEK
TOTALT ansökt belopp	**100 000 SEK**

Tidigare beviljade anslag för detta projekt för 2021: Stohnes stiftelse 10 000 SEK, Medicinska fakultetens stipendium för forskning 50 000 SEK. Anslag har sökts från Hedlunds stiftelse 500 000 SEK.

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Akademiska befattningar och utbildning

MEDICINE DOKTOR 2013-06-14, Examensämne: *Neurokirurgi*
Institutionen för neurovetenskap, enheten för neurokirurgi, Uppsala universitet.
Avhandlingens namn: *Cellular Reactions and Behavioral Changes in Focal and Diffuse Traumatic Brain Injury" – A study in the Rat and Mouse.* Handledare: Lars Hillered.

MAGISTEREXAMEN I BIOLOGI 2003-11-10
Uppsala universitet
Examensarbete: Apoptosis after Traumatic Brain Injury".

NUVARANDE POSITION

2017-
Forskare vid institutionen för folkhälso – och vårdvetenskap, enheten för molekylär geriatrik, Uppsala universitet.
Projekt: Studier av sjukdomsmekanismer och immunterapi vid synukleinopatier

HANDLEDARSKAP

Forskarnivå

2018- Biträdande handledare - Anish Behere, doktorand, Institutionen för folkhälso – och vårdvetenskap, enheten för molekylär geriatrik, Uppsala universitet.

Examensarbeten magister/mastersnivå

- 2018- Huvudhandledare för Sheyma Hassen, examensarbete apotekarprogrammet 30 hp
- 2017- Huvudhandledare för Anish Behere, examensarbete biomedicinarprogrammet 30 hp
- 2016- Huvudhandledare för Anish Behere, laboratorieprojekt biomedicinarprogrammet 30hp
- 2015 - Huvudhandledare - Nina Ågren, examensarbete apotekarprogrammet 30hp

PUBLIKATIONER

Författare till sju förhandsgranskade artiklar (varav fem som första författare och en som sista författare) samt två populärvetenskapliga artiklar. Ytterligare två artiklar har skickats in till

tidskrifter och är under granskning (varav en som sista författare). Fem manus är under slutbearbetning. För publikationslista, se nedan.

TIDIGARE ANSTÄLLNINGAR

2014-2017, (halvtidsanställning under 2014-2016)

Post doc vid institutionen för folkhälso – och vårdvetenskap, enheten för molekylär geriatrik, Uppsala universitet.
Projekt: Immunterapistudier vid Parkinsons sjukdom

2013-2016, halvtidsanställning

Koordinator vid Uppsala University Behavioral Facility (UUBF), vid institutionerna för neurovetenskap och farmaceutisk biovetenskap, Uppsala universitet. Som koordinator vid Uppsala universitets beteendeplattform, UUBF, arbetade jag med uppstart av plattformen 2014 samt stöd i planering, genomförande, analysering och tolkning av prekliniska beteendetester vid Uppsala universitet samt för externa klienter.

2014, deltidsanställning

Webbplatsansvarig vid institutionen för farmaceutisk biovetenskap

2004-2013

Doktorand vid institutionen för neurovetenskap, enheten för neurokirurgi, Uppsala universitet.
Projekt: Cellulära och beteendemässiga förändringar vid experimentell traumatisk hjärnskada.

2003-2004

Forskarassistent vid institutionen för neurovetenskap, enheten för neurokirurgi, Uppsala universitet.

FÖRÄLDRALEDIGHET/AVBROTT I FORSKNINGEN

- **Barn 1:** Juni 2004 – juni 2005, deltidsarbete under ht 2005
- **Barn 2:** April 2009 – maj 2010, deltidsarbete under ht 2010
- **Barn 3:** Oktober 2012 – april 2013, samt juli 2013-oktober 2013, arbete 25% under ht 2013. Föräldraledig 25% under vt 2014.
- Koordinator UUBF 2014-2016, halvtid.

PUBLIKATIONER (FEM SENASTE ÅREN)

Publicerade artiklar

- Age-related increase of alpha-synuclein oligomers is associated with motor disturbances in L61 transgenic mice. Roshanbin S, Aniszewska A, Gumucio A, Masliah E, Erlandsson A, Bergström J, Ingelsson M, **Ekmark-Lewén S.** *Neurobiology of Aging, 2021. doi: https://doi.org/10.1016/j.neurobiolaging.2021.01.010*
- Early fine motor impairment and behavioral dysfunction in transgenic mice expressing human alpha-synuclein with the A30P mutation. **Ekmark-Lewén S**, Lindström V, Gumucio A, Ihse E, Behere A, Erlandsson A, Kahle P, Nordström E, Eriksson M, Bergström J, Ingelsson M. *Beh Brain. 2018. doi: 10.1002/brb3.915. PMID: 29541535.*
- Diffuse traumatic axonal injury in mice induces complex behavioral alterations that are normalized by neutralization of interleukin-1β. **Ekmark-Lewén S**, Flygt J, Fridgeirsdottir GA, Kiwanuka O, Hånell A, Meyerson BJ, Mir AK, Gram H, Lewén A, Clausen F, Hillered L and Marklund N. *Eur J Neurosci. 2016 Apr;43(8):1016-33. doi: 10.1111/ejn.13190. PMID: 27091435.*

Populärvetenskapliga publikationer

- Immunterapi som framtida behandling vid neurodegenerativa sjukdomar. Ingelsson M, **Ekmark-Lewén S,** Sehlin D, Kilander L, Basun H, Lannfelt L. *Neurologi i Sverige (nr4 2019).*
- Aktuell geriatrisk forskning i Uppsala. Ingelsson M, Franzon K, Åberg AC, **Ekmark-Lewén S**, Giedraitis V. *Svensk geriatrik. 2018 (2) 9-12.*

Inskickade manus/under bearbetning

- Impact of midbrain α-synuclein pathology in the (Thy-1)-h[A30P]α-syn transgenic mouse model. Behere A, Thörnqvist PO, Winberg S, Kahle PJ, Ingelsson M, Bergström J, **Ekmark-Lewén S.** *Submitted to Journal of Neuroscience Research.*
- Accumulation of alpha-synuclein within the liver: a potential pathological feature in the pathogenesis of Parkinson's disease and related synucleinopathies. Reyes Juan F., **Ekmark-Lewén S**, Perdiki M, Klingstedt T, Hoffmann A, Wiechec E, Nilsson P, Nilsson K. R. P., Alafuzoff I, Ingelsson M, and Hallbeck M. *Submitted to J Clinical Investigation.*
- Amelioration of early cognitive symptoms is associated with reduced brain pathology in transgenic mice treated with monoclonal antibodies against α-synuclein protofibrils. **Ekmark-Lewén S**, Aniszewska A, Gumucio A, Lindström V, Nordström E, Eriksson M, Kahle P, Bergström J, Ingelsson M. *Manuscript to be submitted.*
    - Alpha-synuclein affects SNARE protein distribution. Persson E, Almandoz-Gil L, Rofo F, Goedkoop M, **Ekmark-Lewén S**, Ingelsson M, Bergström J. *Manuscript.*
- A novel proximity ligation assay recognizing phosphorylated α-syn reveals previously undetected α-syn pathology in synucleinopathy brains. Behere A., Ingelsson M, **Ekmark-Lewén S**, Bergsröm J. *Manuscript.*
- Preserved gene expression patterns in midbrain dopamine neurons of two different transgenic mouse lines over-expressing the human α-synuclein (SNCA) gene. Vlcek B, Dumas S, **Ekmark-Lewén S,** Ingelsson M and Wallén-Mackenzie Å. *Manuscript.*
- In vivo PET imaging of alpha-synuclein in a fibril deposition mouse model Roshanbin S, Xiong M, Hultqvist G, **Ekmark-Lewén S**, Ingelsson M, Bergström B, Sehlin D, Syvänen S. *Manuscript.*