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Königin-Elisabeth-Stiftung für Medizin
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Publicaties – Publications – Publikationen – Publications

2020

G.S.K.E. – F.M.R.E. – K.E.S.M. – Q.E.M.F.

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Projets de recherche interuniversitaire 2020–2022 subventionnés par la F.M.R.E.

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Universités ayant des programmes de recherche subventionnés par la F.M.R.E.

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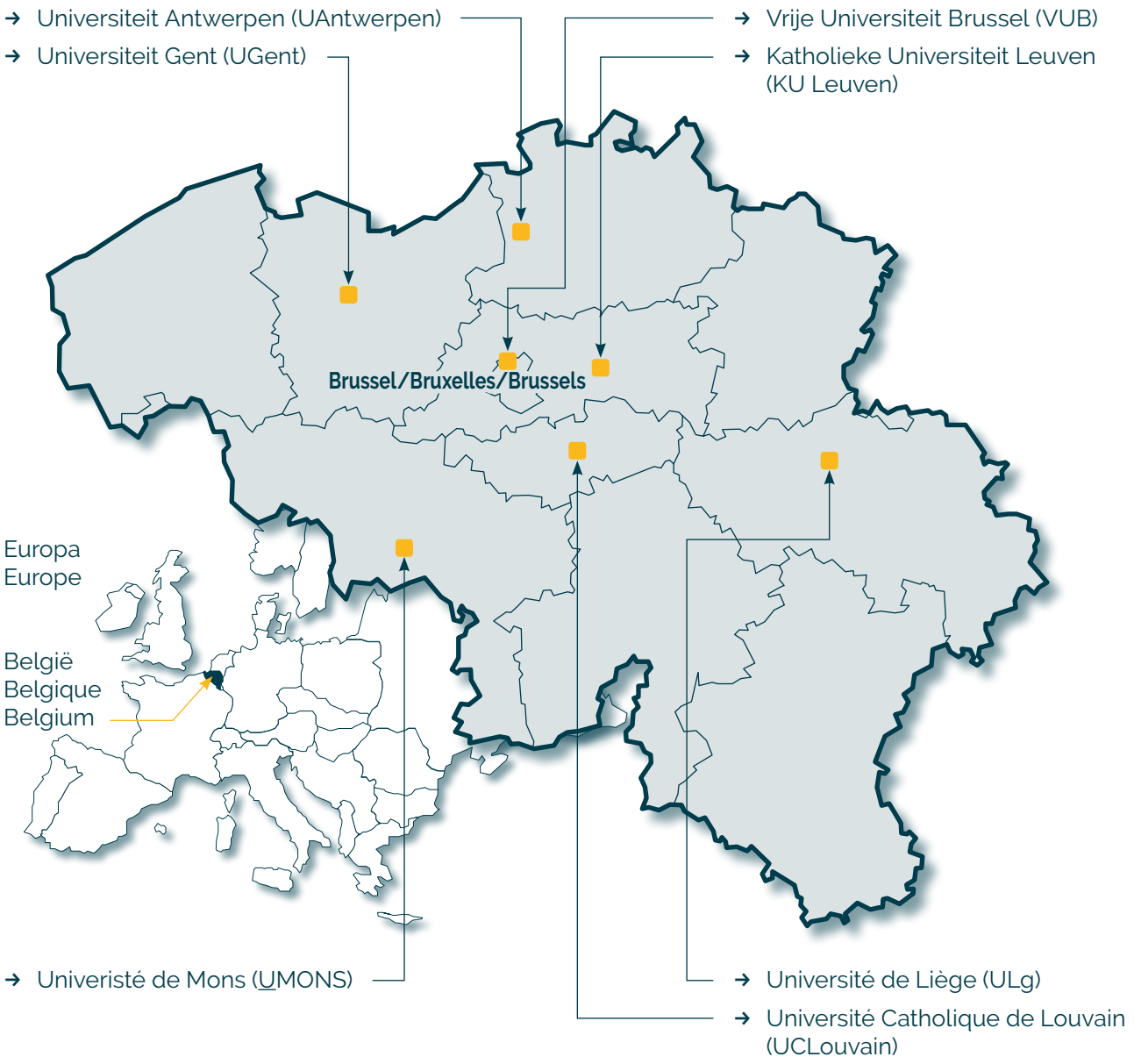

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Publicaties – Publications – Publikationen – Publications

VUB

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Publications in 2020 (first or last author)

- *Wiels W.,* ***Baeken C., Engelborghs S.***
  Depressive Symptoms in the Elderly – An Early Symptom of Dementia? A Systematic Review.
  Front Pharmacol. 2020;11:34. Published 2020 Feb 7. doi:10.3389/fphar.2020.00034.

Abstract

Background: Depression and dementia are common incapacitating diseases in old age. The exact nature of the relationship between these conditions remains unclear, and multiple explanations have been suggested: depressive symptoms may be a risk factor for, a prodromal symptom of, or a coincidental finding in dementia. They may even be unrelated or only connected through common risk factors. Multiple studies so far have provided conflicting results.

Objectives: To determine whether a systematic literature review can clarify the nature of the relation between depressive symptoms and dementia.

Methods: Using the Patient/Problem/Population, Intervention, Comparator, Outcome or PICO paradigm, a known framework for framing healthcare and evidence questions, we formulated the question "whether depressive symptoms in cognitively intact older adults are associated with a diagnosis of dementia later in life." We performed a systematic literature review of MEDLINE and PsycINFO in November 2018, looking for prospective cohort studies examining the aforementioned question.

Results: We critically analysed and listed 31 relevant papers out of 1,656 and grouped them according to the main hypothesis they support: depressive symptoms as a risk factor, not a risk factor, a prodromal symptom, both, or some specific other hypothesis. All but three studies used clinical diagnostic criteria for dementia alone (i.e., no biomarkers or autopsy confirmation). Several studies contain solid arguments for the hypotheses they support, yet they do not formally contradict other findings or suggested explanations and are heterogeneous.

Conclusions: The exact nature of the relationship between depressive symptoms and dementia in the elderly remains inconclusive, with multiple studies supporting both the risk factor and prodromal hypotheses. Some provide arguments for common risk factors. It seems unlikely that there is no connection at all. We conclude that at least in a significant part of the patients, depressive symptoms and dementia are related. This may be due to common risk factors and/or depressive symptoms being a prodromal symptom of dementia and/or depression being a risk factor for dementia. These causal associations possibly overlap in some patients. Further research is warranted to develop predictive biomarkers and to develop interventions that may attenuate the risk of "conversion" from depressive symptoms to dementia in the elderly.

Keywords: Alzheimer; aging; biomarkers; cognitive decline; dementia; depression.

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Publications with Q.E.M.F. acknowledgement (2020)

- *Van Horebeek L.,* ***Goris A.*** *(corresponding author)*
  Transcript-specific regulation in T-cells in multiple sclerosis susceptibility.
  European Journal of Human Genetics (invited Comment), 28 (7), 849-850. IF 3.65.

- *Janssens I. (corresponding author),* ***Cools N.*** *(2020)*
  Regulating the regulators: Is introduction of an antigen-specific approach in regulatory T cells the next step to treat autoimmunity?
  Cell Immunol; 358:104236. IF 4.078.

  Abstract
  In autoimmunity, the important and fragile balance between immunity and tolerance is disturbed, resulting in abnormal immune responses to the body's own tissues and cells. $CD4^+CD25^{hi}FoxP3^+$ regulatory T cells (Tregs) induce peripheral tolerance in vivo by means of direct cell-cell contact and release of soluble factors, or indirectly through antigen-presenting cells (APC), thereby controlling auto-reactive effector T cells. Based on these unique capacities of Tregs, preclinical studies delivered proof-of-principle for the clinical use of Tregs for the treatment of autoimmune diseases. To date, the first clinical trials using ex vivo expanded polyclonal Tregs have been completed. These pioneering studies demonstrate the feasibility of generating large numbers of polyclonal Tregs in a good manufacturing practices (GMP)-compliant manner, and that infusion of Tregs is well tolerated by patients with no evidence of general immunosuppression. Nonetheless, only modest clinical results were observed, arguing that a more antigen-specific approach might be needed to foster a durable patient-specific clinical cell therapy without the risk for general immunosuppression. In this review, we discuss current knowledge, applications and future goals of adoptive immune-modulatory Treg therapy for the treatment of autoimmune disease and transplant rejection. We describe the key advances and prospects of the potential use of T cell receptor (TCR)- and chimeric antigen receptor (CAR)-engineered Tregs in future clinical applications. These approaches could deliver the long-awaited breakthrough in stopping undesired autoimmune responses and transplant rejections.
  Keywords: Adoptive immune-modulatory regulatory T cell therapy; Autoimmunity; Chimeric antigen receptor; Immune modulation; Regulatory T cells; T cell receptor.

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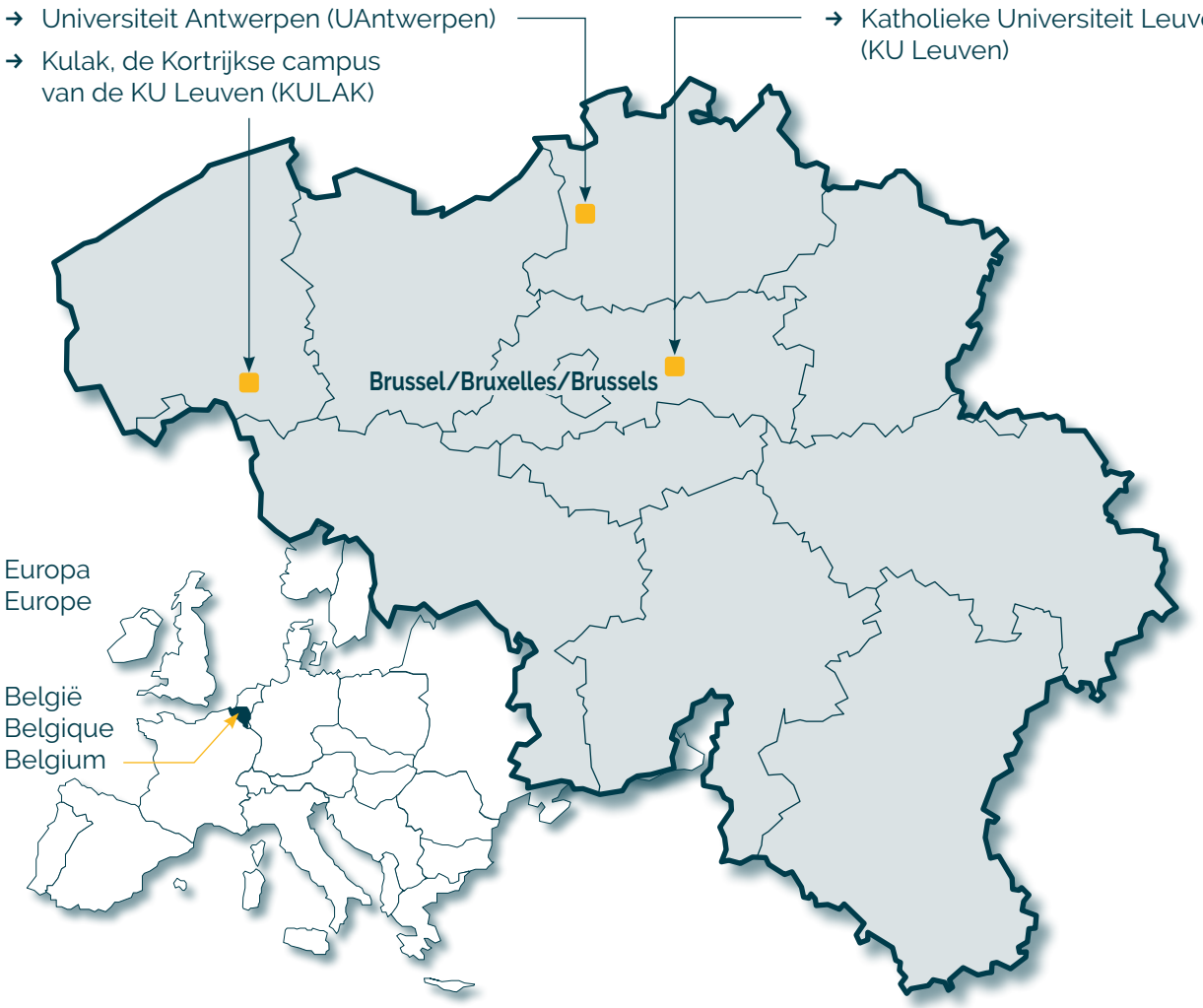

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Publicaties – Publications – Publikationen – Publications

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Publications with Q.E.M.F. acknowledgement (2020)

- *Denorme F., Martinod K., Vandenbulcke A., etal.*
  The von Willebrand FactorA1domainmediatesthromboinflammation, aggravating ischemic stroke outcome in mice.
  Haematologica. 2020. In press.

- *Staessens S.,* ***De Meyer SF.***
  Thrombus heterogeneity in ischemic stroke.
  Platelets. 2020. In press.

- *Staessens S.,* ***De Meyer SF.*** *(2020)*
  Thrombus heterogeneity in ischemic stroke.
  Platelets. doi: 10.1080/09537104.2020.1748586 (Impact factor: 3.38).
  Abstract
  The structure of stroke thrombi has gained an increasing amount of interest in recent years. The advent of endovascular thrombectomy has offered the unique opportunity to provide and analyze thrombi removed from ischemic stroke patients. It has become clear that the composition of ischemic stroke thrombi is relatively heterogenous and various molecular and cellular patterns become apparent. Good understanding of the histopathologic characteristics of thrombi is important to lead future advancements in acute ischemic stroke treatment. In this review, we give a brief overview of the main stroke thrombus components that have been recently characterized in this rapidly evolving field. We also summarize how thrombus heterogeneity can affect stroke treatment.
  Keywords: Stroke; Thrombectomy; Thrombosis; Thrombus.

- *Senna Staessens, Frederik Denorme, Olivier François, Linda Desender, Tom Dewaele, Peter Vanacker, Hans Deckmyn, Karen Vanhoorelbeke, Tommy Andersson and* ***Simon F. De Meyer***
  Structural analysis of ischemic stroke thrombi: histological indications for therapy resistance.
  Haematologica. 2020 Volume 105(2):498-507.
  Abstract
  Ischemic stroke is caused by a thromboembolic occlusion of cerebral arteries. Treatment is focused on fast and efficient removal of the occluding thrombus, either via intravenous thrombolysis or via endovascular thrombectomy. Recanalization, however, is not always successful and factors contributing to failure are not completely understood. Although the occluding thrombus is the primary target of acute treatment, little is known about its internal organization and composition. The aim of this study, therefore, was to better understand the internal organization of ischemic stroke thrombi on a molecular and cellular level. A total of 188 thrombi were collected from endovascularly treated ischemic stroke patients and analyzed histologically for fibrin, red blood cells (RBC), von Willebrand factor (vWF), platelets, leukocytes and DNA, using bright field and fluorescence microscopy. Our results show that stroke thrombi are composed of two main types of areas: RBC-rich areas and platelet-rich areas. RBC-rich areas have limited complexity as they consist of RBC that are entangled in a meshwork of thin fibrin. In contrast, platelet-rich areas are characterized by dense fibrin structures aligned with vWF and abundant amounts of leukocytes and DNA that accumulate around and in these platelet-rich areas. These findings are important to better understand why platelet-rich thrombi are resistant to thrombolysis and difficult to retrieve via thrombectomy, and can guide further improvements of acute ischemic stroke therapy.

- *Staessens S., Fitzgerald S., Andersson T., Clarençon F., Denorme F., Gounis MJ., Hacke W., Liebeskind DS., Szikora I., van Es A., Brinjikji W., Doyle KM.,* ***De Meyer SF.***
  Histological stroke clot analysis after thrombectomy: Technical aspects and recommendations.
  Int J Stroke. 2020 Jul;15(5):467-476.
  Abstract
  The recent advent of endovascular procedures has created the unique opportunity to collect and analyze thrombi removed from cerebral arteries, instigating a novel subfield in stroke research. Insights into thrombus characteristics and composition could play an important role in ongoing efforts to improve acute ischemic stroke therapy. An increasing number of centers are collecting stroke thrombi. This paper aims at providing guiding information on thrombus handling, procedures, and analysis in order to facilitate and standardize this emerging research field.
  Keywords: Stroke; clot; histology; thrombectomy; thrombus.

- *Senna Staessens, Olivier François, Waleed Brinjikji, Karen Doyle, Peter Vanacker, Tommy Andersson and* ***Simon De Meyer***
  Studying stroke thrombus composition after thrombectomy: what can we learn?
  Submitted in the journal 'Stroke'.
  Abstract
  The composition of ischemic stroke thrombi has gained an increasing amount of interest in recent years. The implementation of endovascular procedures in standard stroke care has granted researchers the unique opportunity to examine patient thrombus material. Increasing evidence indicates that stroke thrombi are complex and heterogenous, consisting of various biochemical (eg, fibrin, von Willebrand Factor, and neutrophil extracellular traps) and cellular (eg, red blood cells, platelets, leukocytes, and bacteria) components. This complex composition may explain therapeutic limitations and also offer novel insights in several aspects of stroke management. Better understanding of thrombus characteristics could, therefore, potentially lead to improvements in the management of patients with stroke. In this review, we provide a comprehensive overview of the lessons learned by examining stroke thrombus composition after endovascular thrombectomy and its potential relevance for thrombectomy success rates, thrombolysis, clinical outcomes, stroke etiology, and radiological imaging.
  Keywords: Fibrin; ischemic stroke; leukocytes; thrombectomy; von Willebrand Factor.

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Publications with Q.E.M.F. acknowledgement (2020)

- *Iwata R., Casimir P.,* ***Vanderhaeghen P.***
  Mitochondria dynamics in postmitotic cells regulate neurogenesis.
  Science 2020, 369 (6505):858-862.
  Abstract
  The conversion of neural stem cells into neurons is associated with the remodeling of organelles, but whether and how this is causally linked to fate change is poorly understood. We examined and manipulated mitochondrial dynamics during mouse and human cortical neurogenesis. We reveal that shortly after cortical stem cells have divided, daughter cells destined to self-renew undergo mitochondrial fusion, whereas those that retain high levels of mitochondria fission become neurons. Increased mitochondria fission promotes neuronal fate, whereas induction of mitochondria fusion after mitosis redirects daughter cells toward self-renewal. This occurs during a restricted time window that is doubled in human cells, in line with their increased self-renewal capacity. Our data reveal a postmitotic period of fate plasticity in which mitochondrial dynamics are linked with cell fate.

Key recent publications: Scientific output

- *Iwata R.,* ***Vanderhaeghen P.***
  Tempus fugit: How time flies during development.
  Science 2020 369 (6510):1431-1432.
  No abstract

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Publications with Q.E.M.F. acknowledgement (2020)

- *A.C.N. Freitas,* ***T. Voets***
  Why the emperor penguin reigns where elephants shiver.
  Cell Calcium 91 (2020) 102263.
  Abstract
  Accurate sensing of the environmental temperature is crucial for the survival and wellbeing of organisms. In vertebrates, the cold- and menthol-activated ion channel TRPM8 acts as the prime molecular sensor of cool temperatures. By comparing TRPM8 in vertebrates with different habitat temperatures, from elephants to penguins, Yang et al. identify key residues within the pore domain that determine the channel's cold sensitivity. Strikingly, mice engineered to express penguin TRPM8 show a remarkable tolerance to cold.

- *K. Held, V.D. Aloi, A.C.N. Freitas, A. Janssens, A. Segal, J. Przibilla, S.E. Philipp, Y.T. Wang,* ***T. Voets,*** *J. Vriens*
  Pharmacological properties of TRPM3 isoforms are determined by the length of the pore loop.
  Br J Pharmacol (2020).
  Abstract
  Background and purpose: Transient receptor potential melastatin 3 (TRPM3) is a non-selective cation channel that plays a pivotal role in the peripheral nervous system as a transducer of painful heat signals. Alternative splicing gives rise to several TRPM3 variants. The functional consequences of these splice isoforms are poorly understood. Here, the pharmacological properties of TRPM3 variants arising from alternative splicing in the pore-forming region were compared.
  Experimental approach: Calcium microfluorimetry and patch clamp recordings were used to compare the properties of heterologously expressed TRPM31 (long pore variant) and TRPM32-6 (short pore variants). Furthermore, site-directed mutagenesis was done to investigate the influence of the length of the pore loop on the channel function.
  Key results: All short pore loop TRPM3 variants (TRPM32-6) were activated by the neurosteroid pregnenolone sulphate (PS) and by nifedipine, whereas the long pore loop variant TRPM31 was insensitive to either compound. In contrast, TRPM31 was robustly activated by clotrimazole, a compound that does not directly activate the short pore variants but potentiates their responses to PS. Clotrimazole-activated TRPM31 currents were largely insensitive to established TRPM32 antagonists and were only partially inhibited upon activation of the μ opioid receptor. Finally, by creating a set of mutant channels with pore loops of intermediate length, we showed that the length of the pore loop dictates differential channel activation by PS and clotrimazole.
  Conclusion and implications: Alternative splicing in the pore-forming region of TRPM3 defines the channel's pharmacological properties, which depend critically on the length of the pore-forming loop.
  Keywords: Splice variants TRP channels; TRPM3; nociception.

- *M. Mulier, N. Van Ranst, N. Corthout, S. Munck, P. Vanden Berghe, J. Vriens,* ***T. Voets*** *(corresponding and shared last author), L. Moilanen*
Upregulation of TRPM3 in nociceptors innervating inflamed tissue.
Elife 9 (2020).
Abstract
Genetic ablation or pharmacological inhibition of the heat-activated cation channel TRPM3 alleviates inflammatory heat hyperalgesia, but the underlying mechanisms are unknown. We induced unilateral inflammation of the hind paw in mice, and directly compared expression and function of TRPM3 and two other heat-activated TRP channels (TRPV1 and TRPA1) in sensory neurons innervating the ipsilateral and contralateral paw. We detected increased Trpm3 mRNA levels in dorsal root ganglion neurons innervating the inflamed paw, and augmented TRP channel-mediated calcium responses, both in the cell bodies and the intact peripheral endings of nociceptors. In particular, inflammation provoked a pronounced increase in nociceptors with functional co-expression of TRPM3, TRPV1 and TRPA1. Finally, pharmacological inhibition of TRPM3 dampened TRPV1- and TRPA1-mediated responses in nociceptors innervating the inflamed paw, but not in those innervating healthy tissue. These insights into the mechanisms underlying inflammatory heat hypersensitivity provide a rationale for developing TRPM3 antagonists to treat pathological pain.
Keywords: TRP channels; inflammatory pain; mouse; neuroscience; sensory neurons.

- *M. Mulier, I. Vandewauw, J. Vriens,* ***T. Voets***
Reply to: Heat detection by the TRPM2 ion channel.
Nature 584(7820) (2020) E13-E15.
No abstract

- *E. Persoons, K. De Clercq, C. Van den Eynde, S. Pinto, K. Luyten, R. Van Bree, C. Tomassetti,* ***T. Voets,*** *J. Vriens*
Mimicking Sampson's Retrograde Menstrual Theory in Rats: A New Rat Model for Ongoing Endometriosis - Associated Pain.
Int J Mol Sci 21(7) (2020).
Abstract
Endometriosis is a prevalent gynecologic disease, defined by dysfunctional endometrium-like lesions outside of the uterine cavity. These lesions are presumably established via retrograde menstruation, i.e., endometrial tissue that flows backwards during menses into the abdomen and deposits on the organs. As ongoing pain is one of the main pain symptoms of patients, an animal model that illuminates this problem is highly anticipated. In the present study, we developed and validated a rat model for ongoing endometriosis-associated pain. First, menstrual endometrial tissue was successfully generated in donor rats, as validated by gross examination, histology and qPCR. Next, endometriosis was induced in recipient animals by intraperitoneal injection of menstrual tissue. This resulted in neuro-angiogenesis as well as established endometriosis lesions, which were similar to their human counterparts, since epithelial and stromal cells were observed. Furthermore, significant differences were noted between control and endometriosis animals concerning bodyweight and posture changes, indicating the presence of ongoing pain in animals with endometriosis. In summary, a rat model for endometriosis was established that reliably mimics the human pathophysiology of endometriosis and in which signs of ongoing pain were detected, thus providing a new research tool for therapy development.
Keywords: Endometriosis; menstrual tissue; menstruating rat; ongoing pain.

- *L. Vangeel, M. Benoit, Y. Miron, P.E. Miller, K. De Clercq, P. Chaltin, C. Verfaillie, J. Vriens,* ***T. Voets***
  Functional expression and pharmacological modulation of TRPM3 in human sensory neurons.
  Br J Pharmacol 177(12) (2020) 2683-2695.

Abstract

Background and purpose: The transient receptor potential (TRP) ion channel TRPM3 functions as a noxious heat sensor, plays a key role in acute pain sensation and inflammatory hyperalgesia in rodents. Despite its potential as a novel analgesic drug target, little is known about the expression, function and modulation in the humans.

Experimental approach: We studied TRPM3 in freshly isolated human dorsal root ganglion (hDRG) neurons and human stem cell-derived sensory (hSCDS) neurons. Expression was analysed at the mRNA level using RT-qPCR. Channel function was assessed using Fura-2-based calcium imaging and whole-cell patch-clamp recordings.

Key results: TRPM3 was detected at the mRNA level in both hDRG and hSCDS neurons. The TRPM3 agonists pregnenolone sulphate (PS) and CIM0216 evoked robust intracellular Ca^{2+} responses in 52% of hDRG and 58% of hSCDS neurons. Whole-cell patch-clamp recordings in hSCDS neurons revealed pregnenolone sulphate (PS)- and CIM0216-evoked currents exhibiting the characteristic current-voltage relation of TRPM3. PS-induced calcium responses in hSCDS neurons were reversed in a dose-dependent manner by the flavonoid isosakuranetin and by antiseizure drug primidone. Finally, the μ-opioid receptor agonist DAMGO and the $GABA_B$ receptor agonist baclofen inhibited PS-evoked TRPM3 responses in a subset of hSCDS neurons.

Conclusion and implications: These results provide the first direct evidence of functional expression of the pain receptor TRPM3 in human sensory neurons, largely mirroring the channel's properties observed in mouse sensory neurons. hSCDS neurons represent a valuable and readily accessible *in vitro* model to study TRPM3 regulation and pharmacology in a relevant human cellular context.

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Projecten jonge onderzoekers 2020–2022 gefinancierd door de G.S.K.E.

Projets de recherche de jeunes chercheurs 2020–2022 subventionnés par la F.M.R.E.

Research projects of young researchers 2020–2022 funded by the Q.E.M.F.

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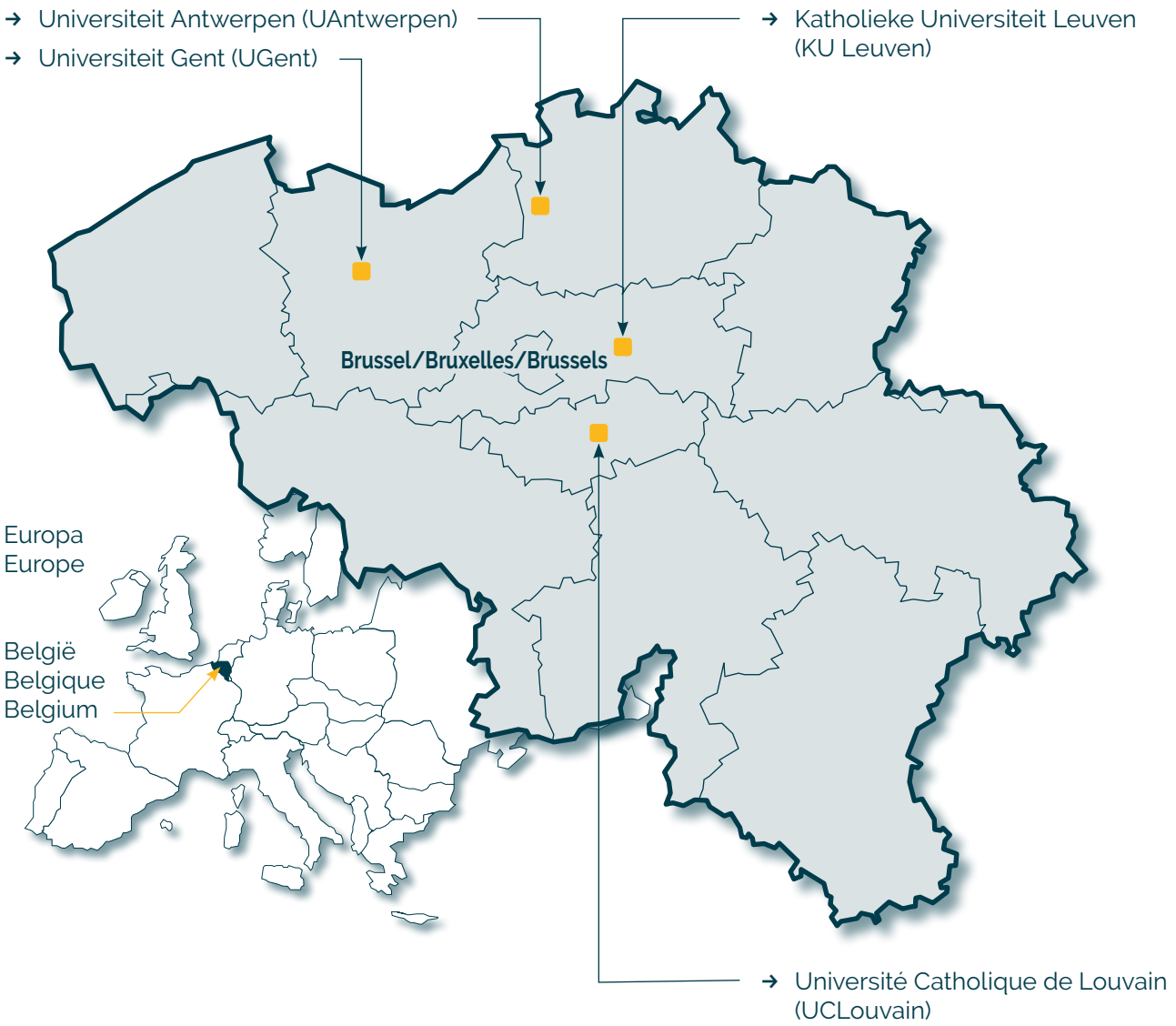

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Publicaties – Publications – Publikationen – Publications

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Publications with Q.E.M.F. acknowledgement (2020)

- *Mertens A., Naert L., Miatton M., Poppa T.,* ***Carrette E.****, Gadeyne S., Raedt R., Boon P., Vonck K.* ***Transcutaneous Vagus Nerve Stimulation Does Not Affect Verbal Memory Performance in Healthy Volunteers.***
  Front Psychol. 2020 Apr 15;11:551. doi: 10.3389/fpsyg.2020.00551. PMID: 32351421; PMCID: PMC7174665.

  Abstract

  Introduction: Invasive vagus nerve stimulation (VNS) improves word recognition memory in patients with epilepsy. Recent studies with transcutaneous VNS (tVNS) have also shown positive effects on various subdomains of cognitive functioning in healthy volunteers. In this randomized, controlled, crossover study, we investigated the effect of tVNS on a word recognition memory paradigm in healthy volunteers to further investigate the potential of tVNS in the treatment of cognitive disorders.

  Methods: We included 41 healthy participants aged between 18 and 30 years (young age group) and 24 healthy participants aged between 45 and 80 years (older age group). Each participant completed a word recognition memory paradigm during three different conditions: true tVNS, sham, and control. During true tVNS, stimulation was delivered at the cymba conchae. Sham stimulation was delivered by stimulating the earlobe. In the control condition, no stimulation was given. In each condition, participants were asked to remember highlighted words from three test paragraphs. Accuracy scores were calculated for immediate recall after each test paragraph and for delayed recognition at the end of the paradigm. We hypothesized that highlighted words from paragraphs in the true tVNS condition would be more accurately recalled and/or recognized compared to highlighted words from paragraphs in the sham or control condition.

  Results: In this randomized study, tVNS did not affect the accuracy scores for immediate recall or delayed recognition in both age groups. The younger group showed significantly higher accuracy scores than the older group. The accuracy scores improved over time, and the most recently learned words were better recognized. Participants rated true tVNS as significantly more painful; however, pain was not found to affect accuracy scores.

  Conclusion: In this study, tVNS did not affect verbal memory performance in healthy volunteers. Our results could not replicate the positive effects of invasive VNS on word recognition memory in epilepsy patients. Future research with the aim of improving cognitive function should focus on the rational identification of optimized and individualized stimulation settings primarily in patients with cognitive deficits.

  Keywords: Transcutaneous vagus nerve stimulation, verbal memory performance, word recognition memory paradigm, cognition, immediate recall, delayed recognition.

Publications with Q.E.M.F. acknowledgement (2020): in preparation

On behalf of tVNS-ConsensusGroup – Farmer AD, et al.International Consesnus Based Review and **Recommendations for Minimum Reporting Standards in Research on Transcutaneous Vagus Nerve Stimulation** (Version 2020).
Frontiers in Human Neuroscience

- *Stefanie Gadeyne,* ***Evelien Carrette****, Ann Mertens, Freek Van Den Bossche, Paul Boon, Robrecht Raedt and Kristl Vonck*
  Can we target cognition in healthy volunteers using transcutaneous auricular vagus nerve stimulation?
  (Paper in preparation).

- *Ann Mertens, Debby Klooster, Sofie Carrette, Emma Lescrauwaet,* ***Evelien Carrette****, Robrecht Raedt, Kristl Vonck, Paul Boon*
  High output current transcutaneous vagus nerve stimulation modulated cortical excitability in healthy participants: a TMS-EMG/EEG study.
  (Paper in preparation).

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Key recent publications: Scientific output (recently, a review was published related to this project)

- *Spanoghe J., **L. E. Larsen**, E. Craey, S. Manzella, A. Van Dycke, P. Boon and R. Raedt (2020)* ***The Signaling Pathways Involved in the Anticonvulsive Effects of the Adenosine A1 Receptor.*** *Int J Mol Sci* 22(1).

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Publicaties – Publications – Publikationen – Publications

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Key recent publications: Scientific output

- ***Hanseeuw B.J.**, Malotaux V., Dricot L., Quenon L., Sznajer Y., Cerman J., Woodard J.L., Buckley C., Farrar G., Ivanoiu A. and Lhommel R. (2020)*
  Defining a Centiloid scale threshold predicting long-term progression to dementia in patients attending the memory clinic: An [18F] flutemetamol amyloid PET study.
  European Journal of Nuclear Medicine and Molecular Imaging.

- *Mormont E., Bier J.C., Bruffaerts R., Cras P., DeDeyn P., Deryck O., Engelborghs S., Petrovic M., Picard G., Segers K., Thiery E., Versijpt J. and **Hanseeuw B.J.** (2020)*
  Practices and opinions about disclosure of the diagnosis of Alzheimer's disease to patients with MCI or dementia: A survey among Belgian medical experts in the field of dementia.
  Acta Neurologica Belgica.

- ***Hanseeuw B.J.**, Scott M.R., Sikkes S.A.M., Properzi M., Gatchel J.R., Salmon E., Marshall G.A. and Vannini P. (2020)*
  Evolution of anosognosia in alzheimer's disease and its relationship to amyloid.
  Annals of Neurology, 87(2), 267280.

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- ***Uytterhoeven V.*** *et al. (2020)*
  Increased HSC704/HSPA8 regulated autophagy reduces taumediated synaptic dysfunction.
  Alzheimer's Dement. 16 (suppl 3). Doi: 10.1002/alz.037892.

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Publications with Q.E.M.F. acknowledgement (2020)

- ***vandenBroeke EN.**, Vanmaele T., Mouraux A., Stouffs A., Biurrun-Manresa J. and Torta DM.*
  Perceptual correlates of homosynaptic long-term potentiation in human nociceptive pathways: a replication study R.
  Soc. Open. Sci. 2021; 8200830. http://doi.org/10.1098/rsos.200830.
  Abstract
  Animal studies have shown that high-frequency stimulation (HFS) of peripheral C-fibres induces long-term potentiation (LTP) within spinal nociceptive pathways. The aim of this replication study was to assess if a perceptual correlate of LTP can be observed in humans. In 20 healthy volunteers, we applied HFS to the left or right volar forearm. Before and after applying HFS, we delivered single electrical test stimuli through the HFS electrode while a second electrode at the contralateral arm served as a control condition. Moreover, to test the efficacy of the HFS protocol, we quantified changes in mechanical pinprick sensitivity before and after HFS of the skin surrounding both electrodes. The perceived intensity was collected for both electrical and mechanical stimuli. After HFS, the perceived pain intensity elicited by the mechanical pinprick stimuli applied on the skin surrounding the HFS-treated site was significantly higher compared to control site (heterotopic effect). Furthermore, we found a higher perceived pain intensity for single electrical stimuli delivered to the HFS-treated site compared to the control site (homotopic effect). Whether the homotopic effect reflects a perceptual correlate of homosynaptic LTP remains to be elucidated.
  Keywords: High-frequency stimulation; homotopic hyperalgesia; long-term potentiation.

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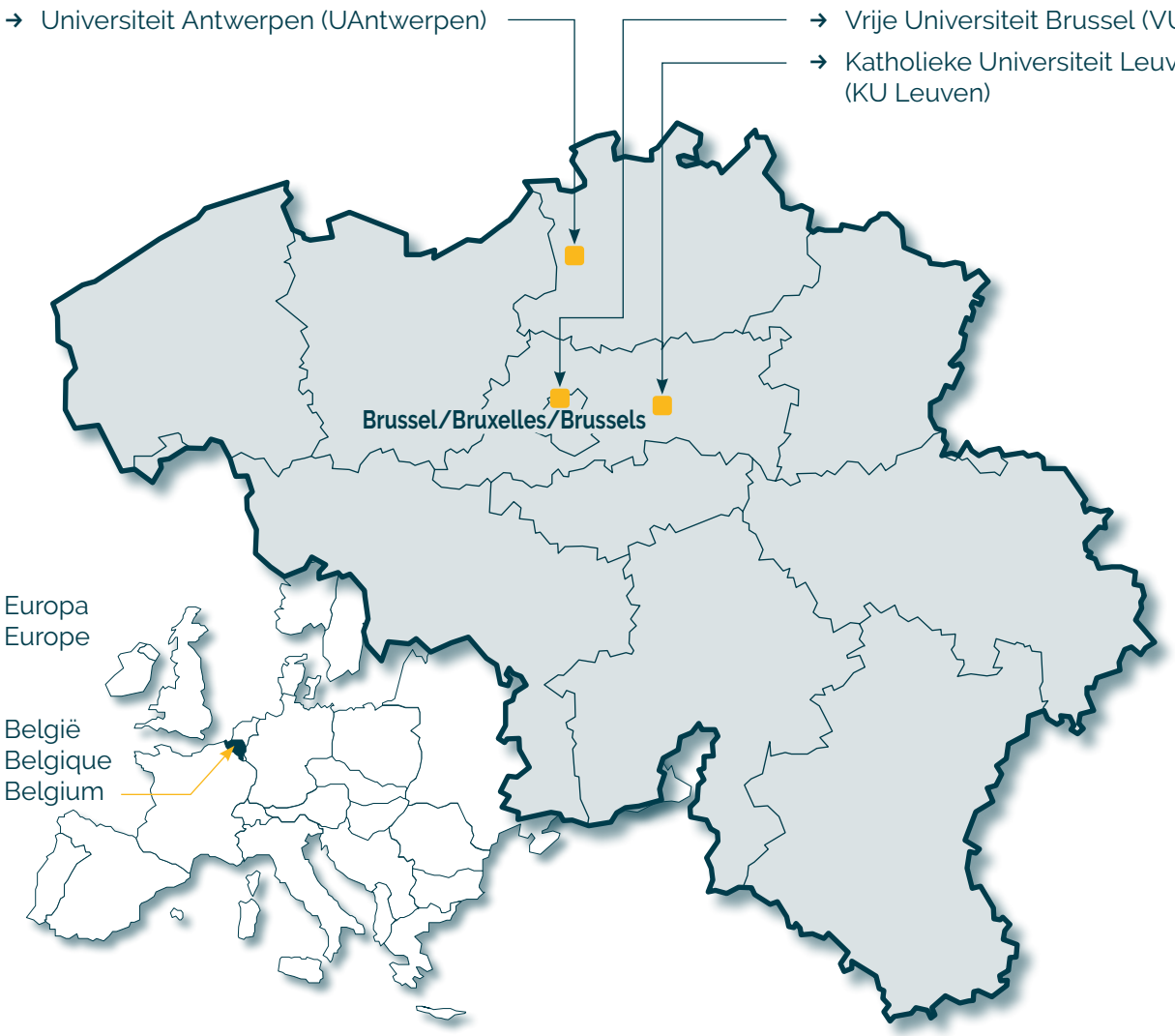

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Publications with Q.E.M.F. acknowledgement (2020)

- *Martin S., Smolders S., Van den Haute C., Heeman B., van Veen S., Crosiers D., Beletchi I., Verstraeten A., Gossye H., Gelders G., Pals P., Hamouda NN., Engelborghs S., Martin JJ., Eggermont J., De Deyn PP., Cras P.,* ***Baekelandt V.****, Vangheluwe P., Van Broeckhoven C. (2020)*
  Mutated ATP10B increases Parkinson's disease risk by compromising lysosomal glucosylceramide export.
  Acta Neuropathol., 139(6):1001-1024 (IF 18.2).
  The Elisabeth Medical Foundation for Neurosciences (PV) and Ernest Solvay Award (PV).
  Abstract
  Parkinson's disease (PD) is a progressive neurodegenerative brain disease presenting with a variety of motor and non-motor symptoms, loss of midbrain dopaminergic neurons in the substantia nigra pars compacta and the occurrence of -synuclein-positive Lewy bodies in surviving neurons. Here, we performed whole exome sequencing in 52 early-onset PD patients and identified 3 carriers of compound heterozygous mutations in the ATP10B P4-type ATPase gene. Genetic screening of a Belgian PD and dementia with Lewy bodies (DLB) cohort identified 4 additional compound heterozygous mutation carriers (6/617 PD patients, 0.97%; 1/226 DLB patients, 0.44%). We established that ATP10B encodes a late endo-lysosomal lipid flippase that translocates the lipids glucosylceramide (GluCer) and phosphatidylcholine (PC) towards the cytosolic membrane leaflet. The PD associated ATP10B mutants are catalytically inactive and fail to provide cellular protection against the environmental PD risk factors rotenone and manganese. In isolated cortical neurons, loss of ATP10B leads to general lysosomal dysfunction and cell death. Impaired lysosomal functionality and integrity is well known to be implicated in PD pathology and linked to multiple causal PD genes and genetic risk factors. Our results indicate that recessive loss of function mutations in ATP10B increase risk for PD by disturbed lysosomal export of GluCer and PC. Both ATP10B and glucocerebrosidase 1, encoded by the PD risk gene GBA1, reduce lysosomal GluCer levels, emerging lysosomal GluCer accumulation as a potential PD driver.
  Keywords: ATP10B P-type ATPase; Endo-lysosomal lipid flippase; Glucosylceramide; Loss-of-function; Massive parallel sequencing; Parkinson's disease.

- *Van der Perren A.*, Gelders G.*, Fenyi A.*, Bousset L., Brito F., Peelaerts W., Van den Haute C., Gentleman S., Melki R.*,* ***Baekelandt V.****** (2020)*
  The structural differences between patient-derived -synuclein strains dictate characteristics of Parkinson's disease, multiple system atrophy and dementia with Lewy bodies.
  Acta Neuropathologica, 139(6):977-1000. (IF 18.2).
  Abstract
  Synucleinopathies, such as Parkinson's disease (PD), multiple system atrophy (MSA), and dementia with Lewy bodies (DLB), are defined by the presence of -synuclein (SYN) aggregates throughout the nervous system but diverge from one another with regard to their clinical and pathological phenotype. The recent generation of pure fibrillar SYN polymorphs with noticeable differences in structural and phenotypic traits has led to the hypothesis that different SYN strains may be in part responsible for the heterogeneous nature of synucleinopathies. To further characterize distinct SYN strains in the human brain, and establish a structure-pathology relationship, we pursued a detailed comparison of SYN assemblies derived from well-stratified patients with distinct synucleinopathies. We exploited the capacity of SYN aggregates found in the brain of patients suffering from PD, MSA or DLB to seed and template monomeric human SYN *in vitro* via a protein misfolding cyclic amplification assay. A careful comparison of the properties of total brain homogenates and pure *in vitro* amplified SYN fibrillar assemblies upon inoculation in cells and in the rat brain demonstrates

that the intrinsic structure of SYN fibrils dictates synucleinopathies characteristics. We report that MSA strains show several similarities with PD strains, but are significantly more potent in inducing motor deficits, nigrostriatal neurodegeneration, SYN pathology, spreading, and inflammation, reflecting the aggressive nature of this disease. In contrast, DLB strains display no or only very modest neuropathological features under our experimental conditions. Collectively, our data demonstrate a specific signature for PD, MSA, and DLB-derived strains that differs from previously described recombinant strains, with MSA strains provoking the most aggressive phenotype and more similarities with PD compared to DLB strains.

Keywords: Neurodegenerative disorders; Strains; Synucleinopathies; -synuclein.

- *van Veen S.#, Martin S.#, Van den Haute C., Benoy V., Lyons J., Vanhoutte R., Kahler JP., Decuypere J-P., Gelders G., Lambie E., Swinnen JV., Annaert W., Agostinis P., Ghesquière B., Verhelst S.,* ***Baekelandt V.****, Eggermont J., Vangheluwe P. (2020)*

  ATP13A2 deficiency disrupts lysosomal polyamine export.

  Nature, 578 (7795):419-424 (IF 43.1).

  Abstract

  ATP13A2 (PARK9) is a late endolysosomal transporter that is genetically implicated in a spectrum of neurodegenerative disorders, including Kufor-Rakeb syndrome-a parkinsonism with dementia[1]-and early-onset Parkinson's disease[2]. ATP13A2 offers protection against genetic and environmental risk factors of Parkinson's disease, whereas loss of ATP13A2 compromises lysosomes3. However, the transport function of ATP13A2 in lysosomes remains unclear. Here we establish ATP13A2 as a lysosomal polyamine exporter that shows the highest affinity for spermine among the polyamines examined. Polyamines stimulate the activity of purified ATP13A2, whereas ATP13A2 mutants that are implicated in disease are functionally impaired to a degree that correlates with the disease phenotype. ATP13A2 promotes the cellular uptake of polyamines by endocytosis and transports them into the cytosol, highlighting a role for endolysosomes in the uptake of polyamines into cells. At high concentrations polyamines induce cell toxicity, which is exacerbated by ATP13A2 loss due to lysosomal dysfunction, lysosomal rupture and cathepsin B activation. This phenotype is recapitulated in neurons and nematodes with impaired expression of ATP13A2 or its orthologues. We present defective lysosomal polyamine export as a mechanism for lysosome-dependent cell death that may be implicated in neurodegeneration, and shed light on the molecular identity of the mammalian polyamine transport system.

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Publications with Q.E.M.F. acknowledgement (2020)

- *Elias Adriaenssens, Barbara Tedesco, Laura Mediani, Bob Asselbergh, Valerie Crippa, Francesco Antoniani, Serena Carra, Angelo Poletti,* ***Vincent Timmerman***
  BAG3 Pro209 mutants associated with myopathy and neuropathy relocate chaperones of the CASA-complex to aggresomes.
  Scientific Reports 2020;10(1):8755. PMID: 32472079;
  Impact Factor: 3.998;
  OPEN ACCESS PUBLICATIE: DOI: 10.1038/s41598-020-65664-z
  Abstract
  Three missense mutations targeting the same proline 209 (Pro209) codon in the co-chaperone Bcl2-associated athanogene 3 (BAG3) have been reported to cause distal myopathy, dilated cardiomyopathy or Charcot-Marie-Tooth type 2 neuropathy. Yet, it is unclear whether distinct molecular mechanisms underlie the variable clinical spectrum of the rare patients carrying these three heterozygous Pro209 mutations in BAG3. Here, we studied all three variants and compared them to the BAG3_Glu455Lys mutant, which causes dilated cardiomyopathy. We found that all BAG3_Pro209 mutants have acquired a toxic gain-of-function, which causes these variants to accumulate in the form of insoluble HDAC6- and vimentin-positive aggresomes. The aggresomes formed by mutant BAG3 led to a relocation of other chaperones such as HSPB8 and Hsp70, which, together with BAG3, promote the so-called chaperone-assisted selective autophagy (CASA). As a consequence of their increased aggregation-proneness, mutant BAG3 trapped ubiquitinylated client proteins at the aggresome, preventing their efficient clearance. Combined, these data show that all BAG3_Pro209 mutants, irrespective of their different clinical phenotypes, are characterized by a gain-of-function that contributes to the gradual loss of protein homeostasis.

- *Vendredy L., Adriaenssens E.,* ***Timmerman V.***
  Small heat shock proteins in neurodegenerative diseases.
  Cell Stress and Chaperones 2020;25(4):679-699. PMID: 32323160;
  Impact Factor: 2.760;
  OPEN ACCESS PUBLICATIE: DOI: 10.1007/s12192-020-01101-4.
  Abstract
  Small heat shock proteins are ubiquitously expressed chaperones, yet mutations in some of them cause tissue-specific diseases. Here, we will discuss how small heat shock proteins give rise to neurodegenerative disorders themselves while we will also highlight how these proteins can fulfil protective functions in neurodegenerative disorders caused by protein aggregation. The first half of this paper will be focused on how mutations in HSPB1, HSPB3, and HSPB8 are linked to inherited peripheral neuropathies like Charcot-Marie-Tooth (CMT) disease and distal hereditary motor neuropathy (dHMN). The second part of the paper will discuss how small heat shock proteins are linked to neurodegenerative disorders like Alzheimer's, Parkinson's, and Huntington's disease.
  Keywords: Diseases of the central nervous system; Hereditary peripheral neuropathies; Neurodegeneration; Protein aggregation; Small heat shock proteins.

- *Reid Alderson, Elias Adriaenssens, Bob Asselbergh, Iva Pritišanac, Jonas Van Lent, Heidi Y. Gastall, Marielle A. Wälti, John M. Louis,* ***Vincent Timmerman****, Andrew J. Baldwin*, Justin L.P. Benesch**

  The neuropathy-causing P182L mutation dysregulates 2 interactions of HSP27.

  EMBO Journal, in final revision. * corresponding authors (IF: 9.963)

  Abstract

  HSP27 is a human molecular chaperone that forms large, dynamic oligomers and functions in many aspects of cellular homeostasis. Mutations in HSP27 cause Charcot-Marie-Tooth (CMT) disease, the most common inherited disorder of the peripheral nervous system. A particularly severe form of CMT disease is triggered by the P182L mutation in the highly conserved IxI/V motif of the disordered C-terminal region, which interacts weakly with the structured core domain of HSP27. Here, we observed that the P182L mutation disrupts the chaperone activity and significantly increases the size of HSP27 oligomers formed in vivo, including in motor neurons differentiated from CMT patient-derived stem cells. Using NMR spectroscopy, we determined that the P182L mutation decreases the affinity of the HSP27 IxI/V motif for its own core domain, leaving this binding site more accessible for other IxI/V-containing proteins. We identified multiple IxI/V-bearing proteins that bind with higher affinity to the P182L variant due to the increased availability of the IxI/V-binding site. Our results provide a mechanistic basis for the impact of the P182L mutation on HSP27 and suggest that the IxI/V motif plays an important, regulatory role in modulating protein-protein interactions.

  Keywords: NMR spectroscopy; charcot-marie-tooth disease; intrinsically disordered regions; molecular chaperones; short linear motif.

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Publications with Q.E.M.F. acknowledgement (2020)

- *De Prins A., Allaoui W., Medrano M., Van Eeckhaut A., Ballet S., Smolders I.,* ***De Bundel D.***
  Effects of neuromedin U-8 on stress responsiveness and hypothalamus-pituitary-adrenal axis activity in male C57BL/6J mice.
  Horm Behav. 2020 May;121:104666. doi: 10.1016/j.yhbeh.2019.104666. Epub 2020 Mar 2.;
  PMID: 31899262;
  Impact Factor: 3.684 (2019)

  Abstract
  Neuromedin U (NMU) is a highly conserved neuropeptide that has been implicated in the stress response. To better understand how it influences various aspects of the stress response, we studied the effects of intracerebroventricular NMU-8 administration on stress-related behavior and activity of the hypothalamus-pituitary-adrenal (HPA) axis in male C57BL/6J mice. We investigated these NMU-8 effects when mice remained in their home cage and when they were challenged by exposure to forced swim stress. NMU-8 administration resulted in increased grooming behavior in mice that remained in their home cage and in a significant increase in c-Fos immunoreactivity in the paraventricular hypothalamus (PVH) and arcuate nucleus (ARC). Surprisingly, NMU-8 administration significantly decreased plasma corticosterone concentrations. Furthermore, NMU-8 administration increased immobility in the forced swim test in both naïve mice and mice that were previously exposed to swim stress. The effect of NMU-8 on c-Fos immunoreactivity in the PVH was dependent on previous exposure to swim stress given that we observed no significant changes in mice exposed for the first time to swim stress. In contrast, in the ARC we observed a significant increase in c-Fos immunoreactivity regardless of previous stress exposure. Interestingly, NMU-8 administration also significantly decreased plasma corticosterone concentrations in mice that were exposed to single forced swim stress, while this effect was no longer observed when mice were exposed to forced swim stress for a second time. Taken together, our data indicate that NMU-8 regulates stress responsiveness and suggests that its effects depend on previous stress exposure.
  Keywords: Arcuate nucleus (ARC); C-Fos immunoreactivity; Forced swim test; Hypothalamus-pituitary-adrenal axis; Neuromedin U (NMU); Paraventricular nucleus (PVH); Stress-related behavior.

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