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Final report of the research group of

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Team members who have contributed to this GSKE project report:
Postdoc: Manisha Juneja, PhD (period covered 2017-2018)
PhD students: Michiel Krols (2017), Mansour Haidar (2018), Elias Adriaenssens (2017-2019), Leen Vendredy (2018-2019), Angela Sisto (2019) and Jonas Van lent (2019)
Lab technician: Vicky De Winter (2017-2019)

Unravelling the novel molecular pathways contributing to distal Hereditary Motor Neuropathy caused by mutant HSPB8, with the aim to identify potential therapeutic targets

1. Research report:

1.1. State of the art:

Charcot-Marie-Tooth (CMT) neuropathies comprise a clinically diverse and genetically heterogeneous group of monogenic disorders affecting the peripheral nervous system. It is a progressive debilitating condition that can result in muscle weakness, sensory loss, foot deformities, fatigue, pain and injury. Found in both genders and in all populations, it affects an estimated 1 in 2500 (or 2.8 million) people worldwide. An important group of genes mutated in CMT are those coding for small heat shock proteins (HSPBs). Although constitutively expressed by every cell, mutations in three of these HSPBs cause exclusive degeneration of the peripheral nervous system. As molecular chaperones, HSPBs are regulated by various types of stress and responsible for protein quality control and protein folding. In addition, they play a role in many essential cellular processes such as apoptosis, autophagy, splicing and translation, cytoskeleton dynamics and neuronal survival.

1.2. Aims of the GSKE project 2017-2018-2019:

The development of induced pluripotent stem cells (iPSC) has brought together the genetic accuracy of a patient-derived model and the possibility of having the disease-specific cell type. This model promises to influence modern medicine and drug development particularly for neurological disorders by providing an unlimited access to patient-derived neurons. We will take advantage of the iPSC model along with a knock-in/knock-out mouse model that we have recently developed for distal hereditary motor neuropathy (dHMN), caused by mutations in the small heat shock protein HSPB8, to identify and validate translationally relevant pathway(s) leading to axonal degeneration, and with the ambition to select and test promising therapeutic targets.

1.3. Results obtained:

We made a survey of all published mutations in HSPBs; so far 32 mutations occur in HSPB1 and were reported in 169 patients, nine mutations have been described in HSPB8 in 68 patients, and one mutation in HSPB3 in two patients. The phenotypic spectrum of HSPB mutations may not be restricted to axonal CMT disease (CMT2) or distal hereditary motor neuropathy (dHMN), but mutations were also reported in three sporadic patients with amyotrophic lateral sclerosis (ALS) or distal myopathy. This indicates that mutations in multifunctional HSPBs can give rise to multiple neurodegenerative and neuromuscular phenotypes (Adriaenssens et al 2017).

Since we first described the K141N missense mutation in the *HSPB8* gene (Irobi et al 2004), **we and others reported additional patients and families with *HSPB8* mutations** (Echaniz-Laguna et al 2017a). Interestingly, most mutations target the same lysine residue (K141E, K141M, K141N, K141T) in the highly conserved α-crystallin domain of the HSPB8 protein. The spectrum of diseases caused by mutations in the *HSPB8* gene was recently expanded to myofibrillar myopathy (MFM) (Adriaenssens et al 2017, Echaniz-Laguna et al 2017b, Ghaoui et al 2016). **To delineate the molecular deficits and functional consequences of *HSPB8* mutations, we generated a knock-in mouse model for the K141N mutation mimicking the dHMN phenotype** (Bouhy et al 2018). We observed that homozygous knock-in mice (*Hspb8*$^{K141N/K141N}$) develop a progressive axonopathy resulting in locomotor deficits. At the ultrastructural level, mice accumulate the mutant Hspb8 protein and display degenerative patterns similar to dHMN patients. Interestingly, these animals also develop a progressive MFM phenotype as observed in some patients with HSPB8 mutations (Ghaoui et al 2016).

Our mouse model allowed us to also generate a *Hspb8* knock-out using the same targeting construct. The homozygous *Hspb8* knock-out animals (*Hspb8*$^{-/-}$) do not show any sign of axonopathy and display only minor (subclinical) muscle irregularities. The knock-out mice are indistinguishable from the wild type mice on the Rotarod and therefore present with a much milder phenotype than the *Hspb8* knock-in animals (Bouhy et al 2018). Although we did not expect that deletion of *Hspb8* would be so well tolerated, this may actually offer an attractive therapeutic strategy. Based on these findings, we expect that reducing the expression of Hspb8 in the knock-in mice may improve the dHMN and MFM phenotype (i.e. phenocopying the *Hspb8*$^{-/-}$ mouse). PhD student L. Vendredy was attracted thanks to this GSKE project within our research team and she obtained a competitive FWO-SB fellowship (starting from 1/1/2018) to explore this approach.

To this end, Leen Vendredy tested compounds from a high-throughput screen that was performed in the lab of our collaborator Prof. A. Poletti (Crippa et al 2016). In this published screen, an FDA/EMA approved library was used to identify compounds that, as a side-effect, up- or down-regulate the expression of human HSPB8. **As we aim to downregulate the expression of HSPB8, we performed a dose-response study for the top 10 expression inhibitors.** In HeLa cells, meclofenamate sodium (MFS) treatment resulted in a dose-dependent decrease of HSPB8. Unfortunately, the drug was unable to reproduce and inhibit the HSPB8 expression in NSC34 or C2C12 cells (two murine cell lines). The high-throughput screen mentioned above was performed with the human HSPB8 gene promoter, and although these drugs are working for human cell lines, these might not be effective for the mouse *Hspb8* promoter. Since our aim is to validate these drugs on our published *Hspb8* mouse model, it is a prerequisite for them to be effective on the mouse *Hspb8* promoter. **We therefore started with the development of a novel reporter line that can be used for a new high-throughput screen.** The new screen will be performed on mouse embryonal fibroblasts (MEF) derived from the abovementioned mouse model (CRISPR/Cas9-edited to tag Hspb8 with eGFP-P2A-mCherry) ensuring that the identified compounds are effective on both human and murine cells. We are currently looking for funding sources that would help us to cover the costs of the new high-throughput screening and allow us to embark this last part of the project. Lead compounds that are identified in the high-throughput screen will be validated *in vivo* in the Hspb8 knock-in mouse model as well as on human iPSC-derived motor neurons, thereby ensuring the translation to human.

The careful characterization of the mouse model helped us to identify a first therapeutic target for HSPB8-associated forms of CMT disease. However, **with the help of this GSKE-funding, we were able to identify two more pathways which are altered by mutant HSPB8 and which could form interesting therapeutic targets**. What is appealing about these two additional targets is that they are not only affected by mutations in HSPB8 but also by mutations in HSPB1, another member of the HSPB family. As such, targeting these molecular pathways may allow us to develop a single therapeutic approach that would benefit a spectrum of HSPB-associated CMT patients.

The first shared pathway we identified, highlighted in a review of emerging common pathomechanisms in inherited peripheral neuropathies, is autophagy (Haidar & Timmerman 2017). **Mutations in both HSPB1 and HSPB8 impair the formation and degradation of autophagosomes** (Guilbert et al 2018, Haidar et al 2019). Our molecular work showed that the function of HSPB1 and HSPB8 converges at the formation of *sequestosome 1* (SQSTM1/p62) puncta. The SQSTM1/p62 acts as a selective receptor for ubiquitinated proteins and clusters them in small dense round formations (known as p62 bodies). These p62 bodies undergo phase-separation and thereby promote the engulfment of these toxic aggregates by autophagic membranes. After the engulfment, the cargo is degraded by autophagosomes which fuse with lysosomes. **Our results show that both HSPB1 and HSPB8 interact and/or promote p62-puncta formation and thereby regulate one of the initial steps of autophagosome formation.** CMT causing mutations in HSPB1 and HSPB8 impair this function and lead to a decreased autophagic

flux which could be detrimental for terminally differentiated cells like motor neurons. We found that in both our models, iPSC-derived motor neurons and the knock-in *Hspb8* mouse model, the autophagic pathway was impaired. As this is the first time that the two HSPBs, HSPB1 and HSPB8, evidently share the same pathomechanism (as their activities merge in SQSTM1/p62 modulation), this pathway arises as a promising new drug target for the group of HSPB-related CMT patients.

Thanks to the GSKE project we attracted Angela Sisto as a second PhD student who will proceed with this project and screen for FDA/EMA-approved molecules that are able to reverse the autophagic deficits caused by mutant HSPB1 and HSPB8. We will validate the effect of the selected drugs in motor neurons differentiated from patient-derived iPSCs with the aim to improve the neurodegenerative phenotype. In the perspective of a preclinical study, we also crossed-bred the knock-in *Hspb8* mouse model with the GFP-LC3 mouse (Mizushima 2009), a standard model to monitor autophagy in mammals, to obtain a *Hspb8/GFP-LC3* double transgenic mouse model. This not only allows us to expand the knowledge on the mouse phenotype regarding the autophagic deficits in the murine tissues (muscles and peripheral nerves), it also provides a powerful platform to validate the most effective compound *in vivo.* In parallel with the drug screening, we will characterize the molecular interplay further, thereby providing insights in the mechanism-of-action of new drugs. So Angela Sisto, who recently also obtained a competitive FWO-SB fellowship (starting from 1/11/2019), will proceed to explore autophagy as a novel target and the first shared pathomechanism between these two genes. **Thanks to the support of the GSKE-grant we have every model available to proceed in this direction and we hope to identify a small molecule in the high-throughput screen that can reverse the autophagic deficits.**

In addition to autophagy, we identified one more pathomechanism that is shared by both HSPBs. Data obtained with the support of a previous GSKE-grant demonstrated that mitochondrial transport was severely compromised in a mutant HSPB1 transgenic mouse model (in collaboration with Prof. L. Van Den Bosch, KULeuven). In this study we were able to identify a potential novel treatment for HSPB1-related neuropathy as restoration of mitochondrial transport led to amelioration of the phenotype (d'Ydewalle et al 2011). However, why abundant cytosolic chaperones like HSPB1 would cause a mitochondrial transport defect remained enigmatic. The work from PhD student Elias Adriaenssens now identified a possible answer to this question. **Unexpectedly, HSPBs were found to translocate into mitochondria**. This raised the possibility that mutations in HSPB1 are the direct cause of mitochondrial dysfunction. Indeed, mutations in the highly conserved alpha-crystallin domain of HSPB1 were found to increase mitochondrial residence as a consequence of binding stronger to molecular client proteins inside mitochondria. A mutation (P182L) in the C-terminal domain of HSPB1, which is associated with one of the most severe dHMN phenotypes, even prohibited HSPB1 from being imported into the mitochondria. These results may thus form part of the explanation as for why restoring mitochondrial transport was beneficial in a mutant HSPB1 mouse model (full data are reported in the PhD thesis of Elias Adriaenssens in December 2019, and a manuscript is in preparation).

Our data suggest that HSPB8 is also imported into the mitochondria and mitochondrial transport defects may thus also form a shared disease mechanism. Indeed, patient-derived iPSC-lines show a clear axonal transport problem in HSPB8 mutant neuronal cells (**Figure 1A**). Moreover, in addition to a deficit in their axonal transport, also the morphology of the mitochondria is altered (higher circularity and higher solidity compared to healthy control lines) (**Figure 1B**). Also in our knock-in *Hspb8* mouse, mitochondrial abnormalities were reported further supporting the notion that mitochondrial deficits are an underlying pathomechanism. Given the positive results obtained in a transgenic mutant *HSPB1* mouse model, where we found in collaboration with Prof. L. Van Den Bosch (KULeuven) that the rescue of axonal transport led to an amelioration of the phenotype, we are encouraged to explore this pathway further and evaluate whether restoration of axonal transport in the *Hspb8* knock-in mice can also ameliorate the neuropathy phenotype.

1.4. Outlook for the future:

In summary, where we still had to identify molecular targets for HSPB8-associated CMT at the start of this GSKE-project, **we now have identified three potential therapeutic targets (Figure 2)**. The successful identification of these molecular pathways and therapeutic targets forms a big leap forward for this untreatable disease. With the planned high-throughput screens we hope to identify compounds that can either downregulate the expression of HSPB8, rescue the autophagic deficits, or rescue the mitochondrial defects and as such allow us to test the first treatment strategies for HSPB8-related CMT in our new disease models (knock-in mouse model and patient iPSC-derived motor neurons). The successful identification of such compounds will accelerate the therapy development for this rare disorder and may finally bring the first therapies towards the clinical stage.

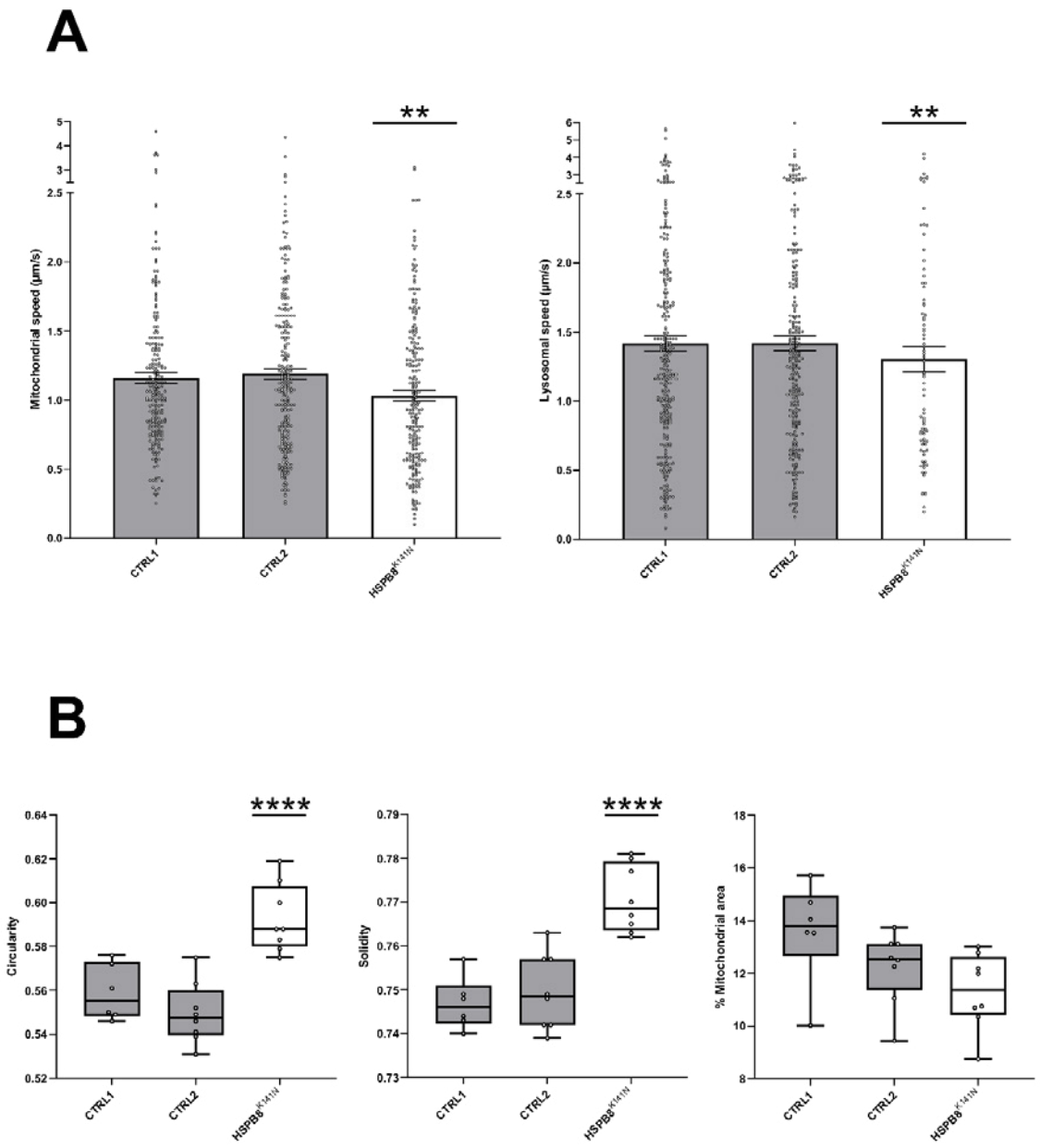

Figure 1: **Characterization of derived motor neurons from the HSPB8^{K141N} patient iPSC line.** (A) Quantification of mitochondrial (left) and lysosomal (right) speed (µm/s). (B) Quantification of mitochondrial morphology parameters: circularity (left), solidity (middle) and % mitochondrial area (right). Data values represent mean ± SEM. One-way ANOVA with post-hoc Dunnett test is used to compare the patient line with the average of both two controls; *, **, *** for *P* values of 0.05, 0.01, and 0.001, respectively (*unpublished results generated by PhD student Jonas Van lent*).

Graphical summary

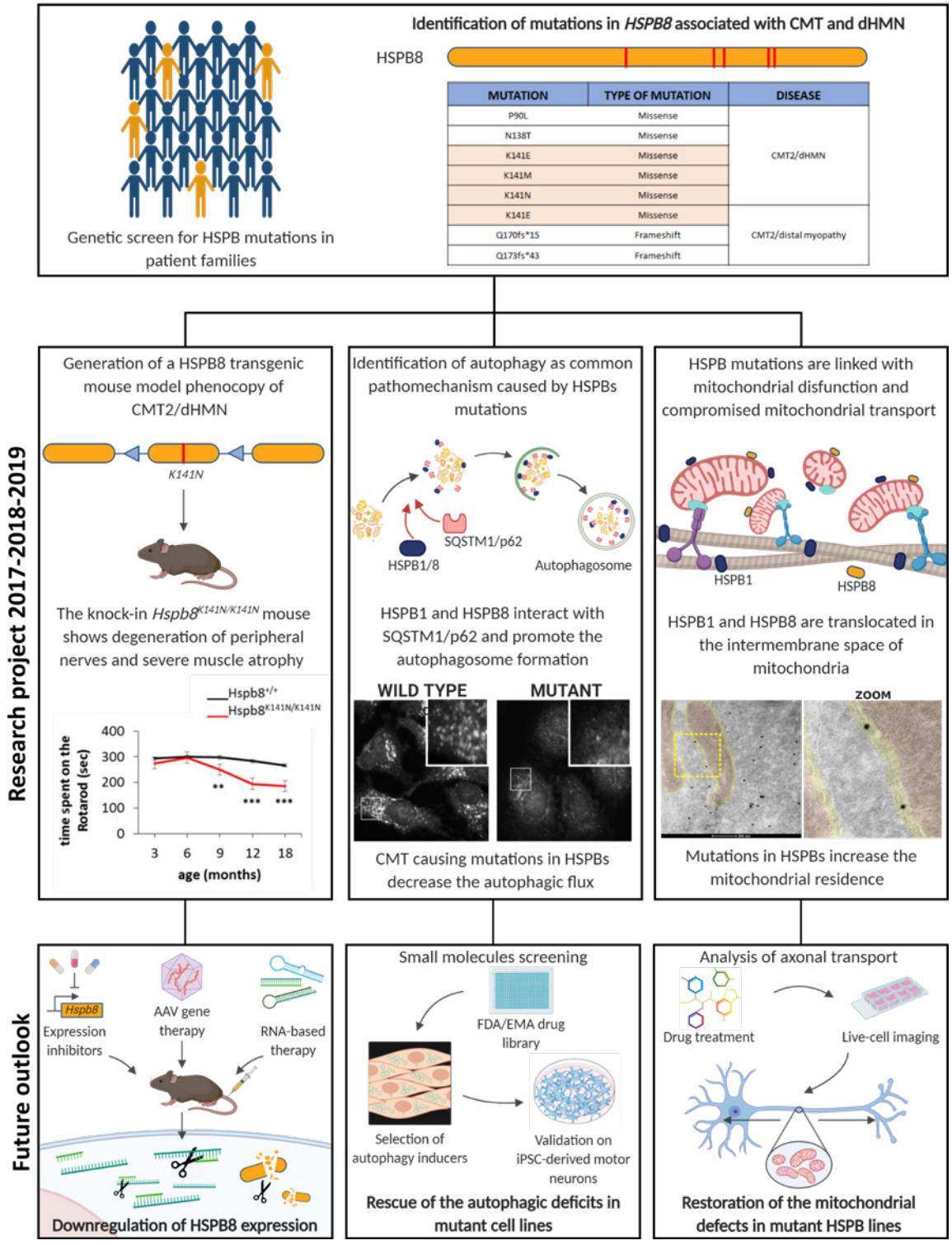

Figure 2: Schematic representation of the main results obtained in the past 3 years of the GSKE project (2017-2018-2019), complemented with future perspectives.

2. Research Activities 2017-2018-2019:

Articles published in International Journals – Acknowledging the GSKE:

1. Beijer D., Sisto A., Van lent J., Baets J., Timmerman V.; Invited Review: Defects in axonal transport in inherited neuropathies. Journal of Neuromuscular Diseases 6:401-419 (2019) Epub: 2019-09-20 (PMID: 31561383) (I.F.: 2.44, new journal and first IF)
2. Wu,J., Ma,S., Sandhoff,R., Ming,Y., Hotz-Wagenblatt,A., Timmerman,V., Bonello-Palot,N., Schlotter-Weigel,B., Auer-Grumbach,M., Seeman,P., Löscher,W., Reindl,M., Weiss,F., Mah,E., Weisshaar,N., Madi,A., Mohr,K., Schlimbach,T., Velasco Cárdenas,R.M-H., Koeppel,J., Grünschläger,F., Müller,L., Baumeister,M., Brügger,B., Schmitt,M., Wabnitz,G., Samstag,Y., Cui,G.: Loss of neurological disease HSAN-I-associated gene SPTLC2 impairs CD8+ T cell responses to infection by inhibiting T cell metabolic fitness. Immunity 50(5): 1218-1231 (2019) Epub: 23-Mar-2019 (PMID: 30952607) (I.F.: 21.522)
3. Haidar,M., Asselbergh,B., Adriaenssens,E., De Winter,V., Timmermans,J-P., Auer-Grumbach,M., Juneja,M., Timmerman,V.: Neuropathy-causing mutations in HSPB1 impair autophagy by disturbing the formation of SQSTM1/p62 bodies. Autophagy 15(6): 1051-1068 (2019) Epub: 23-Jan-2019 (PMID: 30669930) (I.F.: 11.059)
4. Bernard-Marissal,N., van Hameren,G., Juneja,M., Pellegrino,C., Louhivuori,L., Bartesaghi,L., Rochat,C., El Mansour,O., Médard,J-J., Croisier,M., Maclachlan,C., Poirot,O., Uhlén,P., Timmerman,V., Tricaud,N., Schneider,B.L., Chrast,R.: Altered interplay between endoplasmic reticulum and mitochondria in Charcot-Marie-Tooth type 2A neuropathy. PNAS 116(6): 2328-2337 (2019) Epub: 18-Jan-2019 (PMID: 30659145) (I.F.: 9.58)
5. Juneja,M., Burns,J.M., Saporta,M.A., Timmerman,V.: Challenges in modelling the Charcot-Marie-Tooth neuropathies for therapy development. Journal of Neurology, Neurosurgery and Psychiatry 90(1): 58-67 (2019) Epub: 17-Jul-2018 (PMID: 30018047) (I.F.: 8.272)
6. Krols,M.*, Asselbergh,B.*, De Rycke,R., De Winter,V., Seyer,A., Muller,F-J., Kurth,I., Bultynck,G., Timmerman,V.*, Janssens,S.* (* equal contribution): Sensory neuropathy-causing mutations in ATL3 affect ER-mitochondria contact sites and impair axonal mitochondrial distribution. Human Molecular Genetics 28(4): 615-627 (2019) Epub: 18-Oct-2018 (PMID: 30339187) (I.F.: 4.544)
7. Bouhy,D., Juneja,M., Katona,I., Holmgren,A., Asselbergh,B., De Winter,V., Hochepied,T., Goossens,S., Haigh,J.J., Libert,C., Ceuterick-de Groote,C., Irobi,J., Weis,J., Timmerman,V.: A knock-in/knock-out mouse model of HSPB8-associated distal hereditary motor neuropathy and myopathy reveals toxic gain-of-function of mutant Hspb8. Acta Neuropathologica 135(1): 131-148 (2018) Epub: 05-Aug-2017 (PMID: 28780615) (I.F.: 18.174)
8. Juneja,M., Azmi,A., Baets,J., Roos,A., Jennings,M.J., Saveri,P., Pisciotta,C., Bernard-Marissal,N., Schneider,B.L., Verfaillie,C., Chrast,R., Seeman,P., Hahn,A., De Jonghe,P., Maudsley,S., Horvath,R., Pareyson,D., Timmerman,V.: PFN2 and GAMT as common molecular determinants of axonal Charcot-Marie-Tooth disease. Journal of Neurology, Neurosurgery and Psychiatry 89(8): 870-878 (2018) Epub: 15-Feb-2018 (PMID: 29449460) (I.F.: 8.272)
9. Haidar,M., Timmerman,V.: Review: Autophagy as an emerging common pathomechanism in inherited peripheral neuropathies. Frontiers in Molecular Neuroscience 10: 143- (2017) Epub: 11-May-2017 (PMID: 28553203) (I.F.: 5.076)
10. Adriaenssens,E.*, Geuens,T.*, Baets,J., Echaniz-Laguna,A., Timmerman,V. (* equal contribution): Novel insights in the disease biology of mutant small heat shock proteins in neuromuscular diseases. Brain 140(10): 2541-2549 (2017) Epub: 01-Oct-2017 (PMID: 28969372) (I.F.: 10.84)
11. Geuens,T., De Winter,V., Rajan,N., Achsel,T., Mateiu,L., Almeida-Souza,L., Asselbergh,B., Bouhy,D., Auer-Grumbach,M., Bagni,C., Timmerman,V.: Mutant HSPB1 causes loss of translational repression by binding to PCBP1, an RNA binding protein with a possible role in neurodegenerative disease. Acta Neuropathologica Communications 5(1): 5 (2017) Epub: 11-Jan-2017 (PMID: 28077174) (I.F.: 5.414)
12. Echaniz-Laguna,A.*, Geuens,T.*, Petiot,P., Péréon,Y., Adriaenssens,E., Haidar,M., Capponi,S., Maisonobe,R., Fournier,E., Dubourg,O., Degos,B., Salachas,F., Lenglet,T., Eymard,B., Delmont,E., Pouget,J., Morales,R.J., Goizet,C., Latour,P., Timmerman,V.*, Stojkovic,T.* (* equal contribution): Axonal neuropathies due to mutations in small heat shock proteins: clinical, genetic and functional insights into novel mutations. Human Mutation 38(5): 556-568 (2017) Epub: 01-Feb-2017 (PMID: 28144995) (I.F.: 5.359)

Manuscripts under review with acknowledgements to the GSKE:

1. Alderson, R.T., Adriaenssens, E., Asselbergh, B., Pritišanac, I., Gastall, H.Y., Wälti, M., Louis, J.M., Timmerman, V.*, Baldwin, A.J.*, Benesch, J.L.P*. Dysregulation of HSP27 oligomerization and interactions by a neuropathy-causing mutation in the IPV motif. *Manuscript under revision for EMBO Journal (* corresponding authors). bioRxiv preprint first posted online Jul. 19, 2019; doi: http://dx.doi.org/10.1101/708180*
2. Adriaenssens, E. *, Tedesco, B. *, Mediani, L. *, Asselbergh, B., Crippa, V., Antoniani, F., Carra, S., Poletti, A., Timmerman, V. BAG3 Pro209 mutants associated with myopathy and neuropathy sequester chaperones of the CASA-complex in aggresomes. *Manuscript under revision for Scientific Reports. (* equal contributions) bioRxiv preprint first posted online Nov. 24, 2019; http://dx.doi.org/10.1101/853804*

3. Vendredy, L*., Adriaenssens, E*., Timmerman, V. Small heat shock proteins in neurodegenerative diseases. Invited book chapter for: "The big book on small heat shock proteins: II", editor Robert M. Tanguay (* equal contributions).

Awards and fellowships:

1. **Angela Sisto**: FWO-SB PhD fellowship started on 1st November 2019
2. **Jonas Van Lent**: DOCPRO4 PhD fellowship started in 1st October 2019
3. **Leen Vendredy**: FWO-SB PhD fellowship started on 1st January 2018
4. **Angela Sisto**: BOF-FWO-SB "opvangmandaat" from the Research Council of the University of Antwerp started on 1st January 2019 till 30 October 2019
5. **Leen Vendredy, Elias Adriaenssens, Angela Sisto, Jonas Van lent**: PNS fellowships to attend the Annual Meeting of the Peripheral Nerve Society (PNS and CMTR consortium meeting), Genova, Italy 22-25 June 2019
6. **Elias Adriaenssens**: 2019 Rotary 'Hope-in-Head' Grant
7. **Leen Vendredy, Elias Adriaenssens, M. Juneja:** PNS fellowships to attend the Annual Meeting of the Peripheral Nerve Society (PNS and CMTR consortium meeting), Baltimore, 21-25 July 2018
8. **Elias Adriaenssens**: FWO travel fellowship to attend the 3rd CSSI workshop on Small Heat Shock Proteins, Québec City, USA, 26-29 August 2018
9. **Mansour Haidar**: Travel fellowship to attend the Annual Meeting of the Peripheral Nerve Society (PNS and CMTR consortium meeting), Sitges-Barcelona, Spain, 8-12 July 2017

PhD theses:

1. **Elias Adriaenssens**: *The regulation and dysregulation of small heat shock proteins and an associated co-chaperone in health and disease;* PhD in Biochemistry and Biotechnology, Universiteit Antwerpen, 13 December 2019
2. **Mansour Haidar**: *Autophagy in inherited peripheral neuropathies: focus on the small heat shock protein HSPB1*; PhD in Biochemistry and Biotechnology, Universiteit Antwerpen, 29 March 2018
3. **Michiel Krols:** *Mutations in Atlastin-3: implications for ER membrane fusion and crosstalk with mitochondria*; PhD in Biochemistry and Biotechnology, Universiteit Antwerpen, 13 June 2017

Master theses:

1. **Tine Logghe:** *How is mitochondrial import of small heat shock proteins regulated?* Master in Biomedical Sciences, June 2019
2. **Rik van den Boom**: *Exploring the RNAi toolbox to reduce (mutant) HSPB8 expression.* Master in Biochemistry & Biotechnology, June 2019
3. **Jonas Van lent:** *Molecular phenotyping of neurons using CMT2 patient-derived iPSCs.* Master in Biochemistry & Biotechnology, January 2019
4. **Rani Boons:** *The role and import of small heat shock protein HSPB1 in mitochondria*. Master in Bioscience Engineering, Cell- and Gene technology, KULeuven, June 2018
5. **Liedewei Van de Vondel:** *Functional genomics and pathogenic validation of ARHGEF15 de novo mutation in epileptic encephalopathy*. ERASMUS Master in Biochemistry & Biotechnology, University of Barcelona, June 2018
6. **Kim Claes:** *Functional analysis of the interaction between HSPB1 and SLC25A12.* Master in Biomedical Sciences, June 2017
7. **Lotte Conings:** *Investigating the actin cytoskeleton as a common pathomechanism in axonal CMT using iPSC-derived models*. Master in Biomedical Sciences, June 2017

Chair and organizational activities:

1. **Vincent Timmerman**: (re)elected as secretary of the CMTR consortium at the Annual Meetings of the Peripheral Nerve Society (PNS) in Genova, 22-25 June 2019, Baltimore, 21-25 July 2018 and Sitges-Barcelona, Spain, 8-12 July 2017 (will be continued until 2021)
2. **Manisha Juneja**: chair at the CMTR consortium meeting at the Annual Meeting of the Peripheral Nerve Society (PNS), Baltimore, 21-25 July 2018
3. **Manisha Juneja**: co-organizer of the Thermo Fisher Scientific, Technical Stem Cell Workflow and Research Seminar, at the University of Antwerp, with a lecture: *Modeling CMT using iPSC derived motor neurons*, Antwerp, 19 October 2017

Invited research seminars:

1. **Vincent Timmerman**: *Searching for common signatures pathways associated with axonal CMT neuropathies*. Research seminar organized by Prof. R. Martini, University of Wurzburg, Germany, 6 December 2019
2. **Elias Adriaenssens**: *An introduction to CRISPR/Cas9 gene editing*, MosaCell 3rd annual meeting, University of Maastricht (Netherlands), 7 May 2019

3. **Vincent Timmerman:** *Can we find common pathomechanisms in CMT neuropathies?* Research seminar organized by Prof. V. Delague, Université Aix-Marseille, France, 6 December 2018
4. **Vincent Timmerman**: *The pathophysiology of small heat shock protein mutations causing peripheral neuropathy*. Research seminar organized by Prof. R. Horvath at the Institute of Genetic Medicine, Newcastle University, Newcastle, UK, 14 March 2017
5. **Manisha Juneja**: *Identifying common molecular determinants of axonal CMT.* TREAT-NMD, Freiburg, Germany, 27-29th November 2017

Invited lectures at international meetings:

1. **Vincent Timmerman**: *Molecular genetics and mechanisms of hereditary peripheral neuropathies.* Kongress des Medizinisch-Wissenschaftlichen Beirates der Deutschen Gesellschaft für Muskelkranke (DGM) e.V., Göttingen, Germany, 9-10 May 2019
2. **Vincent Timmerman:** *Small heat shock protein (HSPB8) related neuropathy and myopathy.* 246th ENMC workshop on Protein Aggregated Myopathies, Hoofddorp, The Netherlands, 24-26 May 2019.
3. **Vincent Timmerman**: *HSPB1/HSPB8 in axonal neuropathy and distal myopathy*, published in: 234th ENMC International Workshop: Chaperone dysfunction in muscle disease, Naarden, The Netherlands, 8-10 December 2017, by Weihl CC, Udd B, Hanna M; ENMC workshop study group. Workshop report published in Neuromuscular Disorders 2018;28(12):1022-1030.
4. **Vincent Timmerman:** *Identification of common molecular determinants of axonal Charcot-Marie-Tooth disease*. 4th joint meeting of the Belgian-Dutch neuromuscular study club and German Reference Center for Neuromuscular Diseases of the DGNN, Naarden, Netherlands, May 25-26, 2018.
5. **Vincent Timmerman**: *New genes and mechanisms in inherited neuropathies*. Plenary lecture at the 15th International Congress on Neuromuscular Diseases (ICNMD 2018), Vienna, Austria, July 6-10, 2018
6. **Vincent Timmerman**: *Disease mechanisms in the inherited neuropathies*. Educational lecture at the Peripheral Nerve Society (PNS 2018), Baltimore, USA, 21-25 July, 2018

Slide presentations selected at international meetings:

1. **Angela Sisto**: *Mutations in the small heat shock proteins HSPB1 and HSPB8 impair the autophagic flux* (platform session). Peripheral Nerve Society (PNS 2019), Genova, Italy, 22-25 June 2019
2. **Jonas Van Ient:** *Unravelling hallmarks of axonal degeneration in Charcot-Marie-Tooth type 2 using induced pluripotent stem cells* (oral poster). Peripheral Nerve Society (PNS 2019), Genova, Italy, 22-25 June, 2019
3. **Manisha Juneja**: *Molecular phenotyping of neurons derived from CMT2 patient-iPSC lines* (oral poster). Peripheral Nerve Society (PNS 2018), Baltimore, USA, 21-25 July, 2018
4. **Elias Adriaenssens**: *How does mitochondrial dysfunction contribute to the CMT2F pathogenesis caused by HSPB1 mutations.* CMTR and Peripheral Nerve Society (PNS 2018), Baltimore, USA, 21-25 July, 2018
5. **Leen Vendredy:** *A preclinical study to treat neuromuscular diseases caused by mutations in the small heat shock proteins HSPB8*. 3rd CSSI workshop on Small Heat Shock Proteins, Québec City, USA, 26-29 August 2018 [workshop report see below reference]
6. **Elias Adriaenssens:** *Small heat shock proteins operate as chaperones in the mitochondrial intermembrane space*. 3rd CSSI workshop on Small Heat Shock Proteins, Québec City, USA, 26-29 August 2018 [workshop report published in Carra, S., Alberti, S., Benesch, J.L.P., Boelens, W., Buchner, J., Carver, J.A., Cecconi, C., Ecroyd, H., Gusev, N., Hightower, L.E., Klevit, R.E., Lee, H.O., Liberek, K., Lockwood, B., Poletti, A., **Timmerman, V.**, Toth, M.E., Vierling, E., Wu, T., Tanguay, R.M.: Small heat shock proteins: multifaceted proteins with important implications for life. Cell Stress and Chaperones 24(2): 295-308 (2019)]
7. **Manisha Juneja**: *Exploring axonal CMT through iPSC derived neurons*. FP7 NeurOmics final meeting, Berlin, Germany, 3-5th May 2017
8. **Vincent Timmerman**: *A knock-in/knock-out mouse model for small heat shock protein HSPB8 mimicking distal hereditary motor neuropathy and myofibrillar myopathy.* Annual Meeting of the Peripheral Nerve Society, Sitges-Barcelona, Spain, 8-12 July 2017
9. **Mansour Haidar:** *Impairment of autophagy as a possible pathomechanism for CMT causing mutations in HSPB1.* Annual Meeting of the Peripheral Nerve Society, Sitges-Barcelona, Spain, 8-12 July 2017

Poster presentations at international meetings:

1. **Leen Vendredy**: *A CRISPR/Cas9 knock-out screen to identify regulators of Hspb8 expression and stability.* Peripheral Nerve Society (PNS 2019), Genova, Italy, 22-25 June, 2019
2. **Angela Sisto**: *Mutations in the small heat shock proteins HSPB1 and HSPB8 impair the autophagic flux*. Peripheral Nerve Society (PNS 2019), Genova, Italy, 22-25 June 2019
3. **Jonas Van Lent**: *Unravelling hallmarks of axonal degeneration in Charcot-Marie-Tooth type 2 using induced pluripotent stem cells.* Peripheral Nerve Society (PNS 2019), Genova, Italy, 22-25 June, 2019
4. **Elias Adriaenssens**: *Myopathies and neuropathies associated with Pro209 mutations in BAG3 have comparable molecular deficits*. Peripheral Nerve Society (PNS 2019), Genova, Italy, 22-25 June, 2019
5. **Leen Vendredy**: *A CRISPR/Cas9 knock-out screen to identify regulators of Hspb8 expression and stability*. EMBO Workshop-Protein quality control: from mechanisms to disease, Costa de la Calma, Spain, 28 April-3 May 2019
6. **Elias Adriaenssens**: *The small heat shock proteins operate as chaperones in the mitochondrial intermembrane space*. EMBO Workshop-Protein quality control: from mechanisms to disease, Costa de la Calma, Spain, 28 April-3 May 2019
7. **Angela Sisto**: *The shaping function of small heat shock proteins in autophagosome formation.* EMBO Workshop-Protein quality control: from mechanisms to disease, Costa de la Calma, Spain, 28 April-3 May 2019
8. **Angela Sisto**: *The adverse effect of mutations in small heat shock proteins HSPB1 and HSPB8 in autophagosome formation.* 3rd Nordic Autophagy Society (NAS) Conference, 22-24 May 2019
9. **Jonas Van Lent:** *Unravelling hallmarks of axonal degeneration in Charcot-Marie-Tooth type 2 using induced pluripotent stem cells.* 7th Molecular Mechanisms of Axon Degeneration meeting, Loch Lomond, Scotland, 11-14 March, 2019
10. **Manisha Juneja:***Molecular phenotyping of neurons derived from CMT2 patient-iPSC lines.* Peripheral Nerve Society (PNS 2018), Baltimore, USA, 21-25 July, 2018
11. **Elias Adriaenssens:** *How does mitochondrial dysfunction contribute to the CMT2F pathogenesis caused by HSPB1 mutations.* Peripheral Nerve Society (PNS 2018), Baltimore, USA, 21-25 July, 2018
12. **Leen Vendredy:** *A preclinical study to treat neuromuscular diseases caused by mutations in the small heat shock proteins HSPB8.* Peripheral Nerve Society (PNS 2018), Baltimore, USA, 21-25 July, 2018
13. **Manisha Juneja:** *Identification of common molecular players involved in the prognosis and pathogenesis of axonal CMT subtypes*. Annual Meeting of the Peripheral Nerve Society (PNS 2017), Sitges-Barcelona, Spain, 8-12 July 2017
14. **Elias Adriaenssens:** *How does mitochondrial dysfunction contribute to the CMT2F pathogenesis caused by HSPB1 mutations*. EMBO/FEBS Course: Mitochondria in life, death and disease, Fasano, Italy, 9-13 October 2017

Slide presentations selected at national meetings:

1. **Angela Sisto:** *Autophagy: Big responsibility for small molecular chaperones.* Selected speaker at WOG meeting, KU Leuven (Belgium), 28 November 2019
2. **Leen Vendredy:** *Can we treat CMT2L by downregulating HSPB8?* Selected speaker at WOG meeting, KU Leuven (Belgium), 28 November 2019
3. **Elias Adriaenssens:** *A novel role for small heat shock proteins: entering the mitochondrial territory.* Selected speaker at WOG meeting, KU Leuven (Belgium), 28 November 2019
4. **Elias Adriaenssens:** *CRISPR/Cas9: everything you need to know.* Invited speaker at KVCV meeting, KU Leuven (Belgium), 20 February 2019
5. **Elias Adriaenssens:** *CRISPR/Cas9: Everything you need to know.* Invited speaker at KVCV seminar Antwerp, 22 February 2017
6. **Elias Adriaenssens:** *CRISPR/Cas9: Everything you need to know.* Invited speaker at KVCV seminar Ghent, 8 November 2017
7. **Elias Adriaenssens:** *CRISPR/Cas9: recent developments in the field.* ThermoFisher Scientific, Technical Stem Cell Workflow and Research Seminar, at the University of Antwerp, 19 October 2017
8. **Elias Adriaenssens:** *How does mitochondrial dysfunction contribute to the CMT2F pathogenesis caused by HSPB1 mutations.* FBD Faculty Research Day, Universiteit Antwerpen, Antwerp, 27 October 2017

Poster presentations at national meetings:

1. **Manisha Juneja:** *Molecular phenotyping of neurons derived from CMT2 patient-iPSC lines*. Belgian Society for Stem Cell Research (BeSSCR 2018), Leuven, Belgium, 26 October, 2018
2. **Mansour Haidar:** *The role of HSPB1 in autophagy and its implications in peripheral neuropathy.* VIB Conference: ER Stress, Autophagy & Immune System, Bruges, 26-27 January 2017
3. **Elias Adriaenssens:** *How does mitochondrial dysfunction contribute to the CMT2F pathogenesis caused by HSPB1 mutations*. RBSM2017 symposium (P2N), Antwerp, 7 September 2017
4. **Mansour Haidar:** *Impairment of Autophagy as a Possible Pathomechanism for CMT Causing Mutations in HSPB1.* RBSM2017 symposium (P2N), Antwerp, 7 September 2017

Societal activities:

1. **Elias Adriaenssens**: "*Mijn onderzoek naar de ziekte van Charcot-Marie-Tooth*" Rotary Kempenland, Zandhoven, 14/02/2019
2. **Jonas Van lent**: *"Stamcelonderzoek: veelbelovend?"* CMT studie- en contactdag, Antwerpen, 20/10/2018
3. **Vincent Timmerman**: "*Resultaten voorgesteld tijdens het CMT-congres in Baltimore*" CMT studie- en contactdag, Antwerpen, 20/10/2018
4. **Elias Adriaenssens**: "*Het HspB8 muismodel weerspiegelt het ziektebeeld bij de mens*". CMT studie- en contactdag, Antwerpen LO, 4/11/2017

Valorisation of research findings:

1. EP17195108-PTC/EP2018/077133: "*Biomarkers for Charcot-Marie-Tooth disease*" (inventors **M. Juneja** and **V. Timmerman**)

References to the literature cited in the Research Report (part 1).

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