UNIVERSITAT DE BARCELONA

NRF2 and RIP140 as new therapeutic targets for X-linked adrenoleukodystrophy (X-ALD): Control of redox/metabolic homeostasis and inflammation

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NRF2 and RIP140 as new therapeutic targets for X-linked adrenoleukodystrophy (X-ALD): Control of redox/metabolic homeostasis and inflammation

Tesi realitzada al laboratori de malalties neurometabòliques del Institut de investigacions biomèdiques de Bellvitge (IDIBELL)

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Aviso a lectores. Tengo mucha gente a la que agradecer, así que reservad un buen rato para poder leer y cotillear todos los agradecimientos.

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Puede parecer que la tesis se queda en nosotros tres, pero todos sabemos que el trabajo sale adelante en este laboratorio gracias al inmenso equipo humano que forma X-ALD. Sinceramente, no sé cómo hubiera sobrevivido a lo duro que es este trabajo sin todos vosotros, que me habéis hecho sentir como en familia. Desde las más seniors en el lab, como Montse, que ha vivido todo mi cambio personal desde aquel chavalillo que llegó desde Granada y que me ha enseñado múltiples técnicas enzimáticas como una auténtica maestra; o Agatha, de la que aún me sigue flipando todo lo que sabe. Un auténtico diamante que la mayoría no conoce pero sin ella muchos proyectos del lab nunca hubieran surgido. Y qué decir de Nath, una de las mejores personas que he podido conocer (y es que por este laboratorio han pasado algunas de ellas) y de la que estoy orgulloso de poder llevarme su amistad una vez acabe mi etapa aquí. El día en que todo ese trabajo y ese buen trato hacia las personas sean reconocidos justamente está muy cercano, estoy muy seguro. También quiero agradecer a los otros postdocs, como los Xavi (Joya y Ortega), doktor Pohl, el recién llegado Edgard, que siempre han estado ahí para hacer más ameno el día a día en el lab y, muchas veces, fuera de él. Por supuesto gracias a el equipo técnico. Muchas veces no éramos conscientes de todo lo que sacan adelante, aunque últimamente un poco más y creo que aquí puedo hablar en primera persona, desde que se fueron Bárbara y Jordi. Es un gustazo poder trabajar con gente como Laia, Cris y Juanjo. Ya les gustaría a la mayoría de predocs del mundo poder tener a gente así en sus labs. La amabilidad de Cris y su disponibilidad a colaborar siempre hacen el día a día más sencillo y agradable. Sobre Laia, creo que puedo decir

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Un capítulo especial merece Juanjo, no hay duda. Junto con mis compis de piso, la primera persona que me "acogió" aquí en Barcelona, me enseñó infinidad de sitios, siempre dispuesto a ayudar, a ir a cualquier sitio. He aprendido de fotografía, de coches, bueno de todo, ¡porque Juanjo sabe de todo! Una persona especial para mí, y que echaré de menos seguro de aquí a unos meses. ¿Con quién voy a discutir ahora sobre cualquier tema *random*?

No me olvido tampoco de mis compañeros y compañeras de faena, "The Indian Crew", la *famiglia* y la nueva generación. Quien iba a imaginar que acabaría hablando inglés tanto tiempo aquí en Barcelona con mis tres compañeros indios, Janani, Devesh y Sanjib. Thanks people for always being nice to me, and for showing me another culture, for those stories about your cities, the weddings, the traditions, that I always liked. I hope that our paths would meet again somewhere else, eating a berlina in whatever cafetería we may meet. With Janani I spent more years than with the others, and we are finishing at the same time more or less. It has been a great pleasure to share these years with you, Janani. You are unique, extremely brave and I'm sure that you'll get what you want, just keep fighting for it. Kundi power!

¿Qué voy a decir de la *famiglia*? Me faltan las palabras, junto con mis compis de piso, son mi otra familia aquí en Barcelona. Gracias, Patri y Andrea, por tantos buenos ratos, tanto dentro como fuera del laboratorio, por esos viajes con Juanjo y Kelly (que no me olvido tampoco) que nunca olvidaré. Aún tengo pendiente una visita a Catanzaro y a la Puglia a conocer a Beatrice y a saludar a mi colega Lorenzo, que estoy seguro también me echa de menos. Y por supuesto a Ale y Marco, con Ale compartí mis vivencias en Barcelona desde el principio, ya que llegamos casi a la vez, y he podido disfrutar de momentos maravillosos como su boda en Italia con Andrea, y los primeros meses del pequeño Lore. También incluyo aquí en la *famiglia* a Sonia. Otra persona muy especial, a la que hay que conocer y que cuando lo haces descubres a una persona maravillosa y a una buena amiga. Venga, más visitas pendientes, se me llena la agenda en un plis, Valencia y Bogotá (centro), todo a su tiempo.
Y la nueva generación, encabezada por Leire. Ya has demostrado que vales para esto, así que nada, para adelante como haces ahora, que 4 años se pasan volando y hay que aprovechar y disfrutar de todos los momentos. Seguro que ahora van llegando compañeros y compañeras que harán más llevadero todo este viaje. Si son gente como Laura, o como Sergi, seguro tendrás una experiencia tan buena como la mía ;)

Ay, mis maestros y maestras cuando llegué al lab: Jone, Jorge y Laia, ahora todos en el extranjero. Fue una suerte teneros cerca cuando llegué para adaptarme, entender muchas cosas de las que pasaban a mi alrededor y aprender casi todo lo que ahora sé después de 5 años de tesis. Aunque nuestros caminos se hayan separado, siempre pueden volver a cruzarse, fijaos si habrá dado de sí esta tesis que le ha dado tiempo a Jorge a irse a Inglaterra y volver y todo. Siempre lo digo, pero otra de las personas que me han dejado huella ha sido Jone, otro torrente de "buen rollo" con la que hablar de cualquier cosa y de la que se puede aprender y debatir sobre miles de cosas. Estoy seguro que en San Diego están súper contentos de tenerte allí, y espero que volvamos a coincidir pronto.

Ahora que he nombrado Jorge, y ya saliéndome del lab, tengo que mencionar a mis hermanos/as aquí en Barcelona. Mis compañeros de piso Laura y Fran. Llegué por casualidad al piso a través de Jorge cuando visité el laboratorio, y acabo llevándome dos buenísimos amigos, sin los cuales todos estos años en Barcelona no hubieran sido iguales. Una amistad que hace que incluso Fran me perdone la sucesora que le dejé en el piso.

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Ah, cómo no, Brain and Beer. Esa locura que empezó hace cosa de un año, y que ha ayudado a hacer más llevadero este último año y que nos hace levantarnos cada día pensando en nuevas cosas a hacer y que incluso nos ha enseñado a presentar el IVA, quien nos lo iba a decir. Gracias a los componentes de BBB: Maca, IronMarc, Xabi y Sandra, que son otra familia más a unir a las que ya he nombrado. Tengo ya más familias que el rey Juan Carlos, y mira que es complicado llegar a ese nivel, pero eso me hace ver todo lo bueno que ha generado mi paso por aquí.

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LIST OF ABBREVIATIONS

Only those appearing more than once in the text. The rest, as well as the full name of genes and proteins, are indicated in its first appearance in the text

6-OHDA	6-hydroxydopamine
AASA	Aminoadipic semialdehyde
ABC	ATP-binding cassette
ACTH	Adrenocorticotropic hormone
AD	Alzheimer's disease
ADP	Adenosine diphosphate
ALS	Amyotrophic lateral sclerosis
AMN	Adrenomyeloneuropathy
AMP	adenosine monophosphate
ARE	Antioxidant response element
ATP	Adenosine triphosphate
Aβ	Amyloid-β
BAT	Brown adipose tissue
BTB	Broad complex, tramtrack, and bric-a-brac
cAMN	Cerebral adrenomyeloneuropathy
ccALD	Childhood cerebral adrenoleukodystrophy
cDNA	Complementary deoxyribonucleic acid
CEL	Carboxyethyl lysine
CML	Carboxymethyl lysine
CNS	Central nervous system
CoA	Coenzyme A
CTL	Control
DKO	Abcd1/Abcd2double knockout
DMF	Dimethyl fumarate
DNA	Deoxyribonucleic acid
DS	Down syndrome
EAE	Experimental autoimmune encephalomyelitis
EMA	European Medicines Agency
ER	Endoplasmic reticulum
FAD	Flavin adenine dinucleotide
FADH2	Flavin adenine dinucleotide (reduced form)
FDA	Food and Drug Administration
GC/MS	Chromatography/mass spectrometry
GSA	Glutamic semialdehyde
GSH	Reduced glutathione
GSSG	Oxidized glutathione
HD	Huntington's disease
HSCT	Hematopoietic stem cell transplantation
IF	Immunofluorescence
IHC	Immunohistochemistry
IL	Interleukin
IVR	Intervening region
LA	Lipoic acid
LDL	Low-density lipoprotein
LPC	Lysophosphatidylcholine

LSCS	Lumbar spinal cord slices
MDA	Malondialdehyde
MDAL	MDA-lysine
MIM	Mitochondrial inner membrane
miR	Micro RNA
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
mtDNA	Mitochondrial DNA
NAC	N-acetyl-L-cysteine
NAD+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADPH	Nicotinamide adenine dinucleotide phosphate, (reduced form)
NAWM	Normal appearing white matter
OSCSC	Organotypic spinal cord slice cultures
OXPHOS	Oxidative phosphorylation
PBDs	Peroxisome biogenesis disorders
PBMC	Peripheral blood mononuclear cells
PD	Parkinson's disease
PEDs	Single peroxisomal enzyme deficiencies
PEX	Peroxins
PPi	Pyrophosphate
R.C.R	Respiratory control ratio
RCDP	Rhizomelic chondrodysplasia punctata
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
TBS-T	Tris-buffer saline - Tween 20
TMEV-IDD	Theiler's murine encephalomyelitis virus-induced demyelinating disease
UPS	Ubiquitin proteasome system
USA	United States of America
VLCFA	Very long-chain fatty acids
WAT	White adipose tissue
WT	Wild-type
X-ALD	X-linked adrenoleukodystrophy

SUMMARY

Summary

X-linked adrenoleukodystrophy (X-ALD) is a rare neurometabolic disease characterized by the loss of function of the peroxisomal transporter ABCD1, which leads to an accumulation of very long-chain fatty acids, inducing mitochondrial reactive oxygen species. Clinical phenotypes in humans range from adrenal insufficiency to fatal inflammatory cerebral demyelination. *Abcd1*-null mice (*Abcd1*$^{-}$ mice) develop late onset axonal degeneration in the spinal cord and locomotor disability resembling the most common phenotype in humans, adrenomyeloneuropathy (AMN). Oxidative stress and mitochondrial dysfunction are key common features in X-ALD patients as well as in *Abcd1*$^{-}$ mouse. In this thesis, we sought to explore novel therapeutic targets that would contribute to better understand the pathophysiology of the disease, based on the existing knowledge on these hallmarks of X-ALD. First, we studied the nuclear factor erythroid-derived 2, like 2 (NFE2L2, also known as NRF2), in X-ALD mouse models (*Abcd1*$^{-}$ and *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice) and in fibroblasts derived from healthy subjects and X-ALD patients. Here, we identify that NRF2, the master regulator of endogenous antioxidant response, and its target genes are impaired in X-ALD due to an aberrant activity of the AKT/GSK-3β axis. Moreover, GSK-3β inhibitors reactivated the blunted NRF2-dependent response upon oxidative stress in X-ALD fibroblasts (Chapter I). In a second study, we sought to determine the role of RIP140 (receptor interacting protein 140), a transcriptional coregulator essential for metabolic homeostasis and inflammatory response, in X-ALD pathophysiology. To address this objective we studied RIP140 in *Abcd1*$^{-}$ mouse, organotypic spinal cord slice cultures (OSCSC) from mice and normal appearing white matter (NAWM) from healthy subjects and cerebral X-ALD patients. We found out a redox-dependent increase of RIP140 in the spinal cord and OSCSC from *Abcd1*$^{-}$ mice, as well as an induction of RIP140 in the NAWM of childhood cerebral ALD (ccALD) patients (Chapter II). Finally, we explored the therapeutic potential of targeting NRF2 and RIP140, independently, in X-ALD mice (*Abcd1*$^{-}$ and *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice). Regarding NRF2, we followed a pharmacologic approach, by treating *Abcd1*$^{-}$ and *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice with dimethyl fumarate, an FDA-approved NRF2 activator (Chapter I). In the case of RIP140, we followed a genetic approach, by crossing RIP140-deficient mice with X-ALD mouse models (Chapter II). In both cases, the therapeutic intervention led to an amelioration of i) mitochondrial dysfunction, ii) bioenergetic failure, iii) oxidative damage and iv) dysregulated inflammatory profile, and most importantly, halted axonal degeneration and behavioural abnormalities in X-ALD mice (Chapters I and II). Collectively, these findings reveal an impairment of the AKT/GSK-3β/NRF2 axis that controls endogenous response against oxidative stress, as well as point to RIP140 as a candidate for the impaired mitochondrial biogenesis and induction of proinflammatory response in X-ALD. Finally, the results of this doctoral thesis indicate that therapies based on NRF2 activation and RIP140 inhibition may be valuable strategies to treat X-ALD and other neurodegenerative disorders which share impaired redox homeostasis, mitochondrial dysfunction and neuroinflammation among their hallmarks.

Resumen

La adrenoleuocodistrofia ligada al cromosoma X (X-ALD) es una enfermedad neurometabólica rara, que se caracteriza por la pérdida de función del transportador peroxisomal ABCD1. Como consecuencia se acumulan ácidos grasos de cadena muy larga, que inducen la producción de especies reactivas de oxígeno en la mitocondria. El cuadro clínico de X-ALD en humanos es variable, desde la insuficiencia adrenal hasta una desmielinización inflamatoria cerebral que suele ser fatal. Los ratones nulos para el gen *Abcd1* (ratones *Abcd1*$^{-}$) desarrollan una degeneración axonal de aparición tardía en la médula espinal, además de presentar incapacidad locomotora, un fenotipo similar al más común en humanos, la adrenomieloneuropatía (AMN). El estrés oxidativo y la disfunción mitocondrial son unas características claves de X-ALD, tanto en el modelo de ratón como en humanos. En esta tesis, hemos decidido explorar nuevas dianas terapéuticas que contribuyan a una mejor comprensión de la fisiopatología de esta enfermedad, basándonos en el conocimiento existente sobre estas alteraciones en X-ALD. En primer lugar, estudiamos el factor nuclear NRF2 (nuclear factor, erythroid-derived 2, like 2; también NFE2L2) en los modelos animales de X-ALD (ratones *Abcd1*$^{-}$ y *Abcd1*$^{-}$/*Abcd2*$^{-/-}$) y en fibroblastos de pacientes con X-ALD y sujetos sanos. Así, identificamos que NRF2, el regulador maestro de la respuesta antioxidante endógena, así como sus genes diana, están inhibidos en X-ALD, debido a una actividad aberrante del eje AKT/GSK-3β. Además, inhibidores de GSK-3β reactivaron la respuesta frente al estrés oxidativo dependiente de NRF2, que estaba bloqueada en los fibroblastos de pacientes con X-ALD (Capítulo I). En el segundo estudio, pretendemos determinar el papel de RIP140 (receptor interacting protein 140) en la fisiopatología de X-ALD. RIP140 es un coregulador transcripcional esencial para la homeostasis metabólica y la respuesta inflamatoria. Para lograr este objetivo, primero estudiamos RIP140 en los ratones *Abcd1*$^{-}$, en cultivos organotípicos de láminas de médula espinal (OSCSC) de ratón, y en sustancia blanca cerebral de apariencia normal (NAWM) de pacientes X-ALD. De este modo, encontramos una inducción mediada por estrés oxidativo de RIP140 en la médula espinal y en OSCSC de los ratones *Abcd1*$^{-}$, además de una activación de RIP140 en NAWM de pacientes con ALD cerebral infantil (ccALD) (Capítulo II). Por último, investigamos el potencial terapéutico de estas vías para tratar X-ALD, mediante la administración en la dieta de dimetil fumarato, un activador de NRF2 aprobado por la FDA, a los ratones X-ALD (Capítulo I); y a través de la deleción del gen *Rip140* en los ratones X-ALD (Capítulo II). En ambos casos, la intervención terapéutica conllevó una mejora de i) la disfunción mitocondrial, ii) el fallo bioenergético, iii) el daño oxidativo, iv) la alteración del perfil inflamatorio, y sobre todo, detuvo la degeneración axonal y previno las alteraciones en el comportamiento en los ratones X-ALD (Capítulos I y II). En conjunto, estos resultados muestran una disfunción del eje AKT/GSK-3β/NRF2, que controla la respuesta antioxidante endógena, así como apuntan a RIP140 como responsable de la disminuida biogenesis mitocondrial y la inducción de la respuesta pro-inflamatoria que observamos en X-ALD. Finalmente, los resultados derivados de esta tesis doctoral indican que terapias basadas en la activación de NRF2 o la inhibición de RIP140 tienen un valor potencial como estrategias terapéuticas para tratar pacientes con X-ALD u otras enfermedades neurodegenerativas, que compartan como sello distintivo, un fallo en la homeostasis redox, disfunción mitocondrial y neuroinflamación.

Resum

L'adrenoleuocodistròfia lligada al cromosoma X (X-ALD) és una malaltia neurometabòlica rara, que es caracteritza per la pèrdua de funció del transportador peroxisomal ABCD1. Com a conseqüència s'acumulen àcids grassos de cadena molt llarga, que indueixen la producció d'espècies reactives d'oxigen en el mitocondri. El quadre clínic de X-ALD en humans és variable, des de la insuficiència adrenal fins a una desmielinització inflamatòria cerebral que sol ser fatal. Els ratolins nuls per al gen *Abcd1* (ratolins *Abcd1*$^{-}$) desenvolupen una degeneració axonal d'aparició tardana en la medul·la espinal, a més de presentar incapacitat locomotora, un fenotip similar al més comú en humans, l'adrenomieloneuropatia (AMN). L'estrès oxidatiu i la disfunció mitocondrial són unes característiques claus de X-ALD, tant en el model de ratolí com en humans. En aquesta tesi, hem decidit explorar noves dianes terapèutiques que contribueixin a una millor comprensió de la fisiopatologia d'aquesta malaltia, basant-nos en el coneixement existent sobre aquestes alteracions en X-ALD. En primer lloc, hem estudiat el factor nuclear NRF2 (nuclear factor, erythroid-derived 2, like 2; també NFE2L2) en els models animals de X-ALD (ratolins *Abcd1*$^{-}$ i *Abcd1*$^{-}$/ *Abcd2*$^{-/-}$) i en fibroblasts de pacients amb X-ALD i subjectes sans. Així, hem identificat que NRF2, el regulador mestre de la resposta antioxidant endògena, així com els seus gens diana, estan inhibits en X-ALD, a causa d'una activitat aberrant de l'eix AKT/GSK-3β. A més, inhibidors de GSK-3β van reactivar la resposta enfront de l'estrès oxidatiu dependent de NRF2, que estava bloquejada en els fibroblasts de pacients amb X-ALD (Capítol I). En el segon estudi, pretenem determinar el paper de RIP140 (receptor interacting protein 140) en la fisiopatologia de X-ALD. RIP140 és un coregulador transcripcional essencial per a l'homeòstasi metabòlica i la resposta inflamatòria. Per aconseguir aquest objectiu, primer hem estudiat RIP140 en els ratolins *Abcd1*$^{-}$, en cultius organotípics de làmines de medul·la espinal (OSCSC) de ratolí, i en substància blanca cerebral d'aparença normal (NAWM) de pacients X-ALD. D'aquesta manera, hem trobat una inducció de RIP140 intervinguda per estrès oxidatiu en la medul·la espinal i en OSCSC dels ratolins *Abcd1*$^{-}$, a més d'una activació de RIP140 en NAWM de pacients amb ALD cerebral infantil (ccALD) (Capítol II). Finalment, hem investigat el potencial terapèutic d'aquestes vies per tractar X-ALD, mitjançant l'administració en la dieta de dimetil fumarat, un activador de NRF2 aprovat per la FDA, als ratolins X-ALD (Capítol I); i a través de la deleció del gen *Rip140* en els ratolins X-ALD (Capítol II). En tots dos casos, la intervenció terapèutica va comportar una millora de i) la disfunció mitocondrial, ii) la fallada bioenergètica, iii) el dany oxidatiu, iv) l'alteració del perfil inflamatori i, sobretot, va aturar la degeneració axonal i va prevenir les alteracions en el comportament en els ratolins X-ALD (Capítols I i II). En conjunt, aquests resultats mostren una disfunció de l'eix AKT/GSK-3β/NRF2, que controla la resposta antioxidant endògena, així com senyalen a RIP140 com a responsable de la disminuïda biogènesis mitocondrial i la inducció de la resposta pro-inflamatòria que observem en X-ALD. Finalment, els resultats derivats d'aquesta tesi doctoral indiquen que teràpies basades en l'activació de NRF2 o la inhibició de RIP140 tenen un valor potencial com a estratègies terapèutiques per tractar pacients amb X-ALD o altres malalties neurodegeneratives, que comparteixin com a segell distintiu, una fallada en l'homeòstasi redox, disfunció mitocondrial i neuroinflamació.

INTRODUCTION

1 Introduction

1.1 Peroxisomes and related disorders

1.1.1 Peroxisome overview

Peroxisomes are small single-bound membrane subcellular organelles containing a granular matrix and a crystalline core, and present in virtually all eukaryotic cells (Schluter et al., 2010). They were first observed by Johannes Rhodin, during his doctoral thesis on the ultrastructure of proximal tubule cells from mouse kidney (Rhodin, 1954), and then described by Christian de Duve and collaborators, which named them peroxisomes, based on the presence of oxidases, that generate hydrogen peroxide (H_2O_2), and catalase within these organelles (De Duve et al., 1966). Evolutionarily, these organelles were thought to have an endosymbiotic origin similar to mitochondria, as they also contain their own protein import system and share some functions as well as a fission machinery. However, the current view of peroxisomes origin points to the endoplasmic reticulum (Gabaldon et al., 2006; Schluter et al., 2006; van der Zand et al., 2012) and mitochondria (Sugiura et al., 2017) as the organelles from which peroxisomes are formed. Peroxisomes perform essential metabolic functions in the organism, being dynamic organelles that are able to adjust their number and protein content in response to metabolic needs and physiological conditions. The shape and size of peroxisomes also vary in different tissues depending on their needs, with a diameter ranging from 0.1 μm to 1 μm (Smith et al., 2013).

1.1.2 Peroxisome functions

Virtually all types of peroxisomes degrade toxic H_2O_2 by catalase and are involved in fatty acid β-oxidation (Lazarow et al., 1976). Additionally, peroxisomes in higher eukaryotes carry out essential functions for metabolic homeostasis (R. J. Wanders et al., 2006) such as fatty acid α-oxidation; glyoxylate detoxification (Breidenbach et al., 1967); and anabolic functions, for instance the biosynthesis of bile acids, docosahexaenoic acid (C22:6 ω3) and ether phospholipids like plasmalogens (Hajra et al., 1982).

The first function described about peroxisomes was the detoxification of reactive oxygen species (Schrader et al., 2004). These organelles harbour both the oxidases that generate H_2O_2, as well as the enzymes needed for their disposal, such as catalase (De Duve et al., 1966), glutathione peroxidase (A. K. Singh et al., 1994) and peroxiredoxin V (PMP20) (Yamashita et al., 1999). Moreover, superoxide anion can also be generated in the peroxisome by xanthine oxidase (Angermuller et al., 1987), and then inactivated by superoxide dismutases (Cu/Zn-SOD and Mn-SOD), enzymes reported as present in the peroxisome (Dhaunsi et al., 1992; G. A. Keller et al., 1991; A. K. Singh et al., 1999). Together with reactive oxygen species, the detection of inducible nitric oxide synthase (iNOS) enzyme in peroxisomes, suggest that peroxisomes may be also a source of nitric oxide and its subsequent toxic species, such as peroxynitrite (Stolz et al., 2002).

Fatty acid β-oxidation is a universal property of peroxisomes in most organisms, either as the sole site of β-oxidation, like in yeasts and plants, or as a shared function with mitochondria, in higher eukaryotes. Short- and medium-chain fatty acids are exclusively, while long-chain fatty acids are predominantly, β-oxidized in mitochondria, whereas very long-chain fatty acids (VLCFA), particularly hexacosanoic acid (C26:0), can only be oxidized by peroxisomes (I. Singh et al., 1984b). Mitochondrial and peroxisomal fatty acid β-oxidation share a similar mechanism but differ in their function, as suggested by the different clinical signs and symptoms observed in disorders associated with inherited defects in mitochondrial and peroxisomal-β oxidation (R.J. Wanders, 2003). They share a common mechanism of fatty acid substrates chain shortening by 2 carbons through a sequential cycle of dehydrogenation, hydration, dehydrogenation, and thiolytic cleavage that produces acetyl-coenzyme A (Acetyl-CoA) molecules. On the other hand, they differ in how the flavin adenine dinucleotide (reduced form) ($FADH_2$) produced in the first dehydrogenation step is reoxidized. In peroxisomes, the $FADH_2$ reacts with O_2 to produce H_2O_2, which is degraded by peroxisomal catalase into H_2O and O_2, while in mitochondria, the reoxidation of $FADH_2$ is coupled to the electron transport chain to produce energy. Both β-oxidation pathways, together with their similitudes and differences have been illustrated in **Fig. 1**.

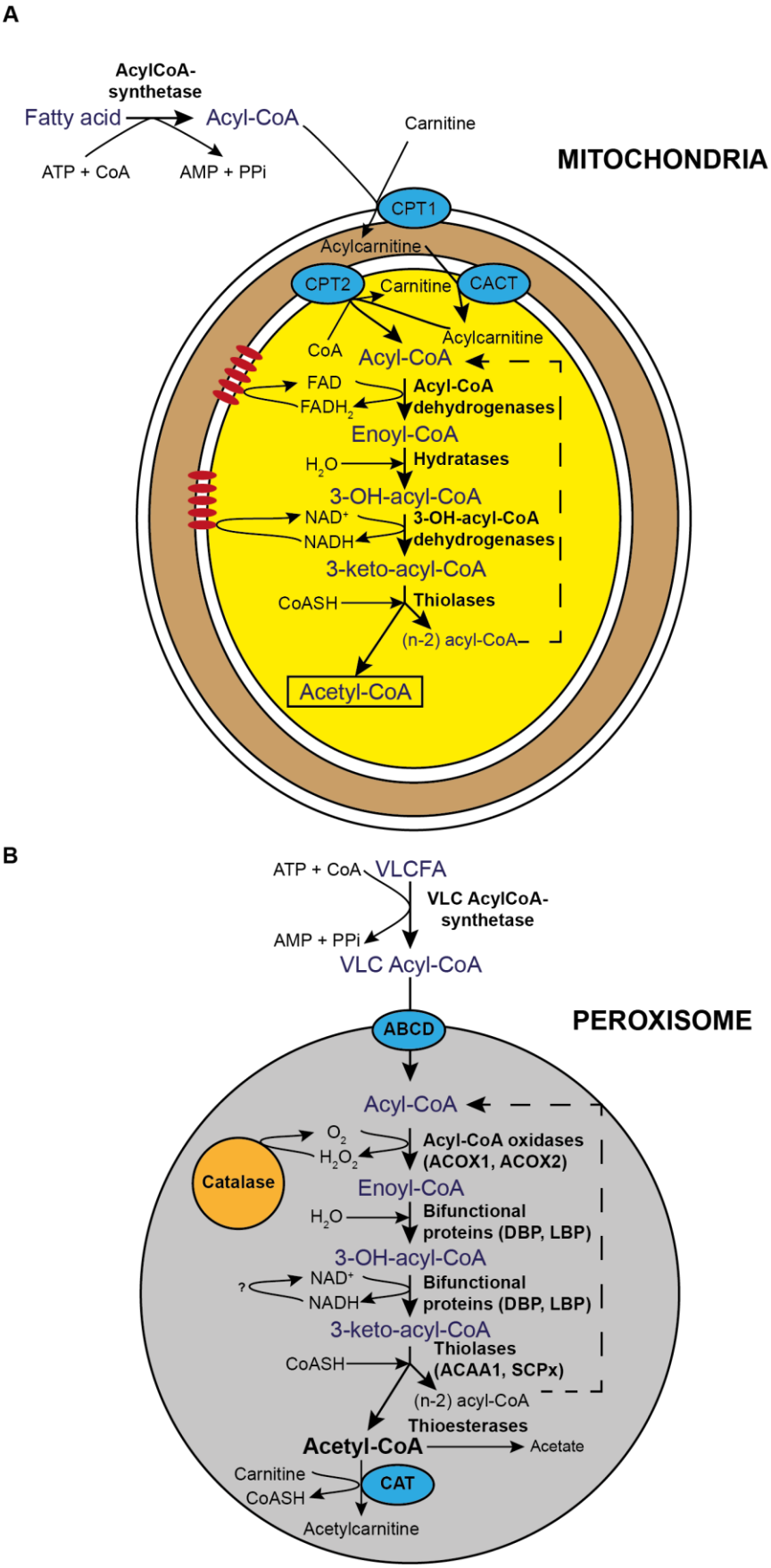


Figure 1 Comparative schematic representation of mitochondrial and peroxisomal fatty acid β-oxidation in humans

A) In the mitochondrion, fatty acids are activated with CoA, but long-chain fatty acids need to be transported into the mitochondrion via the carnitine cycle, which involves CPT1, CACT and CPT2. Then *via* a series of reactions, acyl-CoA chain is shortened by two carbons, releasing acetyl-CoA, which can enter in the Krebs cycle to generate ATP. **B**) On the other hand, in the peroxisomes, VLCFA are activated by VLCS (very-long chain acyl-CoA synthetases) in the cytosol, then transported into the peroxisome by ABCD proteins (ABCD1 in the case of C26:0). Once in the peroxisome, similar reactions are carried out by different enzymes, shortening the acyl chain by two carbons, yielding one acetyl-CoA molecule, which can be exported to the cytosol after being transformed into acylcarnitine, by CAT, or into acetate, by thioesterases. Acylcarnitine and acetate can then enter into the mitochondrion for the complete oxidation of the fatty acid. Abbreviations: CPT1 (carnitine palmitoyltransferase 1), CPT2 (carnitine palmitoyltransferase 2), CACT (carnitine acylcarnitine translocase), ACOX (acyl-CoA oxidase), DBP (D-bifunctional protein), LBP (L-bifunctional protein), ACAA1 (acetyl-CoA acyltransferase 1), SCPx (sterol carrier protein X), CAT (carnitine acyltransferase).

Acetyl-CoA molecules, together with other products of β-oxidation in the peroxisome such as medium-chain acyl-CoA are transferred to the mitochondria for full oxidation to CO_2 and H_2O via carnitine- and thioesterase-mediated pathways, although this process is not well known yet.

1.1.3 Peroxisomal ABC transporters

This dissertation focuses on adrenoleukodystrophy protein (ALDP or ABCD1), as mutations in *ABCD1* lead to X-linked adrenoleukodystrophy (Mosser et al., 1993), the disease that our group has been studying since more than 20 years. ATP-binding cassette (ABC) transporters couple ATP hydrolysis to the transport of substrates across biological membranes. ABCD1 belongs to the mammalian peroxisomal ABC subfamily D (ABCD) half transporters, which also include ALD-related protein (ALDRP or ABCD2) (Lombard-Platet et al., 1996); peroxisomal membrane protein of 70 kDa (PMP70 or ABCD3) (Kamijo et al., 1990) and PMP70-related protein (PMP70R or ABCD4) (Shani et al., 1997). All the four ABCD subfamily genes are conserved in vertebrates whereas in *Drosophila* and yeasts, only two members of the subfamily are present. Particularly, in yeasts, Pxa1p and Pxa2p are the orthologs of *ABCD1* and *ABCD3*, respectively. In the brain, ABCD1 is expressed strongly in the glia (astrocytes, microglia and a subpopulation of oligodendrocytes) and endothelial cells, but not in most neurons, with the exception of a few regions: hypothalamus, basal nucleus of Meynert, periaqueductal grey matter and the locus coeruleus (Fouquet et al., 1997;

Hoftberger et al., 2007; Troffer-Charlier et al., 1998). *ABCD2* is the closest homolog gene to *ABCD1* and is strongly expressed in neurons and the adrenal medulla (Ferrer et al., 2005). ABCD1 and ABCD2 proteins share 66% of identity, with overlapping functions (Pujol et al., 2004), and a mirror expression in most tissues (Troffer-Charlier et al., 1998). ABCD3 is involved in the transport of hydrophilic substrates like long-chain unsaturated-, long branched-chain- and long-chain dicarboxylic fatty acids into the peroxisome (van Roermund et al., 2014), and is also essential for bile acid biosynthesis (Ferdinandusse et al., 2015). ABCD4 is no longer considered a peroxisomal protein, since it is located in lysosomes and endoplasmic reticulum (Coelho et al., 2012; Kashiwayama et al., 2009).

Functional ABC transporters comprise two structurally similar halves, each containing six transmembrane alpha-helices and one hydrophilic nucleotide-binding domain (Dean et al., 2005). Half transporters need to homo- or hetero-dimerize to be functional. Indeed, homo-dimerization of ABCD1 and hetero-dimerization of ABCD1 with ABCD2 and ABCD3, as well as functionally active ABCD1/ACBD2 dimers have been described *in vitro* (Geillon et al., 2014; Genin et al., 2011; L. X. Liu et al., 1999). Conversely, *in vivo* evidence suggests that at least ABCD1 and ABCD3 function as homodimers (Guimaraes et al., 2004). Finally, a recent study has shown that ABCD1 and ABCD2 are organized in tetramers, i.e dimers of full transporters (Geillon et al., 2017).

1.1.4 Peroxisomal disorders

Most of the information known about the essential functions of peroxisomes comes from the phenotype of patients with mutations in genes encoding for peroxisomal proteins. The severity of these diseases varies ranging from early lethality to mild neurosensory defects. These disorders can be classified into peroxisome biogenesis disorders (PBDs) and single peroxisomal enzyme deficiencies (PEDs).

The majority of PBDs are autosomal recessive disorders caused by mutations in any of the 14 PEX genes encoding the peroxins, which are involved in the import of peroxisomal membrane and/or matrix proteins into the peroxisome (Smith et al., 2013).

Within PBDs, there are three distinct subtypes: the Zellweger spectrum disorders; rhizomelic chondrodysplasia punctata (RCDP) type 1 and 5; and the recently described peroxisomal fission defects. Genetic causes as well as phenotypic description of these disorders have been recently reviewed in detail (Waterham et al., 2016).

On the other hand, PEDs include defects of peroxisomal matrix enzymes as well as peroxisomal membrane proteins involved in metabolites transport. The biochemical and clinical consequences of each PED thus depend on the specific function of each affected protein in the peroxisome. Among PEDs, we can find defects in peroxisomal fatty acid α- and β-oxidation [including Refsum disease and X-linked adrenoleukodystrophy (X-ALD)]; glyoxylate metabolism; ether-phospholipid biosynthesis (RCDP type 2, 3 and 4); bile acid synthesis; and catalase metabolism, all of them comprehensively described in a recent review (Waterham et al., 2016).

In summary, peroxisomes play an essential role in metabolism and are crucial for life. Peroxisomal disorders comprise a heterogenous group of disorders that account for a substantial fraction of inborn errors of metabolism, with an estimated combined incidence of 1:5000 individuals and being X-ALD the most common among them, and also the central theme of this thesis.

1.2 The X-ALD case

1.2.1 History

The first report of X-ALD goes back to 1910, when Haberfeld and Spieler described the case of a six-year-old boy who died one year later. He had spastic paraparesis, constant changes of mood, incontinentia, inability to speak, together with other symptoms that would be later described as the prototypicals of the disease. This boy had an older brother who died at 8.5 years old due to a similar illness. In 1913, the neuropathological examination by Paul Schilder reported diffuse involvement of the cerebral hemispheres with severe loss of myelin, relative preservation of axons and accumulation of lymphocytes, fat-laden phagocytes and glial cells (Schilder, 1924). In 1923, Siemerling and Creutzfeldt described a similar case, but with adrenal gland involvement (Siemerling et al., 1923). The disease was first named Schilder's disease and later, in 1970, Blaw coined the term adrenoleukodystrophy.

After some decades with few advances, in 1976 X-ALD research achieved a milestone when Igarashi *et al.* found cholesterol, phospholipids and gangliosides esters within intracytoplasmic lamellae and lamellar-lipid inclusions from the brain and adrenals of X-ALD boys, which had an unusual high proportion of VLCFA (C24-30) (Igarashi et al., 1976; Powers et al., 1974). From that moment, X-ALD is classified as a lipid-storage disease. At that time, first adult patients were described (Budka et al., 1976; J. W. Griffin et al., 1977). The inclusion of X-ALD into the group of peroxisomal disorders arrived in 1984, when Singh *et al*. proved that X-ALD patients are not able to degrade VLCFA (1984b), a reaction localized in the peroxisome (I. Singh et al., 1984a). Before, it was shown that high levels of VLCFA accumulate in cultured skin fibroblasts and plasma from X-ALD patients (H. W. Moser et al., 1981; H. W. Moser et al., 1980), thus VLCFA levels could be used for diagnosis. In 1981 the X-ALD gene is mapped to Xq28, which corroborated the mode of inheritance proposed in the 60s (Fanconi et al., 1963). In 1993, Mosser *et al.* identified the X-ALD gene by positional cloning (1993), coding for a peroxisomal membrane ABC transporter (Mosser et al., 1994), referred to as ALDP protein, and later, as ABCD1.

1.2.2 Clinical features and diagnosis

X-ALD is the most common peroxisomal disease and leukodystrophy, with an estimated incidence of 1:17000 (Bezman et al., 2001). X-ALD affects the nervous system white matter and the adrenal cortex and, as others inborn errors of metabolism, is considered a progressive metabolic disease, as all patients are asymptomatic at birth. X-ALD patients display a complex range of clinical phenotypes, from adrenal insufficiency (Addison disease) to rapidly progressive and fatal cerebral demyelination (cerebral ALD). Overall, adrenal function is abnormal in 80% of patients (Dubey et al., 2005). Apart from other rare described phenotypes of X-ALD, such as "Addison only", "adolescent cerebral" and "adult cerebral" (H. W. Moser et al., 2001), the two main clinical phenotypes in affected males are:

1) Adrenomyeloneuropathy (AMN): Virtually all patients who reach adulthood develop AMN, characterized by peripheral neuropathy and distal axonopathy involving corticospinal tracts of the spinal cord, but neither brain neuroinflammation nor cerebral demyelination. 40%-45% of affected patients present AMN, manifested in the late twenties as progressive spastic paraparesis, sphincter incontinentia, sexual disturbances and impaired adrenocortical function (Kemp et al., 2012). All symptoms progress over decades, leading to the development of additional cerebral demyelination (cerebral AMN, cAMN), in a high proportion of AMN patients at later stages, between 20% (van Geel et al., 2001) and 63% (de Beer et al., 2014) of the AMN patients, depending on the study.
2) Childhood cerebral ALD (ccALD): It is characterized by cerebral demyelination with a strong inflammatory component. Lesions show gadolinium enhancement just behind the leading edge of the lesion (**Fig. 2**). Cerebral white matter lesions in X-ALD can be scored using the Loes classification system on a scale from 0 (normal on magnetic resonance imaging, MRI) to 34 (severely abnormal) (Loes et al., 1994). The onset starts between four and eight years old. It affects approximately 35% of patients. Initially, main symptoms are behavioral or learning deficits, which usually lead to attention deficit or hyperactivity diagnosis. Next, progressive impairment of cognition, behaviour, vision, hearing

and motor function often leads to total disability within two years and eventually death before adolescence (Kemp et al., 2012).

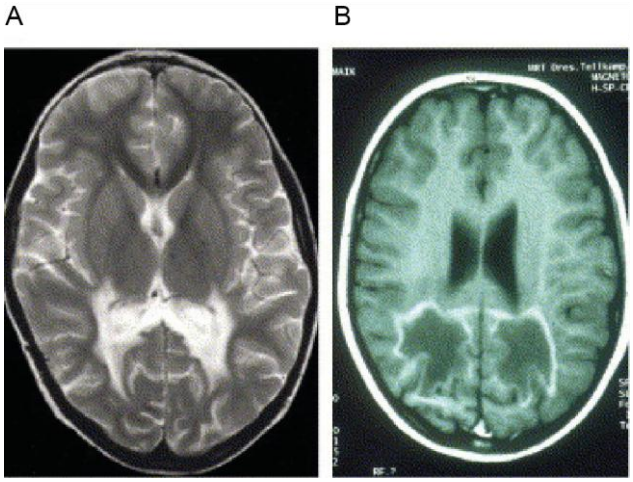


Figure 2 Typical white matter lesions in a ccALD patient

Extensive lesions can be appreciated by magnetic resonance imaging (MRI) in the parieto-occipital white matter by **(A)** increased signal intensity on T2-weighted image and **(B)** extensive gadolinium enhancement in the areas adjacent to demyelination on T1-weighted image. Adapted from Berger *et al* (2006).

Female carriers are also affected, as a recent study reported that >80% of these individuals developed signs and symptoms of myelopathy, although later than in men and with milder severity (Engelen et al., 2014).

Definitive diagnosis of X-ALD is achieved by demonstration of the biochemical defect (accumulation of VLCFA in plasma and organs) and by *ABCD1* mutation analysis. VLCFA are elevated in all male X-ALD patients regardless of age, disease duration, metabolic status, or clinical symptoms. Plasma VLCFA assay detects abnormally high concentrations of C26:0, especially C26:0-lysophosphatidylcholine (C26:0-LPC), and abnormally high ratios of C24:0/C22:0 and C26:0/C22:0, which can be detected at the day of birth, even in most heterozygous females (A. B. Moser et al., 1999a). Combined and highly sensitive mass spectrometry assays are highly accurate methods for diagnosis (Hubbard et al., 2009). Proper genetic counsel of affected families is also of huge importance. To identify the subset of males who will develop ccALD, all males with *ABCD1* mutations require clinical tracking and serial MRI to monitor neuroradiological changes, as well as evaluation of adrenocortical function to guide

treatment. Prenatal diagnosis using the VLCFA assay in cultured amniocytes or cultured chorion villus cells is also available (A. B. Moser et al., 1999b) and more recently direct DNA mutation analysis has become available, being of special interest in the case of heterozygous women, where VLCFA analysis can result in false negatives (Engelen et al., 2014). Recent validation of a method for detecting C26:0-LPC in dried blood spots from newborns (Hubbard et al., 2009) has permitted to include X-ALD in the Recommended Uniform Screening Panel (RUSP) in the United States of America (USA) (Kemper et al., 2017). Therefore, it is currently being applied in four states of the USA and the Netherlands, and it is expected to be implanted in more countries in the coming years. This new screening will allow the identification of the majority of X-ALD patients presymptomatically.

1.2.3 Genetics and biochemistry

1.2.3.1 X-ALD mutations and modifier genes

X-ALD patients carry a mutation in *ABCD1* (Mosser et al., 1993). *ABCD1* contains 10 exons and encodes for an mRNA of 4.3 kb that translates a protein of 745 amino acids. Up to date, over 1500 different mutations in *ABCD1* have been catalogued in the X-ALD database (Kemp et al., 2001) (see www.x-ald.nl), with 4% of the patients affected by *de novo* mutations (Y. Wang et al., 2011). Interestingly, all clinical phenotypes can occur within the same family (Berger et al., 1994; Kemp et al., 1994), indicating that there is no direct phenotype-genotype correlation. Even in monozygotic twins, different clinical phenotypes have been observed (Korenke et al., 1996). These evidences suggest that the defect in *ABCD1* and subsequent accumulation of VLCFA, results in adrenal insufficiency and myelopathy, but for the initiation of cerebral demyelination, further additional environmental triggers and/or genetic factors are required, altogether exemplified by the “three-hit” hypothesis (I. Singh et al., 2010). Initially, a segregation analysis based on 3,862 individuals from 89 kindreds suggested the presence of an autosomal modifier locus (H. W. Moser et al., 1992). The members of the ABCD subfamily, *ABCD2* (Holzinger et al., 1997; Holzinger et al., 1999) and to a lesser extent *ABCD3* (Braiterman et al., 1998; Pujol et al., 2004) seemed to be suitable candidates for modifier genes, as their overexpression compensate for the lack of *ABCD1 in vitro* and *in vivo* (Kemp et al., 1998; Netik et al., 1999; Pujol et al., 2004). However, two

independent association studies comparing *ABCD2* and *ABCD3* polymorphisms within X-ALD patients with different clinical phenotypes failed to determine significant correlations (Maier et al., 2008; Matsukawa et al., 2011). A single nucleotide polymorphism (SNP) in the cluster of differentiation 1 (*CD1*) gene, rs973742, located 4 kb downstream from the *CD1D* gene, arose as another modifier candidate gene, but eventually, the association was no longer significant after Bonferroni correction for multiple testing (Barbier et al., 2012). Polymorphisms in two genes related to methionine metabolism (cystathionine beta-synthase, *CBS;* and transcobalamin 2, *TC2*), which is important for myelination, together with a polymorphism in superoxide dismutase 2 (*SOD2*), which is involved in the antioxidant response, were found associated with phenotypic outcome in X-ALD, although, again, the results were not conclusive (Brose et al., 2012; Linnebank et al., 2006; Semmler et al., 2009). Moreover, a recent report has found a correlation between total antioxidant capacity and SOD levels and activity in samples of monocytes and blood plasma from X-ALD patients with magnetic resonance imaging severity score in these patients (Turk et al., 2017), reinforcing the link between the response against oxidative stress and X-ALD, a topic which is discussed in detail in this thesis. Additionally, none of the human leukocyte antigen (HLA) type II haplotypes, a confirmed risk factor in multiple sclerosis (International Multiple Sclerosis Genetics et al., 2011), are involved in the triggering of inflammation that leads to cerebral demyelination in cerebral ALD (Berger et al., 1995; McGuinness et al., 1997; S. Schmidt et al., 2003). Furthermore, genes involved in fatty acid elongation such as elongation of very long-chain fatty acid-1 (*ELOVL1*) (Ofman et al., 2010) or fatty acid ω-oxidation (Sanders et al., 2006) may represent rational modulatory gene candidates, except for the fact that association studies still have to be performed concerning these genes. As in other rare diseases, the relatively small number of X-ALD patients makes difficult the extraction of definitive conclusions.

When comparing mRNA expression from normal appearing white matter (NAWM) from AMN patients versus ccALD patients, few genes have shown a differential pattern of expression. For instance, *ABCD4* and acyl-CoA synthetase bubblegum family member-1 (*ACSBG1*) were decreased only in NAWM from ccALD patients when compared to controls (Asheuer et al., 2005). Recently, other differentially expressed genes or molecules have been found out between AMN and ccALD, such as 25-hydroxycholesterol and cytochrome P450 family 4 subfamily F member 2 (*CYP4F2*),

associated to cholesterol metabolism (J. Jang et al., 2016; van Engen et al., 2016) and nuclear factor kappa B (NFκB)-dependent inflammatory components regulated by a microRNA, miR-196a (Shah et al., 2017). Viral infections associated to demyelination have been also investigated as a plausible environmental factor in X-ALD, even though, for the moment, this possibility has been ruled out after negative results of an immunohistochemical search for viral antigens in the white matter of cerebral ALD patients (Ito et al., 2001). Complementary to this, some studies suggest that head trauma may be a possible environmental trigger for cerebral ALD (Bouquet et al., 2015; Raymond et al., 2010; Weller et al., 1992).

Taken together, these evidences strongly indicate a highly unlikely existence of a single modifier gene/locus. Thus, clinical phenotype of ALD patients will probably be defined by the intertwin of rare genetic variants and environmental factors. Further research is needed to shed light on these factors.

1.2.3.2 Biochemistry

In X-ALD, VLCFA content is increased, mainly C24:0 and C26:0 fatty acids, in cholesterol esters and complex lipids such as gangliosides, phosphatidylcholine, sphingomyelin, cerebrosides and sulfatides (Igarashi et al., 1976; H. W. Moser et al., 1981; Wiesinger et al., 2013). VLCFA accumulation is caused by an impaired ability of the peroxisomes to degrade VLCFA (I. Singh et al., 1984a; I. Singh et al., 1984b; R. J. Wanders et al., 1987). To initiate peroxisomal β-oxidation, VLCFA need to be activated by thioesterification with CoA. As mentioned above, until the discoverment of *ABCD1* as the mutated gene in X-ALD, VLCFA oxidation defect was first misassigned to a defect in the very long-chain fatty acyl-CoA synthetase (VLCS) enzyme (Lazo et al., 1988; R. J. Wanders et al., 1988).

ABCD1 is an integral peroxisomal membrane protein with the ATP-binding domain located towards the cytoplasmic surface of the peroxisomal membrane (Contreras et al., 1996). Experimental evidence of actual ABCD1 function comes from complementation studies, in which expression of wild-type *ABCD1* cDNA in X-ALD fibroblasts restores VLCFA β-oxidation (Braiterman et al., 1998; Shinnoh et al., 1995) and reduces VLCFA levels (Cartier et al., 1995). Structural and functional features of ABCD1 were also

extracted from studies with the *Saccharomyces cerevisiae* peroxisomal ABC half transporters, Pxa1p and Pxa2p, which form a heterodimer to import acyl-coA esters into the peroxisome (Hettema et al., 1996). Since ABCD1 protein expression in yeast is able to restore the growth of mutants lacking these genes in oleate-enriched medium, it was demonstrated that ABCD1 transports acyl-CoA esters across the peroxisomal membrane (van Roermund et al., 2008). In X-ALD fibroblasts, β-oxidation activity is reduced up to 75%-85% of the levels measured in normal fibroblasts (McGuinness et al., 2001). This fact is explained by the ability of ABCD3 to also transport VLCFA-CoA into the peroxisome (Wiesinger et al., 2013). The impairment in the degradation of VLCFA causes a rise in the levels of cytosolic VLCFA-CoA, and further elongation of the chain length by ELOVL1, the human VLCFA-specific elongase (Ofman et al., 2010). This elongation leads to enhanced incorporation of VLCFA into complex lipids (Kemp et al., 2005). In summary, ABCD1 is active as a homodimer, and *ABCD1* mutations impair the import of VLCFA-CoA into the peroxisome, leading to accumulation of VLCFA.

1.2.4 Animal models for X-ALD

The identification of *ABCD1* as the gene mutated in X-ALD allowed the generation of ABCD1-deficient mice (*Abcd1*$^{-}$), seeking for an animal model which could be used to better understand X-ALD pathophysiology. In 1997, three different groups developed three independent X-ALD mouse models, by inactivating *Abcd1* gene (Forss-Petter et al., 1997; T. Kobayashi et al., 1997; J. F. Lu et al., 1997). The three models display the main biochemical hallmark of the disease, which is accumulation of VLCFA. Despite this, these mice do not develop cerebral demyelination or brain inflammation, characteristic of ccALD. However, around 18 months of age, these mice develop a late-onset AMN-like phenotype. This phenotype is characterized by spastic paraparesis due to degeneration of corticospinal tracts, without signs of inflammatory demyelination in the brain. Axonal pathology is found in sciatic nerves and spinal cord, including slower sciatic nerve conduction and motor behaviour abnormalities in rotarod, bar cross and treadmill tests (Pujol et al., 2002). ABCD1-deficient mice have been instrumental in dissecting pathomechanisms of AMN (Fourcade et al., 2008; Galino et al., 2011; Launay et al., 2015; Launay et al., 2013; Launay et al., 2017; Lopez-Erauskin et al., 2013; Morato et al., 2013; Morato et al., 2015). In an attempt to obtain a more severe

phenotype, ABCD1/ABCD2 double-deficient mice (*Abcd1*$^{-}$/*Abcd2*$^{-/-}$) were generated, although they do not develop brain inflammation or demyelination either (Pujol et al., 2004). The neuropathology in these mice is also restricted mainly to the spinal cord and, due to the contribution of ABCD2, the dorsal root ganglia, resulting in a sensory neuropathy. Nevertheless, these defects are more pronounced and start at 12 months of age, earlier than upon ABCD1 deficiency alone (Pujol et al., 2004), setting up a suitable preclinical model for testing disease-modifying drugs (Launay et al., 2017; Lopez-Erauskin et al., 2011; Mastroeni et al., 2009; Morato et al., 2013; Morato et al., 2015). Furthermore, since *Abcd1*$^{-}$ mouse does not present a progressive phenotype that leads to cerebral demyelination and neuroinflammation, it underscores the hypothesis that considers that genetic and/or environmental factors trigger the onset of cerebral forms of X-ALD.

Drosophila mutants of *bubblegum* (*bbg*) and *double bubble* (*dbb*) very long-chain acyl-CoA synthetases have been also claimed as X-ALD models (Min et al., 1999; Sivachenko et al., 2016), as these mutations provoke profound effects on behaviour and brain morphology, although the mouse model of the same gene does not present neurodegeneration (Heinzer et al., 2003). The ortholog of *ABCD1* and *ABCD2* in *Caenorhabditis elegans*, *pmp-4*, is also being studied as another animal model for X-ALD (data not published).

Collectively, several animal models have been developed to study X-ALD. The most studied one is *Abcd1*$^{-}$ mouse, which develops an AMN-like phenotype. Further efforts are needed to obtain a model for the devastating ccALD.

1.2.5 Therapies for X-ALD

Given the wide clinical spectrum of X-ALD patients, different therapeutic strategies must be considered for individual symptoms. Follow-up in boys and men with X-ALD is important to detect adrenocortical insufficiency and also to early identify first signs of cerebral demyelination to propose allogeneic hematopoietic stem cell transplantation (HSCT), the only available intervention able to arrest the progression of cerebral demyelination.

As 70% of X-ALD patients develop adrenal insufficiency, X-ALD diagnosed boys and men should be monitored by measuring adrenocorticotropic hormone (ACTH) levels in plasma and with the ACTH stimulation test. Then, if necessary, they must be treated with steroid replacement, in the same way as for other forms of primary adrenal insufficiency (H. W. Moser et al., 2007). Therapies addressed to correct VLCFA accumulation and to improve neurologic symptoms are listed below:

1) Lorenzo's oil is a 4:1 mixture of oleic (C18:1ω9) and erucic (C22:1ω9) acids, which competes for the fatty acid elongation system, preventing the synthesis of saturated VLCFA. Oral administration of this oil, combined with moderate reduction of fat in the diet, reduces plasma levels of VLCFA within four weeks (A. B. Moser et al., 1987), so it was a promising therapeutic strategy in the 80s-90s. Nevertheless, VLCFA levels in brain do not change (Rasmussen et al., 1994) and further studies proved that Lorenzo's oil administration does not stop the progression of X-ALD (Aubourg et al., 1993; van Geel et al., 1999). Even though a study with 89 asymptomatic boys with normal MRI showed that Lorenzo's oil might prevent cerebral demyelination (H. W. Moser et al., 2005), there is no definitive evidence for the efficacy of Lorenzo's oil in the prevention of the onset of cerebral ALD.

2) Lovastatin, a cholesterol-lowering drug, normalizes VLCFA levels in X-ALD fibroblasts and in plasma of X-ALD patients (I. Singh et al., 1998a; I. Singh et al., 1998b). However, in a placebo-controlled trial, it did not lower C26:0 levels in peripheral-blood cells, and its effect on plasma VLCFA were attributed to be a consequence of a nonspecific decrease in the level of low-density lipoprotein (LDL)-cholesterol (Engelen et al., 2010).

3) Bezafibrate gave hope for therapy, as it reduces *de novo* C26:0 synthesis, by competitive inhibition of ELOVL1 in X-ALD fibroblasts (Engelen et al., 2012a), but it does not lower VLCFA levels in plasma or lymphocytes of X-ALD patients (Engelen et al., 2012b).

4) Immunomodulators or immunosuppressants aimed to reduce brain inflammation have not worked in X-ALD. Neither cyclophosphamide nor subcutaneous injections of

interferon (IFN)-β1a nor intravenous immunoglobulins injection stopped the progression of the disease (Cappa et al., 1994; Korenke et al., 1997; Naidu et al., 1988).

5) Pharmacological induction of *ABCD2* gene expression: Since overexpression of *ABCD2* in X-ALD fibroblasts and in *Abcd1*$^{-}$ mice prevents VLCFA accumulation (Flavigny et al., 1999; Netik et al., 1999; Pujol et al., 2004), ABCD2-inducing drugs were proposed as a therapeutic option for X-ALD. Nevertheless, 4-phenylbutyrate (4-PBA) (Kemp et al., 1998; McGuinness et al., 2001; H. W. Moser et al., 2000), fenofibrate (Albet et al., 1997; Fourcade et al., 2001) and valproic acid (Fourcade et al., 2010) had little success when tested.

6) Antioxidant supplementation: Given the important role of oxidative stress in X-ALD, antioxidant therapies were tested in the X-ALD animal models. A combination of antioxidants (vitamin E, N-acetylcysteine and lipoic acid) halts axonal degeneration and prevents locomotor deficits in *Abcd1*$^{-}$ mice (Lopez-Erauskin et al., 2011). These results from our group, have led to a phase II clinical trial in AMN patients with a cocktail of antioxidants (NCT01495260). High-dose of antioxidants resulted in the normalization of several protein and DNA oxidative damage biomarkers and a significant reduction of several inflammation markers such as chemokines and lipid mediators. In addition, the results showed a significant reduction in central motor conduction time in both legs (upper motorneuron), and an improvement in the six-minute walking test (6MWT) in eight out of ten patients (Montserrat Ruiz personal communication).

7) Apart from these therapeutic strategies, the only useful therapy for cerebral X-ALD is HSCT (Aubourg et al., 1990). HSCT arrests neuroinflammation, probably through an improvement in microglial functions, as these cells derive from bone marrow progenitors (Eglitis et al., 1997). HSCT can only be performed at an early stage of the disease, meaning a score of ≤9 in the X-ALD MRI Severity Scale (Loes et al., 2003) and no neurologic or neuropsychological deficits. Because of this, monitoring of X-ALD boys is so important. In these conditions the 5-year survival is above 90% (A. Mahmood et al., 2007; Miller et al., 2011). Anyway, HSCT remains associated with high risks of morbidity and mortality, and it is not easy to find a matching donor. The absence of biomarkers that can predict the evolution of cerebral disease together with the fact that HSCT performed in childhood does not seem to prevent myelopathy and

peripheral neuropathy in adulthood (van Geel et al., 2015) makes mandatory the search of new therapies for X-ALD patients.

8) A recent alternative to allogeneic HSCT is gene therapy, by transplantation of genetically corrected autologous $CD34^+$, with encouraging results in the first two treated boys, in which this therapy has impeded the progression of the disease (Cartier et al., 2009).

To sum up, given that the only available therapy (HSCT) has a narrow therapeutic window and elevated risks associated; together with the fact that most of the approaches followed until now have failed, much more research on X-ALD pathophysiology and subsequent therapies needs to be done.

1.2.6 Pathophysiology of X-ALD

The mechanisms of how VLCFA accumulation leads to axonal degeneration in AMN, or cerebral inflammatory demyelination in ccALD are not known yet. First evidences pointed to destabilization of myelin sheats components due to VLCFA accumulation (Bizzozero et al., 1991). Conversely, the wide clinical spectrum of X-ALD phenotypes and the lack of cerebral demyelination in *Abcd1*-null mice, in spite of VLCFA accumulation in brain (Pujol et al., 2002), indicates the presence of additional mechanisms associated to VLCFA accumulation. Examining the phenotype of $Pex7^{-/-}$ mice, which present a defect in the biosynthesis of plasmalogens; these cellular components were associated to the VLCFA-induced damage characteristic of X-ALD (Brites et al., 2009). VLCFA could also alter the permeability of the inner mitochondrial membrane by substituting the lateral chains of phospholipid bilayers and physically interfering with the assembly of oxidative phosphorylation (OXPHOS) system, or by increasing membrane microviscosity (Knazek et al., 1983; Whitcomb et al., 1988) and provoking its disruption (J. K. Ho et al., 1995).

Compelling evidence indicates that X-ALD recapitulates the pathological hallmarks of the main neurodegenerative disorders, exemplified by redox dyshomeostasis; mitochondrial dysfunction; proteostasis impairment, the latter being defined by

proteasomal and autophagy flux dysfunction; and neuroinflammation (Pujol, 2016). The following sections explain and illustrate the different molecular pathways affected in X-ALD, as well as the plausible therapeutic targets derived from them, which would be the focus of this thesis.

1.3 Hallmarks of X-ALD

1.3.1 Redox dyshomeostasis

1.3.1.1 ROS: Hero and villain

Since Denham Harman proposed the free-radical theory of aging in 1956 (Harman, 1956), a large body of evidence has been accumulated showing that living organisms have adapted to coexist with free radicals but also have developed diverse mechanisms to cope with them. Oxygen plays a dual role in the life of organisms. While being essential for life, it also generates by-products that are toxic in high concentrations. Free radicals [such as superoxide ($O_2^{\bullet -}$) and hydroxyl ($HO^{\bullet}$)] and other reactive small molecules (e.g. H_2O_2 and peroxynitrite), previously thought as unregulated species with random and non-specific targets, play important physiological roles in the organisms, including metabolic regulation, immune function, stem cell-renewal and aging (Holmstrom et al., 2014; Pan et al., 2011; W. Yang et al., 2010).

Oxidative stress occurs when redox homeostasis is unbalanced, leading to an excess of reactive oxygen/nitrogen species (ROS/RNS), mainly H_2O_2 and superoxide anions. This excess disrupts the oxidation-reduction cellular balance (redox balance), inducing damage to biomolecules such as DNA, lipids and proteins, perturbing cellular thiol levels, and deregulating cellular signaling pathways implicated in a myriad of human diseases. These disorders range from neurodegenerative diseases to metabolic disorders such as obesity or type 2 diabetes (C. E. Cross et al., 1987; Halliwell, 2006). Oxidative lesions can also transform lipid properties and induce mutations in mitochondrial and nuclear DNA, causing mitochondrial dysfunction and pathology (Richter et al., 1988). Proteins can be directly damaged by ROS or indirectly by reacting with active aldehyde products of lipid peroxidation, e.g. malondialdehyde (MDA) and hydroxynonenal (HNE) (Esterbauer et al., 1991), or as a result of alterations in membrane lipid microenvironment secondary to peroxidative processes (Hecht et al., 1992; Stadtman et al., 2000). These changes are detrimental if the modified residues are essential for the activity or turnover of the protein (Y. Wang et al., 2012).

Apart from exogenous sources such as ionizing radiation, diet, metals, pesticides or other toxic compounds, ROS can be generated endogenously in a variety of sites and conditions (e.g ischemia–reperfusion and enzymatic reactions). These molecules are generated in metabolic processes that consume oxygen in the mitochondria, peroxisomes and endoplasmic retiiculum, although the majority of ROS is produced in mitochondria (Boveris et al., 1972; Halliwell, 2007), where superoxide anion is produced during the flow of electrons along the respiratory chain to produce ATP (Jensen, 1966; Murphy, 2009). Mitochondrial complex I and III are the major sites of superoxide production, both in the matrix site and mitochondrial inner membrane space. Other enzymes contributing to superoxide generation within mitochondria include 2-oxoglutarate dehydrogenase (OGDH), pyruvate dehydrogenase (PDH), mitochondrial glycerol 3-phosphate dehydrogenase (GPDM) and the electron transfer flavoprotein-ubiquinone oxidoreductase system (Brand, 2010). Another modulator of mitochondrial ROS production is the proton motive force (pmf), composed of an electrical gradient (ψ, mitochondrial membrane potential) and a chemical gradient (pH) across the inner mitochondrial membrane. Mitochondrial ROS production and pmf are positively correlated (Echtay et al., 2002). Extra mitochondrial sources of ROS comprise, for instance, the membrane nicotinamide adenine dinucleotide phosphate, (reduced form) oxidase (NADPH); lipoxygenases; cyclooxygenases; peroxidases; other heme proteins; xanthine oxidase; peroxisomal β-oxidation and hepatic P-450 microsomal detoxification. To combat ROS produced from such a vast set of sources, the cell is equipped with its own antioxidant defences.

1.3.1.2 Antioxidant defences

Before formulating therapeutic approaches towards the modulation of ROS levels, a comprehensive understanding of antioxidant pathways is essential. This section comprises, first, a description of the set of antioxidant defences present in the organism, which can be classified in enzymatic and non-enzymatic defences. Then, a review about thetherapeutic approaches based on antioxidant research, and finally, an extensive explanation of the main regulatory pathways of antioxidant response, with a special focus on nuclear factor, erythroid-2 like 2 (NFE2L2, also NRF2) transcription factor.

1.3.1.2.1 Enzymatic antioxidant defences

Enzymatic antioxidant defences include SODs, glutathione peroxidases (GPXs), catalase, peroxiredoxins (PRDXs), thioredoxin system, glutaredoxins (GRLXs) and glutathione S-transferases (GSTs) (**Fig. 3**).

SODs accelerate the dismutation of two molecules of superoxide ($O_2^{\bullet -}$) into one molecule of H_2O_2 and one molecule of O_2, reducing the consequent harmful effects of superoxide anion radical. Animals possess three SOD enzymes, one containing active-site manganese (MnSOD/SOD1), another one containing active-site copper and zinc (CuZnSOD/SOD2) and an extracellular SOD (SOD3) (Fridovich, 1995). SOD1 is localized in the mitochondrial matrix, whereas SOD2 is present in mitochondrial intermembrane space and in the rest of the cell, including the peroxisome, as commented before. The importance of SODs is evidenced by the phenotypes displayed by multiple animal models with mutations in SOD enzymes. Mice lacking SOD2 exhibit extensive mitochondrial damage and neurodegeneration before perinatal death (Lebovitz et al., 1996). Another key evidence of the association between oxidative stress and neurodegenerative diseases came from the identification of pathological mutations in SOD2 in familial amyotrophic lateral sclerosis (ALS) patients (Rosen et al., 1993). Nevertheless, SODs must work together with other enzymes that catalyze H_2O_2.

These enzymes include catalase, mainly a peroxisomal enzyme, and glutathione peroxidases (GPXs), which are another important H_2O_2 scavenger enzymes, especially in the brain (Brigelius-Flohe, 1999). GPXs couple the reduction of H_2O_2 and other peroxides to H_2O or the corresponding alcohols with the oxidation of reduced glutathione (GSH → GSSG). GSSG can be converted back to GSH by glutathione reductase (GR). Among the eight described GPXs (GPX1-8), GPX1-4 are selenoproteins with a selenocysteine (Sec) in the catalytic centre, while GPX6 is a selenoprotein in humans, but not in rats or mice. On the other hand GPX 5, 7 and 8 possess a cysteine (Cys) in the active site (Brigelius-Flohe et al., 2013). Among them, GPX4 plays a crucial neuroprotective function by controlling the production of lipid hydroperoxides and apoptosis (M. H. Yoo et al., 2010; S. E. Yoo et al., 2012).

Peroxiredoxins are another important group of ROS scavengers that reduce H_2O_2 and organic peroxides by a cycle of peroxide-dependent oxidation and thiol-dependent reduction (catalyzed by thioredoxins) of Cys residues (Rhee et al., 2005). The six isoforms of PRDX (I to VI) are classified into three subgroups based on the number and position of the Cys residues involved (2-Cys, atypical 2-Cys and 1-Cys). Peroxiredoxins depend on the thioredoxin system, composed by thioredoxin (TXN) and thioredoxin reductase (TXNRD), and the responsible of the reduction of the 2-Cys PRDXs (Chae et al., 1994a; Chae et al., 1994b; Luthman et al., 1982).

Thioredoxin system comprises TXN, truncated TXN (TXN-80), TXNRD, NADPH, and a natural TXN inhibitor, the thioredoxin-interacting protein (TXNIP). Thioredoxin system is involved in the regulation of redox signaling, being pivotal for growth promotion, neuroprotection, inflammatory regulation, apoptosis, and atherosclerosis (D. F. Mahmood et al., 2013). In humans, three distinct TXNs are present. TXN1 is cytosolic, TXN2 is mitochondrial, while TXN3 is only expressed in spermatozoa. TXN-80 is a truncated form of TXN1, whose functions are not well defined yet (Pekkari et al., 2004). Mammalian TXNRD are selenoproteins that reduce the active site disulfide in thioredoxins' Cys residues, in a NADPH-dependent manner. Three isoforms encoded by three different genes are present in humans, TXNRD1 (cytosolic), TXNRD2 (mitochondrial) and TXNRD3 (present in testis) (Arner, 2009). Finally, thioredoxin interacting protein (TXNIP) is a negative modulator of TXN1 (Junn et al., 2000). Recently, TXN2 deficiency in a patient led to impaired mitochondrial redox homeostasis as well as early-onset neurodegeneration (Holzerova et al., 2016)

The same function of the thioredoxin system can be performed by glutaredoxins (Fernandes et al., 2004). In humans four glutaredoxins have been described (GRLX1, GRLX2, GRLX3 and GRLX5). GRLX1 and GRLX3 reside mainly in the cytoplasm. GRLX2 and GRLX5 are mitochondrial, although two GRLX2 isoforms (GRLX2B and GRLX2C) can be found in the nucleus and cytosol of some testicular and cancer cells (Lillig et al., 2008). Glutaredoxins protect against oxidative stress by catalyzing reduction of protein mixed disulfides with GSH. When oxidized, GRLXs isoforms are regenerated by GSH (Fernandes et al., 2004).

Glutathione S-transferases, also related to GSH, compose a family of cytosolic, mitochondrial and microsomal enzymes, whose main function is detoxifying reactive electrophilic compounds (chemotherapeutic agents, insecticides, herbicides, carcinogens, and by-products of oxidative stress) by conjugation with GSH (Hayes et al., 2005). Mammalian cytosolic GSTs are all dimeric, with at least 17 subunits existing in humans (Board et al., 2013), only one mitochondrial subunit although also identified in peroxisomes (κ subunit) (Morel et al., 2004) and six microsomal GSTs subunits (mostly involved in the synthesis of eicosanoids).

1.3.1.2.2 Nonenzymatic antioxidant defences

Nonenzymatic antioxidants act by scavenging free radicals, embodying molecules such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, flavonoids, and other antioxidants. Most of them are ingested with the diet, although others can be synthesized endogenously (Forman et al., 2014).

Among them, the most important antioxidant system of the organism is glutathione (GSH/GSSG) (**Fig. 3**). Actually, glutathione antioxidant functions are performed by a system composed of i) GSH, a γ-L-Glu-L-Cys-Gly tripeptide; ii) NADPH-dependent glutathione reductase (GR, a pyridine nucleotide disulfide oxidoreductase GSSG reductase); iii) some of the GRLX isoforms described above, that act as thiol-disulfide reductases; iv) and several GPXs (also described above) (S. C. Lu, 2013). GSH is synthesized in the cytosol in two steps that require ATP. The first and rate-limiting step is catalyzed by glutamate-cysteine ligase (also known as γ-glutamylcysteine synthase, GCL). GCL is a heterodimeric enzyme composed by a catalytic subunit, GCLC and a regulatory (modifier) one, GCLM. In the second step, glutathione synthetase (GS) catalyzes the addition of glycine, which availability is rate-limiting too (Dalton et al., 2004). Cytosolic glutathione (the most abundant pool in the cell) is transported into the mitochondrion, the endoplasmic reticulum and the nucleus. GSH is the predominant form, except in the endoplasmic reticulum, where it exists mainly as GSSG (Hwang et al., 1992). GSH is only metabolized extracellularly by γ-glutamyl transpeptidase (GGT), allowing for recycling of GSH into its constituent amino acids (γ-glutamyl cycle) (S. C. Lu, 2013).

GSH/GSSG redox system enables optimum performance of multiple redox sensitive biochemical and biophysical processes, but can also directly scavenge free radicals (Winterbourn, 1993), although the physiological relevance of this latter function is still debated (Forman et al., 2014). The antioxidant function of GSH is thus accomplished by GPX-catalyzed and GRLX-catalyzed reactions, which reduce H_2O_2 and lipid peroxide, or reduce thiol groups in proteins, while GSH is oxidized to GSSG. GSSG is reduced back to GSH by GR at the expense of FAD and NADPH, forming a redox cycle (S. C. Lu, 2013).

GR acts as a dimer, utilizing FAD and NADPH to reduce one GSSG to two GSH (Schulz et al., 1978). GR adopts a central role in glutathione metabolism by linking the cellular NADPH pool with the GSH/GSSG pool, thus collaborating to maintain a reducing intracellular milieu (high GSH and low GSSG levels) (S. C. Lu, 2013).

Moreover, the interaction between GSH and free thiols in Cys residues of proteins, leads to reversible disulfides, in a process called S-glutathionylation. This process may result in the activation or inhibition of protein function (Grek et al., 2013). Thereby S-glutathionylation is able to modulate different cellular pathways, affect gene expression by affecting different transcription factors, and serves as a protective mechanism against irreversible cysteine oxidation. S-glutathionylation can be spontaneous or can be catalyzed by GSTP (Manevich et al., 2004), whereas it is reverted mainly by glutaredoxins (Fernandes et al., 2004). The role of these modifications in neurodegenerative diseases has been extensively reviewed in (Sabens Liedhegner et al., 2012).

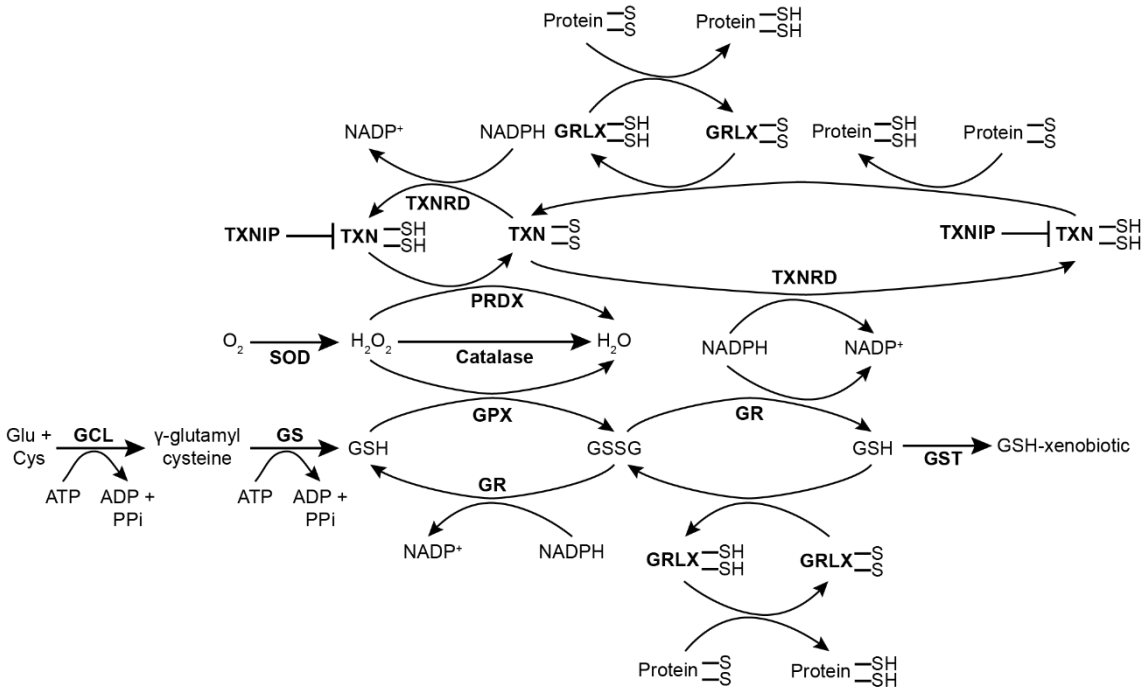


Figure 3 Glutathione and enzymatic antioxidant defence system

This schematic view of the endogenous antioxidant system embodies every arm of this system, as explained in detail in the introduction. Abbreviations: GCL (glutamate-cysteine ligase), GS (glutathione synthetase), GPX (glutathione peroxidase), GR (glutathione reductase), GST (glutathione S-transferase), GRLX (glutaredoxin), PRDX (peroxiredoxin), SOD (superoxide dismutase), TXN (thioredoxin), TXNIP (thioredoxin-interacting protein), TXNRD (thioredoxin reductase). Adapted from Zhang *et al.* (2014).

1.3.1.3 Oxidative stress in X-ALD

Evidences of oxidative stress associated to neurodegeneration are found in the majority of neurodegenerative disorders such as Parkinson's disease (PD) (Chung et al., 2004); ALS (Mitsumoto et al., 2008; Simpson et al., 2004); multiple sclerosis (Haider et al., 2011); Alzheimer's disease (AD) (Lopez et al., 2013); and Huntington's disease (HD) (Browne et al., 1999; C. M. Chen et al., 2007). Neurons are especially sensitive to oxidative damage because of their high metabolic needs (Halliwell, 2001), considering that the brain represents about 2% of the total body weight yet it accounts for 20% of all the energy consumed by the organism. Reversing the ion influxes needed for action potential and synaptic neurotransmission consumes high amounts of energy (A. Ames, 3rd, 2000; Attwell et al., 2001). Other factors determining the higher susceptibility of neurons to oxidative damage are: i) large dependence on oxidative phosphorylation

compared to other cells (Hall et al., 2012); ii) a relatively poor expression of antioxidant defences in comparison with other cells from the CNS (Bell et al., 2015); iii) they are enriched in metals which can act as catalysts for reactive species formation (Ward et al., 2012); and iv) they posess a high content of highly peroxidizable polyunsaturated fatty acids in their membranes (Adibhatla et al., 2010).

In the case of peroxisomal diseases, and in particular, X-ALD, it is currently well established that oxidative stress plays a key role in the pathophysiology of these diseases (Galea et al., 2012; I. Singh et al., 2010). Indeed, peroxisome-deficient mice exhibit an increase of SOD2 in different organs, reflecting oxidative stress (Baumgart et al., 2001). In X-ALD first clues were given by studies showing higher oxidizability of LDL in plasma from ALD patients compared to that of control subjects (Di Biase et al., 2000), and detecting expression of iNOS in astrocytes and microglia from brain tissue of X-ALD patients but not in controls (Gilg et al., 2000). Then, studies by Vargas *et al.* and Powers *et al.* detected oxidative stress in plasma, erythrocytes, fibroblasts (Vargas et al., 2004) and human adrenal cortex and brain sections from ccALD patients (Powers et al., 2005). X-ALD derived lymphoblasts also present higher levels of oxidative stress (Uto et al., 2008). Later, a series of studies from our group have demonstrated that oxidative stress is an early event in X-ALD pathophysiology. Fibroblasts and peripheral blood mononuclear cells (PBMC) derived from X-ALD patients display a twofold increase in the concentration of selected biomarkers of oxidative damage [glutamic semialdehyde (GSA) and aminoadipic semialdehyde (AASA) for carbonylation; Nε-(carboxymethyl)-lysine (CML), Nε-(carboxyethyl)-lysine (CEL) for glycoxidation/lipoxidation; and Nε-MDA-lysine (MDAL) for lipoxidation], analyzed by gas chromatography/mass spectrometry (GC/MS) (Fourcade et al., 2008; Fourcade et al., 2010).

Using ABCD1-deficient mice, our group uncovered an aberrant accumulation of MDAL, a consequence of lipoxidative damage to proteins, in the spinal cord of these mice as early as 3.5 months of age. At 12 months of age, *Abcd1*$^{-}$ mice accumulate additional proteins modified by oxidative damage arising from metal-catalyzed oxidation and glycoxidation/lipoxidation (Fourcade et al., 2008). We also unveiled an impairment of bioenergetic homeostasis due to oxidation of key enzymes of glycolytic pathway and Krebs cycle (Galino et al., 2011; Lopez-Erauskin et al., 2013), together

with an impairment of the mitochondrial oxidative phosphorylation, including mitochondrial DNA (mtDNA) oxidation (see below, in "Mitochondrial dysfunction in X-ALD", for more details) (Lopez-Erauskin et al., 2013). Defects in bioenergetic homeostasis in X-ALD are associated with a decreased activity of pyruvate kinase, and lower levels of ATP, nicotinamide adenine dinucleotide (reduced form) (NADH) and GSH compared to controls (Galino et al., 2011). Alterations in energetic metabolism are also found in other neurodegenerative disorders, such as AD (Rhein et al., 2009), HD (Mochel et al., 2011) or ALS (Dupuis et al., 2004). Finally, a functional genomic analysis of transcriptomic data from the spinal cord of X-ALD mouse model and NAWM of X-ALD patients revealed a dysregulation in a wide array of mitochondrial genes related to the pyruvate and oxoglutarate dehydrogenase complexes, tricarboxylic acid (TCA) cycle, OXPHOS system and antioxidant defences (Schluter et al., 2012). Collectively, all these data provide enough evidences of the key role of oxidative stress in X-ALD.

In addition, VLCFA accumulation has been identified as the causative agent of redox imbalance in X-ALD. Excess of C26:0 generates ROS in human fibroblasts (Fourcade et al., 2008; Fourcade et al., 2010); immortalized neuronal and oligodendrocyte cell cultures (Baarine et al., 2012; Zarrouk et al., 2012); and mitochondria and primary astrocytes from WT and *Abcd1*$^{-}$ mice brain (Kruska et al., 2015). In X-ALD fibroblasts, ROS generated by C26:0 oxidize mtDNA and impair OXPHOS system, triggering mitochondrial ROS production from electron transport chain complexes (Lopez-Erauskin et al., 2013).

Besides an excess of reactive oxygen species, oxidative stress can be caused by a deficit in endogenous antioxidant response. Measurement of the status of antioxidant defences in X-ALD has been done in multiple ways. Total antioxidant reactivity (TAR) is reduced in plasma from symptomatic AMN, ccALD patients (Deon et al., 2007; Turk et al., 2017; Vargas et al., 2004) and carrier women (Deon et al., 2008). Vargas *et al.* found no difference in total radical-trapping antioxidant potential (TRAP), indicative of non-enzymatic antioxidant defences (2004). However, GSH is reduced in the spinal cord of ABCD1-deficient mice (Galino et al., 2011) as well as in lymphocytes and erythrocytes from X-ALD patients (Petrillo et al., 2013). Furthermore, excess of C26:0 in X-ALD fibroblasts induces GSH depletion, and X-ALD fibroblasts present higher

sensitivity to L-buthionine-sulfomixine (BSO), an inhibitor of GSH synthesis (Fourcade et al., 2008). In brief, these data suggest a deficit in the antioxidant defences in X-ALD.

Regarding enzymatic defences, Fourcade *et al.* described changes in the expression of enzymatic antioxidant defences such as catalase, SOD1, SOD2 and GPX1 in the spinal cord of the X-ALD mouse model (Fourcade et al., 2008). VLCFA induce the expression of some enzymatic antioxidant defences like catalase and GPX1 in WT mice-derived organotypic spinal cord slice cultures (OSCSC) (Fourcade et al., 2008), or SOD in 158N murine oligodendrocytes (Baarine et al., 2012). Interestingly, upon C26:0 treatment, control fibroblasts do not show any lesion characteristic of oxidative damage, whereas X-ALD fibroblasts do, suggesting that the mechanisms that should buffer the oxidative stress caused by C26:0 may work less efficiently in X-ALD fibroblasts (Fourcade et al., 2008). Increased activity of GPX1, catalase and SODs has been detected in erythrocytes and fibroblasts from ccALD patients (Vargas et al., 2004), suggesting a pro-oxidative status in these cells compared to control. SOD2 expression is increased in astrocytes (Powers et al., 2005) and fibroblasts (Fourcade et al., 2008) from X-ALD patients, and its level and enzymatic activity have been associated with cerebral demyelination (Brose et al., 2012; Turk et al., 2017).

Considering all these evidences, antioxidants were then suggested as a therapeutic strategy for X-ALD patients. Several studies supported this approach. Vitamin E (α-tocopherol) prevents oxidative lesions to X-ALD fibroblasts induced by excess of C26:0 (Fourcade et al., 2008). N-acetylcysteine (NAC) reverses mitochondrial damage markers and cyclophilin D-mediated opening of the mitochondrial permeability transition pore in X-ALD fibroblasts (Lopez-Erauskin et al., 2012). Lipoic acid (LA), NAC and vitamin E, individually or combined at lower doses, prevent C26:0-dependent ROS accumulation in X-ALD fibroblasts (Lopez-Erauskin et al., 2011). Moreover, *in vivo*, a cocktail of NAC, LA and vitamin E neutralizes oxidative stress and damage to proteins; preserves bioenergetic homeostasis; and prevents axonal degeneration and locomotor deficits in the X-ALD mouse models (Lopez-Erauskin et al., 2011). These preclinical results led to a phase II clinical trial with a cocktail of antioxidants (NCT01495260) in AMN patients, which results were detailed before in this thesis.

1.3.1.4 Antioxidant therapeutics

Since an excess of free radicals and reactive species has proven to be harmful, it seemed reasonable that scavenging them with supplements of antioxidants would protect against oxidative damage. Indeed, first studies in the early 90s with animal models showed the potential of dietary antioxidants such as α-tocopherol (Vit E) (Parola et al., 1992). Studies over these decades have demonstrated the beneficial effects of antioxidants supplements for a myriad of neurodegenerative pathologies in animal models, including X-ALD (Kamat et al., 2008; Lopez-Erauskin et al., 2011).

So, why all these promising *in vitro* and *in vivo* (with animal models) evidences do not usually translate into successful human clinical trials? (Petersen et al., 2005; H. H. Schmidt et al., 2015; Sesso et al., 2008; Shuaib et al., 2007). This contradiction has been defined as the *antioxidant paradox* (Halliwell, 2000). The recent results of a large study investigating the effect of vitamin E or selenium supplements to prevent dementia illustrate perfectly this paradox. The authors concluded that these compounds do not prevent neurodegeneration or dementia, even though it is known that oxidative stress plays a role in the onset of dementia (Kryscio et al., 2017). One possible reason is that rats and mice appear to be more sensitive to dietary antioxidant levels than humans, being therefore more likely to display less oxidative damage after antioxidant supplementation (Halliwell, 2011; Lehr et al., 1999). Furthermore, exposure to oxygen in experiments with cell cultures, isolated organs, and cell-free models represent artifactual conditions due to a very high partial oxygen pressure of about 200 μM, much more than physiological concentrations (Halliwell, 2014; Kojo, 2012). Other reasons have been pointed to play a role in the so called *antioxidant paradox*, for instance the lack of a correct antioxidant efficacy assessment *in vivo* (Cocheme et al., 2010).

The predominating trend in the last decades has been that protection by diets containing antioxidants against different chronic diseases and cancer is due to their antioxidant capacity (B. N. Ames et al., 1993). However, this idea of a free radical scavenger mechanism for antioxidant supplements cannot be substantiated on a kinetic basis *in vivo*, as explained in detail in (Forman et al., 2014). Even when reaching high concentrations of a given antioxidant in cells or plasma, the reactions rates are insignificant in comparison with enzymatic reactions catalyzed by various peroxidase

and peroxiredoxins (Flohe et al., 2011). Hence, endogenous antioxidants provide for biologically relevant antioxidant defences rather than antioxidant supplements.

As we have seen in this section, ROS play a dual role that requires a tight regulation of ROS producing and detoxifying pathways, since low levels are needed for physiological functions, but an excess is harmful. A good example could be found in the muscle, where diverse regulatory systems, both positive and negative, have evolved to enable adaptation to oxidative environments (Y. C. Jang et al., 2010; Ristow et al., 2009). Thereby, and given the unprosperous results of antioxidant supplements in clinical trials, therapeutic efforts should seek the activation of endogenous antioxidant response while leaving ROS physiological functions unaltered. Therefore, one of the main challenges in redox biology is to develop effective antioxidants for human use, particularly in the brain.

1.3.1.5 NRF2/ARE system

At the center of this new focus, i.e targeting endogenous antioxidants, is the transcription factor NRF2, a member of the cap 'n' collar (CNC) subfamily of basic region leucine zipper (bZip) transcription factors, and also considered the master regulator of endogenous antioxidant response (Andrews et al., 1993; Moi et al., 1994). NRF2-mediated signaling activates a whole battery of cytoprotective defences against oxidative stress and phase-II detoxifying enzymes through binding to antioxidant response element (ARE) sequences in the DNA (Favreau et al., 1991; Itoh et al., 1997; Rushmore et al., 1990). In *Caenorhabditis elegans*, skinhead-1 (SKN-1) protein has a significant homology with NRF2 and binds to an ARE-like element to regulate oxidative stress-related genes (An et al., 2003). In *Drosophila* CncA, CncB, and CncC proteins are similar to mammalian CNC bZip proteins (Sykiotis et al., 2008). Hundreds of genes are regulated by this system, including heme oxygenase-1 (*HMOX1*); NAD(P)H:quinone oxidoreductase-1 (*NQO1*) and enzymes involved in the metabolism of glutathione such as GSTs, GCL and GPXs (J. M. Lee et al., 2003; McMahon et al., 2001; Thimmulappa et al., 2002). An extensive list of NRF2-controlled genes has been reported by Ma *et al.* and Tebay *et al.* (Ma, 2013; Tebay et al., 2015).

Among the prototypicals target genes regulated by NRF2, *HMOX1*, *NQO1*, *GCLC* and *GSTA3* can be highlighted, as they were studied in this thesis as readout of NRF2 pathway status. *GCLC* encodes for the catalytic subunit of GCL, the enzyme involved in the rate-limiting step of glutathione synthesis, where *GSTA3* encodes for one of the glutathione S-transferases. The functions of these proteins are detailed in the "Non-enzymatic antioxidant defences" section. *HMOX1* encodes for HO-1 protein, which catalyzes the rate-limiting step in the degradation of free heme [iron (Fe) protoporphyrin (IX)] into biliverdin, carbon monoxide (CO) and free iron (Fe^{2+}). HO-1 expression is induced upon oxidative stress due to NRF2-dependent signaling (Alam et al., 1999), and it provides cytoprotection against oxidative stress caused by potentially toxic free heme (not contained within the heme pockets of hemoproteins), as less reactive iron can be stored as ferritin, which is a non toxic form (Gozzelino et al., 2010). NQO1 protein is a cytosolic homodimeric FAD-dependent flavoprotein, also regulated by NRF2/ARE signaling (Favreau et al., 1991; Nioi et al., 2003). NQO1 provides cytoprotection by different mechanisms. First, it catalyzes two electron-reduction and detoxification of a

myriad of substrates, in particular quinones and its derivatives, using NADH or NADPH as electron donors (Talalay et al., 2004). This reduction, bypassing the toxic semiquinone intermediate, generates hydroquinone, which is relatively stable and can be further conjugated and excreted (R. Li et al., 1995). In addition, oxidoreductase activity maintains the endogenous lipid-soluble antioxidants, such as α-tocopherol-hydroquinone and coenzyme Q10 in their reduced and active forms (Landi et al., 1997; Siegel et al., 1997). Finally, NQO1 regulates the stability of p53 tumour suppressor protein in response to DNA damage (Asher et al., 2001).

Antioxidant response induction requires a common DNA sequence called ARE, 5′-RTGASnnnGCR-3′ (Rushmore et al., 1991), later updated to 5′-TMAnnRTGAYnnnGCR-3′ (M = A or C; R = A or G; Y = C or T; W = A or T; S = C or G; n = any nucleotide) (Nioi et al., 2003). NRF2 binding to the promoter of its target genes requires small musculoaponeurotic fibrosarcoma (MAF) proteins (MAFF, MAFG and MAFK), forming heterodimers (Itoh et al., 1997; Katsuoka et al., 2005; Toki et al., 1997). On the other hand, BTB (Broad complex, tramtrack, and bric-a-brac) domain and CNC homolog proteins (BACH1 and BACH2) inhibit NRF2 signaling by competitive binding of small MAF proteins (Oyake et al., 1996). In general NRF2 activates an endogenous response against oxidative stress embodying hundreds of genes by binding to ARE sequences in the DNA forming heterodimers with small MAF proteins.

NRF2 is a modular protein comprising 597 amino acids in rodents, and 605 amino acids in humans, structurally divided into seven Neh (NRF2-ECH homology) domains. ECH terminology derives from the name given by Yamamoto and collaborators when they described the chicken NRF2 orthologous protein, which was called erythroid cell-derived protein with CNC homology (ECH) (Itoh et al., 1995). NRF2 regulation is determined by two separate protein domains, one based on KEAP1-dependent cysteine thiol–based redox signaling, and another domain regulated by a redox-insensitive mechanism (McMahon et al., 2004), that are detailed in the following section.

1.3.1.5.1 KEAP1-dependent regulation of NRF2

Under non-stress conditions, Kelch ECH associating protein 1 (KEAP1) sequesters NRF2 in the cytoplasm for its degradation by the ubiquitin-proteasome system (Itoh et

al., 1999), thus inhibiting NRF2-dependent antioxidant response (**Fig. 4**). Half life of NRF2 is around 20 min, due to its rapid degradation by proteasomes at basal conditions, resulting in low levels of NRF2 protein in most of the cells (A. Kobayashi et al., 2004; D. D. Zhang et al., 2004). The two main domains of KEAP1 are BTB and Kelch/DGR (double glycine repeat).

BTB domain in the N-terminal region of KEAP1 interacts with a Cullin-3-based E3 ubiquitin ligase complex that recognizes NRF2, delivering it to the proteasome. Kelch/DGR (double glycine repeat) domain in the C-terminal region mediates binding of KEAP1 to the Neh2 domain of NRF2. Intervening region (IVR) or linker region (LR), which is rich in cysteines, is found between BTB and DGR domains (X. Li et al., 2004; Ogura et al., 2010). The proposed mechanism of NRF2-KEAP1 interaction is denoted as "hinge-and-latch", in which a dimer of KEAP1 interacts with the ETGE and DLG domains of one molecule of NRF2. DLG domain interaction with KEAP1 is the first disrupted upon oxidative stress, preventing more NRF2 to bind KEAP1, and thus allowing newly synthesized NRF2 to translocate to the nucleus (McMahon et al., 2006; Ogura et al., 2010; Tong et al., 2006). (**Fig. 4**)

By the mechanism just explained before, oxidative stress or electrophiles modify redox-sensitive cysteine residues in KEAP1 (especially Cys 151 in BTB domain, and Cys 273/Cys 288 in IVR domain), provoking derepression of NRF2 and its subsequent nuclear translocation to the nucleus. Electrophiles such as sulforaphane, dexamethasone mesylate, synthetic triterpenoids or dimethyl fumarate (Dinkova-Kostova et al., 2002; Dinkova-Kostova et al., 2005; Dinkova-Kostova et al., 2001; Linker et al., 2011) modify cysteines residues on KEAP1, disrupting its interaction with NRF2 (T. Yamamoto et al., 2008; D. D. Zhang et al., 2003). *In vivo* data from *Keap1* knockout mice supports this NRF2/KEAP1 interaction, as these mice show constitutive hyperactivation of NRF2 leading to postnatal lethality (Wakabayashi et al., 2003). However, apart from this redox sensititive KEAP1-dependent regulation, there are several upstream signaling pathways that can also regulate NRF2-dependent response.

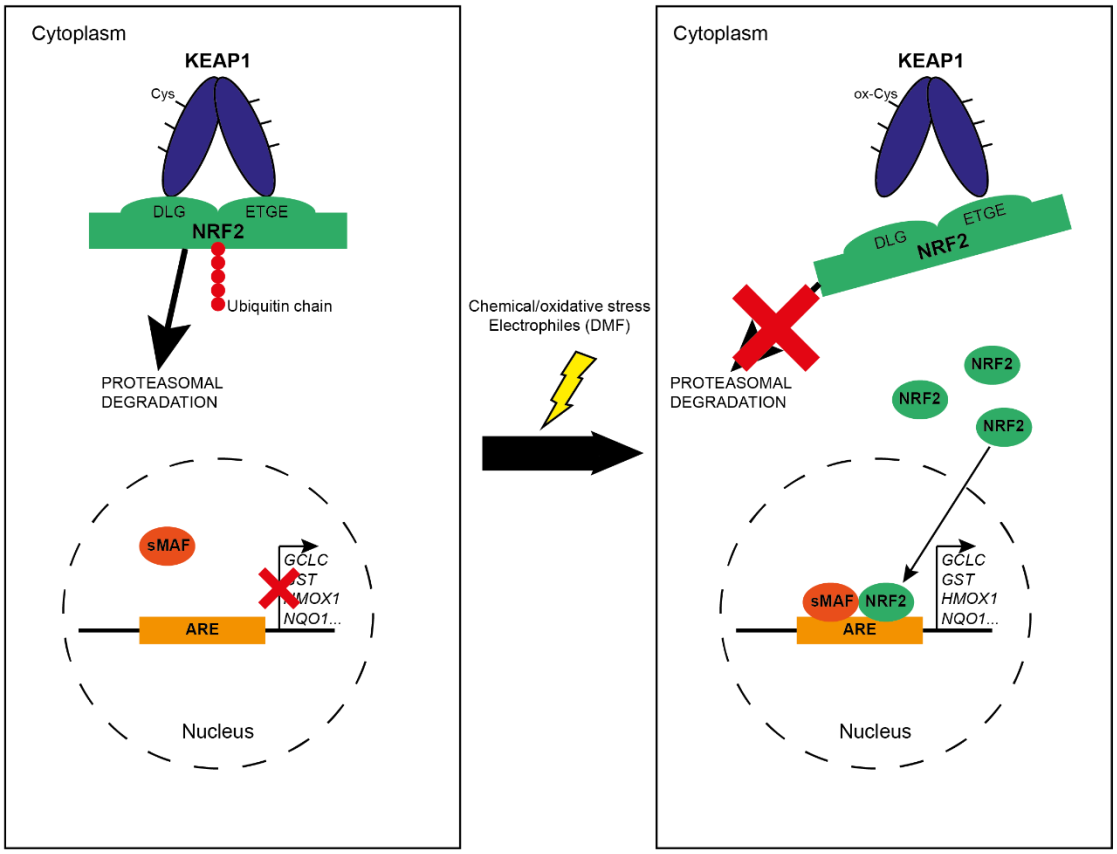


Figure 4 Schematic overview of the KEAP1-dependent NRF2 regulation

Under basal conditions (left side), NRF2 is bound to its cytoplasmic repressor KEAP1, and is degraded by the ubiquitin-proteasome system. Upon exposure to a stressor or electrophiles, which modify cysteine residues on KEAP1, newly synthesized NRF2 can translocate to the nucleus. Once in the nucleus, NRF2 dimerizes with small MAF proteins (sMAF) and binds to antioxidant response element (ARE) sequences in the DNA, and then activates the transcription of hundreds of genes involved in the endogenous antioxidant response, inflammation and many other functions. Abbreviations: DMF (dimethyl fumarate), KEAP1 (Kelch ECH associating protein 1), NRF2 (nuclear factor, erythroid-2 like 2), GCLC (glutamate-cysteine ligase, catalytic), GST (glutathione S-transferase), HMOX1 (heme oxygenase-1), NQO1 (NAD(P)H:quinone oxidoreductase-1). Adapted from Bryan et al. (2013).

1.3.1.5.2 KEAP1-independent regulation of NRF2 pathway

In this category, the most important and better described regulatory mechanism is mediated by signaling pathways that regulate glycogen synthase kinase-3 beta (GSK-3β) (Martin et al., 2004; Rada et al., 2011; Rojo et al., 2008b; Salazar et al., 2006). Among them, phosphoinositide 3-kinase (PI3K)/AKT and canonical WNT signaling pathways are involved in NRF2 regulation. The interaction between GSK-3 and NRF2 was first described in *C. elegans*, where SKN-1 interacts with GSK-3 (An et al., 2005)

and AKT (Tullet et al., 2008). PI3K/AKT association with NRF2 is particularly interesting in the case of neurodegenerative diseases because it connects antioxidant cytoprotective response with other essential cellular functions in the CNS, such as pro-survival signaling (Dudek et al., 1997). Activation of PI3K/AKT protects PC12 cells against several stresses/toxins such as 1-methyl-4-phenylpyridinium (MPP^+) (Salinas et al., 2001), 6-hydroxydopamine (6-OHDA) (Salinas et al., 2003) and amyloid-β (Aβ) fragment Aβ(25-35) (Martin et al., 2001) while reducing ROS levels and inducing the expression of *HMOX1*, a prototypical NRF2 target gene (Salinas et al., 2003). Other stressors such as peroxynitrite and sulfur amino acid deprivation lead to PI3K/AKT-mediated NRF2 activation (Kang et al., 2002; Kang et al., 2000). However, the link between these two pathways was elusive until GSK-3β mediated phosphorylation of NRF2 was described (Rada et al., 2011), providing a conceptual frame that embodies both pathways.

Modulation of GSK-3β by pharmacologic modulators and genetic variants has led to the demonstration that GSK-3β modulates NRF2-dependent antioxidant response (Salazar et al., 2006). In particular, GSK-3β phosphorylates serine residues in the Neh6 domain of NRF2, creating a recognition motif for beta-transducin containing protein (β-TrCP), an adaptor protein for the Cul1-dependent SCF (Skp1-Cullin-F-box) E3 ubiquitin ligase, and leading to the ubiquitination and proteasomal degradation of NRF2 independently of KEAP1 (Rada et al., 2011; Rada et al., 2012) (**Fig. 5**). The functional relevance of this pathway *in vivo* was demonstrated in the hippocampus of mice with postnatal neuronal deficiency of GSK-3β. These neurons display increased levels of NRF2, phase-II gene products and GSH, together with lower levels of carbonylated proteins and MDA (Rada et al., 2012). In addition, oxidative damage activates GSK-3β leading to NRF2 inhibition in neurons (Rojo et al., 2008a; Rojo et al., 2008b). To sum up, GSK-3β plays a fundamental role in the regulation of NRF2-dependent response.

GSK-3 is a highly conserved serine/threonine protein kinase encoded by two similar genes encoding GSK-3α (51 kDa) and GSK-3β (47 kDa) proteins (Woodgett, 1990). GSK-3 is expressed ubiquitously in virtually all mammalian tissues, with high expression in the brain, in particular in neurons and glia (Ferrer et al., 2002; Pandey et al., 2009; Perez-Costas et al., 2010; Yao et al., 2002).

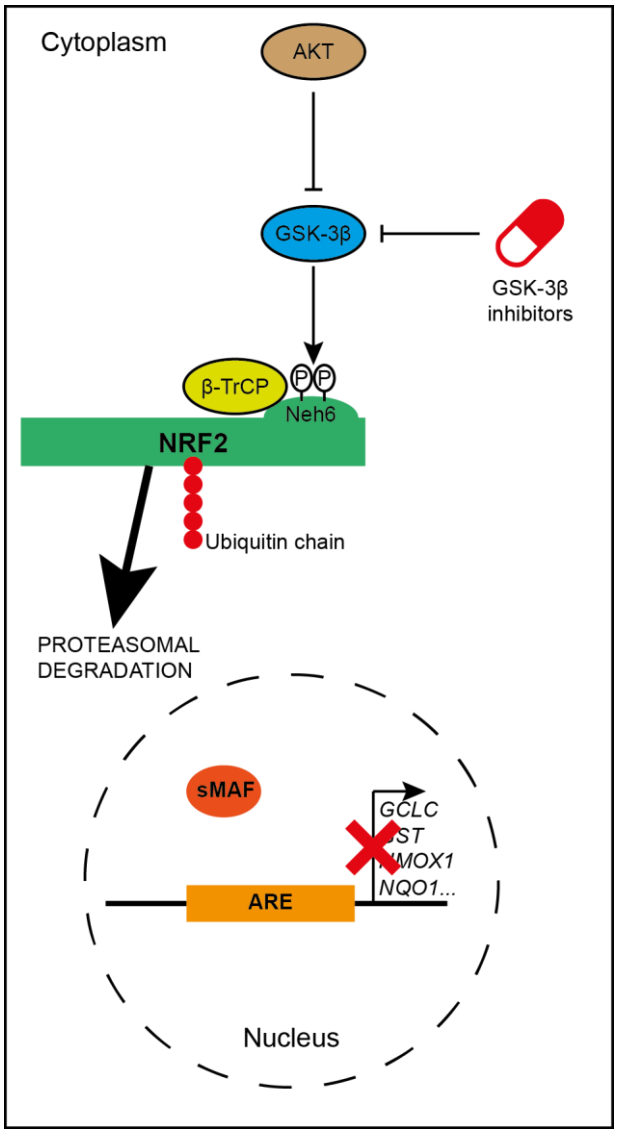


Figure 5 Schematic overview of the GSK-3β dependent negative regulation of NRF2

GSK-3β, which is inhibited by AKT-mediated phosphorylation, phosphorylates several amino acids in Neh6 domain of NRF2, creating a recognition motif. Then, β-TrCP, an adaptor protein, recognizes this motif, and allows ubiquitination of NRF2 and its subsequent proteasomal degradation. GSK-3β inhibitors induce NRF2 pathway by preventing NRF2 phosphorylation by GSK-3β. Abbreviations: GSK-3β (glycogen synthase kinase-3 beta), β-TrCP (beta transducing containing protein).

GSK-3 is a unique kinase due to its high activity under resting conditions (Sutherland et al., 1993) and its inhibition, mediated by extracellular signals that phosphorylate a serine residue (Ser9 in GSK-3β, Ser21 in GSK-3α), leading to a decrease in its enzymatic activity (Stambolic et al., 1994; Welsh et al., 1993). GSK-3 is constitutively phosphorylated in resting cells on a Tyr residue (Y216 in GSK-3β and Y279 in GSK-3α), associated with facilitation of substrate phosphorylation (Hughes et al., 1993). Insulin, for instance, inactivates GSK-3 *via* PI3K/AKT-mediated phosphorylation of

Ser9/21 residues (D. A. Cross et al., 1995). Other regulators of GSK-3β include the mammalian target of rapamycin (mTOR) pathway and mitogen-activated protein kinase (MAPK) cascades. Another particularity compared with other protein kinases is the preference of GSK-3 for primed substrates, i.e. substrates previously phosphorylated by another kinase (Dajani et al., 2001; ter Haar et al., 2001).

Regarding its function, GSK-3 is involved in multiple cellular pathways including glycogen metabolism, inflammation, apoptosis, cell cycle regulation and proliferation (Diehl et al., 1998; S. Shin et al., 2011; H. Wang et al., 2011). Dysregulation of signaling pathways involving GSK-3 is associated with the pathogenesis of human diseases such as diabetes, AD, bipolar disorder and cancer (Amar et al., 2011; Freyberg et al., 2010; Medina et al., 2014).

Focusing on the nervous system, GSK-3β has attracted a lot of attention after being pointed as the kinase responsible for Tau phosphorylation (Hanger et al., 1992; Mandelkow et al., 1992). Since then, functional studies of GSK-3 through the use of knockout mice, have revealed fundamental roles for this kinase in memory, behavior, and neuronal fate determination (Kaidanovich-Beilin et al., 2009; W. Y. Kim et al., 2009; Y. C. Li et al., 2011; K. J. Liu et al., 2007; Peineau et al., 2008). *Gsk-3α*-null mice are viable (MacAulay et al., 2007), but *Gsk-3β* knockout mice die *in utero* (Hoeflich et al., 2000), thus studies in mice are performed on heterozygous mice (*Gsk-3β*$^{+/-}$) or in cellular-specific knockouts. These *Gsk-3β*$^{+/-}$ mice display an antidepressant phenotype which mimics the effect of lithium (O'Brien et al., 2004), a GSK-3 inhibitor drug widely used as mood stabilizer in psychiatric disorders such as schizophrenia and bipolar disorder (P. S. Klein et al., 1996; Stambolic et al., 1996). On the other hand, GSK-3 overexpressing mice present hyperactivity and mania (Prickaerts et al., 2006), reinforcing the central role of GSK-3 in psychiatric disorders. Altogether, these studies have unveiled a key role of GSK-3 in the CNS.

1.3.1.5.3 NRF2/ARE in neurodegenerative diseases: a promising target

The relevance of NRF2 pathway in physiology is evidenced by the phenotype of *Nrf2* knockout mice, which are more susceptible to a broad range of chemical toxicity and disease conditions associated with oxidative stress (Chanas et al., 2002; Chowdhry et

al., 2010; Itoh et al., 1997; Yoh et al., 2001) including neurodegenerative diseases (Hubbs et al., 2007; Johnson et al., 2010; Lastres-Becker et al., 2012). In the recent years, NRF2/ARE has emerged as a promising therapeutic target against neurodegenerative disorders, not only because NRF2 induces a whole battery of antioxidant genes, but also because NRF2 regulates genes involved in proteostasis (Kapeta et al., 2010; Komatsu et al., 2010; Kwak et al., 2003; Pajares et al., 2016; Pickering et al., 2012), neuroinflammation (Innamorato et al., 2008; Rojo et al., 2010) and bioenergetics (Holmstrom et al., 2013; Ludtmann et al., 2014) in the nervous system. Alterations in these pathways are common in neurodegenerative disorders. Consequently, activating NRF2-dependent response should lead to a sustained neuroprotective effect, as it has been demonstrated in other models of neurodegenerative diseases (Kaidery et al., 2013; Kanninen et al., 2009; Lastres-Becker et al., 2016; Neymotin et al., 2011; Stack et al., 2010).

Pharmacologic targeting of NRF2 can be achieved by a dual strategy through KEAP1 or GSK-3β/TrCP. Single-hit activation of NRF2-dependent response is usually achieved by electrophilic compounds that react with specific redox-sensitive cysteine residues described before (Zenkov et al., 2013). However, some of these compounds might also be targeting GSK-3β pathway through other pathways, such as activation of PI3K/AKT or inhibition of phosphatases like phosphotyrosine phosphatase 1B (PTP1B) (Mobasher et al., 2013) and phosphatase and tensin homolog (PTEN) (Pitha-Rowe et al., 2009; Rojo et al., 2014). Therefore, GSK-3β inhibitors open new avenues of research on NRF2 activation.

1.3.1.5.4 Dimethyl fumarate as NRF2 inducer

Among NRF2 activators, I would like to highlight, given the interest for this thesis, dimethyl fumarate (DMF), a drug derived from the simple organic acid fumaric acid. Although free fumaric acid is poorly absorbed by the gastrointestinal tract, its ester derivatives, namely monomethyl fumarate (MMF) and DMF have been proved to be beneficial in treating psoriasis since the late 50s (Schweckendiek, 1959). Fostered by the safe profile and its immunomodulatory properties, the role of DMF in other immune diseases, such as multiple sclerosis, has been explored (Schimrigk et al., 2006). Indeed, DMF (Tecfidera) has been recently approved by the US Food and Drug Administration

(FDA) and the European Medicines Agency (EMA) for the treatment of relapsing remitting multiple sclerosis (Fox et al., 2012; Gold et al., 2012; Kappos et al., 2008). DMF has also been successfully tested in preclinical studies for other neurodegenerative diseases, e.g. multiple sclerosis (Schilling et al., 2006), HD (Ellrichmann et al., 2011), PD (Ahuja et al., 2016; Lastres-Becker et al., 2016) and Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD) (K. Kobayashi et al., 2015).

First studies on DMF mechanism of action were focused on its immunomodulatory properties. DMF induces pro-apoptotic effects on T cells *in vitro* (Treumer et al., 2003), although the proposed mechanism is associated with a Th2 response shift, characterized by production of anti-inflammatory cytokines, such as interleukin(IL)4, IL5 and IL10 (de Jong et al., 1996; Litjens et al., 2004; Wilms et al., 2010). DMF also target other immunological cell types like neutrophils, monocytes/macrophages, endothelial cells and keratinocytes (Loewe et al., 2002; Nibbering et al., 1993; Schilling et al., 2006; Stoof et al., 2001). Recent evidences from multiple sclerosis patients show that DMF reduces lymphocyte counts about 50% after one year of treatment (Fox et al., 2012; Gold et al., 2012; Longbrake et al., 2016; Spencer et al., 2015), as well as alters circulating T helper cell subsets *in vivo* (Gross et al., 2016). Collectively, these evidences show that immunomodulation is an important feature in the mechanism of action of DMF.

Interestingly, DMF may exert neuroprotective, and possibly also myelin-restoring effects beyond its immunomodulatory mechanisms (Arnold et al., 2014; MacManus et al., 2011). On a molecular level, seminal contributions on DMF mechanism of action have involved NRF2-dependent antioxidant response (Linker et al., 2011; Scannevin et al., 2012). DMF modifies Cys 151 residue of KEAP1, allowing NRF2 translocation to the nucleus and ultimately leading to the transcriptional activation of prototypical target genes including *HMOX1* and *NQO1*. These protective effects are lost in NRF2-deficient mice (Linker et al., 2011; Scannevin et al., 2012).

Clinically, oral DMF is well tolerated by the patients, although 30-40% of them experiment transient gastrointestinal irritations and flushing, typically within the first 2-3 months of treatment. Thereafter, these adverse effects disappear (Phillips et al., 2015).

Lymphocytopenia is another side effect of DMF treatment (Longbrake et al., 2015), and related to this, rare cases of progressive multifocal leukoencephalopathy (PML) have been associated to DMF treatment (Nieuwkamp et al., 2015; Rosenkranz et al., 2015). Hence, the latest Committee for Medicinal Products for Human Use (CHMP) decision includes testing for total number of lymphocytes every 3 months after starting DMF treatment (Linker et al., 2016), to minimize the risks of immunosuppression.

To sum up, DMF is an immunomodulatory drug and also activates NRF2 antioxidant pathway, being a promising drug candidate for those diseases presenting oxidative stress and neuroinflammation among their hallmarks.

1.3.2 Mitochondrial dysfunction: X-ALD as a secondary mitochondrial disease

Mitochondria derive from an α-proteobacterial endosymbiont (Gray et al., 1999). These organelles are comprised of two separate membranes, the mitochondrial outer membrane (MOM) and the mitochondrial inner membrane (MIM), that encapsulate the intermembrane space and matrix compartments. They contain a circular genome, mtDNA, which encodes 13 out the 1500 mitochondrial proteins, while the rest are encoded in the nucleus. Mitochondria form a dynamic, interconnected network that is intimately integrated with other cellular compartments, like the peroxisome (Schrader et al., 2015). The main function of mitochondria is the production of energy through the OXPHOS system in the form of ATP, which is the end product of catabolic processes such as glycolysis and tricarboxylic acid cycle (Krebs cycle). During these processes NAD^+ or FAD are reduced to form NADH and $FADH_2$, respectively. Electron transport chain (ETC) consist of four complexes (I-IV) in which electrons from NADH/$FADH_2$ are transferred, generating a proton gradient across the mitochondrial inner membrane, which drives the ATP-synthase (complex V) to transform ADP into ATP. Mitochondria regulate other important functions such as β-oxidation of fatty acids; biosynthesis of amino acids, heme group and steroids; calcium homeostasis; cell death; thermogenesis; and cellular stress responses (McBride et al., 2006). Thereby, mitochondrial dysfunction is associated with a myriad of human inherited disorders and is implicated in neurodegenerative disorders, cardiomyopathies, metabolic syndrome, cancer, and obesity (Nunnari et al., 2012).

1.3.2.1 Mitochondrial dysfunction associated to neurodegeneration

Due to the high metabolic needs of the central nervous system, explained before, improper functioning of mitochondria have devastating effects on neuronal survival (Court et al., 2012). Additionally, mitochondria are essential for non-neuronal cells in the CNS. For instance, mitochondrial function is critical for the differentiation of oligodendrocytes, as well as to provide the lipids necessary for myelination (Bonora et al., 2014; Schoenfeld et al., 2010). Furthermore, lack of functional mitochondria in oligodendrocytes or Schwann cells in mice lead to a severe neuropathy (Funfschilling et

al., 2012). Astrocytes' functions, including support of neurotransmission and supply of energetic substrate, e.g. lactate, to neurons, also require mitochondrial function (Cambron et al., 2012; Hayakawa et al., 2016), even though a recent report claims that astrocytes are naturally glycolytic cells (Supplie et al., 2017). Inflammatory responses mediated by microglia are also modulated by mitochondria (Ferger et al., 2010; Park et al., 2013). Thus, mitochondrial function is essential in neuronal and glial cells.

There are plenty of evidences supporting an important role of mitochondrial dysfunction in the pathogenic cascade of the majority of neurodegenerative diseases. For more details, there are extensive reviews on this topic (Johri et al., 2012a; M. T. Lin et al., 2006). Briefly, it has been described in AD (Swerdlow et al., 2010), PD (Franco-Iborra et al., 2016), HD (Bossy-Wetzel et al., 2008; Browne et al., 2004), ALS (Magrane et al., 2009) and in white matter disorders (Morato et al., 2014). It is worth to note that some mitochondrial diseases, caused by mutations in genes encoding mitochondrial proteins, are, in fact, neurodegenerative diseases such as Leber's hereditary optic neuropathy or Leigh syndrome (Ylikallio et al., 2012), underscoring the importance of this organelle in the central nervous system. Alterations in mitochondrial dynamics are also common in the majority of neurodegenerative diseases, although this aspect of mitochondria is not commented in this section, given that it was not studied in this thesis.

1.3.2.1.1 Mitochondrial dysfunction in X-ALD

Although X-ALD cannot be considered a primary mitochondrial disease, due to its peroxisomal origin, recent evidences point at mitochondria as a central hub in the pathophysiology of the disease. Even though one study found no alterations in respiratory chain from mitochondria isolated from 9-month-old *Abcd1*$^{-}$ mice muscle and brain, despite the accumulation of VLCFA (Oezen et al., 2005); further studies have reported mitochondrial abnormalities in the adrenal cortex and the spinal cord of mice lacking ABCD1 (Lopez-Erauskin et al., 2013; McGuinness et al., 2003; Morato et al., 2013) and ABCD2 (Ferrer et al., 2005). Furthermore, the results from the transcriptomic functional analysis in X-ALD mice and human patients unravelled a common metabolic abnormality signature, characterized, among other pathways, by mitochondrial dysregulation (Schluter et al., 2012). In addition, evidences of mitochondrial structural alterations are present in AMN patients (Powers et al., 2001; Schroder et al., 1996) as

well as in other peroxisomal disorders, like in Zellweger patients (Goldfischer et al., 1973) or *Pex5*$^{-/-}$ mice (Baumgart et al., 2001; Peeters et al., 2015). These results are consistent with mitochondrial alterations caused by a peroxisome dysfunction.

Evidences of oxidative stress have been detailed in the former section. In our group, we have demonstrated that VLCFA-derived ROS are mainly originated in the mitochondria and that oxidative stress induces mitochondrial membrane depolarization (Fourcade et al., 2008; Lopez-Erauskin et al., 2013), as well as triggers the opening of the mitochondrial permeability transition pore due to Cyclophilin D oxidation (Lopez-Erauskin et al., 2012). Using *ex vivo* spinal cord slices from *Abcd1*$^{-}$ mice, excess of VLCFA induced mitochondrial ROS formation from complexes I and II, and impaired complex V function by oxidation (Lopez-Erauskin et al., 2013). Similar mechanisms have been observed *in vitro* using SK-NB-E neuroblastoma cell line (Zarrouk et al., 2012); B12 oligodendrocytes and U87 astrocytes (Baarine et al., 2015); and primary astrocytes from WT and *Abcd1*$^{-}$ mice (Kruska et al., 2015). In the latter study, Kruska and collaborators also found VLCFA-dependent impairment of Ca^{2+} retention capacity, as well as higher Ca^{2+} retention capacity in brain mitochondria from 6-month-old *Abcd1*$^{-}$ mice (Kruska et al., 2015). These results has permitted our group to hypothesize on how VLCFA excess triggers oxidative stress from the mitochondria in X-ALD, based also on other data which suggest that C26:0 may replace the lateral chain of phospholipids of the MIM, increasing membrane microviscosity (Whitcomb et al., 1988), together with interfering with OXPHOS system and inducing ROS production (Lopez-Erauskin et al., 2013). In short, these studies demonstrate the interplay between mitochondrial dysfunction and oxidative stress in the pathophysiology of X-ALD.

In addition, mitochondrial amount is also altered in X-ALD. *Abcd1*$^{-}$ mice as well as X-ALD patients present mitochondrial depletion in the spinal cord and in the affected white matter, respectively (Morato et al., 2013). This depletion comprises reduction of mtDNA levels as well as decreased expression of mitochondrial proteins such as cytochrome c (Cyt C), complex I subunit NADH:ubiquinone oxidoreductase subunit B8 (NDUFB8), complex II subunit succinate dehydrogenase complex iron sulfur subunit B (SDHB) and voltage-dependent anion channel (VDAC), concomitant with a downregulation of the mitochondrial biogenesis pathway driven by sirtuin 1/peroxisome proliferator-activated receptor, gamma, coactivator 1 alpha/peroxisome proliferator-

activated receptor, gamma (SIRT1/PGC-1α/PPARγ) pathway. Indeed, boosting mitochondrial generation *via* PPARγ activation with pioglitazone (Morato et al., 2013) or by SIRT1 activation with resveratrol or SIRT1 overexpression (Morato et al., 2015) normalizes redox balance and prevents axonal degeneration in X-ALD mice. The next section includes detailsregarding what is known about the association between mitochondrial biogenesis impairment and neurodegeneration, and how this pathway can be targeted to ameliorate X-ALD.

1.3.2.2 Mitochondrial biogenesis and neurodegeneration: SIRT1/PGC-1α/PPARγ pathway and a new player, RIP140

Maintenance of mitochondrial network through the balance of mitochondrial turnover by mitophagy; mitochondrial shape by fission and fusion; and generation of new mitochondria *via* mitochondrial biogenesis is critical for metabolic homeostasis (Whitaker et al., 2016). The metabolic flexibility needed for the adaptation to different nutritional status is given, in part, by mitochondrial biogenesis, which is governed in large part by PGC-1α, a transcriptional coactivator that interact with transcription factors and nuclear receptors to regulate a network of approximately 1,500 genes, coordinating mitochondrial biogenesis and oxidative metabolism (Scarpulla, 2011). Thereby, this pathway regulates energy production. Another essential point is that only 13 out of these genes are encoded by mtDNA, thus this gene-network is comprised mainly by nuclear-encoded genes that control mitochondrial function. These nuclear-encoded mitochondrial proteins are translated on cytosolic ribosomes and imported into the mitochondrion through various mitochondrial protein import systems (Neupert et al., 2007).

1.3.2.2.1 Upstream metabolic sensors

To conform the tight regulatory system that allows adaptations to changes in environmental conditions, upstream sensor of these variations are essential. Among them, I would like to highlight mTOR, adenosine monophosphate (AMP)-activated protein kinase (AMPK) and sirtuin 1 (SIRT1). mTOR stimulates anabolic pathways and suppress autophagy when energy is abundant (Saxton et al., 2017). AMPK is activated

by a decrease in ATP production, which increases AMP/ATP ratio, and increased ADP concentrations, both of them go along with a reduction in caloric intake or increase in energy expenditure (Mihaylova et al., 2011). SIRT1 is a NAD^+-dependent deacetylase (Imai et al., 2000; Vaziri et al., 2001) that together with AMPK, coordinately regulates mitochondrial mass, nutrient oxidation, and ATP production *via* PGC-1α (Canto et al., 2009). In X-ALD these upstream sensors are altered, as mTOR signalling is aberrant, inhibiting autophagy (Launay et al., 2015). AMPK levels are decreased in fibroblasts and lymphoblasts from ccALD patients compared with cells from AMN patients, as well as in NAWM from ccALD patients compared with controls (J. Singh et al., 2014; J. Singh et al., 2016). SIRT1 is also inhibited, and its activation by resveratrol or transgenic overexpression in *Abcd1*$^-$ mice is neuroprotective (Morato et al., 2015).

1.3.2.2.2 Regulation of mtDNA transcription and replication

Apart from nuclear-encoded proteins imported into the mitochondrion, 13 mitochondrial proteins and encoded by the mitochondrial genome (mtDNA). Human mtDNA (16 kb) encodes 37 genes, including: 7 of the 46 subunits of complex I (ND1, 2, 3, 4, 4L, 5, and 6); one of the 11 subunits of complex III (cytochrome b, CYTB); 3 of the 13 subunits of complex IV (COX I, II, and III); 2 of the 16 subunits of complex V (ATP6 and ATP8); 2 rRNAs (12S and 16S); and 22 tRNAs. All 13 proteins encoded by mtDNA are essential subunits of respiratory complexes I, III, IV and V. These subunits are transcribed from mtDNA through multigenic transcripts that are processed and translated into individual mRNAs (Taanman, 1999). Mitochondrial transcription machinery consists of an RNA polymerase (POLRMT), two transcription factors including transcription factor A, mitochondrial (TFAM) (Larsson et al., 1998) and one of the two transcription factor B paralogues (TFB1M and TFB2M); together with a family of termination factors (mTERFs) (Bonawitz et al., 2006). In addition, the replication machinery contains the heterotrimeric mtDNA polymerase gamma (POLG) (Y. S. Lee et al., 2009), the hexameric DNA helicase TWINKLE (Milenkovic et al., 2013) and the tetrameric mitochondrial single-stranded DNA-binding protein (mtSSB) (Korhonen et al., 2004).

1.3.2.2.3 Regulation of nuclear-encoded mitochondrial genes: PGC-1α related pathways

However, the majority of genes involved in mitochondrial biogenesis are encoded in the nucleus, controlled by a series of transcriptional coactivators and corepressors, in which, PGC-1α is the best-known component. PGC-1α belongs to a small family of transcriptional coactivators composed of PGC-1α, PGC-1β (J. Lin et al., 2002) and PGC-1-related coactivator (PRC) (Andersson et al., 2001). PGC-1α was discovered in adaptive thermogenesis, due to its interaction with PPARγ, increasing its transcriptional activity upon cold exposure, as well as inducing uncoupling protein-1 (UCP1) expression (Puigserver et al., 1998). In addition to PPARγ, PGC-1α binds several transcription factors and nuclear receptors, such as nuclear respiratory factors 1 and 2 (NRF-1, NRF-2), ERRα, YY1, myocyte enhancer factor 2C (MEF2C), PPARα and many others (Scarpulla, 2006; Schreiber et al., 2004). NRF-1, ERRα, YY1 and MEF2C regulate the expression of the respiratory chain components while PPARα and ERRα control fatty acid oxidation. Moreover, PGC-1α network is involved in multiple metabolic functions, including the OXPHOS system, antioxidant defences (St-Pierre et al., 2006), adaptive thermogenesis, glucose/fatty acid metabolism and muscle fiber type switching (Scarpulla et al., 2011). Furthermore, the expression of PGC-1 family is integrated with the cellular energetic status, as distinct environmental factors such as temperature, growth factors, nutrient availability or energy deprivation, modulate the expression of these proteins (Scarpulla et al., 2011).

Mitochondrial biogenesis is also controlled by a set of transcriptional coregulators which fine tune the network of genes involved in energetic homeostasis (Feige et al., 2007). Indeed, more than 350 coregulators have been identified (http://www.nursa.org). Among the most important, steroid receptor co-activator protein 1 (SRC1) acts as a coactivator, whereas nuclear receptor corepressor 1 (NCOR1), NCOR2 (also known as silencing mediator of retinoid and thyroid hormone receptors, SMRT) and receptor interacting protein 140 (RIP140; also called nuclear receptor interacting protein 1, NRIP1) are members of the corepressor counterpart. SRC1 coactivator recruitment instead of SRC2 or SRC3 leads to an oxidative programme mediated by PPARγ activity (Picard et al., 2002). On the other hand, inhibition of the corepressors enhances mitochondrial function. For instance, muscle-specific NCOR1-deficient mice and gei-8-

mutant worms (NCOR1 homolog) present increased mitochondrial number and activity in muscle (H. Yamamoto et al., 2011). NCOR1 deletion in mice adipocytes leads to adipogenesis, reduced inflammation, and enhanced systemic insulin sensitivity (P. Li et al., 2011). RIP140 is a unique ligand-dependent coregulator that can also exert some functions as a coactivator. RIP140 interacts with multiple nuclear receptors to modulate important metabolic functions (Christian et al., 2006). Since the case of RIP140 is of special interest for this thesis, the following section is dedicated to describe the current knowledge about this particular transcriptional coregulator.

1.3.2.2.4 RIP140: A dual-faced coregulator

RIP140 is a transcriptional coregulator of nuclear receptors (NRs) such as estrogen-related receptors (ERRs), liver X receptor (LXR), retinoic acid/X receptors (RAR/RXR), thyroid hormone receptors (TRα/β), glucocorticoid receptor (GR), and peroxisome proliferator-activated receptors (PPAR-α/δ/γ) and other transcriptions factors including NFκB and E2F1 (Augereau et al., 2006; Nautiyal et al., 2013). Although not much is known about RIP140 in comparison with other coregulators, it was one of the first transcriptional coregulators to be discovered, by its interaction with estrogen receptor alpha (ERα) (Cavailles et al., 1995). *RIP140* mRNA is transcribed from multiple promoters, although the open-reading frame is confined to a single exon (exon 4) (Augereau et al., 2006). RIP140 interacts with its partners by 9 LXXLL motifs and a 10th LXXML motif in the C-terminal of RIP140 protein that is required for the interaction of retinoic acid receptor (RAR) with retinoid X receptor (RXR) transcription factors (Wei et al., 2001). Even though the first reports reported a coactivator function of RIP140 (Joyeux et al., 1997), soon it was unveiled that RIP140 can act both as a coactivator and as a corepressor (Chuang et al., 1997). Then, a series of studies demonstrated that RIP140 mainly acts as a corepressor. RIP140 recruits histone deacetylases (HDACs) for its repressive actions (Wei et al., 2000), by four repressive domains (RD) on its structure (Castet et al., 2004; Christian et al., 2004). HDACs remove acetyl groups on lysines, leading to a closed chromatin state, and thus repressing transcription (Perissi et al., 2010). In conclusion, RIP140 is a particular transcriptional coregulator, mainly acting by repressing the transcription of multiple nuclear receptors and transcription factors.

RIP140 can be regulated post-translationally. Acetylation of Lys446 of RIP140 prevents the interaction of RIP140 with one of its partner required for repressive action, C-terminal binding protein (CtBP) (Vo et al., 2001). Other eight acetylated lysines identified in RIP140 might be important for the repressive function and subcellular localization (Huq et al., 2005). RIP140 can be phosphorylated in 11 residues, although not well characterized. One example is MAPK-mediated phosphorylation of Thr202 and Thr207 residues of RIP140, which enhances the recruitment of HDAC3 and thus, the repressive action of RIP140 (Gupta et al., 2005). In addition, phosphorylation modulates the interaction of RIP140 with 14-3-3 protein, inducing a cytoplasmic location of RIP140 (Zilliacus et al., 2001). Protein kinase C epsilon (PKCε) phosphorylation of RIP140's Ser-102 and Ser-1003 residues triggers its export to the cytoplasm in adipocytes (Gupta et al., 2008; Huq et al., 2006). Then, RIP140 interacts with Akt substrate of 160 KDa (AS160), impeding its phosphorylation by AKT, and thus negatively regulates GLUT4 trafficking and glucose uptake in adipocytes (P. C. Ho et al., 2009). In brief, RIP140 is modified post-translationally as well as exerts some of its functions in the cytoplasm, although these are not well described yet. Thus, further investigations should be addressed to understand better RIP140 regulation and its subcellular localization implications.

RIP140 is considered a master regulator of metabolic homeostasis, by regulating glucose and lipid metabolism in metabolic tissues such as skeletal muscle, liver, heart and adipose tissue. RIP140-deficient mice provide the evidence for a central role of RIP140 in metabolic homeostasis, as these mice are leaner and present 70% reduction in body fat than their control littermates, resistance to diet-induced obesity and increased clearance and insulin sensitivity (Berriel Diaz et al., 2008; Herzog et al., 2007; Leonardsson et al., 2004; Seth et al., 2007). However, the first phenotype noted in *Rip140*-null mice, apart of the 20% reduction in body weight, was female infertility (R. White et al., 2000). In other metabolic tissues, adipocytes lacking RIP140 and white adipose tissue (WAT) of *Rip140*-null animals present a marked increase in UCP1 expression, typically expressed in brown adipose tissue (BAT), which display much more mitochondria than WAT (Christian et al., 2005; Leonardsson et al., 2004). Moreover, RIP140 inhibits genes related to fatty acid oxidation, mitochondrial biogenesis and oxidative phosphorylation in adipocytes (Powelka et al., 2006). In short, RIP140 inhibits multiple pathways associated to metabolic functions (**Fig. 6**).

Another essential of choosing RIP140 as a target of study for this thesis is given by its role in NFκB-dependent proinflammatory response, which is induced in *Abcd1*$^{-}$ mice spinal cord (Schluter et al., 2012). In this case, RIP140 exerts a coactivator role by interacting with RELA (p65) subunit of NFκB and promoting the transcription of pro-inflammatory cytokines such as IL1β, IL6 and tumour necrosis factor alpha (TNFα) (P. C. Ho et al., 2012; Zschiedrich et al., 2008). The role of RIP140 in inflammation is also illustrated in studies using mice in which RIP140 has been knocked-down in macrophages. Upon exposure to high-fat diet, these mice present an increase of alternatively polarized M2 macrophages which improves insulin resistance and a decrease of classically polarized M1 macrophages, which usually represents a state of chronic inflammation. This chronic inflammation correlates with an increased in RIP140 expression in macrophages (P. S. Liu et al., 2014). Subsequently, injection of macrophages extracted from WAT of the animals in which RIP140 was knocked-down, leads to a reduction of pro-inflammatory response and ameliorates diet-induced insulin resistance (P. S. Liu et al., 2015). Altogether, these evidences highlight the role of RIP140 in inflammation as a coactivator (**Fig. 6**).

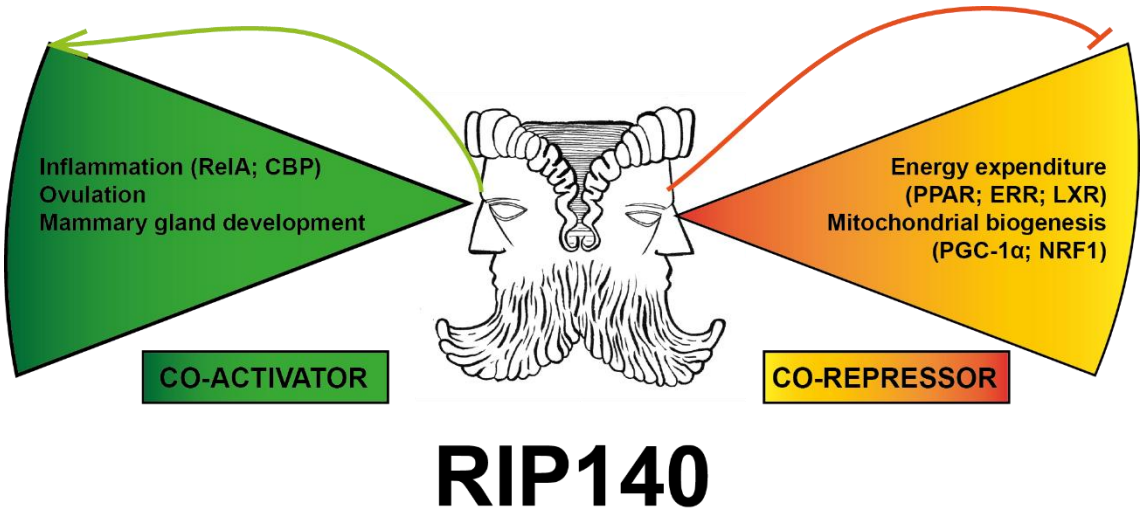


Figure 6 Janus-like dual function of RIP140 as a co-activator and a co-repressor

Transcriptions factors involved in the corresponding role of RIP140 are specified between brackets. Abbreviations: RelA (p65), CBP (CREB-binding protein), PPAR (Peroxisome proliferator-activated receptor), ERR (estrogen-related receptor), LXR (liver X receptor), PGC-1α (peroxisome proliferator-activated receptor, gamma, coactivator 1 alpha); NRF1 (nuclear respiratory factor 1).

In the transcriptomics analysis of X-ALD that we have commented before, it emerges that a metabolic and inflammatory interplay; characterized by mitochondrial dysregulation, insulin desensitization and an NFκB-mediated proinflammatory response; has an important role in the pathophysiology of X-ALD (Schluter et al., 2012). Moreover, our group have also found that most of the genes involved in mitochondrial biogenesis are down-regulated in X-ALD, as evidenced in our recent studies with pioglitazone and resveratrol in the X-ALD model, in which an inhibition in SIRT1/PGC-1α/PPARγ underlies biochemical defects and axonal degeneration (Morato et al., 2013; Morato et al., 2015). Additionally, RIP140 is considered the antagonist coregulator of PGC-1α, as they share common targets (Hallberg et al., 2008). Hence, RIP140 could provide a plausible link between metabolic impairment and induction of inflammation in X-ALD.

1.3.2.2.5 Mitochondrial biogenesis impairment in neurodegeneration

Given the importance of mitochondria in metabolism, especially in the CNS, a tight regulation in the PGC-1α mediated biogenesis of these organelles is mandatory. The phenotype of PGC-1α null mice reflect the important functions of this coactivator in the nervous system, as these mice are hyperactive and present lesions in the striatum (J. Lin et al., 2004). The mRNA expression of the genes involved in mitochondrial biogenesis is decreased in most of the tissues of these mice, including the brain (Leone et al., 2005; J. Lin et al., 2004). Furthermore, loss of PGC-1α leads to oxidative phosphorylation impairment and bioenergetics deficit in many tissues, being the mice intolerant to exercise (Handschin et al., 2007). Moreover, PGC-1α-deficient mice are more sensitive to chemically-induced neurodegeneration of the substantia nigra and hippocampus, and increasing PGC-1α levels protects neural cells against oxidative stress (St-Pierre et al., 2006).

Evidences about the role of RIP140 in nervous system and/or neurodegenerative diseases are scarce (Katsouri et al., 2012). RIP140 is expressed in the mouse brain, particularly in neurons from cortex, hippocampus, and pituitary gland (Duclot et al., 2012), recently reproduced in another study (Blondrath et al., 2016). Studies about the functions of RIP140 in nervous system describe a role of RIP140 in memory formation (Duclot et al., 2012), aging (Yuan et al., 2012), modulation of neuronal ER stress (Feng

et al., 2014) and generation of Aβ peptide (Blondrath et al., 2016). In addition, RIP140 levels are altered in the heart (Conti et al., 2007) and fibroblasts (Piccoli et al., 2013) from Down syndrome (DS) fetuses, as well as in the hippocampus from DS patients (Gardiner, 2006). Intriguingly, gene pathways altered in RIP140 KO mice (Powelka et al., 2006), resemble dysregulated pathways identified by transcriptomics in the spinal cord of *Abcd1*$^{-}$ mice (Schluter et al., 2012). Overall, even though RIP140 controls a myriad of pathways involved in neurodegeneration, not much is known about its role in the CNS.

Besides the limited data about RIP140 in neurodegeneration, there are plenty of evidences of dysregulation of the pathways that regulate mitochondrial biogenesis in neurodegenerative diseases. In animal models and patients of PD, PGC-1α target genes are downregulated (Zheng et al., 2010). In addition, restoration of PGC-1α levels protects neural cells against oxidative stress (Zheng et al., 2010), whereas PGC-1α-deficient mice are more susceptible to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurodegeneration (St-Pierre et al., 2006). Parkin interacting substrate (PARIS), an upstream regulator of parkin, a protein mutated in genetic forms of PD, represses PGC-1α by binding to its promoter, leading to a progressive loss of dopaminergic neurons (J. H. Shin et al., 2011). In HD, mutant huntingtin (HTT) interacts with the promoter of PGC-1α, repressing downstream pathways (Cui et al., 2006). Lack of PGC-1α in HD knockin mice provokes a more severe neurodegeneration, which is reversed by lentiviral-mediated delivery of PGC-1α into the striatum (Cui et al., 2006). In addition, PGC-1α expression is reduced in the striatum of HD patients and several HD mouse models (Weydt et al., 2006), as well as in muscle tissue (Chaturvedi et al., 2009). PGC-1α expression is also decreased in the brain of AD patients, in M17 cells harbouring the swedish mutation in human amyloid precursor protein (APPswe) and in neurons derived from Tg2576 mice (AD model) (Qin et al., 2009; Rice et al., 2014; Sheng et al., 2012). Equally, this pathway is inhibited in ALS, a fatal neurodegenerative disease characterized by motor neuron loss, in which decreased levels of PGC-1α are found in the SOD1G93A ALS mouse model and in human sporadic ALS (Thau et al., 2012). In the same way, multiple sclerosis patients display lower levels of PGC-1α in the brain than controls (Witte et al., 2013). On the whole, PGC-1α and its downstream targets are dysregulated in neurodegenerative disorders.

Since PGC-1α is downregulated in all these neurodegenerative disorders, multiple efforts have been made to overexpress this protein *in vivo* as a therapeutic target. Nevertheless, in few cases, the results have rendered unexpected outcomes. On one hand, PGC-1α overexpression have led to neuroprotection in several studies using MPTP-induced PD mouse model (Mudo et al., 2012), SOD1-G93A ALS mice (Zhao et al., 2011), R6/2 and N171-82Q HD mice (Johri et al., 2012b; Tsunemi et al., 2012), and APP23 AD mice (Katsouri et al., 2016). Conversely, when PGC-1α is delivered into the rat nigrostriatal system by an adenovirus, it leads to degeneration of dopaminergic neurons, as well as does not prevent neurodegeneration induced by α-synuclein overexpression (Ciron et al., 2012). Similar result was obtained in an AD mouse model, in which overexpression of PGC-1α exacerbates Aβ and tau deposition (Dumont et al., 2014). In addition, another study found out that PGC-1α overexpression in skeletal muscle leads to muscle atrophy with depletion of ATP (Miura et al., 2006). Consequently, a word of caution has to be established about this kind of approach, seeking for new pharmacological targets that can better fine tune the transcriptional network of mitochondrial biogenesis.

Likewise, modulation of upstream regulators of PGC-1α also exerts neuroprotection. Activation of SIRT1 results in amelioration of motor function, reduction in brain atrophy and attenuation of mutant-HTT-mediated metabolic abnormalities in HD mice (Jiang et al., 2011), whereas SIRT1 brain-specific deletion leads to exacerbation of brain pathology (Jeong et al., 2011). Lentiviral-mediated injection of SIRT1 in the hippocampus of p25 transgenic mice also confers protection against neurodegeneration (D. Kim et al., 2007). Therapeutic strategies directed at increasing the supply of NAD^+, such as NAD^+ boosters or inhibitors of poly(ADP-ribose) polymerase 1 (PARP1), an enzyme that competes with SIRT1 for NAD^+, also seem promising in providing neuroprotective effects (Bai et al., 2015; Verdin, 2015). For instance, induction of nicotinamide mononucleotide adenylyl-transferase 1 (NMNAT1) activity, involved in the synthesis of NAD^+ in the nucleus (Magni et al., 2004), leads to axonal protection (Araki et al., 2004). In patients with xeroderma pigmentosum group A (XPA), both PARP1 inhibition and NAD^+ supplementation reverse mitochondrial phenotype (Fang et al., 2014; Pirinen et al., 2014). In addition, pharmacological stimulation of PGC-1α expression with nicotinamide riboside, the precursor of NAD^+, results in reduced Aβ levels and attenuated cognitive deterioration in Tg2576 AD mice (Gong et al., 2013). In

our group we have recently explored this pathway, by treating X-ALD mice with a PPARγ agonist (pioglitazone) and with resveratrol, which among other targets, induces SIRT1 activity. Both compounds, as well as genetic overexpression of SIRT1 reverses biochemical defects and, more importantly, prevents axonal degeneration and locomotor deficits (Morato et al., 2013; Morato et al., 2015). To sum up, therapies addressed to replenish NAD^+ and thereby, activating SIRT1/PGC-1α axis, have been proved to be neuroprotective.

1.3.3 Other hallmarks of X-ALD: A focus on neuroinflammation

Apart from redox dyshomeostasis and mitochondrial dysfunction, which constitute the central core of this thesis, other hallmarks of X-ALD and the majority of neurodegenerative diseases deserve a special mention, with a special focus on neuroinflammation.

1.3.3.1 Proteostasis impairment

Disruption in protein homeostasis (proteostasis), evidenced by the presence of misfolded or aggregated proteins, is prevalent in most neurodegenerative disorders, and has been documented in animal models as well as in the brain of the patients (Douglas et al., 2010; Selkoe, 2003). Within this hallmark, we can embody malfunction of the proteasome, alterations in the autophagic flux and endoplasmic reticulum stress. All of them are different pathological conditions arising from an improper response against misfolded proteins. Indeed, in our group we have described alterations in these three pathways in X-ALD (Launay et al., 2015; Launay et al., 2013; Launay et al., 2017).

1.3.3.1.1 Ubiquitin-proteasome system

Most soluble misfolded proteins are cleared through the ubiquitin-proteasome system (UPS), the major eukaryotic proteolytic pathway (Ciechanover, 1998). Proteins assigned to be degraded are tagged by polyubiquitination, and then sent for destruction to the 26S proteasome (Wilkinson, 2000). The link between UPS and neurodegeneration is clearly exemplified by juvenile forms of PD caused by mutation in Parkin, an ubiquitin-ligase (Kitada et al., 1998). Alterations in this pathway are commonly found in the majority of neurodegenerative disorders (Hyun et al., 2003; J. N. Keller et al., 2000; McNaught et al., 2001). Moreover oxidative stress and protein aggregates inhibit the UPS (Bence et al., 2001; Davies et al., 2007). Indeed, in X-ALD chronic and progressive malfunctioning of the UPS is caused by the accumulation of oxidatively modified proteins. Furthermore, excess of C26:0 induces the appearance of immunoproteasomes, together with their translocation to mitochondria in X-ALD fibroblasts (Launay et al., 2013).

1.3.3.1.2 Autophagic flux

Autophagy represents an alternative way of degrading misfolded proteins, by their delivery to the lysosome (Ravikumar et al., 2002). It is worth to note that deletion of genes involved in the autophagic pathway causes neurodegeneration in mice (Hara et al., 2006; Komatsu et al., 2006). Moreover, alterations in autophagy, as well as in mTOR, a key upstream regulator or this process, and also one of the most important nutrient sensors, as stated above, have been described elsewhere (Cuervo et al., 2004; J. H. Lee et al., 2010). Again, we have recently reported impaired autophagy together with mTOR pathway dysregulation in X-ALD, which, together with the neuropathology, is reversed with administration of temsirolimus, an mTOR inhibitor analog of rapamycin (Launay et al., 2015).

1.3.3.1.3 ER stress

The ER is an essential organelle for the maturation and processing of proteins folded through the secretory pathway. For that purpose, ER contains a dynamic network of chaperones, foldases and co-factors in the lumen (Matus et al., 2011). When homeostasis in the ER is disrupted, abnormally folded proteins accumulate, leading to ER stress, which engages the unfolded protein response (UPR) (Ron et al., 2007), a physiological response addressed to restore the capacity to generate properly folded proteins and reduce the amount of misfolded proteins. Signs of ER stress have been detected in most animal models of neurodegeneration and more recently in the brain of human patients, for example in ALS (Nassif et al., 2010; Saxena et al., 2009), PD (Hoozemans et al., 2007), HD (Vidal et al., 2011), AD (Hoozemans et al., 2005), and in diseases associated with myelin (W. Lin et al., 2009). Unlike the cases mentioned, in X-ALD no aggregated protein has been described up to date. Instead, oxidative stress (van der Vlies et al., 2003) and lipid dyshomeostasis (Hou et al., 2014) are the culprit of ER stress activation and UPS engagement in X-ALD (Launay et al., 2017), a relationship that has been also described in other peroxisomal deficiency models (J. Huang et al., 2011; Kovacs et al., 2012) and in neurodegenerative disorders, e.g ALS (Ilieva et al., 2007), AD and PD (Holtz et al., 2006; Uehara et al., 2006).

1.3.3.2 (Neuro)Inflammation

In a thesis about X-ALD we need to talk about inflammation, mainly because is a primary differential factor between AMN and ccALD, as inflammation is present in the cerebral demyelination of the ccALD phenotype. However, the molecular mechanisms that govern disease progression, and its transformation to the cerebral inflammatory form, remain unknown. In many ways, X-ALD resembles multiple sclerosis, since infiltrating inflammatory cells are mainly macrophages and T cells rather than B cells (D. E. Griffin et al., 1985; Powers et al., 1992), but an autoimmune component has not been identified in X-ALD. In contrast to multiple sclerosis lesions, where these cells lead the lesion edge, inflammatory cells in X-ALD trail the demyelinating edge, suggesting that they emerge as a secondary feature after white matter degeneration (Eichler et al., 2008; Schaumburg et al., 1975). Classical pro-inflammatory cytokines, such as TNFα (Powers et al., 1992) and IL6 (McGuinness et al., 1997), among others, have been detected in inflammatory lesions in ccALD.

Nevertheless, is actually the adult form, AMN, a pure non-inflammatory phenotype? Recent data do not seem to support this hypothesis. In the transcriptomics analysis with samples from the X-ALD mouse model and cerebral X-ALD patients, induction of NFκB-dependent pro-inflammatory response represents one the main pathways dysregulated, together with increased expression of several proinflammatory cytokines (Schluter et al., 2012). Moreover, a recent study from our group unveils a low-grade inflammatory induction in plasma and PBMC from AMN patients, with a mixed profile of M1 and M2 components induction (Ruiz et al., 2015). PBMC from cerebral and AMN patients secrete higher amounts of TNF-α than PBMC obtained from controls (Lannuzel et al., 1998). Therefore, AMN cannot be considered anymore as a non-inflammatory disease.

Microglia are one of the main players in the abnormal inflammatory response of X-ALD. These cells are the immune-resident cells in the brain, performing a myriad of physiological function in the CNS (Tremblay et al., 2011), but also involved in the pathogenesis of almost all CNS diseases (Cartier et al., 2014). The key role of microglia in inflammatory demyelination in X-ALD is evidenced by the fact that bone marrow transplantation and gene therapy are able to arrest demyelination (Cartier et al., 2009).

As microglial cells derive from the same origin than monocytes and macrophages (Ginhoux et al., 2010), these are the only cells of central nervous system that come from the bone marrow after transplantation. In the case of gene therapy, genetically-corrected $CD34^+$ cells differentiate into microglia in the brain (Asheuer et al., 2004). In addition, studies from our group using *Abcd1*$^-$ and *Abcd1*$^-$/*Abcd2*$^{-/-}$ mice show that microgliosis and astrogliosis are consistent features in the neuropathology of these mice (Lopez-Erauskin et al., 2011; Pujol et al., 2004). Furthermore, VLCFA triggers inflammatory response mainly by abnormal activation of microglia and apoptosis in mice injected with C24:0-LPC (Eichler et al., 2008). Subsequently, activated microglia produce higher amounts of the proinflammatory cytokine TNFα, that together with IL1β, induce further accumulation of VLCFA (Khan et al., 1998), creating a vicious cycle that worsens the pathology of X-ALD. The role of microglia in neurodegeneration is not limited to X-ALD, being common in other neurodegenerative disorders, such as ALS (Boillee et al., 2006a; Boillee et al., 2006b). Recently, microglial-like cells have been obtained from human pluripotent stem cells, expanding the therapeutic potential of targeting these cells in central nervous system disorders (Muffat et al., 2016).

To conclude, X-ALD pathophysiology displays most of the features of neurodegenerative disorders, meaning that data coming from research in this rare disease could be applied to a vast number of patients suffering from these disorders. This introductory chapter describes what is known about the main hallmarks of X-ALD, that are oxidative stress, mitochondrial dysfunction (these two explained in more detail as required for a better understanding of the approaches followed in the studies that compose this thesis), proteostasis impairment and neuroinflammation. These hallmarks have been represented in **Fig. 7** as a general scheme of the pathophysiology of X-ALD.

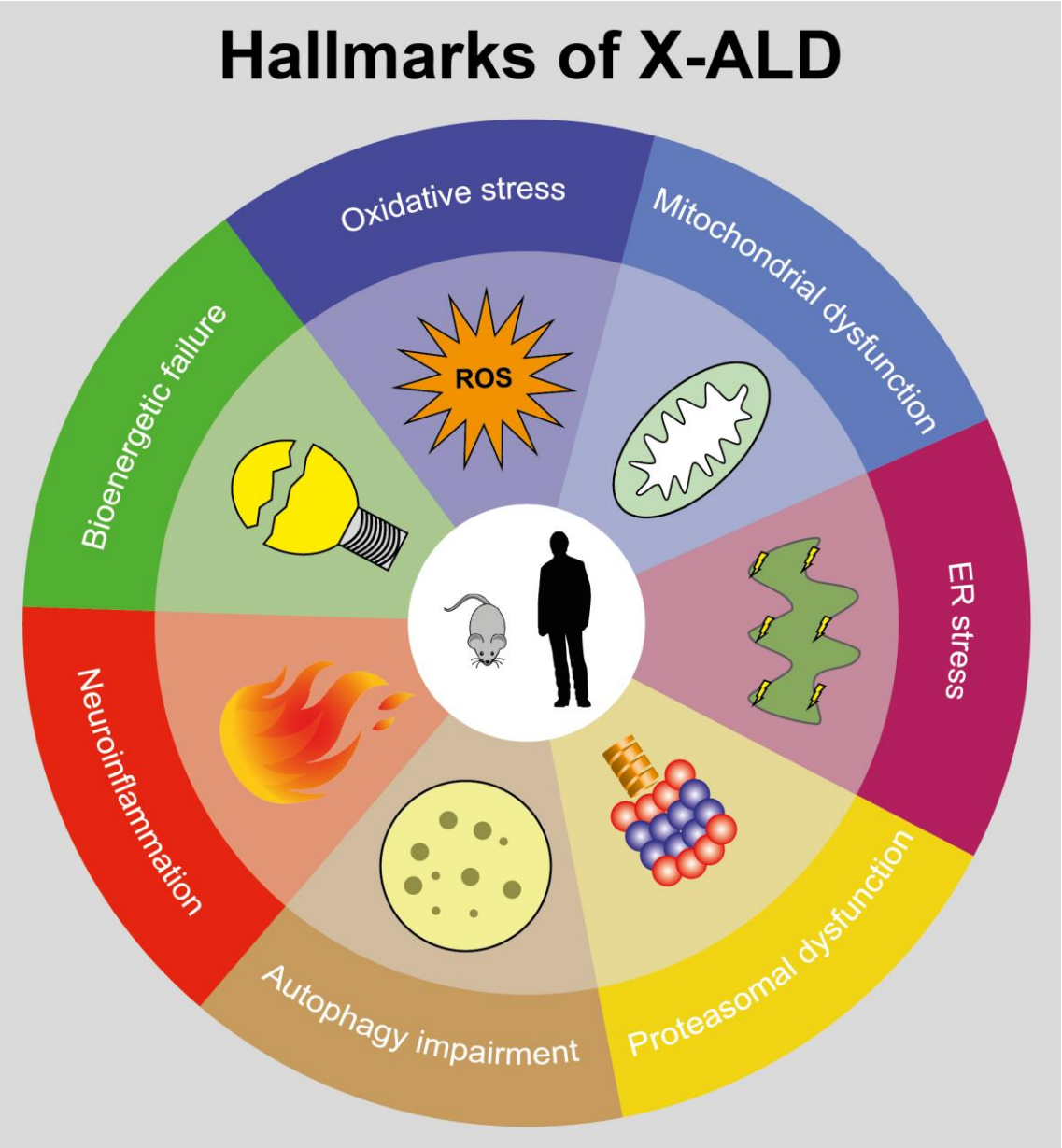


Figure 7 The hallmarks of X-linked adrenoleukodystrophy (X-ALD)

The scheme lists the seven hallmarks of X-ALD described in this thesis: oxidative stress; mitochondrial dysfunction; bioenergetic failure; neuroinflammation; and loss of proteostasis due to autophagy impairment, endoplasmic reticulum (ER) stress and proteasomal malfunction. All of them have been described in the animal models as well as in the human patients of X-ALD.

AIM AND OBJECTIVES

2 Aim and objectives

The main aim of this study is to identify new therapeutic targets for X-ALD that also contribute to understand the pathophysiology of this disease, based on the existing knowledge on the hallmarks of X-ALD that are also shared with other neurodegenerative disorders. This aim was accomplished by fulfilling the following objectives:

1) To determine the role of **endogenous antioxidant response pathway mediated by NRF2** in the pathophysiology of X-ALD, and its applicability as a pharmacologic target in X-ALD. To complete this objective, we:
    a) Studied **NRF2 pathway and its associated regulatory signalling pathway AKT/GSK-3β** in WT and *Abcd1*$^{-}$ mice, as well as in skin fibroblasts derived from healthy subjects and X-ALD patients
    b) Evaluated the **therapeutic potential of DMF**, an NRF2 activator, for X-ALD. For this purpose, we tested the effect of DMF treatment on the classical biochemical defects found in *Abcd1*$^{-}$ mice, as well as on axonal degeneration and locomotor impairment in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice.
2) To explore the **role of RIP140**, a transcriptional coregulator essential for metabolic homeostasis and inflammatory response, in X-ALD pathophysiology, and its possible application as a therapeutic target for X-ALD. To fulfil this objective, we:
    a) Analysed **RIP140 expression, localization and its regulation by oxidative stress**, *in vivo* and *ex vivo* in WT, *Abcd1*$^{-}$ and *Rip140*$^{-/-}$ mice, and normal-appearing white matter (NAWM) from the brain of healthy subjects and cerebral X-ALD patients
    b) Assessed **whether RIP140 deficiency can exerts neuroprotection** in X-ALD mice, by crossing RIP140-deficient mice with *Abcd1*$^{-}$ and *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice. We therefore determined the effect of RIP140 loss on the classical biochemical defects found in *Abcd1*$^{-}$ mice, but also on axonal degeneration and behavioural abnormalities present in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice.

MATERIALS AND METHODS

3 Materials and Methods

3.1 Reagents and antibodies

The following chemicals were used: DMF (Ref. 242926), hexacosanoic acid (C26:0, Ref. H0388), oligomycin (Ref. O4876), hydrogen peroxide (H_2O_2) were from Sigma-Aldrich (Steinheim, Germany). Trolox (Ref. 648871) was from Calbiochem (Billerica, MA, USA). GSK-3β inhibitors CHIR99021 (6-[[2-[[4-(2,4-Dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)-2-pyrimidinyl]amino]ethyl]amino]-3-pyridinecarbonitrile; Ref.361559), SB216763 (3-(2,4-Dichlorophenyl)-4-(1-methyl-1H-indol-3-yl)-1H-pyrrole-2,5-dione; Ref.1616) were from Calbiochem and Tocris Biosciences (Bristol, UK), respectively. Detailed information on antibodies is summarized in **Tables 1 and 2**.

3.2 Human samples

3.2.1 Post-mortem brain samples

Brain tissue samples from X-ALD patients and age-matched controls were obtained from the National Institutes of Health (NIH) NeuroBioBank. Frozen blocks of normal-appearing white matter (NAWM) were dissected from frontal or parietal lobes from controls and ccALD and cAMN patients. All children and adults with cerebral ALD had the more frequent parieto-occipital form of cerebral ALD. Informed written consent was obtained from all patients or their legal representatives, and the local ethics committee approved the studies. Detailed information about the brain samples including age and post-mortem interval until processing of the sample is summarized in **Table 3**.

Table 1 List of primary antibodies used in this thesis

Antibody	Source	Reference	Dilution
8-oxo-dG	Abcam	62623	1:1000
AKT	Cell Signaling	4685	1:1000
pSer473 AKT	Cell Signaling	4060	1:1000
pThr308 AKT	Cell Signaling	13038	1:1000
Aldolase A	Novus Biologicals	600-915	1:5000
APP	AbD Serotec	AHP538	1:100
Cytochrome C	BD Biosciences Pharmigen	55643	1:500
GFAP	Dako	Z0334	1:500
GFP	Aves Lab	GFP-1020	1:1000
GSK-3β	Cell Signaling	9315	1:1000
pSer9/21 GSK-3α/β	Cell Signaling	9331	1:1000
pTyr216/279 GSK-3α/β	EMD Millipore	05-413	1:1000
IBA1	Wako	019-19741	1:1000
IκBα	Santa Cruz	sc-1643	
P-IκBα (Ser32/36)	Cell Signaling	9246S	
IKKα	Oncogen	OP-133	
IKKβ	Cell Signaling	-	
P-IKKα/β (Ser180/181)	Santa Cruz	sc-23470	
Lamin B1	Abcam	16048	1:10000
MDA	-	Hall et al. 2007[1]	1:1000
Neurofilament H non-phophorylated (SMI32)	Covance Antibody-Biolegend	801701	1:3000
NRF2	Abcam	31163	1:500
p50	Santa Cruz	sc-7178	
p65	Santa Cruz	sc-109	
P-p65 (Ser536)	Cell Signaling	3036S	
p100/p52	EMD Millipore	05-361	
RIP140	Cancer Research UK	6D7	1:50
SDHB	Invitrogen	459230	1:500
Synaptophysin	Leica Biosystems	299-L-CE	1:500
α-Tubulin	Sigma-Aldrich	T6074	
γ-Tubulin	Sigma-Aldrich	T6557	1:20000

[1] Hall, E. D., Oostveen, J. A., Andrus, P. K., Anderson, D. K., & Thomas, C. E. (1997). Immunocytochemical method for investigating in vivo neuronal oxygen radical-induced lipid peroxidation. *J Neurosci Methods, 76*(2), 115-122.

Table 2 List of secondary antibodies used in this thesis

Antibody	Source	Reference	Dilution
Goat anti-chicken Alexa 488	Invitrogen	A11039	1:500
Goat anti-rabbit HRP	Dako	P0448	1:10000
Goat anti-mouse HRP	Dako	P0447	1:15000
Rabbit anti-goat HRP	Dako	P0449	1:15000

3.2.2 Primary human fibroblasts

Primary human fibroblasts were prepared from skin biopsies collected from healthy individuals (n=5) and AMN patients (n=5) according to the IDIBELL guidelines for sampling, including informed consent from the persons involved or their representatives. The fibroblasts were grown in Dulbecco's Modified Eagle Medium (Gibco, Thermo Fisher Scientific Inc., Rockford, IL, USA) containing 10% foetal bovine serum (Cultek, Ref. 91S1800; Madrid, Spain), 100 U/ml penicillin and 100 µg/ml streptomycin (Pen Strep; Gibco, Ref. 15140-122) and maintained at 37 °C in humidified 95% air / 5% CO_2 incubator. The compounds tested were added at 80–90% cell confluence with the following concentrations: 15 µM oligomycin or 50 µM C26:0 (diluted in Ethanol) for 18 and 24 hours, respectively. GSK-3β inhibitors (CHIR99021 and SB216763) were dissolved in dimethylsulfoxide (DMSO) and added 18 hours after C26:0 treatment for 6 hours at 3 and 10 µM, respectively. All experiments were performed with fibroblasts at passage 10 to 20.

Table 3 List of human brain samples used in this study

Phenotype	Sex	Age	Post-mortem interval (hours)	Phenotype	Sex	Age	Post-mortem interval (hours)
Control	Male	7	12	Control	Male	32	7
Control	Male	8	12	Control	Male	34	6
Control	Male	12	13	Control	Male	35	12
Control	Male	13	5	Control	Male	35	23
Control	Male	13	18	Control	Male	37	12
Control	Male	13	16	Control	Male	37	9
ccALD	Male	6	12	Control	Male	39	14
ccALD	Male	8	1	Control	Male	39	24
ccALD	Male	8	22	Control	Male	31	15
ccALD	Male	9	9	Control	Male	31	13
ccALD	Male	9	15	Control	Male	31	13
ccALD	Male	13	14	Control	Male	32	12
				Control	Male	33	8
				Control	Male	52	13
				Control	Male	53	17
				Control	Male	57	16
				Control	Male	57	16
				Control	Male	58	17
				Control	Male	59	18
				Control	Male	61	21
				Control	Male	62	6
				Control	Male	64	22
				Control	Male	68	13
				cAMN	Male	27	11
				cAMN	Male	28	18
				cAMN	Male	33	6
				cAMN	Male	35	40
				cAMN	Male	36	2
				cAMN	Male	38	4
				cAMN	Male	39	5
				cAMN	Male	39	2
				cAMN	Male	39	7
				cAMN	Male	43	6
				cAMN	Male	44	8
				cAMN	Male	45	19
				cAMN	Male	47	22
				cAMN	Male	48	14
				cAMN	Male	52	20
				cAMN	Male	58	17
				cAMN	Male	63	23

3.3 Mouse experiments

We used male mice of a pure C57BL/6J background. All of the methods employed in this study were in accordance with the Guide for the Care and Use of Laboratory Animals (Guide, 8th edition, 2011, NIH) and European (2010/63/UE) and Spanish (RD 53/2013) legislation. Experimental protocol had been approved by IDIBELL, IACUC (Institutional Animal Care and Use Committee) and regional authority (3546 DMAH, Generalitat de Catalunya, Spain). IDIBELL animal facility has been accredited by The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC, Unit 1155). Animals were housed at 22ºC on specific-pathogen free conditions, in a 12-hour light/dark cycle, and *ad libitum* access to food and water. Cages contained 3 to 5 animals.

We used two X-ALD mouse models in this study. We characterized the effect of DMF administration or RIP140 deletion on the biochemical signs of adult AMN in *Abcd1*$^{-}$ mice at 12 months of age. These mice show biochemical signs of pathology, including oxidative stress (Fourcade et al., 2008) and energetic homeostasis impairment (Galino et al., 2011), before the first clinical signs of AMN-like pathology (axonopathy and locomotor impairment) appear at 20 months (Pujol et al., 2002).

To address the therapeutic effect of DMF administration or RIP140 deletion, we assessed the clinical signs of AMN in *Abcd1*$^{-}$*/Abcd2*$^{-/-}$ (DKO) mice, which display increased VLCFA accumulation in the spinal cord (Pujol et al., 2004), higher levels of oxidative damage to proteins (Fourcade et al., 2008; Galino et al., 2011), and a more severe AMN-like pathology with an earlier onset at 12 months of age (Pujol et al., 2004).

Antioxidant cocktail (NAC, α-LA and α-tocopherol) treatment was performed as described before (Galino et al., 2011). Specifically, 430 mg/kg/day of α-LA (0.5% wt/wt) and 90IU/kg/day (65mg/kg/day) of α-tocopherol (1050IU/kg of chow) were mixed into AIN-76A chow from Dyets (Bethlehem, PA). 850mg/kg/day of NAC (1%) were dissolved in drinking water (pH 3.5).

For biochemical analysis, we killed the mice and stored the tissues at -80ºC after snap-freezing them in liquid nitrogen, except for mitochondrial respiration, in which the tissue was used fresh. For histological analysis, spinal cord and brain were harvested from the mice after perfusing them with 4% paraformaldehyde (PFA; Sigma-Aldrich, Ref. 441244) in 0.1 M phosphate buffer pH 7.4. Histological and behavioural tests were performed in a blind way with respect to the animal's genotype and also for the treatment administered.

3.3.1 DMF administration to mice

DMF was mixed into AIN-76A chow from Dyets to provide a dose of 100 mg/kg/day. Human equivalent dose would be 8 mg/kg/day (240 mg in a typical 60 kg person). This is equivalent to the starting dose of BG-12/Tecfidera that is 120 mg twice a day for multiple sclerosis patients (EMA/204830/2013).

For the characterization of biochemical signs on adult X-ALD mice, 8-months-old animals were randomly assigned to one of the following dietary groups for 4 months. Group I: wild-type (WT) mice received normal AIN-76A chow (n=12); group II: *Abcd1*$^{-}$ mice received normal AIN-76A chow (n=12); group III: *Abcd1*$^{-}$ mice received AIN-76A chow containing DMF (n=12). To evaluate the effect of DMF on the clinical signs of AMN-like pathology, 12-months-old animals were randomly assigned to one of the following dietary groups for 6 months. Group I: WT mice received normal AIN-76A chow (n=14); group II: *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice received normal AIN-76A chow (n=16); group III: *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice received AIN-76A chow containing DMF (n=14). DMF had no effect on weight or food intake under any treatment protocol, and none of the mice administered with DMF experienced any adverse events or death during treatment.

3.3.2 Generation of *Rip140* knockout mice with knock-in of eGFP

A conditional knockout mouse of the *Rip140* gene with knock-in of eGFP was generated by Ozgene (Bentley DC, Australia). The conditional allele was based on Cre-mediated inversion of sequence between lox66 and lox71 sites. The inversion was used

to disrupt the exon 4 splice acceptor sequence and introduce a gene-trap cassette that consisted of a splice acceptor sequence, the eGFP coding sequence and a polyadenylation signal. Thereby, a lox66 site was placed in intron 3 and a lox71 site in exon 4, upstream of the start codon in the untranslated region of the gene. The gene-trap cassette was in reverse orientation relative to transcription of the *Rip140* gene. The PGK-neo selection cassette was inserted between the gene-trap cassette and exon 4. The PGK-neo cassette was flanked by FRT (flippase recognition target) sites and was deleted using FLP-recombinase (conditional allele (neo removed)) (**Fig. 8a**).

The targeting vector was constructed using three genomic fragments; the 5' homology arm, the exon 4 splice acceptor fragment and the 3' homology arm; all of which was generated by PCR. Both homology arms are approximately 5-6 kb in length. For the conditional allele (neo removed), splicing from exon 3 to exon 4 occurred normally. The splice acceptor and the polyA signal in the gene-trap cassette were not recognized because they were in inverted orientation (**Fig. 8a**). Cre-mediated recombination between the lox66 and lox71 sites resulted in the generation of one loxP site and one double-mutant lox site. In the inverted locus, splicing occurred from exon 3 to the splice acceptor in the gene-trap cassette, resulting in expression of eGFP (**Fig. 8a**). Mice heterozygous for the *Rip140* floxed allele (conditional *Rip140* mice) were crossed with mice expressing Cre recombinase under the control of CMV promoter to generate *Rip140* heterozygous ($Rip140^{+/-}$) mice.

Specific primers used for genotyping mouse tail-tip genomic DNA were as follows: a common primer (5′-TGAGCGTTGTGTTGGATGA-3′) used together with specific primers for the WT- (5′-ATAGCAGTCGGGCAGAGATC-3′), conditional- (5'-CCACTACCTGAGCACCCAGT-3') or KO-allele (5′-TGGATGTGGCTGTCATGTGT-3′). These combinations of primers amplified a 504 bp segment for the conditional allele, a 704 bp segment for the WT allele, and a 530 bp segment for the KO allele (**Fig. 8b**), under the following PCR conditions: 94ºC for 30 s, 54ºC for 30 s, 72ºC for 60 s (WT allele); and 94ºC for 30 s, 62ºC for 30 s, 72ºC for 90 s (conditional and knockout alleles).

$Rip140^{-/-}$ females obtained were infertile, as already described for another RIP140-deficient mouse (R. White et al., 2000). Furthermore, $Rip140^{-/-}$ mice offspring did not

have expected Mendelian ratios, as the proportion of null offspring coming from matings of *Rip140*$^{-/-}$ male mice with *Rip140*$^{+/-}$ heterozygous female mice was less than the expected 50% (data not shown). This same observation is found in the other *Rip140* KO mice (R. White et al., 2000).

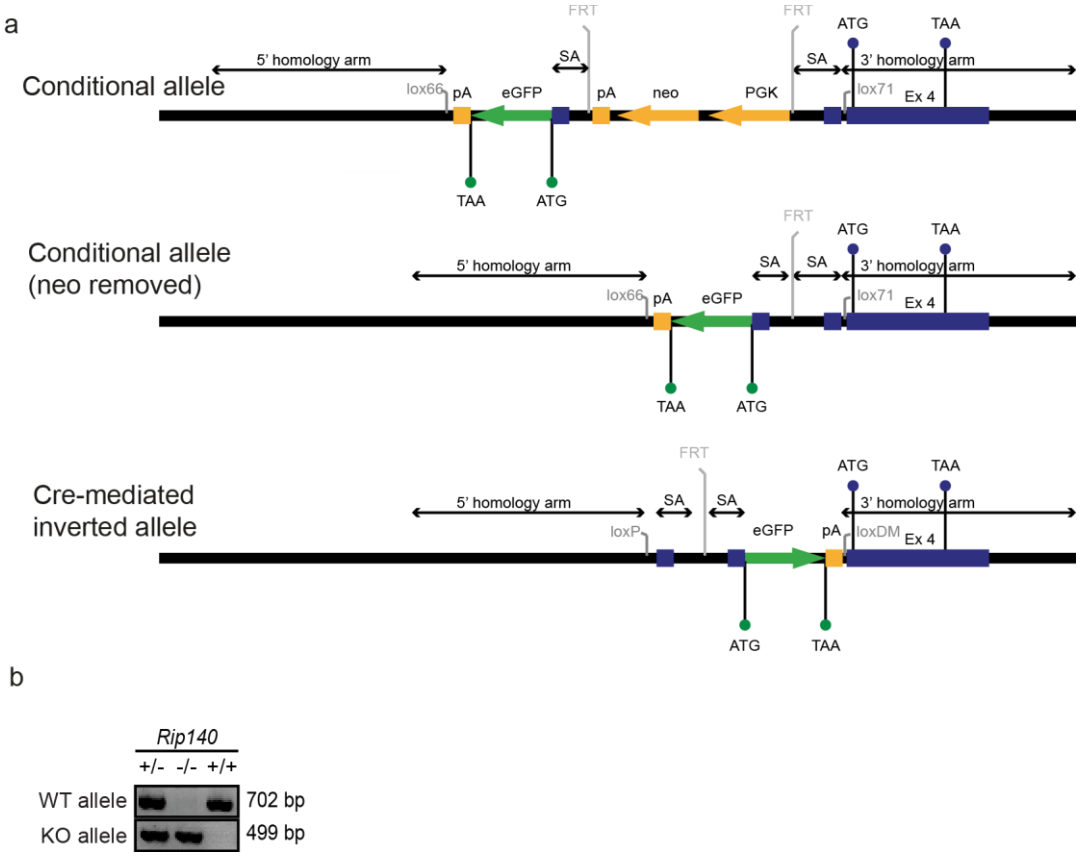


Figure 8 Generation of RIP140 knockout mice

a) Schematic view of the procedure used to disrupt coding exon of *Rip140* in mice. [SA (splice acceptor), pA (polyA), neo (neomycin), PGK (phosphoglucokinase)]. **b**) PCR bands obtained after genotyping RIP140 wild-type (*Rip140*$^{+/+}$), heterozygous (*Rip140*$^{+/-}$) and knockout (*Rip140*$^{-/-}$) mice tails

3.4 Organotypic spinal cord slice culture (OSCSC)

The spinal cord from 18-day-old WT and *Abcd1*$^{-}$ mice were immediately removed after mice dissection and placed in ice-cold dissecting media (pH 7.15). Next, the spinal cord was cut into 350 µm-thick slices using a McIlwain tissue chopper to generate the

organotypic spinal cord slice cultures (OSCSC) and placed into a sterile Petri dish with Grey's balanced salt solution. Spinal cord slices were then transferred onto Millicell-CM cultured plate inserts (Millipore, MA, USA). The inserts were placed into wells of a 6-well plate containing 1 mL of medium containing 50% minimum essential media (MEM) with Earl's salts and glutamine, 25% Hanks balanced salt solution and 25% horse serum supplemented with 20 mM of HEPES acid–salt and D-glucose (6 mg/mL) (Thermo Fisher Inc.). Slices were incubated at 37°C, changing the media twice a week (Krassioukov et al., 2002). After two weeks of culture, OSCSC were treated with C26:0 (100 μM) dissolved in ethanol (Fourcade et al., 2008), hydrogen peroxide (H_2O_2) (10 μM) and/or Trolox (500 μM) (Calbiochem).

3.5 Nuclear-cytoplasmic fractionation in human fibroblasts

We performed subcellular fractionation of human skin fibroblasts from X-ALD patients and age-matched healthy subjects (CTL) to study NRF2 translocation to the nucleus, using a non-ionic detergent lysis method with slight modifications (Abmayr et al., 2006; Suzuki et al., 2010). Briefly, cells grown in 100 mm diameter dishes (Nunc Dish, Thermo Fisher Inc.) were washed in ice-cold phosphate buffer saline (PBS) pH 7.4, and collected by trypsinization (0.25% Trypsin-EDTA Solution A; Biological Industries USA, Cromwell, CT, USA) in a 15-mL falcon tube. After a centrifugation and a new ice-cold PBS wash, cell-pellet was resuspended with 140 μL of lysis buffer (0.1% Nonidet P-40 in PBS, plus proteases (Complete Mini, Ref. 11836153001; Roche Diagnostics GmbH; Mannheim; Germany) and phosphatases (PhosSTOP, Ref. 04906845001; Roche Diagnostics GmbH) inhibitors) and scratched twenty times to lyse the cells. At this step, 20 μL of the homogenate were taken as total extract. After 10 minutes incubation in ice, we centrifuged at 300 x g during 5 min at 4ºC in an Eppendorf® microcentrifuge. Supernatant was collected as cytoplasmic fraction. Then, we added 70 μL of RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl, 12 mM deoxycholic acid and 1% Nonidet P-40; complemented with proteases/phosphatases inhibitors) to the pellet to obtain nuclear fraction. After homogenising the pellet through a syringe with a 25G needle, and 30 min of shaking at 4ºC, we centrifuged for 10 min at 16,100 x g at 4ºC. Supernatant was collected as nuclear fraction. After sonicating all the subcellular fractions, we performed immunoblot procedure as described below. Lamin

B1 and Aldolase A were used as markers for nuclear and cytoplasmic fraction, respectively.

3.6 Quantitative real-time PCR

Total RNA was extracted from human brains, human fibroblasts and mouse tissues using RNeasy Kit (Qiagen, Hilden, Germany). Total DNA was extracted from mouse spinal cord using Gentra Puregene Tissue Kit (Qiagen, Hilden, Germany). One µg of RNA was transcribed into cDNA using Superscript II reverse transcription reagents (Invitrogen, Thermo Fisher Scientific Inc.) in a final volume of 25 µL. Dilutions ranging from 1/75 to 1/10 of cDNA or 100 ng of DNA were used to measure mRNA or mtDNA levels, respectively. The expression of the genes of interest was analyzed by Q-PCR using TaqMan® Gene Expression Assays (Thermo Fisher Scientific Inc.) and standardized Taqman® probes (**Table 4**) on a LightCycler® 480 Real-Time PCR System (Roche Diagnostics GmbH). Relative quantification was carried out using the 'Delta-Delta Ct' (ΔΔCt) method with m*Rplp0/hRPLP0* as endogenous control. To quantify mouse mtDNA content, primers for mouse cytochrome b (*Cytb*) were designed (Custom TaqMan Gene Expression Assays; Thermo Fisher Scientific Inc.). The sequences for *Cytb* primers were: ATGACCCCAATACGCAAAATTA (forward) and GGAGGACATAGCCTATGAAGG (reverse) and the FAM-labeled probe sequence was TTGCAACTATAGCAACAG. Quantification of mtDNA was referred to nuclear DNA (nDNA) as determined by the amplification of the intron-less mouse nuclear gene *C/ebpα* (Morato et al., 2013). Transcript quantification was performed in triplicates for each sample.

3.7 Immunoblot

Human brains, human fibroblasts and mouse tissues were homogenized in radioimmunoprecipitation assay (RIPA) buffer, then sonicated, centrifuged and heated for 10 min at 70ºC after adding 4X NuPAGE® LDS Sample Buffer (Invitrogen, Thermo Fisher Scientific Inc.). Twenty to 100 µg of proteins were loaded onto 8% Novex NuPAGE® SDS-PAGE gel system (Invitrogen, Thermo Fisher Scientific Inc.), and run for 60-90 min at 120 V in NuPAGE® MOPS SDS Running Buffer (Invitrogen, Thermo

Fisher Scientific Inc.) supplemented with 5 mM sodium bisulfite (Ref. 243973, Sigma-Aldrich). SeeBlue® Plus2 Pre-stained (Invitrogen, Thermo Fisher Scientific Inc.) was used as a ladder.

Resolved proteins were transferred onto nitrocellulose membranes using iBlot® 2 Gel Transfer Device (Invitrogen, Thermo Fisher Scientific Inc.). After blocking in 5% bovine serum albumin (BSA, Sigma-Aldrich) in 0.05% TBS-Tween (TBS-T) for 1 h at room temperature, membranes were incubated with corresponding diluted primary antibodies (**Table 1**) in 5% BSA in 0.05% TBS-T overnight at 4ºC. Following incubation with diluted secondary antibody (**Table 2**) in 0.05% TBS-T for 1 hour at room temperature, proteins were detected with ECL western blotting analysis system (GE Healthcare, Buckinghamshire, UK), followed by exposure to CL-XPosure Film (Thermo Fisher Scientific Inc.) as earlier described (Galino et al., 2011)**.** Immunoblots were quantified by densitometry using ImageJ v1.50i (U. S. National Institutes of Health, Bethesda, MD, USA).

Table 4 List of Q-PCR probes used in this thesis

Gene	Species	Taqman ref.
Arg1	Mouse	Mm00475991
Cebpα	Mouse	Mm00514283
Ccl5	Mouse	Mm01302427
Ccr6	Mouse	Mm99999114
Chil3	Mouse	Mm00657889
Cxcl9	Mouse	Mm00434946
Cxcl10	Mouse	Mm00445235
Cxcl12	Mouse	Mm00457276
Gstα3	Mouse	Mm01233706
Hmox1	Mouse	Mm00516005
Igf1	Mouse	Mm00439560
Il1β	Mouse	Mm01336189
Il10	Mouse	Mm00439614
Il6	Mouse	Mm00446190
Mif	Mouse	Mm01611157
Nfκb2	Mouse	Mm00479807
Nqo1	Mouse	Mm01253561
Nrf1	Mouse	Mm00447996
Pgc-1α	Mouse	Mm00447183
Pparγ	Mouse	Mm01184322
Retnlα	Mouse	Mm00445109
Rip140	Mouse	Mm01343437
Rplp0	Mouse	Mm01974474
Sirt1	Mouse	Mm00490758
Tfam	Mouse	Mm00447485
Tgfβ1	Mouse	Mm01178820
Tnfα	Mouse	Mm00443258
Tnfrsf1a	Mouse	Mm01182929
RIP140	Human	Hs00942766
GCLC	Human	Hs00155249
HMOX1	Human	Hs01110250
NQO1	Human	Hs00168547
RPLP0	Human	Hs99999902

3.8 High resolution respirometry

O_2 consumption was measured in sets of five permeabilized lumbar spinal cord slices (LSCS) (n=5 mice per genotype) at 37°C in MiR05 medium pH 7.4 by high-resolution respirometry using an Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) as previously described (Lopez-Erauskin et al., 2013).

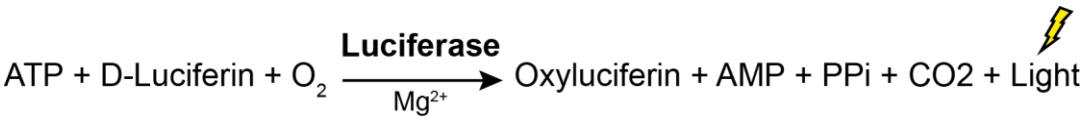
In detail, fresh spinal cords were rinsed in ice-cold normal saline and cut into slices with a tissue chopper adjusted to a cut width of 300 μm. O_2 consumption was measured in sets of 5 LSCS at 37°C with high-resolution respirometry in permeabilized conditions (including substrates and inhibitors of specific respiratory complexes) using an Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) with chamber volumes set at 2 mL. Spinal cord permeabilization was a modification of a previously published method (Safiulina et al., 2004). About 12-15 slices of the lumbar region were collected and transferred quickly into an individual well of a 6-well tissue culture plate with 2 mL of ice-cold permeabilization medium (7.23 mM potassium ethylene glycol-bis{b-aminoethyl ether}N,N,N',N'-tetraacetate [K_2-EGTA]; 2.77 mM CaK_2-EGTA; 60 mM N,N-bis{2-hydroxyethyl}-2-aminoethanesulfonic acid [BES]; 5.69 mM MgATP, 20 mM taurine; 3 mM K_2HPO_4; 0.5 mM dithiothreitol [DTT] and 81 mM potassium methanesulfonate, pH 7.1 at 25ºC), rinsed and immediately transferred into another well with the same medium containing 20 μL of saponin stock solution (5 mg/mL; final concentration 50 μg/mL). LSCS were then shaken by gentle agitation at 4ºC for 30 min. Afterwards, all samples were quickly transferred from the saponin permeabilization medium into 2 mL of respiration medium (7.23 mM K_2-EGTA; 2.77 mM CaK_2-EGTA; 100 mM potassium salt of 2-{N-morpholino}ethanesulfonic acid [K-MES], 1.38 mM $MgCl_2$; 20 mM taurine; 3 mM K_2HPO_4; 0.5 mM DTT; 20 mM imidazole and 5 mg/mL BSA, pH 7.1 at 25ºC), then shaken by gentle agitation for 10 min in the cold room (on ice) before performing respirometry.

In order to avoid tissue disaggregation, LSCS required setting a slow bar stirring speed (150 rpm), but not too low to compromise homogeneity of substrate and oxygen concentrations in the measuring chambers, and, therefore, signal stability. DatLab software (Oroboros Instruments, Innsbruck, Austria) was used for data acquisition (2 s

time intervals) and analysis, which includes calculation of the time derivative of oxygen concentration and correction for instrumental background oxygen flux (Gnaiger, 2001). Respiration of LSCS required tissue or cell permeabilization before placing the sample in the measurement chamber. Initially, we measured endogenous respiration in the absence of additional substrates. For evaluation of relative contributions of mitochondrial complexes to oxygen consumption, several specific mitochondrial inhibitors and substrates were added sequentially and calculated as steady-state respiratory flux in the time interval between 5 and 10 min after their addition. First, we added glutamate (10 mM) and malate (5 mM) to increase NADH levels in order to measure the complex I non-phosphorylative activity, or state 2. ADP (10 mM) was added to quantify the complex I-dependent phosphorylative activity, or state 3. Immediately afterwards, we added succinate (10mM), which is the substrate for complex II. At this point, the level of oxygen consumption corresponded to complex I- and II-dependent phosphorylative activity. The addition of rotenone (0.5 µM; Sigma-Aldrich) inhibits complex I; therefore, oxygen consumption measured after the addition of rotenone only reflects complex II-dependent phosphorylative activity (in the absence of electron back flux to complex I). Then, complex III activity was inhibited with antimycin A (2.5 µM; Sigma-Aldrich) to obtain residual oxygen consumption. In order to avoid oxygen limitations, all the experiments were performed above 50% oxygen saturation. Oxygen consumption was normalized for actual protein content in the respirometer chambers.

3.9 ATP measurement

ATP levels were measured by a chemiluminescence system using ATPlite 1step (PerkinElmer, Inc., Waltham, MA, USA). Measurement of ATP was performed according to the manufacturer's instructions, and relative ATP levels were calculated by normalizing the luminescence obtained to the total protein concentration of the sample measured by BCA (bicinchoninic acid assay). The ATPlite 1step assay system is based on the production of light caused by the reaction of ATP with added luciferase and D-luciferin, as depicted in the following reaction:



3.10 NAD-NADH determination

NAD^+ and NADH were quantified in the mice spinal cord by the NAD cycling assay as previously described (Galino et al., 2011). For NAD^+ determination, ~10 mg of frozen tissue were homogenized with a motor-driven grinder in 200 µL of ice-cold 0.05 M HCl. The homogenate was centrifuged (16,000 x g, 5 min, 4ºC), and supernatant was collected. After the second centrifugation (16,000 x g, 5 min, 4ºC), the supernatant was kept at 4ºC for 30 min. For the NADH determination, ~10 mg of frozen tissue were homogenized with a motor-driven grinder in 200 µL of ice-cold 0.02 M NaOH. The homogenate was centrifuged (16,000 x g, 5 min, 4ºC), and supernatant was collected. After the second centrifugation (16,000 x g, 5 min, 4ºC), the supernatant was incubated at 60ºC for 30 min to eliminate NAD^+. Next, we neutralized both extracts with 0.2 M potassium phosphate buffer pH 7.5 on ice, then homogenates were centrifuged for 5 min at 16,000 x g and the supernatants were collected for NAD^+ or NADH measurement. Fifteen µL of supernatant containing NAD^+ or NADH extract were added to 300 µL of cycling buffer (100 mM Tris-HCl; 5 mM EDTA; 0.5 mM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT]; 1 mM phenazine ethosulfate [PES], and 0.15 mg/mL alcohol dehydrogenase [ADH]) and incubated for 5 min at 37ºC. Then, 15 µL of EtOH (0.6 M final concentration) were added to start the reaction, and then the mixture was centrifuged for 30 s at 16,000 x g. The absorbance of supernatant was measured at 570 nm after 10 min in a microplate spectrophotometer (PowerWave Microplate Spectrophotometer, BioTek). For quantification, values of the linear increase in absorbance were compared with values of NAD^+ or NADH standards. Data were normalized to milligram of tissue.

3.11 Pyruvate kinase activity

Pyruvate kinase (PK) activity in mice spinal cord was determined by a spectrophotometric method as previously described (Galino et al., 2011). Briefly, 15 µg of mitochondria-free supernatant were added to 0.2 mL of reaction buffer (50 mM Tris-HCl pH 7.4, 100 mM KCl, 20 mM $MgCl_2$, 0.3 mM NADH, 4 mM ADP, 1 mM phosphoenolpyruvate (PEP), and 5 units/mL of lactate dehydrogenase [LDH]). NADH was spectrophotometrically recorded every two minutes at 340 nm during 16 minutes in

a microplate spectrophotometer (PowerWave Microplate Spectrophotometer, BioTek). The measurement recorded at sixth minute was used for the analysis. All assays were performed in triplicate at room temperature. PK activity (µmol/min) was normalized to milligram of tissue.

3.12 Evaluation of oxidative lesions

AASA, CML, CEL and MDAL concentrations in total proteins from spinal cord homogenates were measured with GC/MS, as reported (Fourcade et al., 2008). The amounts of products were expressed as the ratio of micromole of AASA, CML, CEL, or MDAL per mol of lysine.

3.13 Immunohistochemistry (IHC)/Immunofluorescence (IF)

Mice were anaesthetized by intraperitoneal injection of Sodium Pentobarbital (Dolethal®; Vetoniquol, Alcobendas, Spain) diluted 1/10 in physiological serum (Braun, Rubí, Spain). Spinal cord and brain were harvested after perfusing the mice with 4% paraformaldehyde (PFA; Sigma-Aldrich, Ref. 441244) in 0.1 M phosphate buffer pH 7.4.

For studying axonal degeneration, spinal cord was embedded in paraffin and serial sections (4 µm thick) were cut in a transversal or longitudinal (1 cm long) plane with a microtome (Microm HM 340E, Thermo Fisher Scientific). IHC studies performed in WT; *Abcd1*$^{-}$/*Abcd2*$^{-/-}$; *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ + DMF; *Abcd1*$^{-}$/*Abcd2*$^{-/-}$/*Rip140*$^{-/-}$ and *Rip140*$^{-/-}$ mice were carried out with the avidin–biotin peroxidase method.

After antigen retrieval step using 10 mM citrate buffer pH 6.0 and endogenous peroxidases blocking step using 3% H_2O_2 (H1009, Sigma-Aldrich), the rehydrated sections were stained with 3% Sudan black (Ref. 199664, Sigma-Aldrich) or processed for IHC. At least three spinal cord sections were analysed per animal and per stain. After primary antibody incubation (**Table 1**), the sections were incubated with the Labelled Streptavidin-Biotin2 System (LSAB2, Ref. K0675, Dako). Staining was visualized after incubation with 3-3'-diaminobenzidine (DAB) substrate chromogen

(Ref. D5637, Sigma-Aldrich) which results in a brown-coloured precipitate at the antigen site. After dehydrating the sections, slides were mounted with DPX (Ref. 06522, Sigma-Aldrich).

Images were acquired using Olympus BX51 microscope (20x/N.A 0.50 Ph 1 UPlan FL N; Olympus Corporation, Tokyo, Japan) connected to an Olympus DP71 camera and Cell^B software (Olympus Corporation). The researcher was blinded to both the genotype and the treatment of the sample when analysing the results.

For GFP immunofluorescence (IF) studies, spinal cord and brain from one-month-old WT and *Rip140*$^{-/-}$ mice were equilibrated in 30% sucrose after perfusion. Then tissues were embedded with tissue freezing medium (O.C.T, Sakura) inside disposable plastic molds (Sakura; Torrance, CA, USA). Serial sections (5 µM thick) were cut in coronal and sagittal planes with a cryostat (1950M; Leica Biosystems, Wetzlar, Germany). IF was performed using a chicken anti-GFP (GFP-1020; Aves Lab, Tigard, OR, USA) as primary antibody and an Alexa 488-conjugated goat anti-chicken IgG (Thermo Fisher Inc.) as secondary antibody. Nuclei were stained by DAPI (Thermo Fisher Inc.). Confocal images were collected using a Leica TCS SP5 confocal microscope (Leica Biosystems), and processed with ImageJ v1.50i (U. S. National Institutes of Health, Bethesda, MD, USA). Since there was some background present as a result of the IF procedure, sections from WT mice were used to set the background where no eGFP signal was observed, to determine specific eGFP signal in *Rip140*$^{-/-}$ mice. The researcher was blinded to the genotype of the sample when analysing the images.

3.14 Behavioural tests

3.14.1 Horizontal bar cross test

Bar cross test uses a wooden bar of 100 cm in length and 2 cm in width (diameter). This bar is just wide enough for mice to stand on with their hind feet hanging over the edge such that any slight lateral misstep will result in a slip. The bar was elevated 50 cm from the bench surface, so that animals did not jump off, yet were not injured upon falling from the bar. The mice were put on one end of the bar and expected to cross to the other end. To eliminate the novelty of the task as a source of slips, all animals were given two

trials on the bar the day before and at the beginning of the testing session. In the experimental session, the numbers of hind limbs lateral slips and the time to cross the bar were counted on three consecutive trials. The researchers that performed this experiment were blinded for the genotype or treatment of the mice.

3.14.2 Treadmill test

Treadmill apparatus (Panlab, Barcelona, Spain) consist of a belt (50 cm long and 20 cm wide) varying in terms of speed (5–30 cm/s) and slope (0–25º) enclosed in a plexiglass chamber. An electrified grid is located to the rear of the belt on which foot shocks (0.5 mA) were administered whenever the mice fell down the belt. The mice were placed on the top of the belt in the opposite direction to the movement of the belt. Thus, to avoid the foot shocks, the mice had to move forward. The latency to falling off the belt (time of shocks in seconds) and the number of shocks received were measured. The mice were evaluated in five trials in a single-day session, always between 2 p.m. and 6 p.m. In the first trial, the belt speed was set at 20 cm/s and the inclination at 5º. In the second and third trial, the belt speed was 10 cm/s and the slope was increased to 10º and 20º, respectively. Then, for the fourth and the fifth trials, the slope was maintained at 20º and the belt speed was increased to 20 and 30 cm/s, respectively. For the three first trials, mice ran for 1 min. For the fourth and fifth tests, times of the experiment were 3 minutes, and 7 minutes and 30 seconds, respectively. Intervals between each test were 1, 1, 5 and 20 minutes, respectively. When the mice were subjected to consecutive trials at increasing speeds of up to 20 cm/s and a 20° slope, no differences were detected from one session to another between the WT and *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice. However, when the belt speed was increased up to 30 cm/s and the slope was 20°, differences were detected between the *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice and the controls because this task requires greater coordination. These conditions were therefore chosen to assess the effects of the treatment or the genotype.

3.14.3 Hind limbs clasping test

Assessment of hind limbs clasping was done by suspending WT, *Abcd1*$^{-}$/*Abcd2*$^{-/-}$, *Abcd1*$^{-}$/*Abcd2*$^{-/-}$/*Rip140*$^{-/-}$ and *Rip140*$^{-/-}$ mice from their tails until they reached a vertical

position. The hind limbs extension reflex was analysed for 10 seconds in three consecutive trials separated by 5 min rest. The hind limbs extension reflex was scored as described in **Table 5**, adapted from Dumser *et al*. (2007). The researcher was blinded for the genotype of the mice while performing these behavioural tests.

3.15 Statistical analysis

The values were expressed as the mean ± standard deviation (SD), unless explicitly noted otherwise, in that case values were expressed as the mean ± standard error of the mean (SEM). The significant differences (*/$/# $p<0.05$, **/$$/## $p<0.01$, ***/$$$/### $p<0.001$) when comparing two groups were determined by a two-tailed unpaired Student's t test. When comparing more than two groups, significant differences were determined by one-way or two-way ANOVA followed by Tukey's *post hoc* test or Kruskal Wallis non-parametric test followed by Dunn's *post hoc* test, after verifying normality (Shapiro-Wilk test). Statistical analyses were performed using SPSS for Windows version 12.0.

Table 5 Scoring system for the clasping test

SCORE	PHENOTYPE
0	Total paralysis of the hindlimbs
0.5	Both hindlimbs are entirely retracted and touching the abdomen during 100% of the time suspended
0.75	Both hindlimbs are entirely retracted toward the abdomen during 100% of the time suspended
1	Both hindlimbs are partially retracted toward the abdomen during 100% of the time suspended
1.25	alternating clasping and flexion of hindlimbs, although clasping occurs for more than 50% of the time suspended
1.5	Alternating clasping and flexion, equally occurring during 100% of the time suspended
1.75	Alternating clasping and flexion of hindlimbs, although flexion occurs for more than 50% of the time suspended
2	Flexion of hindlimbs during 100% of the time suspended
2.25	Alternating flexion and extension of hindlimbs, although flexion occurs for more than 50% of the time suspended
2.5	Alternating flexion and extension, equally occurring during 100% of the time suspended
2.75	Alternating flexion and extension of hindlimbs, although extension occurs for more than 50% of the time suspended
3	Extension of hindlimbs in an angle <90º during 100% of the time suspended
3.5	Alternating extension of hindlimbs in an angle <90° and ≥90° during 100% of the time suspended
4	Extension of hind limbs in an angle ≥90° during 100% of the time suspended

RESULTS

4 Results

4.1 Chapter I: Study of AKT/GSK-3β/NRF2 antioxidant pathway in X-ALD pathophysiology: Neuroprotective effect of dimethyl fumarate in a mouse model of X-ALD

4.1.1 NRF2-dependent antioxidant pathway is altered in an X-ALD mouse model

Given the central role of NRF2 in the antioxidant cellular defence associated with redox dyshomeostasis in X-ALD (Fourcade et al., 2008), we asked whether NRF2-dependent antioxidant pathway was altered in X-ALD. In the last years, the right size of NRF2 protein in western blot has been a subject of controversy. Using tissue and cell samples from *Nrf2*$^{-/-}$ mice, the specific NRF2 band was detected at around 100 kDa but not at the predicted 68 kDa (Lau et al., 2013). We found out decreased NRF2 protein levels in *Abcd1*$^{-}$ mice spinal cord at 12 months of age (**Fig. 9a**), a presymptomatic stage of the disease in this mouse model, but not earlier at 3 months of age (**Fig. 9b**). The dysregulation of NRF2 protein levels was organ-specific as we did not observe any change in non-affected tissues in this mouse model, such as cortex or liver (**Fig. 9b**). To further address whether NRF2 pathway was altered, we measured mRNA expression of NRF2 classical target genes (*Hmox1*; *Nqo1* and glutathione S-transferase alpha-3, *Gstα3*) at the same age, and we observed a slight significant decrease of their expression in *Abcd1*$^{-}$ mice spinal cord at 12 months of age (**Fig. 9c**), in the line of a downregulated NRF2 pathway.

Several signalling pathways can regulate NRF2-dependent response, in particular, those ones modulating GSK-3β activity, such as AKT/GSK-3β pathway (Rada et al., 2011; Rojo et al., 2008b; Salazar et al., 2006). Since our group previously reported a common dysregulation of insulin signaling pathways in X-ALD mouse model and cAMN and ccALD patients (Schluter et al., 2012), and AKT/GSK-3β axis is one of the main regulators of this pathway, thereby we studied AKT/GSK-3β pathway in *Abcd1*$^{-}$ mice spinal cord. We uncovered less AKT activation, as shown by decreased phosphorylation

of AKT at Threonine 308 residue (pThr308) relative to total AKT levels. Subsequently, one of its target, GSK-3β, was more active, as indicated by reduced phosphorylation of GSK-3β at Serine 9 residue (pSer9) compared with total GSK-3β levels (**Fig. 9d**). We also quantified phosphorylation of other residues such as Serine 473 (pSer473) of AKT, another marker of AKT activation, and Tyrosine 216 (pTyr216) of GSK-3β, which indicates GSK-3β activation. We did not observe any changes neither in these phosphorylated forms of AKT and GSK-3β nor in total levels of AKT and GSK-3β (**Fig. 9d**).

In summary, these data show that AKT/GSK-3β/NRF2 axis was dysregulated in *Abcd1*$^{-}$ mouse spinal cord.

4.1.2 NRF2-dependent antioxidant pathway activation is impaired in X-ALD patients-derived fibroblasts

The fibroblasts of X-ALD patients are a good surrogate cell model for dissecting disease mechanisms, as they recapitulate the main X-ALD hallmarks: (i) accumulation of VLCFA , (ii) higher production of free radicals of mitochondrial origin (Lopez-Erauskin et al., 2013), (iii) loss of energetic homeostasis (Galino et al., 2011), (iv) altered proteostasis (Launay et al., 2015; Launay et al., 2013) and ER stress (Launay et al., 2017). Using this cell system, we sought to determine whether fibroblasts from X-ALD patients had an alteration of the AKT/GSK-3β/NRF2 pathway.

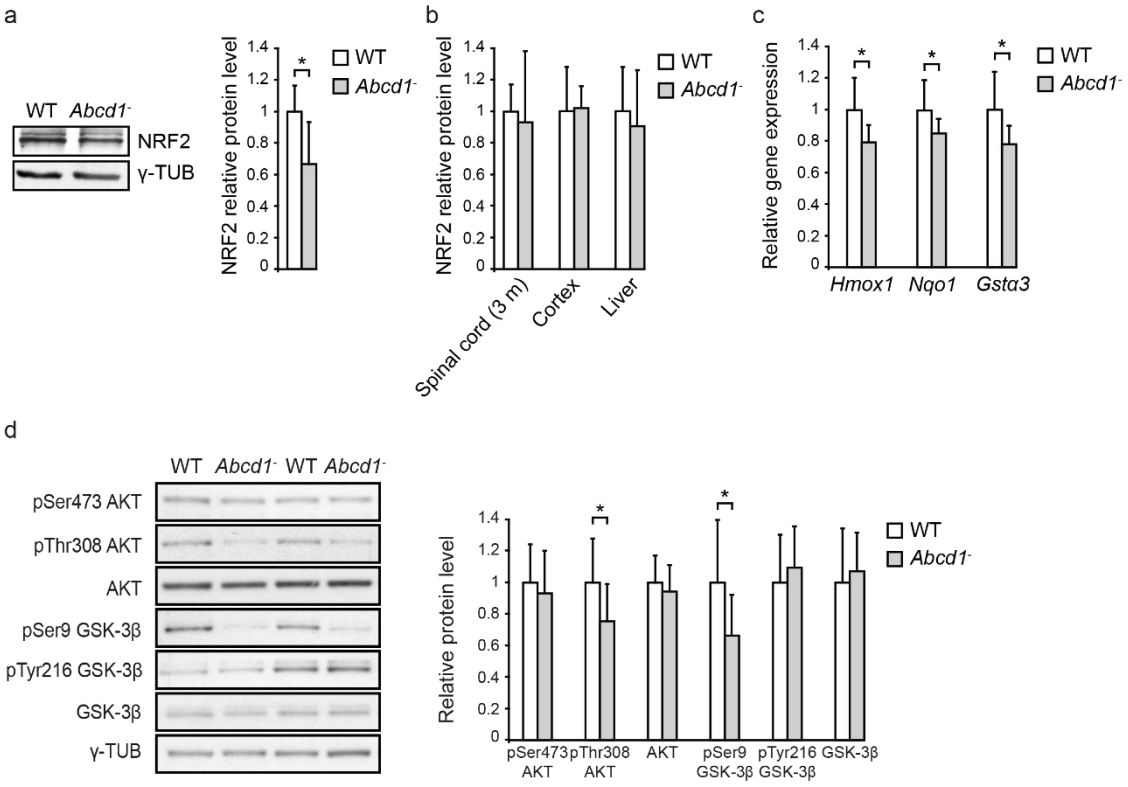


Figure 9 NRF2-dependent antioxidative pathway is altered in X-ALD

a) NRF2 protein levels were measured in WT (n=6) and *Abcd1*$^{-}$ (n=6) spinal cord at 12 months of age. Protein levels were normalized relative to γ-tubulin (γ-TUB) and quantification is depicted as fold change to WT mice. **b)** NRF2 protein levels were measured in WT (n=8) and *Abcd1*$^{-}$ (n=8) spinal cord at three months of age (3m), as well as in cortex and liver at 12 months of age. Protein levels were normalized relative to γ-tubulin (γ-TUB) and quantification is depicted as fold change to WT mice. **c)** NRF2-dependent antioxidative genes (*Hmox1, Nqo1* and *Gstα3*) expression in WT (n=8) and *Abcd1*$^{-}$ (n=8) mice spinal cord at 12 months of age. Gene expression levels were normalized relative to mouse *Rplp0* and depicted as fold change to WT mice. **d)** pSer473 AKT, pThr308 AKT, AKT, pSer9 GSK-3β, pTyr216 GSK-3β and GSK-3β protein levels were measured in WT (n=12) and *Abcd1*$^{-}$ (n=12) mice spinal cord at 12 months of age. Protein levels were normalized relative to corresponding non-phosphorylated proteins or γ-TUB (in the case of AKT and GSK-3β). Quantification is depicted as fold change to WT mice. Data are shown as mean ± SD (* $p<0.05$ after unpaired Student's t test).

At baseline, we noted that nuclear NRF2 protein levels were raised and cytoplasmic NRF2 levels were decreased in X-ALD fibroblasts compared with controls (**Fig. 10a**). In addition, *GCLC* mRNA expression was increased in X-ALD fibroblasts (**Fig. 10b**), reflecting the pro-oxidative status in these cells compared with control fibroblasts (Fourcade et al., 2008; Vargas et al., 2004). Then, we treated control and X-ALD

fibroblasts with C26:0, the main VLCFA accumulated in the patients; and oligomycin, which acts as a mitochondrial stressor (Fourcade et al., 2008; Paupe et al., 2009). Both compounds generate mitochondrial ROS in fibroblasts (Lopez-Erauskin et al., 2013). We showed that both C26:0 and oligomycin activated NRF2-dependent response in control fibroblasts, which was characterized by both higher NRF2 translocation to the nucleus (**Fig. 10c**) and increased expression of NRF2 target genes (*HMOX1*, *NQO1* and *GCLC* mRNA) (**Fig. 10d**). However, this physiological response against oxidative stress was blunted in X-ALD fibroblasts (**Fig. 10c-d**).

Furthermore, upon C26:0 and oligomycin treatment, AKT was activated, which was characterized by increased phosphorylation of Ser473 and Thr308 residues, whereas one of its targets, GSK-3β, was inactivated, as shown by higher pSer9 GSK-3β levels in treated-control fibroblasts (**Fig. 10e**). This physiological response against oxidative stress was impaired in X-ALD fibroblasts, as phosphorylated levels of AKT and GSK-3β did not change upon C26:0 or oligomycin treatment (**Fig. 10e**).

As GSK-3β activation can repress NRF2, we sought to determine the role of GSK-3β in the impaired NRF2 response against oxidative stress in X-ALD fibroblasts. To address this question, we assessed whether specific inhibitors of GSK-3β (CT99021 and SB216763) (Coghlan et al., 2000; Ring et al., 2003) could rescue NRF2-dependent response in X-ALD fibroblasts. We reveal that GSK-3β inhibition by both compounds reactivated NRF2 pathway upon C26:0 treatment, as evidenced by the upregulation of NRF2-target genes in X-ALD fibroblasts (**Fig. 10f**).

Collectively, these data indicate an altered NRF2-dependent response under oxidative conditions in X-ALD patients' fibroblasts and point to GSK-3β activation upstream of NRF2, as responsible for this inhibition of NRF2 pathway.

4.1.3 NRF2 activation by DMF prevents mitochondrial depletion and bioenergetic failure in X-ALD mice

The abovementioned results demonstrate that NRF2 pathway is inhibited in X-ALD. We therefore decided to treat *Abcd1*$^{-}$ mice with DMF, a classical activator of NRF2

(Linker et al., 2011; Scannevin et al., 2012) and we evaluated the possible therapeutic benefits of this drug. Indeed, DMF has shown beneficial effects in clinical trials for relapsing-remitting multiple sclerosis (Fox et al., 2012; Gold et al., 2012) and has recently been approved by the FDA and the EMA for the treatment of relapsing forms of multiple sclerosis. It has also been successfully tested in preclinical studies for other neurodegenerative diseases as HD (Ellrichmann et al., 2011) and PD (Ahuja et al., 2016; Lastres-Becker et al., 2016). In order to verify that the dietary administration of DMF was effective, we first measured NRF2 protein levels and mRNA expression of three classical NRF2-target genes (*Hmox1*, *Nqo1* and *Gstα3*). Interestingly, DMF induced both NRF2 protein levels (**Fig. 11a**) and NRF2 target genes expression in *Abcd1*$^{-}$ mice spinal cord at 12 months of age (**Fig. 11b**).

Next, we examined the effect of DMF on mitochondrial dysfunction in *Abcd1*$^{-}$ mouse spinal cord at 12 months of age (Morato et al., 2013). DMF normalized mtDNA levels (**Fig. 11c**) and mitochondrial biogenesis genes mRNA expression (*Sirt1*, *Pgc-1α, Nrf1* and *Tfam)* (**Fig. 11d**). We previously reported decreased levels of ATP in the spinal cord of *Abcd1*$^{-}$ mice (Galino et al., 2011), suggesting that a deficit in energy homeostasis is a key feature in X-ALD pathology. In the present study, we unveil that DMF prevented bioenergetic failure in X-ALD mouse spinal cord at 12 months of age, as ATP levels were normalized by DMF treatment (**Fig. 11e**).

In summary, DMF activated NRF2-dependent antioxidant pathway, as well as prevented mitochondrial depletion and bioenergetic failure in *Abcd1*$^{-}$ mice spinal cord.

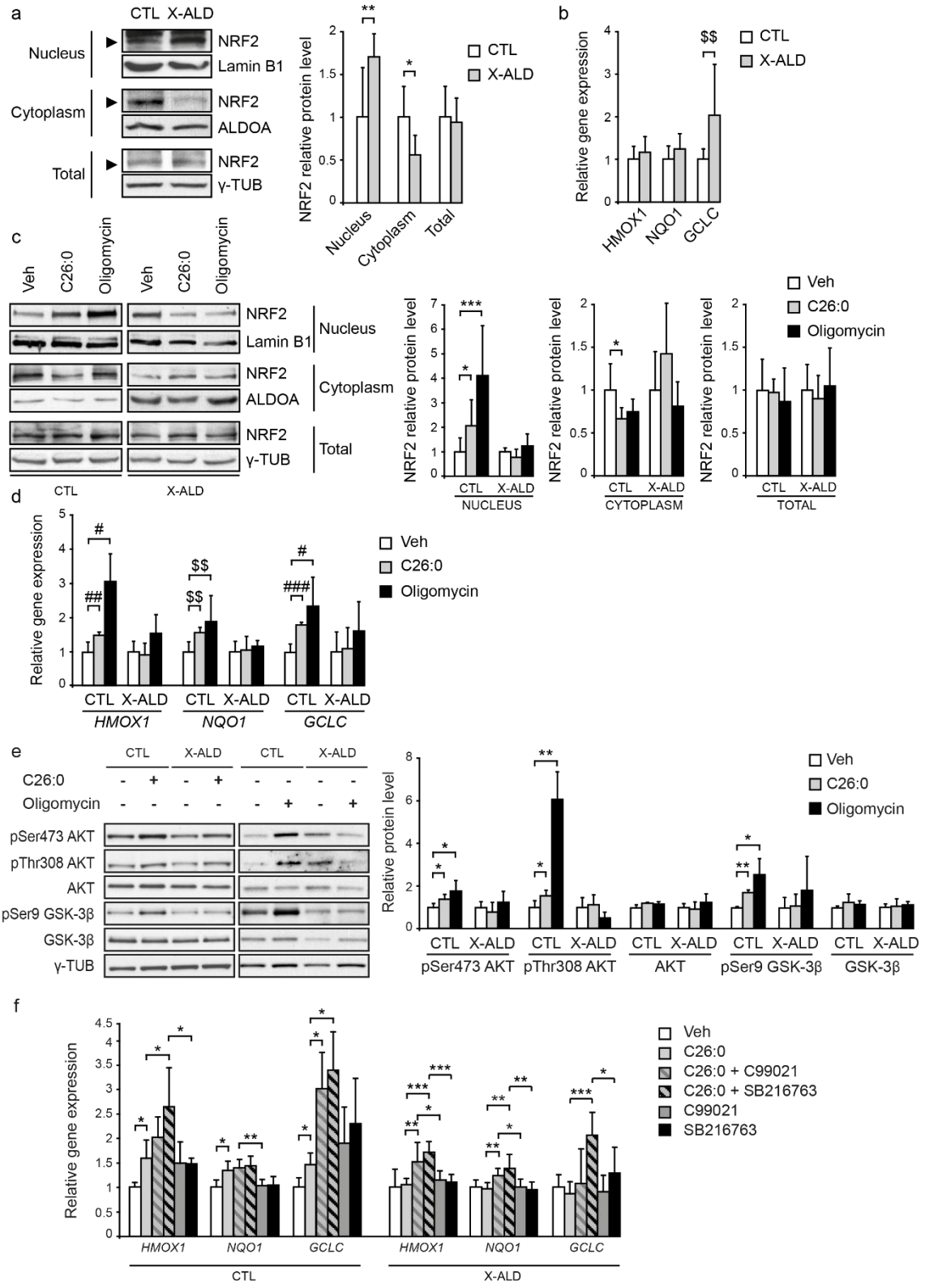


Figure 10 AKT/GSK-3β/NRF2-dependent antioxidative pathway activation upon oxidative stress induced by VLCFA or Oligomycin is impaired in X-ALD patients' fibroblasts

a) NRF2 protein levels in nuclear, cytoplasmic and total extracts from control (CTL) (n=5) and X-ALD (n=5) fibroblasts. Protein levels were normalized relative to Lamin B1 in nuclear fraction, Aldolase A (ALDOA) in cytoplasmic fraction and γ-TUB in total fraction. Quantification is depicted as fold change to control fibroblasts. **b)** NRF2-dependent antioxidative genes (*HMOX1, NQO1* and *GCLC*) expression in CTL (n=5) and X-ALD (n=5) fibroblasts under basal conditions. Gene expression levels were measured by quantitative RT-PCR and normalized relative to *RPLP0*. Quantification is depicted as fold change to control fibroblasts. **c)** NRF2 protein translocation to the nucleus upon VLCFA (C26:0, 50 μM, 24h) or Oligomycin (15 μM, 18h) in control (CTL) (n=5 per condition, left panels) and X-ALD (n=5 per condition, right panels) fibroblasts. Protein levels were normalized relative to Lamin B1 in nuclear fraction, Aldolase A (ALDOA) in cytoplasmic fraction and γ-TUB in total fraction. Quantification is depicted as fold change to vehicle-treated (Veh) fibroblasts. **d)** NRF2-dependent antioxidative genes (*HMOX1, NQO1* and *GCLC*) expression upon oxidative stress in CTL (n=5 per condition) and X-ALD (n=5 per condition) fibroblasts. Gene expression levels were measured by quantitative RT-PCR and normalized relative to *RPLP0*. Quantification is depicted as fold change to vehicle-treated (Veh) fibroblasts. **e)** pSer473 AKT, pThr308 AKT, pSer9 GSK-3β, pTyr216 GSK-3β, AKT and GSK-3β protein levels were measured upon oxidative stress in CTL (n=5 per condition) and X-ALD (n=5 per condition) fibroblasts. Protein levels were normalized relative to corresponding non-phosphorylated proteins or γ-TUB (in the case of AKT and GSK-3β) and quantification is depicted as fold change to vehicle-treated (Veh) fibroblasts. **f)** NRF2-dependent antioxidative genes (*HMOX1, NQO1* and *GCLC*) expression after GSK-3β inhibition in VLCFA-treated CTL (n=5 per condition) and X-ALD (n=5 per condition) fibroblasts. Gene expression levels were measured by quantitative RT-PCR and normalized relative to *RPLP0*. Quantification is depicted as fold change to vehicle-treated (Veh) fibroblasts. Data are shown as mean ± SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ after (**a**) unpaired Student's t test, or (**e,f**) one-way ANOVA test followed by Tukey's post-hoc test. **b)** # $p<0.05$, ## $p<0.01$, ### $p<0.001$ after one-way ANOVA test followed by Dunnett's post-hoc test. (**b, d**) $$ $p<0.01$ after non-parametric Kruskal-Wallis' test.

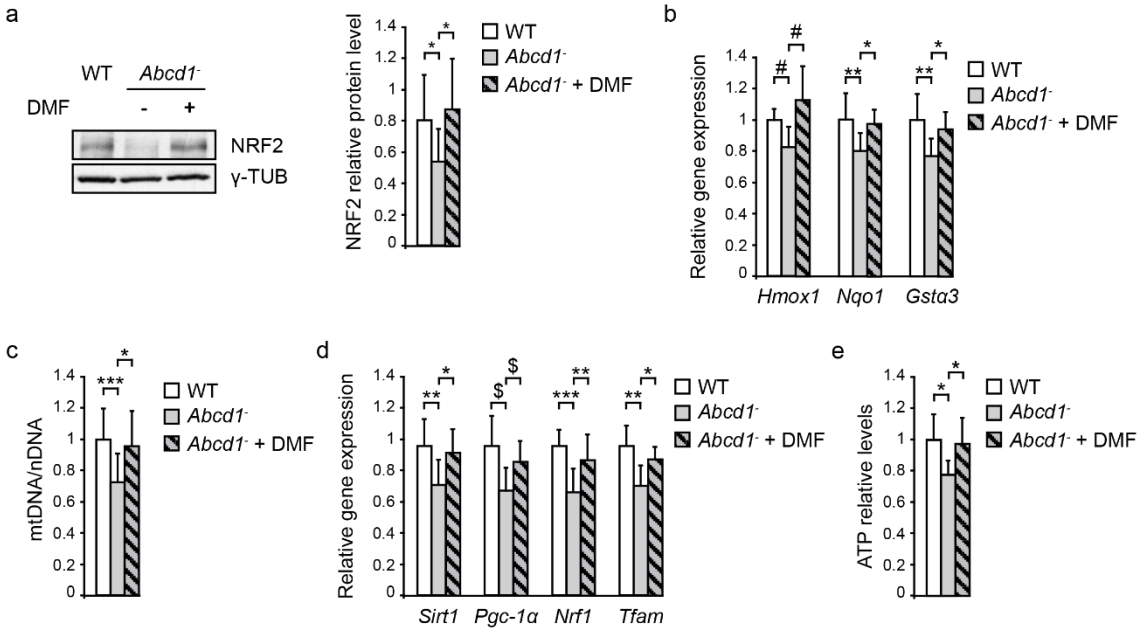


Figure 11 NRF2 activation by DMF prevents mitochondrial depletion and bioenergetic failure in Abcd1¯ mice

a) NRF2 protein levels in WT (n=6), *Abcd1*¯ (n=6) and DMF-treated *Abcd1*¯ mice (*Abcd1*¯ + DMF, n=6) mice spinal cord at 12 months of age. Protein levels were normalized relative to γ-TUB and quantification is depicted as fold change to WT mice. **b)** NRF2-dependent antioxidative genes (*Hmox1, Nqo1* and *Gstα3*) expression in WT (n=8), *Abcd1*¯ (n=8) and *Abcd1*¯ + DMF (n=8) mice spinal cord at 12 months of age. Gene expression levels were normalized relative to *Rplp0*. Quantification is represented as fold change to WT mice. **c)** Mitochondrial DNA (mtDNA) levels in WT (n=8), *Abcd1*¯ (n=8) and *Abcd1*¯ + DMF (n=8) mice spinal cord at 12 months of age. mtDNA content was measured by quantitative RT-PCR and expressed as the ratio of mtDNA (*CytB* levels) to nuclear DNA (nDNA, *Cebpα* levels). Quantification is depicted as fold change to WT mice. **d)** *Sirt1, Pgc-1α, Nrf1* and *Tfam* expression was measured by quantitative RT-PCR in WT (n=8), *Abcd1*¯ (n=8) and *Abcd1*¯ + DMF (n=8) mice spinal cord at 12 months of age. Gene expression levels were normalized relative to *Rplp0*. Quantification is depicted as fold change to WT mice. **e)** ATP levels in WT (n=8), *Abcd1*¯ (n=8) and *Abcd1*¯ + DMF (n=8) mice spinal cord at 12 months of age. Quantification is represented as fold change to WT mice. Data are shown as mean ± SD (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ after one-way ANOVA test followed by Tukey's post-hoc test; # $p<0.05$ after one-way ANOVA test followed by Dunnett's post-hoc test; $ $p<0.05$, $$ $p<0.01$ after non-parametric Kruskal-Wallis' test).

4.1.4 DMF treatment prevents inflammatory imbalance and oxidative damage to proteins and lipids in X-ALD mice

Although AMN patients do not present overt brain inflammation leading to demyelination, we have found a low-grade inflammatory dysregulation in *Abcd1*$^{-}$ mouse spinal cord and in AMN patients. In *Abcd1*$^{-}$ mouse, a functional genomics assay detected NFκB-mediated inflammatory pathway activation in *Abcd1*$^{-}$ mouse spinal cord, and also increased expression of several proinflammatory genes (Schluter et al., 2012). Regarding AMN patients, we have recently described a general dysregulation of inflammatory pathways in PBMC and plasma from AMN patients (Ruiz et al., 2015). As DMF is a classical immunomodulatory drug (Linker et al., 2011; Schilling et al., 2006), we examined its effects on mRNA expression of several inflammation-related genes in *Abcd1*$^{-}$ mice spinal cord.

At 12 months of age, *Abcd1*$^{-}$ mouse exhibited a general imbalance of both M1 and M2 markers in spinal cord. mRNA expression of *Nfκb2*, which belongs to the non-canonical NFκB pathway, as well as mRNA expression of the majority of M1 markers was increased in *Abcd1*$^{-}$ mouse spinal cord, including *Il1β*, *Tnfα*, tumour necrosis factor receptor superfamily member 1a (*Tnfrsf1a*), chemokine (C-C motif) ligand 5 (*Ccl5*), chemokine (C-X-C motif) ligand 9 (*Cxcl9*), *Cxcl10* and chemokine (C-C motif) receptor type 6 (*Ccr6*) (**Fig. 12a**). Also, some M2 markers were upregulated, as, chitinase-like 3 (*Chil3*), *Cxcl12*, insulin-like growth factor 1 (*Igf1*) and transforming growth factor, beta 1 (*Tgfβ1*) (**Fig. 12b**). Only few of them were decreased in *Abcd1*$^{-}$ mouse spinal cord: *Il6*, resistin like alpha (*Retnla*, also called *Fizz1*) and macrophage migration inhibitory factor (*Mif*) (**Fig. 12a-b**).

DMF prevented most of the alterations observed in this inflammatory profile, normalizing mRNA levels of *Nfκb2*, *Il6*, *Tnfα*, *Ccl5*, *Cxcl10*, *Ccr6* (**Fig. 12a**), and *Mif*, *Cxcl12*, *Tgfβ1*, *Igf1* (**Fig. 12b**). However, DMF had no effect on *Tnfrsf1a*, *Cxcl9* and *Fizz1* mRNA (**Fig. 12a-b**). Furthermore, DMF exacerbated the induction of *Il1β* and *Chil3* mRNA in *Abcd1*$^{-}$ mouse spinal cord (**Fig. 12a-b**). Interestingly, *Il10* mRNA, an anti-inflammatory cytokine, was upregulated by DMF (**Fig. 12b**).

Apart of its anti-inflammatory properties, DMF also has antioxidant properties, so we measured the effect of DMF on several quantitative markers of oxidative damage to lipids and proteins, like direct carbonylation of proteins (AASA), glycoxidation and lipoxidation (CEL and CML), and protein lipoxidation (MDAL) (Fourcade et al., 2008). We reported an antioxidant role of DMF, as it normalized AASA, CEL, CML and MDAL levels in *Abcd1*$^{-}$ mice spinal cord (**Fig. 12c**).

Collectively, these data show that DMF was able to normalize inflammatory profile and prevented oxidative damage to lipids and proteins in *Abcd1*$^{-}$ mice.

4.1.5 DMF prevents axonal degeneration in X-ALD mice

To evaluate the effect of DMF on axonal degeneration and locomotor impairment in X-ALD mice, *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ (DKO) mice were fed with chow supplemented with DMF (100 mg/kg). First, we assessed immunohistochemical signs of neuropathology, present in DKO mice, at 18 months of age, and characterized by (i) microgliosis; (ii) astrocytosis; (iii) axonal degeneration, shown by accumulation of APP and synaptophysin in axonal swellings; (iv) lipidic myelin debris, showed by Sudan Black staining (Pujol et al., 2004); (v) oxidative damage to DNA, indicated by increased 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dG) staining (Lopez-Erauskin et al., 2011); (vi) unhealthy motor neurons seen by reduced staining of SMI-32, an antibody that labels a non-phosphorylated epitope of neurofilament proteins and (vii) decreased mitochondrial content observed by cytochrome c (Cyt C) staining in motor neurons (Morato et al., 2013) (**Fig. 13a-y**).

DMF reversed microgliosis and astrocytosis in DKO mice spinal cord (**Fig. 13a-f**), prevented the accumulation of APP and synaptophysin in axons (**Fig. 13g-l, y**), halted the appearance of myelin debris along the spinal cord (**Fig. 13m-o**), and also reduced oxidative damage to DNA shown by a reduced staining by 8-oxo-dG antibody in DKO mice treated with DMF (**Fig. 13p-r**). In addition, mitochondrial levels and motor neurons health were improved with DMF treatment (**Fig. 13s-x**).

Altogether, these data reveal that DMF treatment prevented the presence of the immunohistochemical signs of X-ALD neuropathology in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice.

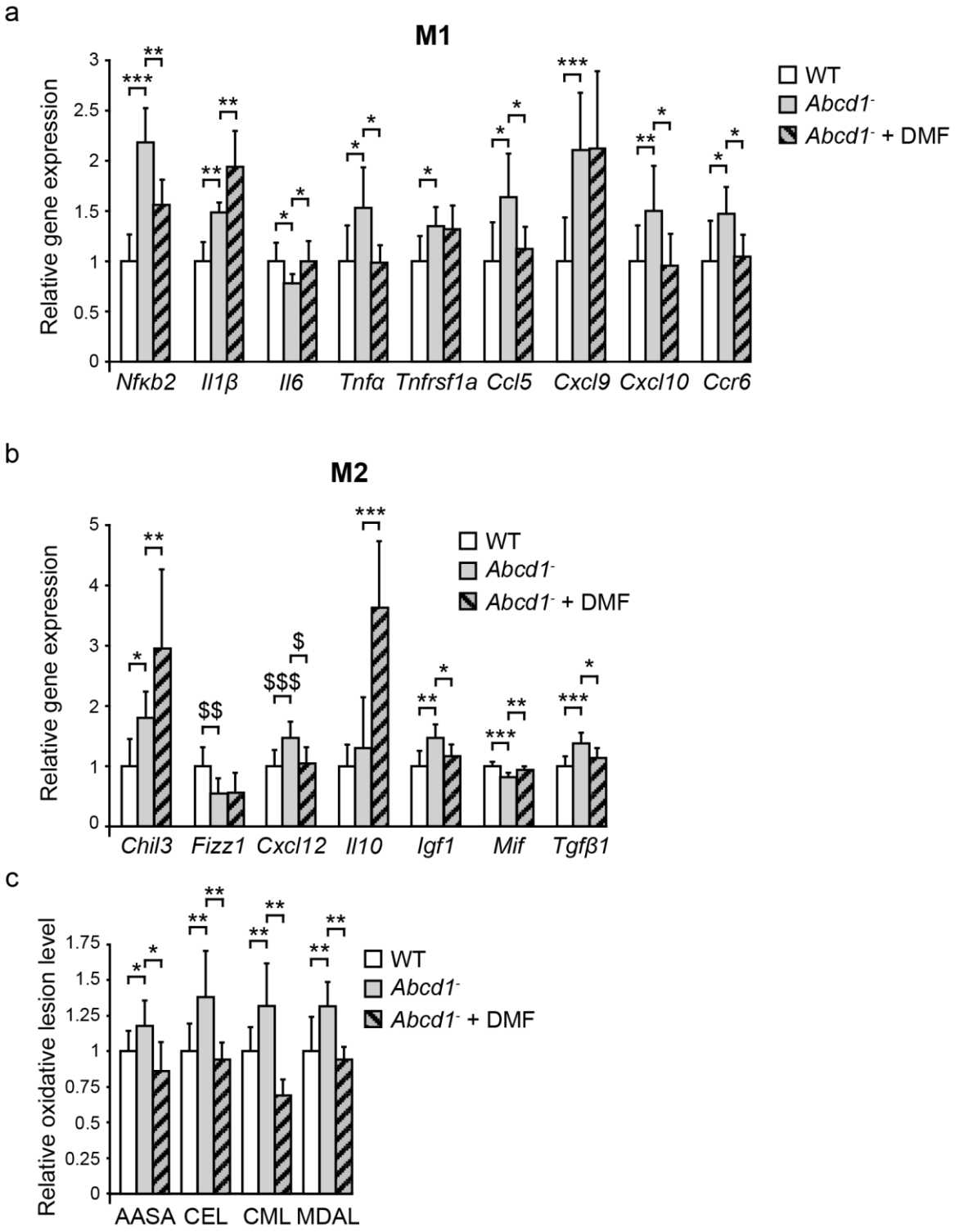


Figure 12 DMF treatment prevents inflammatory imbalance and oxidative damage to proteins and lipids in *Abcd1*$^{-}$ mice

a) Th1 (*Nfκb2, Il1β, Il6, Tnfα, Tnfrsf1a, Ccl5, Cxcl9, Cxcl10, Ccr6*) and **b)** Th2 (*Fizz1, Chil3, Cxcl12, Il10, Igf1, Mif, Tgfβ1*) inflammatory profile in WT (n=8), *Abcd1*$^{-}$ (n=8) and *Abcd1*$^{-}$ + DMF (n=8) mice spinal cord at 12 months of age. Cytokines, chemokines and other inflammation-related genes expression was measured by quantitative RT-PCR. Gene expression levels were normalized relative to *Rplp0*. Quantification is depicted as fold change to WT mice. **c)** Oxidative lesions to lipids and proteins in WT (n=5), *Abcd1*$^{-}$ (n=5) and *Abcd1*$^{-}$ + DMF (n=5) mice spinal cord at 12 months of age. AASA, CEL, CML and MDAL levels were measured by GC/MS. Quantification is represented as fold change to WT mice. Data are shown as mean ± SD (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ after one-way ANOVA test followed by Tukey's post-hoc test; $ $p<0.05$, $$ $p<0.01$, $$$ $p<0.001$ after non-parametric Kruskal-Wallis' test).

4.1.6 DMF reverses locomotor deficits of X-ALD mice

Next, we measured the effect of DMF on the neurological phenotype of DKO mice at 18 months of age, by performing bar cross and treadmill tests at the end of the treatment. As already described (Lopez-Erauskin et al., 2011), DKO mice took longer time to cross the bar, and tended to slip off more times while crossing the bar. After DMF treatment, treated DKO mice behaved similar to WT mice, indicating that DMF ameliorated the ability of DKO mice to cross the bar (**Fig. 13z**). In addition, as earlier described as well (Lopez-Erauskin et al., 2011), DKO behaved worse than WT in the treadmill test. The total number and duration of shocks was higher than in WT. DMF treatment also improved the performance of DKO mice in this test, up to the level of WT mice (**Fig. 13z**).

In summary, these data indicate that DMF treatment halted the progression of locomotor deficits in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice.

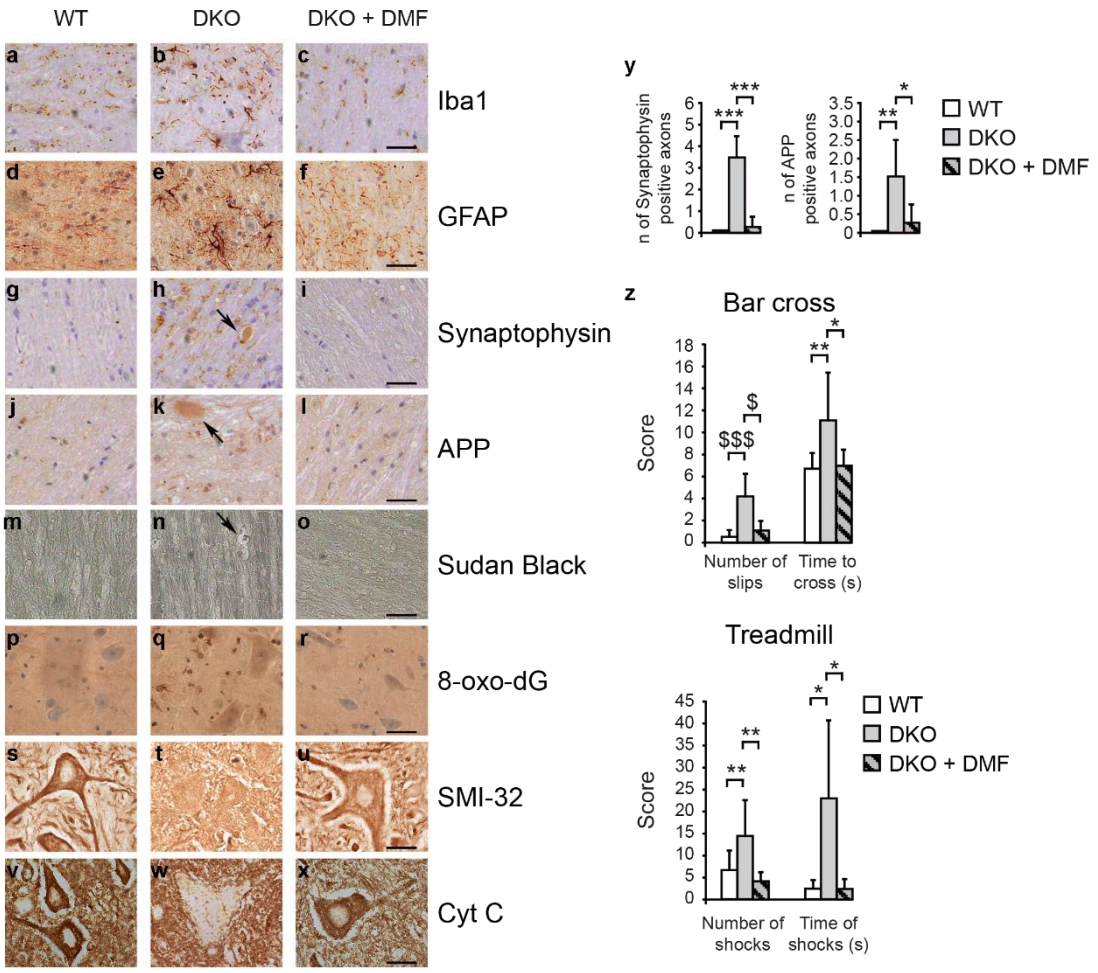


Figure 13 DMF treatment prevents axonal degeneration and locomotor impairment in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice

a-x Immunohistological analysis of axonal pathologies performed in 18-month-old WT, *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ (DKO) and *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice treated with DMF (DKO + DMF) (n= 5 per genotype and condition). Spinal cord immunohistological sections were processed for (**a-c**) Iba1, (**d-f**) GFAP, (**g-i**) Synaptophysin, (**j-l**) APP, (**m-o**) Sudan black, (**p–r**) 8-oxo-dG, (**s-u**) SMI-32 and (**v-x**) Cyt C immunostaining. Representative images for (**a, d, g, j, m, p, s, v**) WT, (**b, e, h, k, n, q, t, w**) DKO, and (**c, f, i, l, o, r, u, x**) DKO + DMF are shown. Scale bars = 25 μm (**a-r**) and 125 μM (**s-x**). **y** Quantification of synaptophysin and APP accumulations in spinal cord immunohistological sections of WT (n=5), DKO (n=5) and DKO + DMF (n=5) mice. **z** Bar-cross and treadmill tests performed on 18-month-old WT (n=14), *Abcd1*$^{-/}$/*Abcd2*$^{-/-}$ (DKO) (n=16) and *Abcd1*$^{-/}$/*Abcd2*$^{-/-}$ mice treated with DMF (DKO + DMF) (n= 14). Data showed refers to number of slips and time (seconds) spent to cross the bar, in the bar cross, as described in Methods. In the treadmill test, data showed refers to number of shocks and time of shocks at the last time point measured (7 minutes and 30 seconds), as described in Methods. Values are expressed as mean ± SD (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ after one-way ANOVA test followed by Tukey's post hoc test; $ $p<0.05$, $$$ $p<0.001$ after non-parametric Kruskal-Wallis' test).

4.2 Chapter II: Study of the role of nuclear receptor coregulator RIP140 in X-ALD pathophysiology: Neuroprotective effect of RIP140 deletion in X-ALD mouse models

4.2.1 RIP140 is induced in X-ALD mouse model spinal cord *via* a redox-dependent mechanism

We previously reported a decrease in mitochondrial content together with an impairment of the signalling pathway governing mitochondrial biogenesis. Indeed, we previously found that *Pgc-1α*, the master regulator of this pathway, was down regulated, while the transcriptional coregulator *Rip140* was induced in 12-month-old *Abcd1*$^{-}$ spinal cord (Schluter et al., 2012). Since RIP140 represses mitochondrial biogenesis among many other metabolic pathways and is considered the antagonist of PGC-1α (Hallberg et al., 2008), we explored the role of RIP140 in X-ALD. First, we studied RIP140 expression in the *Abcd1*$^{-}$ mouse. We found out increased *Rip140* mRNA and RIP140 protein levels in 12-month-old *Abcd1*$^{-}$ mice spinal cord (**Fig. 14a-b**), 6 months prior to the disease onset. The dysregulation of RIP140 protein levels was organ-specific as we did not observe any change in non-affected tissues in this mouse model, such as cortex or liver (**Fig. 14c**).

Next, since oxidative stress plays a decisive role in the pathogenic cascade of X-ALD pathophysiology, we analysed whether oxidative stress regulates RIP140 expression. First, we reported normalization in RIP140 protein levels after administration of a cocktail of antioxidants in 12-month-old *Abcd1*$^{-}$ mice spinal cord (**Fig. 14d**). Then, we explored the molecular mechanism underlying this redox-dependent increase of RIP140 levels. For that purpose, we used an *ex vivo* model, i.e organotypic spinal cord slice cultures (OSCSC) (Fourcade et al., 2008). First, we showed that, just as in the *Abcd1*$^{-}$ mice spinal cord, *Rip140* mRNA expression was increased in *Abcd1*$^{-}$ OSCSC compared with WT OSCSC (**Fig. 14e**), which proved that OSCSC can be used as a good *ex vivo* model to study molecular mechanisms associated to RIP140 dysregulation in mice spinal cord. Using this *ex vivo* model, we revealed a redox-dependent induction of

RIP140 expression, since the addition of 10 μM of hydrogen peroxide (H_2O_2) or excess of C26:0, the fatty acid accumulated in X-ALD, provoked an increase of RIP140 (mRNA expression and protein levels) in OSCSC from WT and *Abcd1*$^-$ mice (**Fig. 14e-f**). We confirmed that the induction of RIP140 was due to a redox-dependent mechanism, as the addition of Trolox together with C26:0 prevented the increase of RIP140 (mRNA expression and protein levels) in OSCSC from WT and *Abcd1*$^-$ mice (**Fig. 14g-h**).

In short, we report an induction of *Rip140* mRNA expression and RIP140 protein levels in *Abcd1*$^-$ mice spinal cord, a phenomenon that was modulated by C26:0 *via* a redox dependent mechanism.

4.2.2 RIP140 is induced in the normal appearing white matter from ccALD patients but not from cAMN patients

To translate these findings obtained with animal models of X-ALD to the human X-ALD pathology, we measured RIP140 mRNA and protein levels in normal appearing white matter (NAWM) from the brain of cAMN patients, ccALD patients and age-matched control subjects. We observed increased mRNA expression and protein levels of RIP140 in the normal appearing white matter (NAWM) of ccALD patients, but not in the NAWM of cAMN patients, when compared to control individuals (**Fig. 14i-j**).

In summary, these data demonstrate that RIP140 was induced in the NAWM from ccALD patients.

4.2.3 RIP140 deletion prevents mitochondrial depletion and bioenergetic failure in X-ALD mice

The abovementioned results demonstrate that RIP140 is induced in X-ALD mouse model and in ccALD patients. We therefore decided to generate a *Rip140* knockout (*Rip140*$^{-/-}$) mouse and crossbred it with our X-ALD mouse models (*Abcd1*$^-$ and *Abcd1*$^-$/*Abcd2*$^{-/-}$ mice), to ascertain the role of RIP140 in the pathophysiology of X-ALD. Initially, we confirmed there was no expression of RIP140 protein in *Rip140*$^{-/-}$ mice

tissues (**Fig. 15a**). Next, given that eGFP is expressed under the *Rip140* promoter in this *Rip140*$^{-/-}$ mouse, we studied the localization of RIP140 protein in mouse central nervous system by IF using an anti-GFP antibody. We identified RIP140 expression in neuronal layers from cortex, cerebellum and hippocampus; together with motor neurons of the spinal cord (**Fig. 15b**).

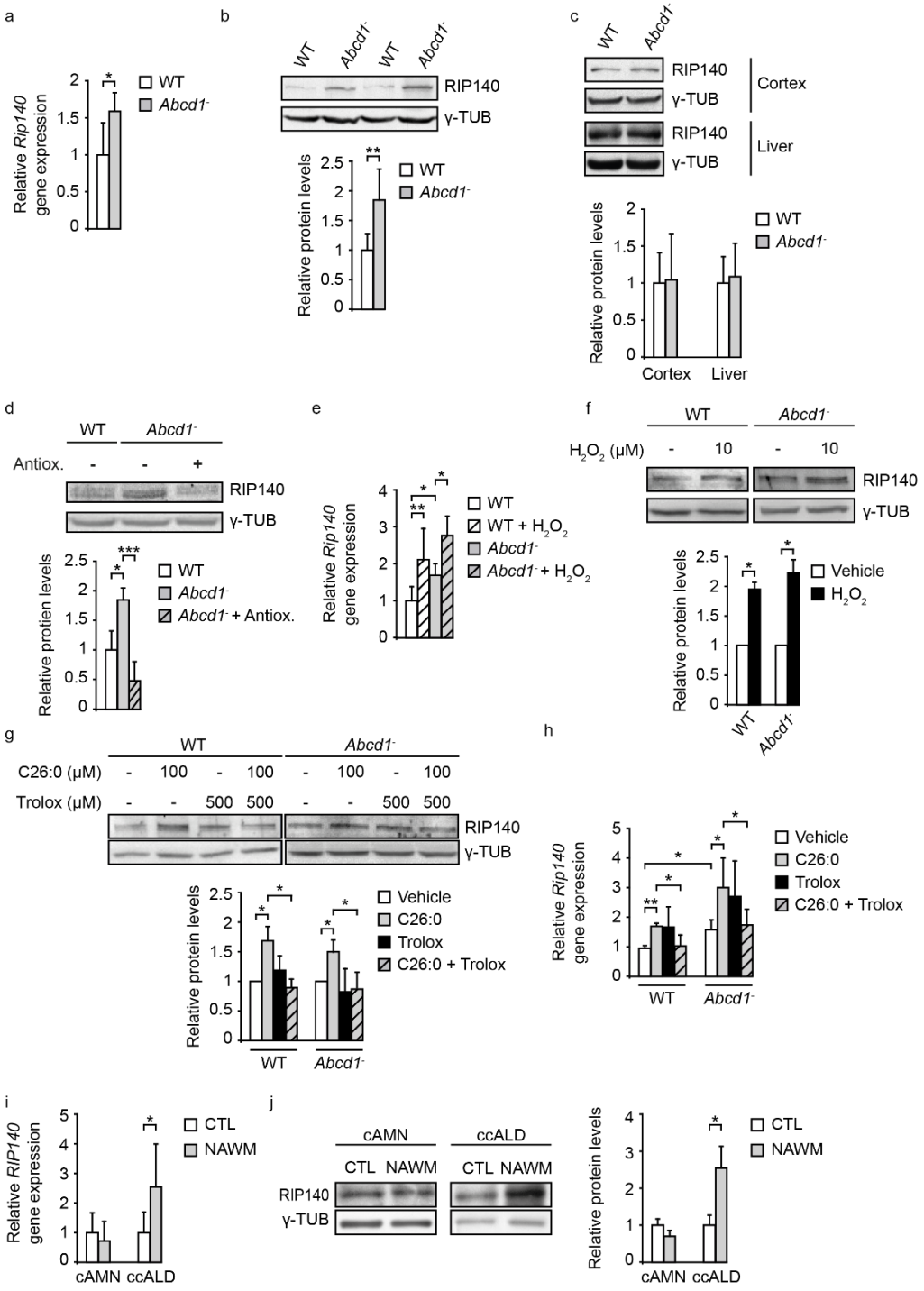


Figure 14 RIP140 is induced in X-ALD mouse model (*Abcd1*$^{-}$ mice) spinal cord *via* a redox-dependent mechanism, as well as in NAWM from the brain of ccALD patients

a) Representative immunoblots showing RIP140 protein levels in WT (n=8) and *Abcd1*$^{-}$ (n=8) mice spinal cord at 12 months of age. Protein levels were normalized relative to γ-TUB and quantification is depicted as fold change to WT mice. **b**) *Rip140* mRNA expression was measured by quantitative RT-PCR in WT (n=8) and *Abcd1*$^{-}$ (n=8) mice spinal cord at 12 months of age. Gene expression levels were normalized relative to *Rplp0*. Quantification is depicted as fold change to WT mice. **c)** Representative immunoblots showing RIP140 protein levels in cortex and liver from WT (n=8) and *Abcd1*$^{-}$ (n=8) mice at 12 months of age. Protein levels were normalized relative to γ-TUB and quantification is depicted as fold change to WT mice. **d**) Representative immunoblots showing RIP140 protein levels in the spinal cord of WT (n=6), *Abcd1*$^{-}$ (n=6) and *Abcd1*$^{-}$ mice treated with an antioxidant cocktail (*Abcd1*$^{-}$ + Antiox.) at 12 months of age. Protein levels were normalized relative to γ-TUB and quantification is depicted as fold change to WT mice. **e**) *Rip140* mRNA expression was measured by quantitative RT-PCR in OSCSC from WT and *Abcd1*$^{-}$ mice treated with 10 µM H_2O_2 or vehicle (n=6 per genotype and treatment). Gene expression was normalized relative to *Rplp0*. Quantification is depicted as fold change to vehicle-treated WT OSCSC. **f**) Representative immunoblots showing RIP140 protein levels in OSCSC from WT and *Abcd1*$^{-}$ mice treated with 10 µM H_2O_2 or vehicle (n=6 per genotype and treatment). Protein levels were normalized relative to γ-TUB and quantification is depicted as fold change to the corresponding vehicle-treated OSCSC. **g**) Representative immunoblots showing RIP140 protein levels in WT and *Abcd1*$^{-}$ OSCSC treated with 100 µM C26:0 and/or 500 µM Trolox, compared with vehicle-treated OSCSC (n=6 per genotype and treatment). Protein levels were normalized relative to γ-TUB and quantification is depicted as fold change to the corresponding vehicle-treated OSCSC. **h**) *Rip140* mRNA expression was measured by quantitative RT-PCR in WT and *Abcd1*$^{-}$ OSCSC treated with 100 µM C26:0 and/or 500 µM Trolox, compared with vehicle-treated OSCSC (n=6 per genotype and condition). Gene expression was normalized relative to *Rplp0*. Quantification is depicted as fold change to vehicle-treated WT OSCSC. **i**) *RIP140* mRNA expression was measured by quantitative RT-PCR in control adults (n=23), control children (n=6), cAMN patients (n=17) and ccALD patients (n=6) cerebral white matter. Gene expression levels were normalized relative to *RPLP0*. Quantification is depicted as fold change to CTL patients (adults for cAMN or children for ccALD). **j**) Representative immunoblots showing RIP140 protein levels in cerebral white matter from controls (CTL) (n=6) and cerebral NAWM from cAMN (n=6) and ccALD (n=6) patients. Protein levels were normalized relative to γ-TUB and quantification is depicted as fold change to corresponding CTL patients (adults for cAMN or children for ccALD). Values are expressed as (**a-h**) mean ± SD, or (**i-j**) mean ± SEM. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ after (**a, b**) unpaired Student's t test, (**d, i-j**) one-way ANOVA test followed by Tukey's post-hoc test or (**e-h**) two-way ANOVA test followed by Tukey's post-hoc test.

Since RIP140 is a master regulator of mitochondrial biogenesis by co-repressing the transcription of the genes involved in this pathway (Powelka et al., 2006), we measured mtDNA levels in different tissues from WT and *Rip140*$^{-/-}$ mice at 3 months of age. We discovered that RIP140 deletion increased mtDNA levels in spinal cord; skeletal muscles as gastrocnemius and soleus; and brown adipose tissue (**Fig. 15c**).

We did not detect any differences neither in other tissues from central nervous system (cortex, cerebellum, pons and hippocampus) nor in other metabolic tissues such as heart, liver or white adipose tissue (data not shown).

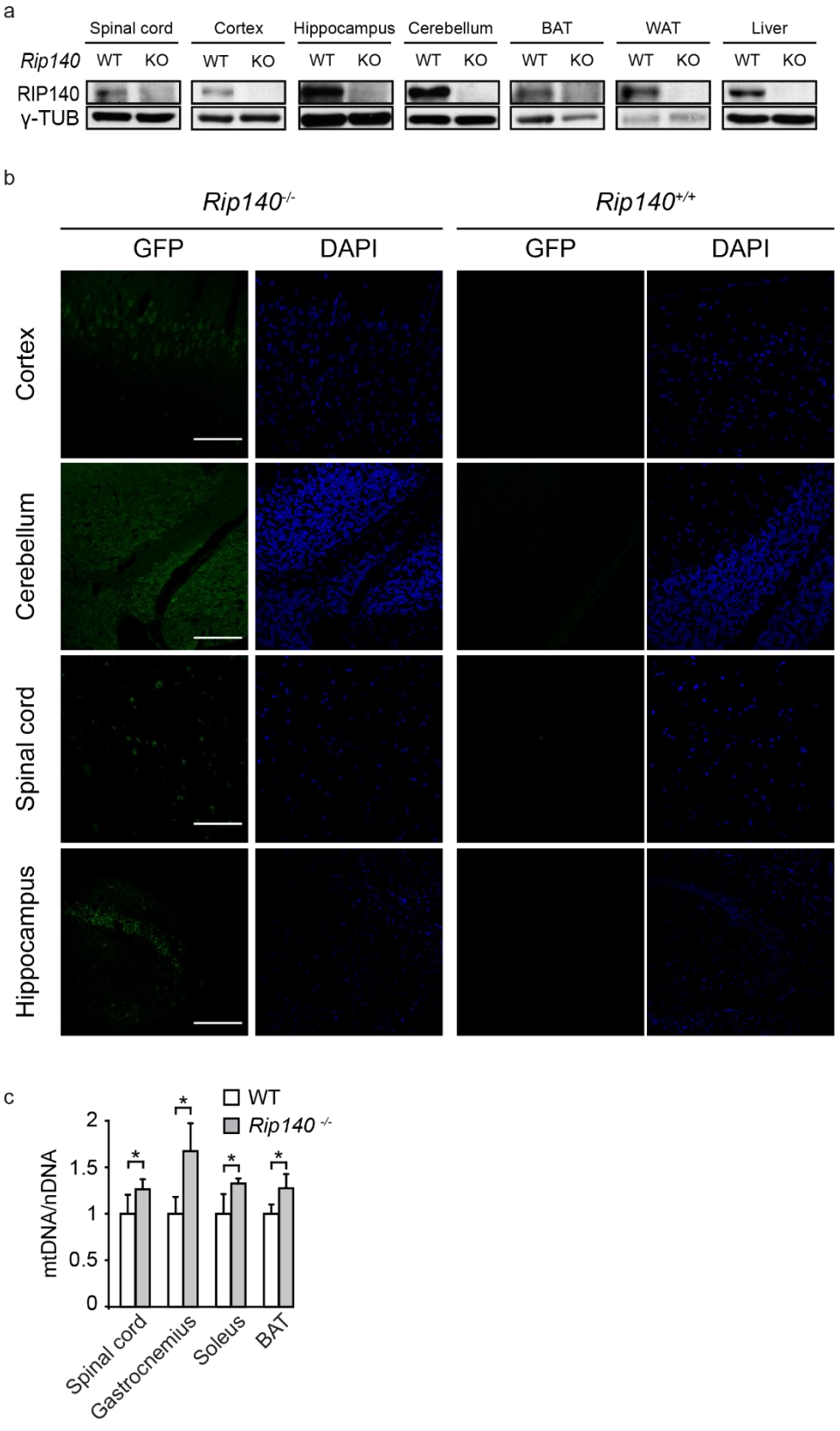


Figure 15 *Rip140*$^{-/-}$ mice characterization

a) RIP140 protein expression in tissues from wild-type and *Rip140*$^{-/-}$ mice. RIP140 immunoblot was performed in spinal cord, cortex, cerebellum, hippocampus, brown adipose tissue (BAT), white adipose tissue (WAT) and liver. **b**) Representative confocal immunofluorescent images from mouse central nervous system structures (cortex, cerebellum, hippocampus and spinal cord) showing eGFP expression (green) and DAPI (blue, nuclei). Immunofluorescence was performed with a GFP antibody on frozen sections from *Rip140*$^{-/-}$ mice and from *Rip140*$^{+/+}$ mice, that served as negative controls for GFP expression. Scale bar = 100 µM. **c**) Mitochondrial DNA (mtDNA) levels in WT (n=5) and *Rip140*$^{-/-}$ (n=4) mice spinal cord, gastrocnemius, soleus and brown adipose tissue at 3 months of age. mtDNA content was measured by quantitative RT-PCR and expressed as the ratio of mtDNA (*CYTB* levels) to nuclear DNA (nDNA, *CEBPα* levels). Quantification is depicted as fold change to WT mice. Values are expressed as mean ± SD * $p<0.05$ after unpaired Student's t test.

Then, we sought to determine the effect of RIP140 deletion on mitochondrial dysfunction in *Abcd1*$^{-}$ mouse spinal cord at 12 months of age. Loss of RIP140 normalized mtDNA levels (**Fig. 16a**); mitochondrial biogenesis genes mRNA expression (*Sirt1*, *Pgc-1α*, *Pparγ*, *Tfam* and *Nrf1*) (**Fig. 16b**); and protein levels of a mitochondrial complex I subunit, NDUFB8 [NADH dehydrogenase (ubiquinone) 1 beta subcomplex 8]; and a complex II subunit SDHB [succinate dehydrogenase complex, subunit B, iron sulfur (Ip)] (**Fig. 16c**). In the 12-month-old *Rip140*$^{-/-}$ mice spinal cord we only detected increased mRNA expression of *Tfam* but no change in the expression of the other genes governing mitochondrial biogenesis (**Fig. 16b**). Moreover, the increased quantity of mtDNA at 3 months of age (**Fig. 15c**) was not maintained at 12 m (**Fig. 16a**).

To determine whether RIP140 can modulate mitochondrial function, measured by oxidative phosphorylation (OXPHOS) activity, we carried out high-resolution respirometry using Oroboros technology. Loss of RIP140 prevented OXPHOS impairment, previously observed in 12-month-old *Abcd1*$^{-}$ mice spinal cord (Morato et al., 2015), normalizing respiratory control ratio (R.C.R), as well as oxygen consumption after the addition of ADP; ADP + succinate; or ADP + succinate + rotenone (State 3) (**Fig. 16d**).

We then analysed the effect of RIP140 deletion on bioenergetics, by measuring ATP, NADH and NAD^+ levels; together with pyruvate kinase activity. Lack of RIP140 prevented decline of ATP (**Fig. 16e**) and NADH levels (**Fig. 16f**); increased NAD^+

levels (**Fig. 16g**) and normalized pyruvate kinase activity (**Fig. 16h**) in 12-month-old *Abcd1*$^{-}$/*Rip140*$^{-/-}$ mice spinal cord.

Altogether, these findings illustrate that loss of RIP140 normalized mitochondrial depletion and malfunction, and prevented alterations of energetic homeostasis, in *Abcd1*$^{-}$ mice spinal cord.

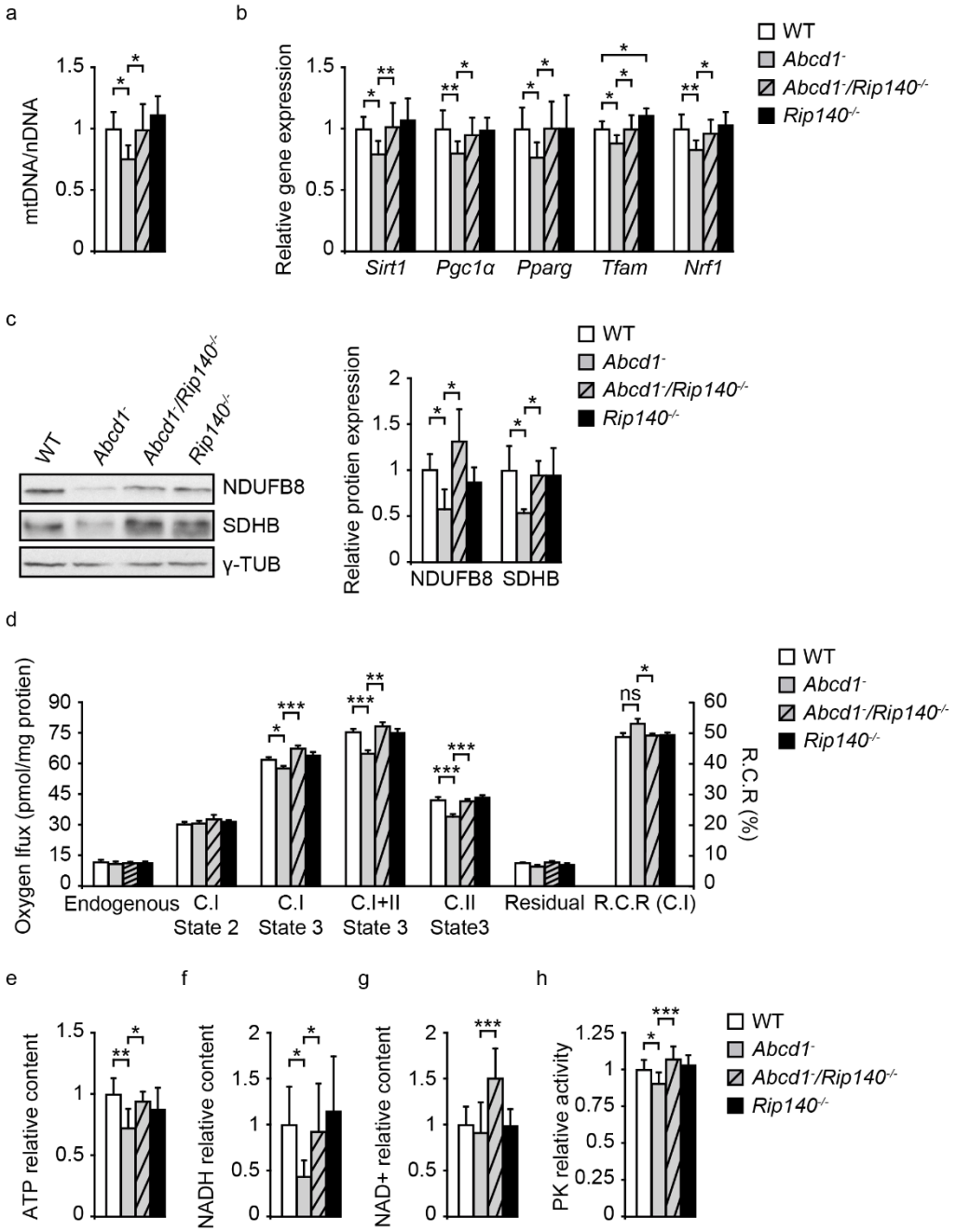


Figure 16 RIP140 deletion restores mitochondrial number and function as well as bioenergetic deficits in X-ALD mouse spinal cord

a) mtDNA levels in WT (n=8), *Abcd1*$^{-}$ (n=8), *Abcd1*$^{-}$/*Rip140*$^{-/-}$ (n=8) and *Rip140*$^{-/-}$ (n=8) mice spinal cord at 12 months of age. mtDNA content was measured by quantitative RT-PCR and expressed as the ratio of mtDNA (*CYTB* levels) to nuclear DNA (nDNA, *CEBPα* levels). Quantification is depicted as fold change to WT mice. **b**) *Sirt1, Pgc-1α, Pparγ, Tfam* and *Nrf1* mRNA expression was measured by quantitative RT-PCR in WT (n=8), *Abcd1*$^{-}$ (n=8) *Abcd1*$^{-}$/*Rip140*$^{-/-}$ (n=8) and *Rip140*$^{-/-}$ (n=8) mice spinal cord at 12 months of age. Gene expression levels were normalized relative to *Rplp0*. Quantification is depicted as fold change to WT mice. **c**) Representative immunoblots showing mitochondrial complex I (NDUFB8) and complex II (SDHB) protein levels in WT (n=6), *Abcd1*$^{-}$ (n=6), *Abcd1*$^{-}$/*Rip140*$^{-/-}$ (n=6) and *Rip140*$^{-/-}$ (n=6) mice spinal cord at 12 months of age. Protein levels were normalized relative to γ-TUB and quantification is depicted as fold change to WT mice. **d**) *Ex vivo* mitochondrial respiration analysis performed on permeabilized sections of 12-month-old spinal cord from WT (n=5), *Abcd1*$^{-}$ (n=5), *Abcd1*$^{-}$/*Rip140*$^{-/-}$ (n=6) and *Rip140*$^{-/-}$ (n=5) mice. (C.I = complex I. C.II = complex II). **e**) ATP levels in 12-month-old spinal cord from WT (n=8), *Abcd1*$^{-}$ (n=6), *Abcd1*$^{-}$/*Rip140*$^{-/-}$ (n=9) and *Rip140*$^{-/-}$ (n=10) mice. Data are shown as fold change respect to WT mice. (**f,g**) NADH and NAD^{+} levels in 12-month-old spinal cord from WT (n=10), *Abcd1*$^{-}$ (n=9), *Abcd1*$^{-}$/*Rip140*$^{-/-}$ (n=9) and *Rip140*$^{-/-}$ (n=9) mice. Data are shown as fold change with respect to WT mice. **h**) PK activity in 12-month-old spinal cord from WT (n=6), *Abcd1*$^{-}$ (n=6), *Abcd1*$^{-}$/*Rip140*$^{-/-}$ (n=7) and *Rip140*$^{-/-}$ (n=7) mice. Data are shown as fold change with respect to WT mice. (**a-h**) Values are expressed as mean ± SD * $p<0.05$; ** $p<0.01$; *** $p<0.001$ after one-way ANOVA test followed by Tukey's post-hoc test

4.2.4 Loss of RIP140 prevents NFκB activation and normalizes inflammatory profile in *Abcd1*$^{-}$ mice spinal cord

RIP140 modulates proinflammatory response *via* NFκB (P. C. Ho et al., 2012; Zschiedrich et al., 2008) as part of its functions as a co-activator. In X-ALD, we have already described a low-grade inflammatory dysregulation in *Abcd1*$^{-}$ mice spinal cord and in AMN patients (Ruiz et al., 2015; Schluter et al., 2012). Hence, we examined the effect of RIP140 deletion on neuroinflammation in *Abcd1*$^{-}$ mice spinal cord. First, we checked protein levels of the components of NFκB pathway, and we found out an activation of canonical (RELA/p65) and non-canonical (NFκB2/p52) NFκB pathway in 12-month-old *Abcd1*$^{-}$ mice spinal cord, as well as their regulators pIκBα and IkBκβ/IKKβ (**Fig. 17a**). RIP140 absence in *Abcd1*$^{-}$ mice impeded NFκB pathway activation, restoring p65, p52, pIκBα and IKKβ levels in *Abcd1*$^{-}$/*Rip140*$^{-/-}$ mice spinal cord at 12 months of age (**Fig. 17a**). Moreover, RIP140 deficiency itself altered NFκB pathway in mice spinal cord, as depicted by decreased levels of phosphorylated-Ser536 p65 in *Rip140*$^{-/-}$ mice spinal cord (Sakurai et al., 1999) (**Fig. 17a**).

Then, we quantified mRNA expression of several cytokines, chemokines and related inflammatory genes in 12-month-old WT, *Abcd1*$^{-}$, *Abcd1*$^{-}$/*Rip140*$^{-/-}$ and *Rip140*$^{-/-}$ mice spinal cord. We revealed that lack of RIP140 normalized the expression of pro-inflammatory M1 genes like *Ccl5*, *Ccr6*, *Cxcl9*, *Cxcl10*, *Nfκb2*, *Tnfα* and *Tnfrsf1a* in the spinal cord of *Abcd1*$^{-}$/*Rip140*$^{-/-}$ mice (**Fig. 17b**). Besides, RIP140 deletion in *Abcd1*$^{-}$ mice restored mRNA expression of some anti-inflammatory M2 genes, such as *Fizz1*, *Igf1* and *Tgfβ1* (**Fig. 17c**). However, lack of RIP140 in *Abcd1*$^{-}$ mice did not normalize levels of *Il6* and arginase 1 (*Arg1*) and indeed, led to a further increase in *Il1β* and *Chil3* (**Fig. 17b-c**). Ablation of RIP140 in WT mice provoked a decrease in the expression of *Il6* and *Nfκb2*, as well as an increase in *Chil3* expression in *Rip140*$^{-/-}$ mice spinal cord (**Fig. 17b-c**).

Collectively, these data evidence that lack of RIP140 precluded NFκB pathway activation and prevented inflammatory profile dysregulation in *Abcd1*$^{-}$ mice spinal cord.

4.2.5 RIP140 deficiency halts axonal degeneration and neurological symptoms in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice

Finally, we assessed the impact of RIP140 absence on axonal degeneration and the behavioural phenotype characteristic of *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice. We analysed these parameters at three different ages: 12, 15 and 18 months of age, which range from the onset of the neurological symptoms up to a more advanced stage of the pathology. We also evaluated these parameters on *Rip140*$^{-/-}$ mice to discard any deleterious consequence of deleting RIP140.

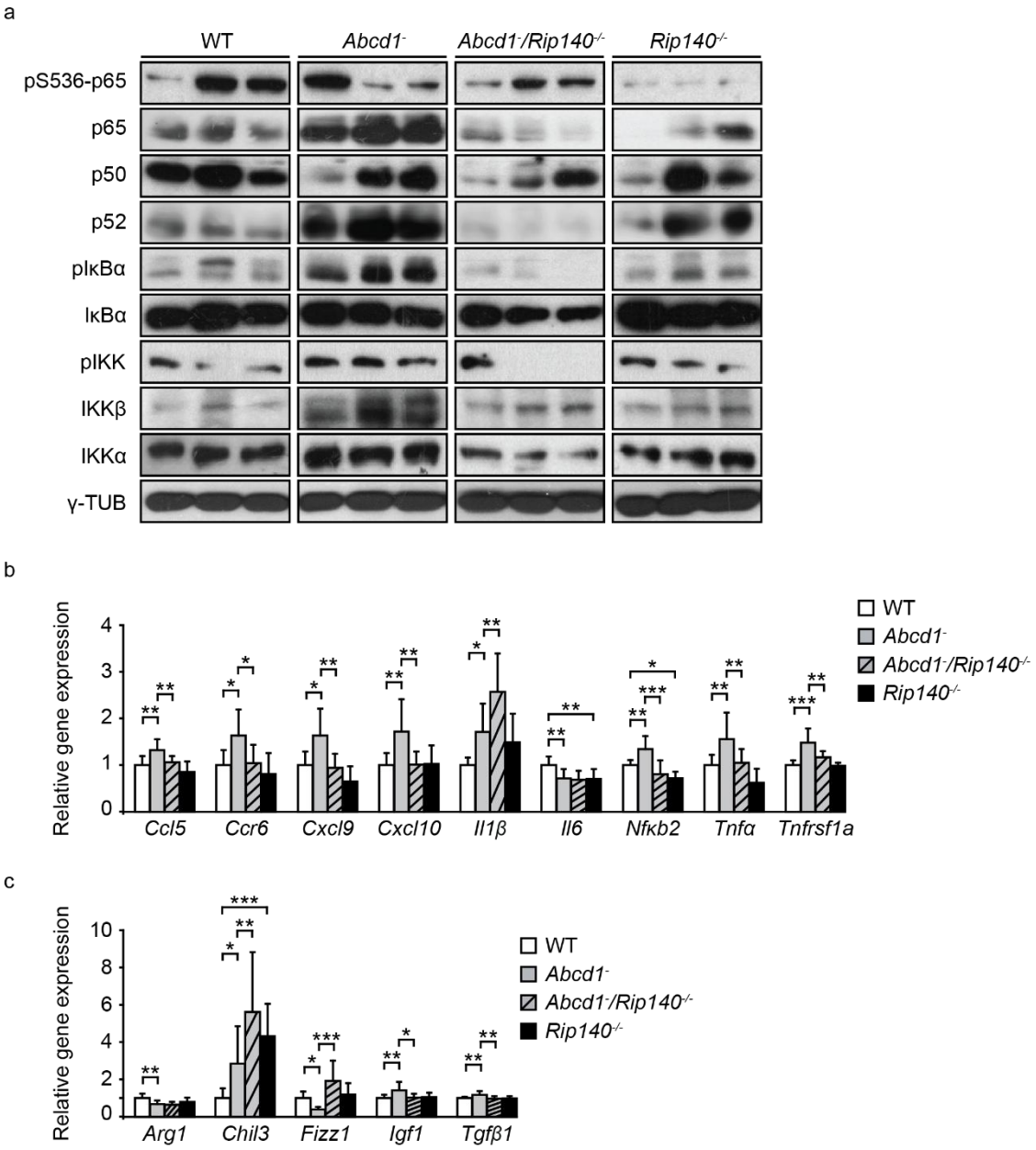


Figure 17 RIP140 deletion prevents NFκB pathway activation and normalizes inflammatory genes expression in X-ALD mouse spinal cord

a) Western blots to monitor levels of pSer536-p65, p65, NFκB1 (p50), NFκB2 (p52), pIκBα, IκBα, pIKKα/β, IKKα and IKKβ were performed in whole spinal cord lysates from 12-month-old WT (n=8), *Abcd1*$^{-}$ (n=8), *Abcd1*$^{-}$/*Rip140*$^{-/-}$ (n=8) and *Rip140*$^{-/-}$ (n=8) mice. Protein levels were normalized relative to γ-TUB. **b)** Th1 (*Ccl5, Ccr6, Cxcl9, Cxcl10, Il1β, Il6, Nfκb2, Tnfα, Tnfrsf1a*) and **c**) Th2 (*Arg1*, *Chil3*, *Fizz1, Igf1, Tgfβ1*) genes expression in WT (n=12), *Abcd1*$^{-}$ (n=12), *Abcd1*$^{-}$/*Rip140*$^{-/-}$ (n=12) and *Rip140*$^{-/-}$ (n=12) mice spinal cord at 12 months of age. mRNA expression was measured by quantitative RT-PCR. Gene expression was normalized relative to *Rplp0*. Quantification is depicted as fold change to WT mice. Data are shown as mean ± SD * $p<0.05$; ** $p<0.01$; *** $p<0.001$ after one-way ANOVA test followed by Tukey's post-hoc test.

We examined the consequences of RIP140 loss on the immunohistochemical signs of X-ALD neuropathology, present in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice at 18 months of age, characterized by (i) microgliosis; (ii) astrocytosis; (iii) axonal degeneration, determined by accumulation of APP and synaptophysin in axons; (iv) lipidic myelin debris, depicted by Sudan Black staining (Pujol et al., 2004); (v) oxidative damage to lipids and proteins, indicated by MDA staining (Lopez-Erauskin et al., 2011); (vi) damage to motor neurons, reflected by a reduced staining of SMI-32, an antibody that labels a non-phosphorylated epitope of neurofilament proteins; and (vii) mitochondrial depletion, featured by decreased cytochrome c (Cyt C) staining in motor neurons (Morato et al., 2013) (**Fig. 18a-g'**).

Lack of RIP140 reversed microgliosis and astrocytosis in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice spinal cord (**Fig. 18a-h**), prevented the accumulation of APP and synaptophysin in axons (**Fig. 18i-p, g'**), blocked the appearance of myelin debris along the spinal cord (**Fig. 18q-t**), as well as attenuated oxidative damage reflected by a reduced staining by MDA antibody (**Fig. 18u-x**). In addition, motor neurons health along with mitochondrial levels were ameliorated in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$/*Rip140*$^{-/-}$ mice, as shown by SMI-32 and Cyt C staining, respectively (**Fig. 18y-f'**). RIP140 ablation did not cause any abnormalities in these stainings in WT mice (**Fig. 18a-g'**)

Complementary, we examined the repercussions of RIP140 deletion on the behavioural phenotype of X-ALD mice. We have previously characterized behavioural abnormalities of X-ALD mice in the bar cross and treadmill tests (Lopez-Erauskin et al., 2011). Here, we also investigated the hind limbs clasping phenotype, which consists in a retraction of the hind limbs towards the midline, when suspending the mouse by its tail for ten seconds. This neurological phenotype has been described before in the X-ALD mouse model and in other animal models of neurodegenerative disorders (Dumser et al., 2007; Lalonde et al., 2011). We uncovered mildly sustained clasping of hind limbs in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice at 12, 15 and 18 months of age (**Fig. 19a**). In addition, we unveiled that lack of RIP140 prevented the appearance of the hind limbs clasping phenotype (**Fig. 19a**) and halted locomotor impairment, measured by bar cross and treadmill tests (**Fig. 19b-c**) in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice at the three ages analysed. Loss of RIP140 did not have any effect on the behavioural parameters analysed (**Fig. 19a-c**).

Overall, RIP140 absence prevented axonal degeneration and halted the onset of neurological symptoms in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice.

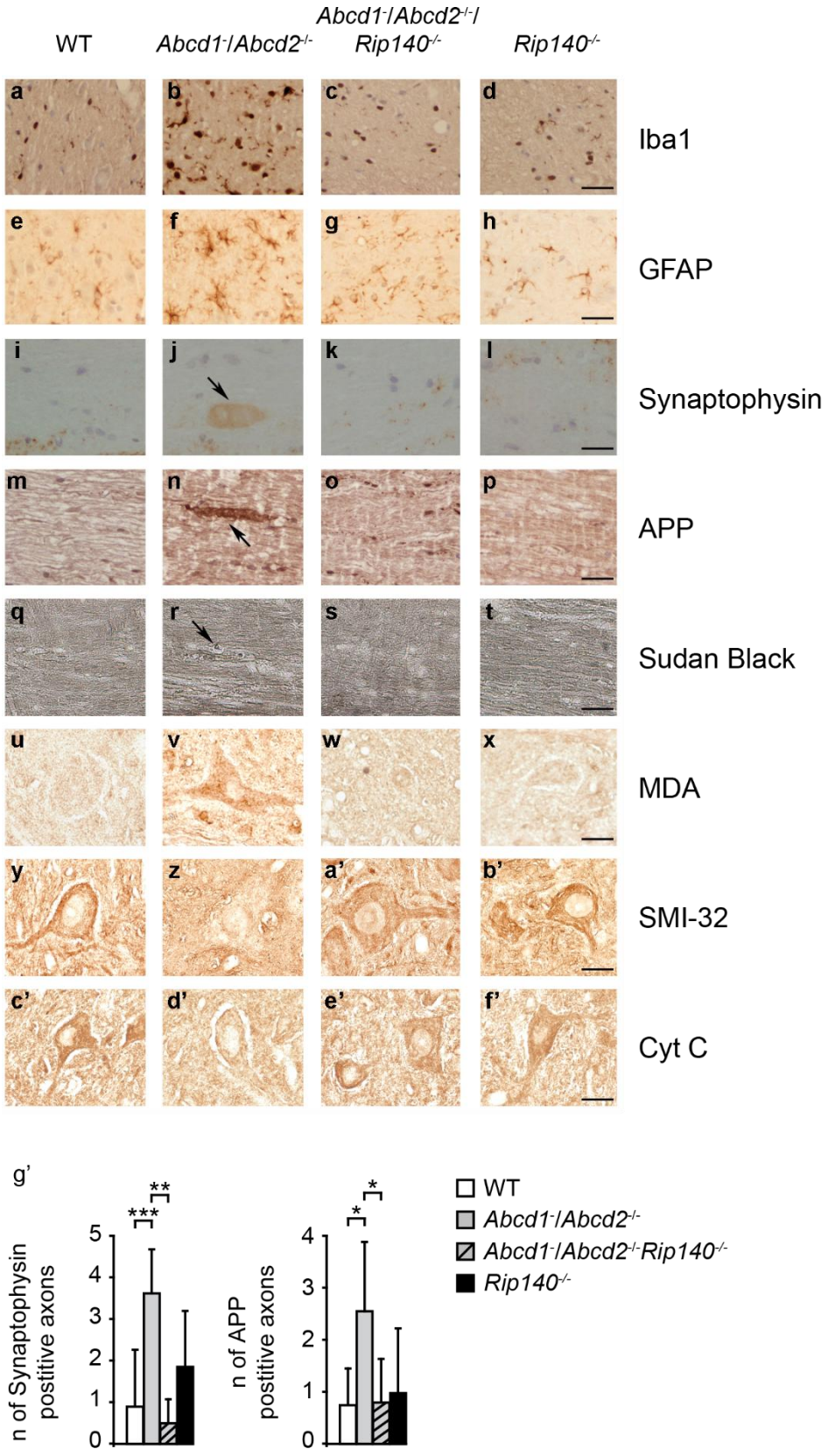


Figure 18 Loss of RIP140 halts axonal degeneration in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice

Immunohistological analysis of axonal pathologies performed in 18-month-old WT, *Abcd1*$^{-}$/*Abcd2*$^{-/-}$, *Abcd1*$^{-}$/*Abcd2*$^{-/-}$/*Rip140*$^{-/-}$ and *Rip140*$^{-/-}$ (n= 5 per genotype) (**A-X**). Spinal cord sections were processed for (**a-d**) Iba1, (**e-h**) GFAP, (**i-l**) Synaptophysin, (**m-p**) APP, (**q-t**) Sudan black, (**u-x**) MDA, (**y-b'**) SMI-32 and (**c'-f'**) Cyt C immunostaining. Representative images for (**a, e, i, m, q, u, y, c'**) WT, (**b, f, j, n, r, v, z, d'**) *Abcd1*$^{-}$/*Abcd2*$^{-/-}$, (**c, g, k, o, s, w, a', e'**) *Abcd1*$^{-}$/*Abcd2*$^{-/-}$/*Rip140*$^{-/-}$ and *Rip140*$^{-/-}$ (**d, h, l, p, t, x, b', f'**) are shown. Scale bars = 25 µm (**a-t**) and 125 µM (**u-f'**). **g'**) Quantification of synaptophysin and APP accumulations in axons. Values are expressed as mean ± SD * $p<0.05$; ** $p<0.01$; *** $p<0.001$ after one-way ANOVA test followed by Tukey's *post hoc* test.

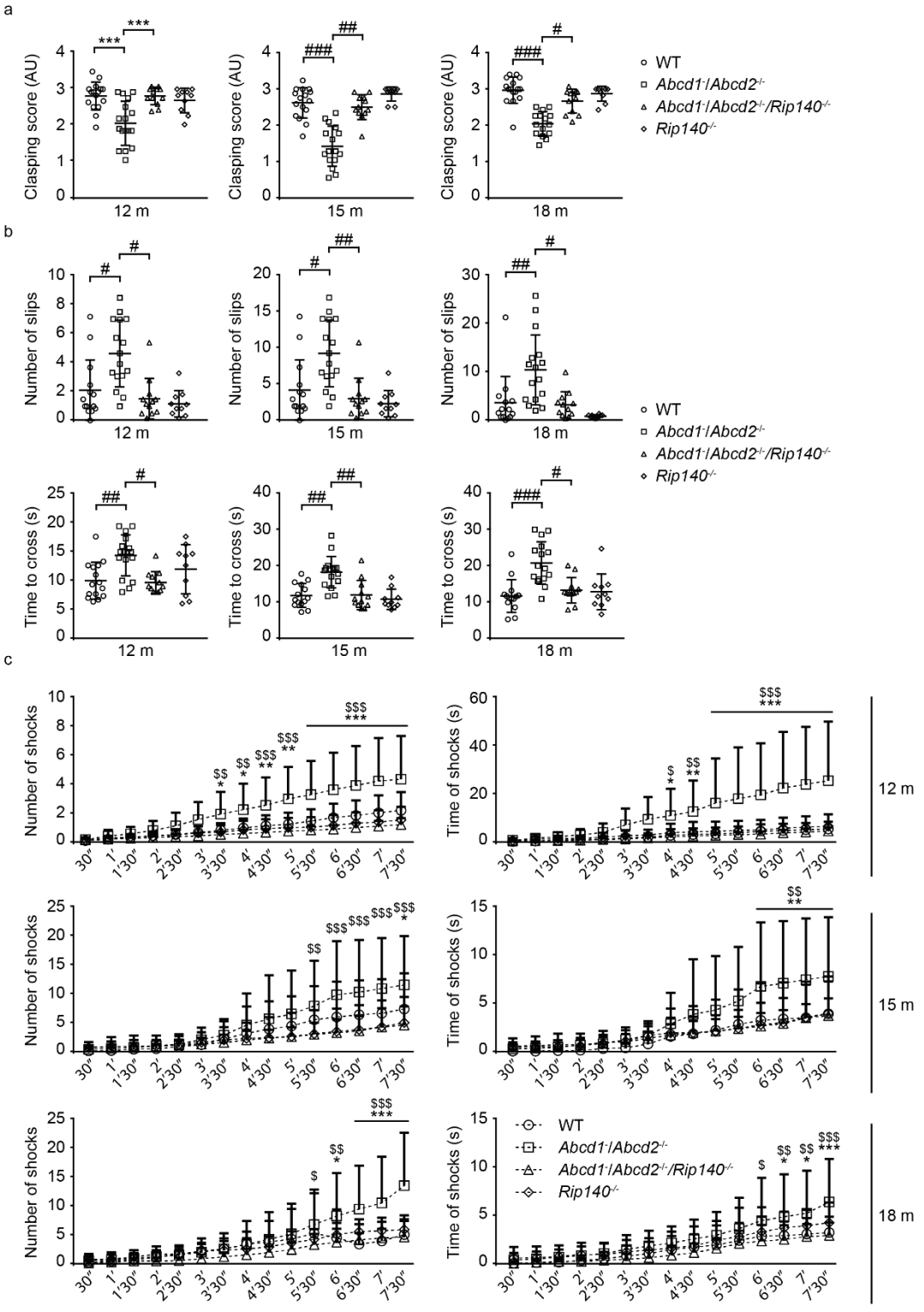


Figure 19 Loss of RIP140 prevents behavioural abnormalities in *Abcd1*$^{-}$*/Abcd2*$^{-/-}$ mice

Clasping, bar-cross and treadmill behavioural tests were performed on 12, 15 and 18-month-old WT (n=15), *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ (n=16), *Abcd1*$^{-}$/*Abcd2*$^{-/-}$/*Rip140*$^{-/-}$ (n=12) and *Rip140*$^{-/-}$ (n=10) mice. Data shown refers to **a**) clasping score, **b**) number of slips and time (seconds) spent to cross the bar, and **c**) number of shocks and time of shocks (in seconds) in the treadmill test. Values are expressed as mean ± SD. (**a, b**) * p<0.05; ** p<0.01; *** p<0.001 after one-way ANOVA test followed by Tukey's *post hoc* test. # p<0.05; ## p<0.001; ### p<0.001 after non-parametric Kruskal-Wallis' test followed by Dunn's *post hoc* test.
(**c**) * p<0.05; ** p<0.01; *** p<0.001 (WT vs *Abcd1*$^{-}$/*Abcd2*$^{-/-}$); $ p<0.05; $$ p<0.001; $$$ p<0.001 (*Abcd1*$^{-}$/*Abcd2*$^{-/-}$ vs *Abcd1*$^{-}$/*Abcd2*$^{-/-}$/*Rip140*$^{-/-}$) after two-way ANOVA test followed by Tukey's *post hoc* test.

DISCUSSION

5 Discussion

5.1 Chapter I

In the present work we identify for the first time an impairment of the AKT/GSK-3β/NRF2 axis in X-ALD. We found an inhibition of the endogenous antioxidant pathway mediated by NRF2 together with an aberrant upstream activation of GSK-3β in the *Abcd1*$^{-}$ mouse spinal cord, as well as a blunted response of AKT/GSK-3β/NRF2 signalling pathway against oxidative stress in X-ALD patients' fibroblasts. This impairment in NRF2-dependent antioxidant response permits us to speculate why C26:0 generates oxidative lesion only in X-ALD fibroblasts but not in control fibroblasts, although it produces ROS in both cells (Fourcade et al., 2008).

The alteration of NRF2 antioxidant pathway here described is characterized by a reduction of *Hmox1*, *Nqo1* and *Gstα3* expression in *Abcd1*$^{-}$ mice spinal cord. These enzymes are involved in cellular defence against oxidative stress and toxic and carcinogenic compounds (Dinkova-Kostova et al., 2010; J. M. Lee et al., 2003). We thus hypothesize that oxidative damage in X-ALD could be a consequence of low HMOX1, NQO1 and GSTα3 activity. Inactivation of NRF2 pathway is not a phenomenon limited to X-ALD, being reported in several neurodegenerative disorders including AD (Kanninen et al., 2008; Ramsey et al., 2007), ALS (Sarlette et al., 2008), Friedrich's ataxia (Paupe et al., 2009; Shan et al., 2013) and experimental autoimmune encephalomyelitis (EAE) mouse models (Morales Pantoja et al., 2016). A recent meta-analysis found NRF2 pathway as a common dysregulated hub in AD and PD patients (Q. Wang et al., 2017). Moreover, NRF2 has been reported to contribute to the pathology of PD and HD (Johnson et al., 2015) Indeed, NRF2-deficient mice are more vulnerable to striatal toxicity resulting from systemic administration of 3-nitropropionic acid (3-NP) (Calkins et al., 2005), MPTP (Burton et al., 2006; P. C. Chen et al., 2009) or 6-OHDA (Jakel et al., 2007). The importance of NRF2 in glial cells is evidenced in *Nrf2* knockout mice, which display astrogliosis and myelinopathy in the cerebellum (Hubbs et al., 2007). NRF2-deficient mice also develop a more severe myelin oligodendrocyte glycoprotein (MOG)-induced EAE with increased oxidative damage in

the CNS finally leading to enhanced demyelination and a more pronounced axonal loss (Johnson et al., 2010).

Several *in vitro* and *in vivo* studies have uncovered the potential of inducing NRF2-mediated transcription to protect from neurodegeneration resulting from mechanisms involving oxidative stress (J. M. Lee et al., 2003; Shih et al., 2005). *Ex vivo* studies and analysis of neurodegenerative models for motor neuron disorders, PD or cerebral ischemia indicated that NRF2-mediated neuroprotection may not only be directly mediated by, but critically involves effects *via* astrocytes (P. C. Chen et al., 2009; Kraft et al., 2004; Shih et al., 2005). Thereby, striatum is protected from MPTP toxicity in transgenic mice which overexpress NRF2 in astrocytes (P. C. Chen et al., 2009). In addition, a direct intrahippocampal gene delivery of NRF2 results in a reduction in diffuse Aβ burden, astrogliosis and oxidative damage which is correlated to spatial learning deficits in aged AD model (APP/PS1 mice) (Kanninen et al., 2009). Other NRF2 activators such as the synthetic triterpenoid, CDDO-methyl amide (2-cyano-*N*-methyl-3,12-dioxooleana-1,9(11)-dien-28 amide) have also neuroprotective effects in ALS (Neymotin et al., 2011), PD (Kaidery et al., 2013) and HD models (Neymotin et al., 2011; L. Yang et al., 2009). Furthermore, genetic variations in NRF2 gene have been associated with increased risk and/or earlier age of onset in AD, PD and ALS (Bergstrom et al., 2014; von Otter et al., 2010a; von Otter et al., 2010b).

Even though inactivation of NRF2 pathway is well renowned in the cascade of neurodegeneration process in these disorders, some studies have shown an induction of NRF2 pathway, either by increased expression of NRF2 targets or by increased NRF2 nuclear expression. Powers *et al.* showed an increased immunoreactivity of HO-1, a classical NRF2 target, in the demyelinating lesion of cAMN and ccALD brains (2005). NRF2 and/or target genes upregulation has been also shown in patients of multiple sclerosis (Licht-Mayer et al., 2015; van Horssen et al., 2010), HD (Browne et al., 1999), AD (Schipper et al., 2006; Tanji et al., 2013; Y. X. Wang et al., 2000) and PD (Ramsey et al., 2007; Schipper et al., 1998; van Muiswinkel et al., 2004). This could be a physiological response against oxidative stress, but limited and then it would not stop the progression of the disease, reinforcing the need for enhancing the expression of NRF2 until protective levels.

Little is known about the link between AKT/GSK-3β and NRF2 in neurodegenerative diseases. Increased GSK-3β has been reported in other neurodegenerative diseases, like tauopathies, in which GSK-3β is one of the kinases responsible for the pathological phosphorylation of Tau (Llorens-Martin et al., 2014); PD (Credle et al., 2015; Duka et al., 2009) or multiple sclerosis (Beurel et al., 2013). Exacerbated GSK-3β activity is also present in other peroxisomal diseases, like rhizomelic chondrodysplasia punctata (RCDP), in which plasmalogens deficiency leads to AKT inactivation and GSK-3β activation (da Silva et al., 2014). Thereby, future works addressed to quantify AKT/GSK-3β/NRF2 axis in other peroxisomal disorders should be very relevant in this field. Indeed, inactivation of the physiological response against oxidative stress mediated by this axis could contribute to the physiopathology of these disorders, thus opening the possibility of novel treatments with DMF or other activators of this pathway to fight these disorders.

In the present work, by inhibiting GSK-3β in X-ALD fibroblasts with two different drugs, we restored NRF2 pathway function, allowing it to activate the transcription of NRF2 target genes upon oxidative stress. Therefore we open a new therapeutic target in X-ALD, which is inhibition of GSK-3β, currently being investigated for other neurodegenerative diseases. In the senescence accelerated mouse-prone 8 (SAMP8) mouse, an AD mouse model, GSK-3β inhibition led to NRF2 activation and decreased oxidative stress, as well as reduced tau phosphorylation and improved learning and memory (Farr et al., 2014). Another study has just uncovered the therapeutic potential of inhibiting the GSK-3β pathway to restore the neurodevelopmental defects in hereditary spastic paraplegia (HSP) patients with SPG11 mutations (Mishra et al., 2016). It is also worth noting that pathways modulating GSK-3β, for instance phosphoinositide 3-kinase (PI3K)/AKT and WNT/β-catenin, also regulate myelination (Fancy et al., 2009; Guo et al., 2016). Unfortunately, GSK-3 inhibitors have had no success in clinical trials for neurodegenerative diseases. Two phase-II clinical trials with a GSK-3β inhibitor (Tideglusib) showed no clinical benefit in AD (Lovestone et al., 2015) and progressive supranuclear palsy (PSP) (Tolosa et al., 2014). However, recent advances with new GSK-3 inhibitors, like L807mts (Licht-Murava et al., 2016), offers new hope for their success in clinical trials. Altogether with our results with GSK-3β inhibitors, we suggest that future research should be directed to study deeper GSK-3β

and associated regulatory pathways in X-ALD, which could end up in new therapeutic options for these patients.

GSK-3β inhibitors have a pleiotropic effect, being upstream of NRF2, and given that the clinical trials with these compounds have not been satisfactories, we chose DMF to activate NRF2 in our X-ALD preclinical models (*Abcd1*$^{-}$ and *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice), in a similar dose as human patients are receiving for multiple sclerosis treatment. DMF has also been successfully tested in preclinical studies for other neurodegenerative diseases as multiple sclerosis (Schilling et al., 2006), HD (Ellrichmann et al., 2011), PD (Ahuja et al., 2016; Lastres-Becker et al., 2016) and TMEV-IDD (K. Kobayashi et al., 2015). Furthermore, DMF treatment has the advantage of giving a single hit that, besides boosting NRF2 antioxidant pathway, also improves proteostasis, mitochondrial function, and neuroinflammation. X-ALD and others neurodegenerative diseases are characterized by oxidative stress, misfolded protein aggregates, and neuroinflammation, the common targets of NRF2 therapeutic strategies (Johnson et al., 2015). DMF also raises GSH levels (S. X. Lin et al., 2011), which are decreased in X-ALD patients (Petrillo et al., 2013) and mouse models (Galino et al., 2011; Morato et al., 2015), by inducing GR activity (Hoffmann et al., 2017)**,** an enzyme that is inhibited in X-ALD (Galino et al., 2011).

Metabolic alterations, mitochondrial dysfunction and neuroinflammation, common features in the majority of neurodegenerative diseases, can be also ameliorated with NRF2 activation. The effects of DMF on mitochondrial function have been described before *in vitro* (Ahuja et al., 2016; Peng et al., 2016; Scannevin et al., 2012). Here, we describe for the first time a positive effect of DMF on mitochondrial levels *in vivo* in the central nervous system, as DMF treatment restored mtDNA and mitochondrial biogenesis regulatory genes (*Sirt1*, *Pgc-1α, Nrf1*, *Tfam*) expression levels, as well as restored ATP levels in the spinal cord of *Abcd1*$^{-}$ mice at 12 months of age.

Neuroinflammation is another common feature of neurodegenerative diseases. In this study, we observed normalization in the expression levels of *Nfκb2* transcription factor and pro-inflammatory cytokines like *Tnfα*, *Ccl5*, *Cxcl10*, and *Ccr6*, as well as an increase in the anti-inflammatory cytokines *Il10* and *Chil3*. This effect of DMF is consistent with previous reports showing *in vivo* anti-inflammatory effects of DMF in

EAE model (Schilling et al., 2006). We also show that DMF prevented microgliosis and astrocytosis in *Abcd1*$^{-}$/*Abcd2*$^{-/-}$ mice, which is consistent with the results of recent studies in PD mouse models (Jing et al., 2015; Lastres-Becker et al., 2016). The immunomodulatory effect of DMF in nervous system can be NRF2-dependent (Linker et al., 2011) or independent (Brennan et al., 2016). G-protein coupled receptor 109A (GPR109A, also known as the hydroxycarboxylic acid receptor 2 (HCA2)) is another DMF target (Parodi et al., 2015). As HCA2 is required for the therapeutic effects of DMF in EAE (H. Chen et al., 2014), future studies on the role of HCA2 in X-ALD and other demyelinating diseases should be performed to deeply understand how DMF ameliorates the disease in X-ALD mouse models.

X-ALD patients and mouse models show peripheral neuropathy, characterized by slower nerve conduction together with myelin abnormalities (Pujol et al., 2002). Recently, a new role of DMF in repairing peripheral nerve regeneration has been reported (Szepanowski et al., 2017), underscoring the multiple beneficial effects that DMF could exert for X-ALD patients. Importantly, a recent proof of NRF2 activation by DMF has been shown in PBMC and glial cells of multiple sclerosis patients treated with DMF from DEFINE and CONFIRM studies (Gopal et al., 2017).

In summary, our data unveil the role of NRF2 in the physiopathogenesis of X-ALD, showing that ABCD1 deficiency leads to an activation of GSK-3β, which impairs the NRF2-mediated antioxidant response. Through the identification of a mechanism by which endogenous antioxidant response is impaired in X-ALD, we then used DMF, a well-known NRF2 activator, in X-ALD mouse models and identify a new therapeutic intervention that overcomes bioenergetic deficits, mitochondrial dysfunction and inflammatory dysregulation, as well as prevents axonal degeneration and locomotor deficits in X-ALD.

5.2 Chapter II

In this study, we sought to determine the role of RIP140, a nuclear receptor coregulator essential for energy homeostasis and NFκB-mediated proinflammatory response, in the pathophysiology of X-ALD. Indeed, we reveal here that loss of RIP140 exerts neuroprotection in X-ALD mouse models, *via* improvement of mitochondrial content and function, together with reversal of bioenergetic deficits and prevention of NFκB-dependent proinflammatory response. By acting on all these pathways, RIP140 inhibition halts axonal degeneration and prevents behavioural abnormalitites in X-ALD mouse models.

Thus, our findings suggest that inhibition of RIP140 is a novel potential therapeutic strategy in disorders that share mitochondrial dysfunction and neuroinflammation as common hallmarks, such as multiple sclerosis, PD or HD (Green et al., 2011; Schapira et al., 2014; Witte et al., 2014). However, the body of evidence present in the literature is controversial about the role of RIP140 in central nervous system (CNS)-related disorders. RIP140 levels are elevated in the hippocampus of DS patients, probably because RIP140 is located in chromosome 21 (Gardiner, 2006). However, inhibition of RIP140 restores mitochondrial function and bioenergetic deficits in DS human foetal fibroblasts (Izzo et al., 2014). On the other hand, several studies have pointed out a protective role of RIP140 in CNS. For instance, nuclear export of RIP140 in hippocampal neurons protects neurons from ER stress-induced cell death (Feng et al., 2014). Given that ER stress is induced in X-ALD (Launay et al., 2017), inhibiting RIP140 would further boost ER stress, thus worsening X-ALD pathology. Our evidence presented here suggests this is not the case, as RIP140 deficiency restored every molecular defect analysed in X-ALD mice. As the implication of RIP140 in neuronal ER stress was determined in an acute state (Feng et al., 2014), we posit that further research should address the role of RIP140 in chronically induced ER stress, which is more relevant to neurodegenerative disorders (Lindholm et al., 2006). In another study, RIP140 expression was reduced in frontal cortex from AD patients compared with healthy controls, as well as associated to modulation of Aβ generation and GSK-3 expression (Blondrath et al., 2016). Interestingly, we describe aberrant GSK-3β activation in X-ALD mice spinal cord in the first study of this thesis, thus the possible

role or RIP140 in GSK-3 regulation in X-ALD deserves further studies. Since several reports have described deleterious impact of RIP140 deletion on CNS (Duclot et al., 2012; Feng et al., 2014), we decided to analyse in *Rip140*$^{-/-}$ mice every altered parameter in X-ALD mouse models. We did not find any defect in mitochondrial function and content; energy metabolism; neuroinflammation; axonal degeneration or behavioural tests; suggesting that RIP140 is not essential for any important function related to these pathways. Indeed, we found increased mtDNA levels in several three-month-old *Rip140*$^{-/-}$ mice tissues, such as brown adipose tissue, skeletal muscles (soleus and gastrocnemius) and spinal cord, although this effect was not sustained at 12 months of age, similar to what have been previously described in skeletal muscles from the other RIP140-deficient mouse (Seth et al., 2007). As formerly described (Herbst et al., 2015), we did not detect increased mtDNA levels in 3-month-old *Rip140*$^{-/-}$ mice cortex (data not shown).

The generation of a RIP140-deficient mouse with knock-in of eGFP, allowed us to study cellular localization of RIP140 in the CNS. We detected RIP140 expression by means of eGFP IF in neurons from cortex, hippocampus and cerebellum which is consistent with previous results using a RIP140-deficient mouse that expresses β-Gal under *Rip140* promoter (Blondrath et al., 2016; Duclot et al., 2012). Furthermore, we uncover here for the first time, expression of RIP140 in motor neurons of the spinal cord. While RIP140 expression in microglia and astrocytes of mouse CNS has been recently reported (Feng et al., 2015; Flaisher-Grinberg et al., 2014), we did not detect eGFP expression in these cells in *Rip140*$^{-/-}$ mice, probably due to the very low expression of RIP140 that we can detect with the available antibodies (data not shown).

One of the main findings of this study is the anti-inflammatory effect of RIP140 deletion in *Abcd1*$^{-}$ mice spinal cord. RIP140 is known to elicit a coactivator function on NFκB-dependent proinflammatory response in macrophages and cardiomyocytes (P. C. Ho et al., 2012; Zschiedrich et al., 2008). Furthermore, in recent studies, RIP140 has been associated with inflammation in type 2 diabetes patients (Xue et al., 2013), as well as with the suppression of M2 macrophages polarization (Y. W. Lin et al., 2016). Here, loss of RIP140 leads to normalization of the inflammatory profile and prevented NFκB pathway induction in X-ALD mice spinal cord. Another essential point that is closely related to the former one is the increment of RIP140 mRNA and protein levels that we

detected in NAWM from ccALD patients compared to controls subjects, but not in NAWM from cAMN patients compared to controls. In the literature, few studies have described plausible biomarkers to discern between the distinct clinical phenotypes present in X-ALD, although most of the findings have not been conclusive (Wiesinger et al., 2015). Recently, 25-hydroxycholesterol and *CYP4F2*, associated to cholesterol metabolism (J. Jang et al., 2016; van Engen et al., 2016) and NFκB-dependent inflammatory components regulated by a microRNA, miR-196a (Shah et al., 2017), have been proposed as differential biomarkers between the different phenotypes of X-ALD. Both pathways are controlled by RIP140 (P. C. Ho et al., 2011). Hence, RIP140 emerges as a candidate responsible for the induction of inflammatory disease in the "second hit" of the proposed "three-hit" hypothesis for the disease pathogenesis of X-ALD (I. Singh et al., 2010). However, the function of RIP140 on neuroinflammation remains elusive, and only two studies have described a correlation between RIP140 inhibition by umbelliferone and salidroside and a decrease in serum proinflammatory cytokines and NFκB pathway proteins in rat's and mouse's brain (T. Chen et al., 2016; Chunhua et al., 2016). These findings presented here underscore the importance of future research on the role of RIP140 in neuroinflammation. For instance, the impact of RIP140 overexpression on inflammatory profile analysing transgenic overexpressing RIP140 mice (Seth et al., 2007) would shed light on this question.

In this study we also unveil another level of regulation of RIP140, as we describe a redox-dependent modulation of RIP140, since H_2O_2 and C26:0 treatments increased RIP140 expression *ex vivo* in OSCSC from WT and *Abcd1*$^{-}$ mice. In addition, trolox administration and antioxidant treatment prevented RIP140 induction in WT OSCSC and *Abcd1*$^{-}$ mice spinal cord, respectively, confirming the role of oxidative stress in the induction of RIP140 mediated by the excess of C26:0. Other saturated fatty acids, such as palmitic acid (C16:0), increase RIP140 expression and the secretion of inflammatory cytokines in cultured control PBMCs (Xue et al., 2013). The expression of PGC-1α, which possesses antagonist functions to RIP140, is decreased upon exposure to saturated fatty acids, for example stearic acid (C18:0) (Crunkhorn et al., 2007). Thus, our findings suggest that RIP140 could be involved in the fatty-acid mediated PGC-1α inhibition, suggesting as well a link between fatty acid accumulation, the inhibition of mitochondrial function and the induction of proinflammatory cytokines, which would place RIP140 in a central position in the pathophysiology of metabolic and

neurodegenerative disorders sharing these features. Furthermore, given the important role of PGC-1α pathway in the response against oxidative stress, in particular in the central nervous system (St-Pierre et al., 2006), these results open new avenues of research on the molecular mechanisms underlying neurodegeneration caused by oxidative stress and PGC-1α/RIP140 dysregulation.

After ablating RIP140 in *Abcd1*$^{-}$ mice, we observed raised levels of NAD^{+} in the spinal cord, thus we could speculate that RIP140 may activate AMPK (Canto et al., 2009) and/or inhibit NAD^{+} consuming enzymes, such as CD38 and poly(ADP-ribose) polymerases (PARPs) (Bai et al., 2015; Barbosa et al., 2007) in X-ALD mice spinal cord. In fact, RIP140 modulates AMPK activity/phosphorylation, at least in skeletal muscle (Fritah et al., 2012). Increased NAD^{+} availability activates SIRT1 (Imai et al., 2000), which could explain restored SIRT1/PGC-1α/PPARγ pathway and subsequent increase of mitochondrial biogenesis observed in X-ALD mice after RIP140 deletion. A recent study reported that suppression of another NAD^{+}-dependent sirtuin, SIRT3, by RIP140 facilitated the development of cardiomyocyte hypertrophy, mitochondrial dysfunction and energy metabolic dysfunction in rat neonatal cardiomyocytes (You et al., 2017). Since SIRT3 has a main role in mitochondrial function (Giralt et al., 2012), it may be worthy to address further research on the relation between RIP140 and sirtuins in the CNS.

In conclusion, we show RIP140 induction in the spinal cord of X-ALD mice and in NAWM from ccALD patients. In addition, we uncover a new redox-dependent mechanism of RIP140 regulation. Finally, RIP140 deficiency exerts neuroprotection by inhibiting neuroinflammation and preventing mitochondrial and bioenergetic dysfunction in X-ALD mice. Since RIP140 deletion also halts axonal degeneration and improves the performance of X-ALD mice in behavioural tests, this study provides enough evidences to develop RIP140-inhibiting strategies to block neurodegeneration in X-ALD and related disorders that share mitochondrial damage and neuroinflammation as common features.

5.3 Global discussion

In this thesis, by modulating two single proteins, NRF2 and RIP140, pharmacologically and genetically, respectively, we provide proofs of a neuroprotective effect of these two approaches in X-ALD mouse models. Neuroprotection was achieved by modulation of multiple pathways related to antioxidant response; mitochondrial biogenesis and function; and neuroinflammation, among others. Thereby, these results lay the basis for new endeavours in developing therapies for X-ALD. Furthermore, given that these pathways are commonly altered in other neurodegenerative and metabolic disorders as it is detailed in the introductory section, these findings, obtained by studying a rare disease, can be expanded to millions of patients suffering these diseases, underscoring the applicability of rare diseases research in the biomedical field.

This thesis also provides enough evidences to consider oxidative stress, mitochondrial dysfunction and neuroinflammation as a central core in the pathophysiology of X-ALD, underlying axonal degeneration. Moreover, this work unveils the therapeutic potential of NRF2 activators and mitochondrial boosters acting by RIP140 inhibition for this devastating disease.

5.3.1 Antioxidant therapies and neurodegeneration: Time for a change of scope

Research in the recent decades has been focused on preventing the deleterious effect of oxidative stress produced by an excess of reactive species. The reasons of this monocentric vision probably derive from the central role of the mitochondrial theory of aging proposed by Harmam in 1956 (Harman, 1956). With this aim, thousands of studies have tested antioxidant supplements able to scavenge reactive species, and thus reducing oxidative stress. Nevertheless, the increasing evidences about the essential physiological roles of reactive species have increased exponentially (Holmstrom et al., 2014; Pan et al., 2011; W. Yang et al., 2010), so nowadays these molecules and their effects are seen in a new light. To illustrate this new paradigm, recent studies on cancer have shown that an excess in the endogenous antioxidant response, instead of preventing cancer as previously thought, accelerates the progression of some

cancerogenous processes. NRF2, the transcription factor studied in this thesis, was usually considered a tumour suppressor due to its cytoprotective functions. Indeed, loss of NRF2 makes mice more prone to carcinogenesis and metastasis (Iida et al., 2004; Rachakonda et al., 2010), whereas NRF2 activation have been described to be beneficial in cancer chemoprevention (Hayes et al., 2010). However, aberrant hyperactivation of NRF2 signaling also protects malignant cells against oxidative stress, chemotherapeutic agents and radiotherapy (DeNicola et al., 2011). The mechanisms involved in NRF2 activation in cancer comprise mutations in NRF2 or KEAP1, and alterations in the regulation of this pathway by epigenetic silencing of KEAP1 promoter or by dysregulation of proteins implicated in the interaction between NRF2 and KEAP1, as explained in detail in (Menegon et al., 2016). Since activation of NRF2 could provoke deleterious effects, e.g carcinogenesis, timing and dosage of NRF2 activators, such as DMF, must be strictly controlled in order to avoid malignant transformation of cells in the patients. In the first study of this thesis, the degree of NRF2 activation observed in X-ALD mice is moderated, similar to the levels present in WT mice. Thereby, and given that the mice receiving DMF in the diet during 4 to 6 months did not present any adverse effects, together with the safety profile of DMF shown in multiple sclerosis clinical trials (Fox et al., 2012; Gold et al., 2012; Phillips et al., 2015), we recommend DMF to be tested in X-ALD patients. Anyway, similar recommendations to multiple sclerosis treatment regarding lymphocyte counts (Linker et al., 2016) should be addressed in these X-ALD patients when receiving DMF.

5.3.2 GSK-3β: A connection to the mitochondrial network in X-ALD

The findings obtained in the first study highlight the role of GSK-3β and associated signaling pathways in the pathophysiology of X-ALD. From our knowledge, this is the first report of an association of this protein with X-ALD. Furthermore, we show here the potential of GSK-3β inhibitors to restore the antioxidant response in X-ALD patients, as we observed a normalization of this response in X-ALD fibroblasts upon GSK-3β inhibitors treatment. This opens lots of possibilities to investigate about, since GSK-3β regulates multiple pathways involved in a myriad of physiological functions (Diehl et al., 1998; S. Shin et al., 2011; H. Wang et al., 2011). One of the most promising, from my point of view, is the relation of GSK-3β with mitochondria, in

particular with the mitochondrial network and the fission process regulated by dynamin-1-like protein (known as DRP1). Mitochondrial network maintenance is crucial for mitochondrial function. DRP1 controls one of the main processes in shaping the mitochondrial network: fission, the other being fusion (Smirnova et al., 1998). Indeed, alterations in DRP1 levels have been associated to neurodegeneration (B. Cho et al., 2013). Although it is not part of this thesis, in our laboratory, we are studying mitochondrial dynamics in X-ALD, and we have found that induction of DRP1 mediates increased mitochondrial fission in X-ALD (data not published). For more details on this topic, I would refer to the doctoral thesis of Patrizia Bianchi entitled "Impairment of mitochondrial dynamics in X-linked adrenoleukodystrophy" from this same group (Bianchi, 2016). Multiple post-translational modifications are implicated in the regulation of DRP1 including phosphorylation, ubiquitination, sumoylation and S-nitrosylation (D. H. Cho et al., 2009). GSK-3β can modulate DRP1 by phosphorylation, either by the activation DRP1 when phosphorylating Ser40, Ser44 (Yan et al., 2015) and Ser616 residues (S. Huang et al., 2015), or by the inhibition of DRP1 thus promoting mitochondrial fusion when Ser693 is phosphorylated (Chou et al., 2012). Moreover, GSK-3β inhibition impedes mitochondrial fragmentation exerting neuroprotective effects in AD and a diabetes mouse model (S. Huang et al., 2015; Yan et al., 2015). Furthermore, DRP1 is implicated in peroxisomal fission (Koch et al., 2003), a process that remains to be studied in X-ALD. In conclusion, the GSK-3β/DRP1 axis offers an exciting new field of research on mitochondrial and peroxisomal fission in neurodegeneration.

5.3.3 RIP140 and neurodegeneration: The first step towards new therapies

From the results of our second study of this essay, we identify RIP140, a transcriptional coregulator with a central role in metabolic homeostasis and inflammatory response, as a new therapeutic target in X-ALD. However, as already discussed before, the functions of RIP140 in the CNS are not well known yet. To address those discrepancies we are generating mice overexpressing RIP140, to study the physiological consequences of a ubiquitous and moderate overexpression of RIP140, in particular in the CNS. By using these mice, we will be able to study the effect of RIP140 induction in mitochondrial

function and neuroinflammation, among other parameters. The results coming from that project will shed light on the potential of targeting this transcription factor. Furthermore, no specific inhibitor of RIP140 has been described up to date. Given the results obtained in this work, the discovery of such inhibitor would have tremendous possibilities for its use in a preclinical set to prevent neurodegeneration, and then translate it into the clinics. Our group will try also to advance in the quest of this inhibitor, by testing a library of FDA-approved compounds on HEK293T cells that overexpress RIP140 tagged to FLAG. This tag will permit us to conduct a low-throughput screening of this library to identify the most active compounds inhibiting RIP140, so then test them in mice.

Among the many functions of RIP140, this protein is also involved in cell-cycle regulation. RIP140, by interacting with RAR and estrogen receptors, and depending on the conditions, either promotes cell proliferation (K. A. White et al., 2005) or cell-cycle arrest (K. A. White et al., 2005). An essential clarification here is that postmitotic neurons cannot divide but they can re-enter in the cell cycle, often leading to apoptosis (Herrup et al., 2007). The role of alterations in the cell cycle in neurodegeneration is supported by studies which show an increase in cell-cycle proteins levels in the brain of patients who were suffering these diseases (Herrup et al., 2007; Lim et al., 2003; Ranganathan et al., 2003). In addition, the main mechanism behind this activation is oxidative stress (J. A. Klein et al., 2003). I propose that, since RIP140 has a role in this cell-cycle reentry and we have found in this study a redox-dependent regulation of RIP140, it would be worth to investigate this function of RIP140 in X-ALD. In particular, I propose to focus on E2F transcription factor 1 (E2F1), the key transcription factor of cell cycle re-entry, and also a known interactor of RIP140. E2F1 overexpression induces RIP140 expression, whereas RIP140 represses E2F1 expression, in a negative regulatory feedback (Docquier et al., 2012; Docquier et al., 2010). Once cell cycle is activated, hyperphosphorylation of retinoblastoma protein (pRB) allows E2F1 to activate the transcription of genes involved in the cell-cycle reentry that finally leads to neuronal apoptosis (Folch et al., 2012). Increased E2F1 levels have been found, for instance, in the brain of DS patients showing AD features (Motonaga et al., 2001), which also present an induction of RIP140 (Gardiner, 2006). Furthermore, loss of E2F1 protects against MPTP-induced neurotoxicity *in vivo*, underscoring the therapeutic potential of targeting RIP140/E2F1 axis. mTOR, which is also induced in X-ALD

(Launay et al., 2015), induces neuronal cell-cycle reentry and subsequent neurodegeneration (Khurana et al., 2006; Norambuena et al., 2017). Hence, studies on the role of E2F1 in X-ALD, using available E2F1-deficient (Blanchet et al., 2011), RIP140-overexpressing and RIP140-deficient mice, could provide insights on the role of RIP140/E2F1 axis in neurodegeneration and offer new therapeutic possibilities for X-ALD patients.

After the explanation of the results and its correspondent discussion and possible future works derived from them, the final conclusions of this thesis are stated in the next section.

CONCLUSIONS

6 Conclusions

1. ABCD1 (ALD protein) loss of function impairs NRF2-dependent antioxidant response in mouse spinal cord and primary human fibroblasts.

2. AKT/GSK-3β axis, which modulates NRF2 pathway, is altered in X-ALD in *Abcd1*$^{-}$ mouse spinal cord and X-ALD fibroblasts.

3. GSK-3β inhibitors reactivate NRF2-dependent response in X-ALD fibroblasts upon C26:0-induced oxidative stress, unveiling a plausible molecular mechanism of antioxidant defence impairment in X-ALD

4. DMF, a NRF2 activator, reverses molecular defects as mitochondrial dysfunction, bioenergetics deficits, oxidative damage and inflammatory dysregulation in *Abcd1*$^{-}$ mouse; and halts axonal degeneration and locomotor disability in *Abcd1*$^{-}$*/Abcd2*$^{-/-}$ mouse. Therefore, DMF is a good candidate drug for X-ALD patients

5. RIP140, a master regulator of mitochondrial biogenesis and inflammation is increased in both the spinal cord of *Abcd1*$^{-}$ mouse and the brain of ccALD patients

6. RIP140 is localized in neurons of cortex, cerebellum, hippocampus and spinal cord in mouse central nervous system

7. C26:0 modulates RIP140 expression via a redox-dependent mechanism, increasing its expression in mouse organotypic spinal cord slice cultures (OSCSC).

8. We uncover for the first time a neuroprotective effect of RIP140 deletion in a mouse model of X-ALD. Loss of RIP140 improves mitochondrial function, normalized bioenergetics deficit and inflammatory dysregulation; and prevents axonal degeneration and locomotor deficits in X-ALD mouse models.

BIBLIOGRAPHY

7 Bibliography

Abmayr, S. M., Yao, T., Parmely, T., & Workman, J. L. (2006). Preparation of nuclear and cytoplasmic extracts from mammalian cells. *Curr Protoc Mol Biol, Chapter 12*, Unit 12 11. doi:10.1002/0471142727.mb1201s75

Adibhatla, R. M., & Hatcher, J. F. (2010). Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal, 12*(1), 125-169. doi:10.1089/ARS.2009.2668

Ahuja, M., Ammal Kaidery, N., Yang, L., Calingasan, N., Smirnova, N., Gaisin, A., . . . Thomas, B. (2016). Distinct Nrf2 Signaling Mechanisms of Fumaric Acid Esters and Their Role in Neuroprotection against 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Induced Experimental Parkinson's-Like Disease. *J Neurosci, 36*(23), 6332-6351. doi:10.1523/JNEUROSCI.0426-16.2016

Alam, J., Stewart, D., Touchard, C., Boinapally, S., Choi, A. M., & Cook, J. L. (1999). Nrf2, a Cap'n'Collar transcription factor, regulates induction of the heme oxygenase-1 gene. *J Biol Chem, 274*(37), 26071-26078.

Albet, S., Causeret, C., Bentejac, M., Mandel, J. L., Aubourg, P., & Maurice, B. (1997). Fenofibrate differently alters expression of genes encoding ATP-binding transporter proteins of the peroxisomal membrane. *FEBS Lett, 405*(3), 394-397.

Amar, S., Belmaker, R. H., & Agam, G. (2011). The possible involvement of glycogen synthase kinase-3 (GSK-3) in diabetes, cancer and central nervous system diseases. *Curr Pharm Des, 17*(22), 2264-2277.

Ames, A., 3rd. (2000). CNS energy metabolism as related to function. *Brain Res Brain Res Rev, 34*(1-2), 42-68.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci U S A, 90*(17), 7915-7922.

An, J. H., & Blackwell, T. K. (2003). SKN-1 links C. elegans mesendodermal specification to a conserved oxidative stress response. *Genes Dev, 17*(15), 1882-1893. doi:10.1101/gad.1107803

An, J. H., Vranas, K., Lucke, M., Inoue, H., Hisamoto, N., Matsumoto, K., & Blackwell, T. K. (2005). Regulation of the Caenorhabditis elegans oxidative stress defense protein SKN-1 by glycogen synthase kinase-3. *Proc Natl Acad Sci U S A, 102*(45), 16275-16280. doi:10.1073/pnas.0508105102

Andersson, U., & Scarpulla, R. C. (2001). Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. *Mol Cell Biol, 21*(11), 3738-3749. doi:10.1128/MCB.21.11.3738-3749.2001

Andrews, N. C., Erdjument-Bromage, H., Davidson, M. B., Tempst, P., & Orkin, S. H. (1993). Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein. *Nature, 362*(6422), 722-728. doi:10.1038/362722a0

Angermuller, S., Bruder, G., Volkl, A., Wesch, H., & Fahimi, H. D. (1987). Localization of xanthine oxidase in crystalline cores of peroxisomes. A cytochemical and biochemical study. *Eur J Cell Biol, 45*(1), 137-144.

Araki, T., Sasaki, Y., & Milbrandt, J. (2004). Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. *Science, 305*(5686), 1010-1013. doi:10.1126/science.1098014

Arner, E. S. (2009). Focus on mammalian thioredoxin reductases--important selenoproteins with versatile functions. *Biochim Biophys Acta, 1790*(6), 495-526. doi:10.1016/j.bbagen.2009.01.014

Arnold, D. L., Gold, R., Kappos, L., Bar-Or, A., Giovannoni, G., Selmaj, K., . . . Dawson, K. T. (2014). Magnetization transfer ratio in the delayed-release dimethyl fumarate DEFINE study. *J Neurol, 261*(12), 2429-2437. doi:10.1007/s00415-014-7504-7

Asher, G., Lotem, J., Cohen, B., Sachs, L., & Shaul, Y. (2001). Regulation of p53 stability and p53-dependent apoptosis by NADH quinone oxidoreductase 1. *Proc Natl Acad Sci U S A, 98*(3), 1188-1193. doi:10.1073/pnas.021558898

Asheuer, M., Bieche, I., Laurendeau, I., Moser, A., Hainque, B., Vidaud, M., & Aubourg, P. (2005). Decreased expression of ABCD4 and BG1 genes early in the pathogenesis of X-linked adrenoleukodystrophy. *Hum Mol Genet, 14*(10), 1293-1303. doi:10.1093/hmg/ddi140

Asheuer, M., Pflumio, F., Benhamida, S., Dubart-Kupperschmitt, A., Fouquet, F., Imai, Y., . . . Cartier, N. (2004). Human CD34+ cells differentiate into microglia and express recombinant therapeutic protein. *Proc Natl Acad Sci U S A, 101*(10), 3557-3562. doi:10.1073/pnas.0306431101

Attwell, D., & Laughlin, S. B. (2001). An energy budget for signaling in the grey matter of the brain. *J Cereb Blood Flow Metab, 21*(10), 1133-1145. doi:10.1097/00004647-200110000-00001

Aubourg, P., Adamsbaum, C., Lavallard-Rousseau, M. C., Rocchiccioli, F., Cartier, N., Jambaque, I., . . . et al. (1993). A two-year trial of oleic and erucic acids ("Lorenzo's oil") as treatment for adrenomyeloneuropathy. *N Engl J Med, 329*(11), 745-752.

Aubourg, P., Blanche, S., Jambaque, I., Rocchiccioli, F., Kalifa, G., Naud-Saudreau, C., . . . et al. (1990). Reversal of early neurologic and neuroradiologic manifestations of X-linked adrenoleukodystrophy by bone marrow transplantation. *N Engl J Med, 322*(26), 1860-1866. doi:10.1056/NEJM199006283222607

Augereau, P., Badia, E., Fuentes, M., Rabenoelina, F., Corniou, M., Derocq, D., . . . Cavailles, V. (2006). Transcriptional regulation of the human NRIP1/RIP140 gene by estrogen is modulated by dioxin signalling. *Mol Pharmacol, 69*(4), 1338-1346. doi:10.1124/mol.105.017376

Baarine, M., Andreoletti, P., Athias, A., Nury, T., Zarrouk, A., Ragot, K., . . . Lizard, G. (2012). Evidence of oxidative stress in very long chain fatty acid--treated oligodendrocytes and potentialization of ROS production using RNA interference-directed knockdown of ABCD1 and ACOX1 peroxisomal proteins. *Neuroscience, 213*, 1-18. doi:10.1016/j.neuroscience.2012.03.058

Baarine, M., Beeson, C., Singh, A., & Singh, I. (2015). ABCD1 deletion-induced mitochondrial dysfunction is corrected by SAHA: implication for adrenoleukodystrophy. *J Neurochem, 133*(3), 380-396. doi:10.1111/jnc.12992

Bai, P., Nagy, L., Fodor, T., Liaudet, L., & Pacher, P. (2015). Poly(ADP-ribose) polymerases as modulators of mitochondrial activity. *Trends Endocrinol Metab, 26*(2), 75-83. doi:10.1016/j.tem.2014.11.003

Barbier, M., Sabbagh, A., Kasper, E., Asheuer, M., Ahouansou, O., Pribill, I., . . . Aubourg, P. (2012). CD1 gene polymorphisms and phenotypic variability in X-linked adrenoleukodystrophy. *PLoS One, 7*(1), e29872. doi:10.1371/journal.pone.0029872

Barbosa, M. T., Soares, S. M., Novak, C. M., Sinclair, D., Levine, J. A., Aksoy, P., & Chini, E. N. (2007). The enzyme CD38 (a NAD glycohydrolase, EC 3.2.2.5) is necessary for the development of diet-induced obesity. *Faseb J, 21*(13), 3629-3639. doi:10.1096/fj.07-8290com

Baumgart, E., Vanhorebeek, I., Grabenbauer, M., Borgers, M., Declercq, P. E., Fahimi, H. D., & Baes, M. (2001). Mitochondrial alterations caused by defective peroxisomal biogenesis in a mouse model for Zellweger syndrome (PEX5 knockout mouse). *Am J Pathol, 159*(4), 1477-1494. doi:10.1016/S0002-9440(10)62534-5

Bell, K. F., Al-Mubarak, B., Martel, M. A., McKay, S., Wheelan, N., Hasel, P., . . . Hardingham, G. E. (2015). Neuronal development is promoted by weakened intrinsic antioxidant defences due to epigenetic repression of Nrf2. *Nat Commun, 6*, 7066. doi:10.1038/ncomms8066

Bence, N. F., Sampat, R. M., & Kopito, R. R. (2001). Impairment of the ubiquitin-proteasome system by protein aggregation. *Science, 292*(5521), 1552-1555. doi:10.1126/science.292.5521.1552

Berger, J., Bernheimer, H., Fae, I., Braun, A., Roscher, A., Molzer, B., & Fischer, G. (1995). Association of X-linked adrenoleukodystrophy with HLA DRB1 alleles. *Biochem Biophys Res Commun, 216*(2), 447-451. doi:10.1006/bbrc.1995.2643

Berger, J., & Gartner, J. (2006). X-linked adrenoleukodystrophy: clinical, biochemical and pathogenetic aspects. *Biochim Biophys Acta, 1763*(12), 1721-1732. doi:10.1016/j.bbamcr.2006.07.010

Berger, J., Molzer, B., Fae, I., & Bernheimer, H. (1994). X-linked adrenoleukodystrophy (ALD): a novel mutation of the ALD gene in 6 members of a family presenting with 5 different phenotypes. *Biochem Biophys Res Commun, 205*(3), 1638-1643. doi:10.1006/bbrc.1994.2855

Bergstrom, P., von Otter, M., Nilsson, S., Nilsson, A. C., Nilsson, M., Andersen, P. M., . . . Zetterberg, H. (2014). Association of NFE2L2 and KEAP1 haplotypes with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener, 15*(1-2), 130-137. doi:10.3109/21678421.2013.839708

Berriel Diaz, M., Krones-Herzig, A., Metzger, D., Ziegler, A., Vegiopoulos, A., Klingenspor, M., . . . Herzig, S. (2008). Nuclear receptor cofactor receptor interacting protein 140 controls hepatic triglyceride metabolism during wasting in mice. *Hepatology, 48*(3), 782-791. doi:10.1002/hep.22383

Beurel, E., Kaidanovich-Beilin, O., Yeh, W. I., Song, L., Palomo, V., Michalek, S. M., . . . Jope, R. S. (2013). Regulation of Th1 cells and experimental autoimmune encephalomyelitis by glycogen synthase kinase-3. *J Immunol, 190*(10), 5000-5011. doi:10.4049/jimmunol.1203057

Bezman, L., Moser, A. B., Raymond, G. V., Rinaldo, P., Watkins, P. A., Smith, K. D., . . . Moser, H. W. (2001). Adrenoleukodystrophy: incidence, new mutation rate, and results of extended family screening. *Ann Neurol, 49*(4), 512-517. doi:10.1002/ana.101

Bianchi, P. (2016). *Impairment of mitochondrial dynamics in X-linked adrenoleukodystrophy*. Universitat de Barcelona.

Bizzozero, O. A., Zuniga, G., & Lees, M. B. (1991). Fatty acid composition of human myelin proteolipid protein in peroxisomal disorders. *J Neurochem, 56*(3), 872-878.

Blanchet, E., Annicotte, J. S., Lagarrigue, S., Aguilar, V., Clape, C., Chavey, C., . . . Fajas, L. (2011). E2F transcription factor-1 regulates oxidative metabolism. *Nat Cell Biol, 13*(9), 1146-1152. doi:10.1038/ncb2309

Blondrath, K., Steel, J. H., Katsouri, L., Ries, M., Parker, M. G., Christian, M., & Sastre, M. (2016). The nuclear cofactor receptor interacting protein-140 (RIP140) regulates the expression of genes involved in Abeta generation. *Neurobiol Aging, 47*, 180-191. doi:10.1016/j.neurobiolaging.2016.08.003

Board, P. G., & Menon, D. (2013). Glutathione transferases, regulators of cellular metabolism and physiology. *Biochim Biophys Acta, 1830*(5), 3267-3288. doi:10.1016/j.bbagen.2012.11.019

Boillee, S., Vande Velde, C., & Cleveland, D. W. (2006a). ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron, 52*(1), 39-59. doi:10.1016/j.neuron.2006.09.018

Boillee, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., . . . Cleveland, D. W. (2006b). Onset and progression in inherited ALS determined by motor neurons and microglia. *Science, 312*(5778), 1389-1392. doi:10.1126/science.1123511

Bonawitz, N. D., Clayton, D. A., & Shadel, G. S. (2006). Initiation and beyond: multiple functions of the human mitochondrial transcription machinery. *Mol Cell, 24*(6), 813-825. doi:10.1016/j.molcel.2006.11.024

Bonora, M., De Marchi, E., Patergnani, S., Suski, J. M., Celsi, F., Bononi, A., . . . Pinton, P. (2014). Tumor necrosis factor-alpha impairs oligodendroglial differentiation through a mitochondria-dependent process. *Cell Death Differ, 21*(8), 1198-1208. doi:10.1038/cdd.2014.35

Bossy-Wetzel, E., Petrilli, A., & Knott, A. B. (2008). Mutant huntingtin and mitochondrial dysfunction. *Trends Neurosci, 31*(12), 609-616. doi:10.1016/j.tins.2008.09.004

Bouquet, F., Dehais, C., Sanson, M., Lubetzki, C., & Louapre, C. (2015). Dramatic worsening of adult-onset X-linked adrenoleukodystrophy after head trauma. *Neurology, 85*(22), 1991-1993. doi:10.1212/WNL.0000000000002173

Boveris, A., Oshino, N., & Chance, B. (1972). The cellular production of hydrogen peroxide. *Biochem J, 128*(3), 617-630.

Braiterman, L. T., Zheng, S., Watkins, P. A., Geraghty, M. T., Johnson, G., McGuinness, M. C., . . . Smith, K. D. (1998). Suppression of peroxisomal membrane protein defects by peroxisomal ATP binding cassette (ABC) proteins. *Hum Mol Genet, 7*(2), 239-247.

Brand, M. D. (2010). The sites and topology of mitochondrial superoxide production. *Exp Gerontol, 45*(7-8), 466-472. doi:10.1016/j.exger.2010.01.003

Breidenbach, R. W., & Beevers, H. (1967). Association of the glyoxylate cycle enzymes in a novel subcellular particle from castor bean endosperm. *Biochem Biophys Res Commun, 27*(4), 462-469.

Brennan, M. S., Patel, H., Allaire, N., Thai, A., Cullen, P., Ryan, S., . . . Scannevin, R. H. (2016). Pharmacodynamics of Dimethyl Fumarate Are Tissue Specific and Involve NRF2-Dependent and -Independent Mechanisms. *Antioxid Redox Signal, 24*(18), 1058-1071. doi:10.1089/ars.2015.6622

Brigelius-Flohe, R. (1999). Tissue-specific functions of individual glutathione peroxidases. *Free Radic Biol Med, 27*(9-10), 951-965.

Brigelius-Flohe, R., & Maiorino, M. (2013). Glutathione peroxidases. *Biochim Biophys Acta, 1830*(5), 3289-3303. doi:10.1016/j.bbagen.2012.11.020

Brites, P., Mooyer, P. A., El Mrabet, L., Waterham, H. R., & Wanders, R. J. (2009). Plasmalogens participate in very-long-chain fatty acid-induced pathology. *Brain, 132*(Pt 2), 482-492. doi:10.1093/brain/awn295

Brose, R. D., Avramopoulos, D., & Smith, K. D. (2012). SOD2 as a potential modifier of X-linked adrenoleukodystrophy clinical phenotypes. *J Neurol, 259*(7), 1440-1447. doi:10.1007/s00415-011-6371-8

Browne, S. E., & Beal, M. F. (2004). The energetics of Huntington's disease. *Neurochem Res, 29*(3), 531-546.

Browne, S. E., Ferrante, R. J., & Beal, M. F. (1999). Oxidative stress in Huntington's disease. *Brain Pathol, 9*(1), 147-163. doi:10.1111/j.1750-3639.1999.tb00216.x

Bryan, H. K., Olayanju, A., Goldring, C. E., & Park, B. K. (2013). The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation. *Biochem Pharmacol, 85*(6), 705-717. doi:10.1016/j.bcp.2012.11.016

Budka, H., Sluga, E., & Heiss, W. D. (1976). Spastic paraplegia associated with Addison's disease: adult variant of adreno-leukodystrophy. *J Neurol, 213*(3), 237-250.

Burton, N. C., Kensler, T. W., & Guilarte, T. R. (2006). In vivo modulation of the Parkinsonian phenotype by Nrf2. *Neurotoxicology, 27*(6), 1094-1100. doi:10.1016/j.neuro.2006.07.019

Calkins, M. J., Jakel, R. J., Johnson, D. A., Chan, K., Kan, Y. W., & Johnson, J. A. (2005). Protection from mitochondrial complex II inhibition in vitro and in vivo by Nrf2-mediated transcription. *Proc Natl Acad Sci U S A, 102*(1), 244-249. doi:10.1073/pnas.0408487101

Cambron, M., D'Haeseleer, M., Laureys, G., Clinckers, R., Debruyne, J., & De Keyser, J. (2012). White-matter astrocytes, axonal energy metabolism, and axonal degeneration in multiple sclerosis. *J Cereb Blood Flow Metab, 32*(3), 413-424. doi:10.1038/jcbfm.2011.193

Canto, C., Gerhart-Hines, Z., Feige, J. N., Lagouge, M., Noriega, L., Milne, J. C., . . . Auwerx, J. (2009). AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature, 458*(7241), 1056-1060. doi:10.1038/nature07813

Cappa, M., Bertini, E., del Balzo, P., Cambiaso, P., Di Biase, A., & Salvati, S. (1994). High dose immunoglobulin IV treatment in adrenoleukodystrophy. *J Neurol Neurosurg Psychiatry, 57 Suppl*, 69-70; discussion 71.

Cartier, N., Hacein-Bey-Abina, S., Bartholomae, C. C., Veres, G., Schmidt, M., Kutschera, I., . . . Aubourg, P. (2009). Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science, 326*(5954), 818-823. doi:10.1126/science.1171242

Cartier, N., Lewis, C. A., Zhang, R., & Rossi, F. M. (2014). The role of microglia in human disease: therapeutic tool or target? *Acta Neuropathol, 128*(3), 363-380. doi:10.1007/s00401-014-1330-y

Cartier, N., Lopez, J., Moullier, P., Rocchiccioli, F., Rolland, M. O., Jorge, P., . . . et al. (1995). Retroviral-mediated gene transfer corrects very-long-chain fatty acid metabolism in adrenoleukodystrophy fibroblasts. *Proc Natl Acad Sci U S A, 92*(5), 1674-1678.

Castet, A., Boulahtouf, A., Versini, G., Bonnet, S., Augereau, P., Vignon, F., . . . Cavailles, V. (2004). Multiple domains of the Receptor-Interacting Protein 140 contribute to transcription inhibition. *Nucleic Acids Res, 32*(6), 1957-1966. doi:10.1093/nar/gkh524

Cavailles, V., Dauvois, S., L'Horset, F., Lopez, G., Hoare, S., Kushner, P. J., & Parker, M. G. (1995). Nuclear factor RIP140 modulates transcriptional activation by the estrogen receptor. *Embo J, 14*(15), 3741-3751.

Chae, H. Z., Chung, S. J., & Rhee, S. G. (1994a). Thioredoxin-dependent peroxide reductase from yeast. *J Biol Chem, 269*(44), 27670-27678.

Chae, H. Z., Robison, K., Poole, L. B., Church, G., Storz, G., & Rhee, S. G. (1994b). Cloning and sequencing of thiol-specific antioxidant from mammalian brain: alkyl hydroperoxide reductase and thiol-specific antioxidant define a large family of antioxidant enzymes. *Proc Natl Acad Sci U S A, 91*(15), 7017-7021.

Chanas, S. A., Jiang, Q., McMahon, M., McWalter, G. K., McLellan, L. I., Elcombe, C. R., . . . Hayes, J. D. (2002). Loss of the Nrf2 transcription factor causes a marked reduction in constitutive and inducible expression of the glutathione S-transferase Gsta1, Gsta2, Gstm1, Gstm2, Gstm3 and Gstm4 genes in the livers of male and female mice. *Biochem J, 365*(Pt 2), 405-416. doi:10.1042/BJ20020320

Chaturvedi, R. K., Adhihetty, P., Shukla, S., Hennessy, T., Calingasan, N., Yang, L., . . . Beal, M. F. (2009). Impaired PGC-1alpha function in muscle in Huntington's disease. *Hum Mol Genet, 18*(16), 3048-3065. doi:10.1093/hmg/ddp243

Chen, C. M., Wu, Y. R., Cheng, M. L., Liu, J. L., Lee, Y. M., Lee, P. W., . . . Chiu, D. T. (2007). Increased oxidative damage and mitochondrial abnormalities in the peripheral blood of Huntington's disease patients. *Biochem Biophys Res Commun, 359*(2), 335-340. doi:10.1016/j.bbrc.2007.05.093

Chen, H., Assmann, J. C., Krenz, A., Rahman, M., Grimm, M., Karsten, C. M., . . . Schwaninger, M. (2014). Hydroxycarboxylic acid receptor 2 mediates dimethyl fumarate's protective effect in EAE. *J Clin Invest, 124*(5), 2188-2192. doi:10.1172/JCI72151

Chen, P. C., Vargas, M. R., Pani, A. K., Smeyne, R. J., Johnson, D. A., Kan, Y. W., & Johnson, J. A. (2009). Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. *Proc Natl Acad Sci U S A, 106*(8), 2933-2938. doi:10.1073/pnas.0813361106

Chen, T., Ma, Z., Zhu, L., Jiang, W., Wei, T., Zhou, R., . . . Yan, T. (2016). Suppressing Receptor-Interacting Protein 140: a New Sight for Salidroside to Treat Cerebral Ischemia. *Mol Neurobiol, 53*(9), 6240-6250. doi:10.1007/s12035-015-9521-7

Cho, B., Choi, S. Y., Cho, H. M., Kim, H. J., & Sun, W. (2013). Physiological and pathological significance of dynamin-related protein 1 (drp1)-dependent mitochondrial fission in the nervous system. *Exp Neurobiol, 22*(3), 149-157. doi:10.5607/en.2013.22.3.149

Cho, D. H., Nakamura, T., Fang, J., Cieplak, P., Godzik, A., Gu, Z., & Lipton, S. A. (2009). S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. *Science, 324*(5923), 102-105. doi:10.1126/science.1171091

Chou, C. H., Lin, C. C., Yang, M. C., Wei, C. C., Liao, H. D., Lin, R. C., . . . Hong, Y. R. (2012). GSK3beta-mediated Drp1 phosphorylation induced elongated mitochondrial morphology against oxidative stress. *PLoS One, 7*(11), e49112. doi:10.1371/journal.pone.0049112

Chowdhry, S., Nazmy, M. H., Meakin, P. J., Dinkova-Kostova, A. T., Walsh, S. V., Tsujita, T., . . . Hayes, J. D. (2010). Loss of Nrf2 markedly exacerbates nonalcoholic steatohepatitis. *Free Radic Biol Med, 48*(2), 357-371. doi:10.1016/j.freeradbiomed.2009.11.007

Christian, M., Kiskinis, E., Debevec, D., Leonardsson, G., White, R., & Parker, M. G. (2005). RIP140-targeted repression of gene expression in adipocytes. *Mol Cell Biol, 25*(21), 9383-9391. doi:10.1128/MCB.25.21.9383-9391.2005

Christian, M., Tullet, J. M., & Parker, M. G. (2004). Characterization of four autonomous repression domains in the corepressor receptor interacting protein 140. *J Biol Chem, 279*(15), 15645-15651. doi:10.1074/jbc.M313906200

Christian, M., White, R., & Parker, M. G. (2006). Metabolic regulation by the nuclear receptor corepressor RIP140. *Trends Endocrinol Metab, 17*(6), 243-250. doi:10.1016/j.tem.2006.06.008

Chuang, F. M., West, B. L., Baxter, J. D., & Schaufele, F. (1997). Activities in Pit-1 determine whether receptor interacting protein 140 activates or inhibits Pit-1/nuclear receptor transcriptional synergy. *Mol Endocrinol, 11*(9), 1332-1341. doi:10.1210/mend.11.9.9978

Chung, K. K., Thomas, B., Li, X., Pletnikova, O., Troncoso, J. C., Marsh, L., . . . Dawson, T. M. (2004). S-nitrosylation of parkin regulates ubiquitination and compromises parkin's protective function. *Science, 304*(5675), 1328-1331. doi:10.1126/science.1093891

Chunhua, M., Lingdong, K., Hongyan, L., & Zhangqiang, M. (2016). Umbelliferone reverses depression-like behavior in chronic unpredictable mild stress-induced mice via RIP140/NF-kappaB pathway. *IUBMB Life*. doi:10.1002/iub.1570

Ciechanover, A. (1998). The ubiquitin-proteasome pathway: on protein death and cell life. *Embo J, 17*(24), 7151-7160. doi:10.1093/emboj/17.24.7151

Ciron, C., Lengacher, S., Dusonchet, J., Aebischer, P., & Schneider, B. L. (2012). Sustained expression of PGC-1alpha in the rat nigrostriatal system selectively impairs dopaminergic function. *Hum Mol Genet, 21*(8), 1861-1876. doi:10.1093/hmg/ddr618

Cocheme, H. M., & Murphy, M. P. (2010). Can antioxidants be effective therapeutics? *Curr Opin Investig Drugs, 11*(4), 426-431.

Coelho, D., Kim, J. C., Miousse, I. R., Fung, S., du Moulin, M., Buers, I., . . . Baumgartner, M. R. (2012). Mutations in ABCD4 cause a new inborn error of vitamin B12 metabolism. *Nat Genet, 44*(10), 1152-1155. doi:10.1038/ng.2386

Coghlan, M. P., Culbert, A. A., Cross, D. A., Corcoran, S. L., Yates, J. W., Pearce, N. J., . . . Holder, J. C. (2000). Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. *Chem Biol, 7*(10), 793-803. doi:10.1016/S1074-5521(00)00025-9

Conti, A., Fabbrini, F., D'Agostino, P., Negri, R., Greco, D., Genesio, R., . . . Nitsch, L. (2007). Altered expression of mitochondrial and extracellular matrix genes in the heart of human fetuses with chromosome 21 trisomy. *BMC Genomics, 8*, 268. doi:10.1186/1471-2164-8-268

Contreras, M., Sengupta, T. K., Sheikh, F., Aubourg, P., & Singh, I. (1996). Topology of ATP-binding domain of adrenoleukodystrophy gene product in peroxisomes. *Arch Biochem Biophys, 334*(2), 369-379. doi:10.1006/abbi.1996.0467

Court, F. A., & Coleman, M. P. (2012). Mitochondria as a central sensor for axonal degenerative stimuli. *Trends Neurosci, 35*(6), 364-372. doi:10.1016/j.tins.2012.04.001

Credle, J. J., George, J. L., Wills, J., Duka, V., Shah, K., Lee, Y. C., . . . Sidhu, A. (2015). GSK-3beta dysregulation contributes to parkinson's-like pathophysiology with associated region-specific phosphorylation and accumulation of tau and alpha-synuclein. *Cell Death Differ, 22*(5), 838-851. doi:10.1038/cdd.2014.179

Cross, C. E., Halliwell, B., Borish, E. T., Pryor, W. A., Ames, B. N., Saul, R. L., . . . Harman, D. (1987). Oxygen radicals and human disease. *Ann Intern Med, 107*(4), 526-545.

Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M., & Hemmings, B. A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature, 378*(6559), 785-789. doi:10.1038/378785a0

Crunkhorn, S., Dearie, F., Mantzoros, C., Gami, H., da Silva, W. S., Espinoza, D., . . . Patti, M. E. (2007). Peroxisome proliferator activator receptor gamma coactivator-1 expression is reduced in obesity: potential pathogenic role of saturated fatty acids and p38 mitogen-activated protein kinase activation. *J Biol Chem, 282*(21), 15439-15450. doi:10.1074/jbc.M611214200

Cuervo, A. M., Stefanis, L., Fredenburg, R., Lansbury, P. T., & Sulzer, D. (2004). Impaired degradation of mutant alpha-synuclein by chaperone-mediated autophagy. *Science, 305*(5688), 1292-1295. doi:10.1126/science.1101738

Cui, L., Jeong, H., Borovecki, F., Parkhurst, C. N., Tanese, N., & Krainc, D. (2006). Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell, 127*(1), 59-69. doi:10.1016/j.cell.2006.09.015

da Silva, T. F., Eira, J., Lopes, A. T., Malheiro, A. R., Sousa, V., Luoma, A., . . . Brites, P. (2014). Peripheral nervous system plasmalogens regulate Schwann cell differentiation and myelination. *J Clin Invest, 124*(6), 2560-2570. doi:10.1172/JCI72063

Dajani, R., Fraser, E., Roe, S. M., Young, N., Good, V., Dale, T. C., & Pearl, L. H. (2001). Crystal structure of glycogen synthase kinase 3 beta: structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell, 105*(6), 721-732.

Dalton, T. P., Chen, Y., Schneider, S. N., Nebert, D. W., & Shertzer, H. G. (2004). Genetically altered mice to evaluate glutathione homeostasis in health and disease. *Free Radic Biol Med, 37*(10), 1511-1526. doi:10.1016/j.freeradbiomed.2004.06.040

Davies, J. E., Sarkar, S., & Rubinsztein, D. C. (2007). The ubiquitin proteasome system in Huntington's disease and the spinocerebellar ataxias. *BMC Biochem, 8 Suppl 1*, S2. doi:10.1186/1471-2091-8-S1-S2

de Beer, M., Engelen, M., & van Geel, B. M. (2014). Frequent occurrence of cerebral demyelination in adrenomyeloneuropathy. *Neurology, 83*(24), 2227-2231. doi:10.1212/WNL.0000000000001074

De Duve, C., & Baudhuin, P. (1966). Peroxisomes (microbodies and related particles). *Physiol Rev, 46*(2), 323-357.

de Jong, R., Bezemer, A. C., Zomerdijk, T. P., van de Pouw-Kraan, T., Ottenhoff, T. H., & Nibbering, P. H. (1996). Selective stimulation of T helper 2 cytokine responses by the anti-psoriasis agent monomethylfumarate. *Eur J Immunol, 26*(9), 2067-2074. doi:10.1002/eji.1830260916

Dean, M., & Annilo, T. (2005). Evolution of the ATP-binding cassette (ABC) transporter superfamily in vertebrates. *Annu Rev Genomics Hum Genet, 6*, 123-142. doi:10.1146/annurev.genom.6.080604.162122

DeNicola, G. M., Karreth, F. A., Humpton, T. J., Gopinathan, A., Wei, C., Frese, K., . . . Tuveson, D. A. (2011). Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature, 475*(7354), 106-109. doi:10.1038/nature10189

Deon, M., Sitta, A., Barschak, A. G., Coelho, D. M., Pigatto, M., Schmitt, G. O., . . . Vargas, C. R. (2007). Induction of lipid peroxidation and decrease of antioxidant defenses in symptomatic and asymptomatic patients with X-linked adrenoleukodystrophy. *Int J Dev Neurosci, 25*(7), 441-444. doi:10.1016/j.ijdevneu.2007.08.008

Deon, M., Sitta, A., Barschak, A. G., Coelho, D. M., Terroso, T., Schmitt, G. O., . . . Vargas, C. R. (2008). Oxidative stress is induced in female carriers of X-linked adrenoleukodystrophy. *J Neurol Sci, 266*(1-2), 79-83. doi:10.1016/j.jns.2007.08.043

Dhaunsi, G. S., Gulati, S., Singh, A. K., Orak, J. K., Asayama, K., & Singh, I. (1992). Demonstration of Cu-Zn superoxide dismutase in rat liver peroxisomes. Biochemical and immunochemical evidence. *J Biol Chem, 267*(10), 6870-6873.

Di Biase, A., Salvati, S., Vari, R., Avellino, C., Sforza, F., Cappa, M., & Masella, R. (2000). Susceptibility to oxidation of plasma low-density lipoprotein in X-linked adrenoleukodystrophy: effects of simvastatin treatment. *Mol Genet Metab, 71*(4), 651-655. doi:10.1006/mgme.2000.3100

Diehl, J. A., Cheng, M., Roussel, M. F., & Sherr, C. J. (1998). Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev, 12*(22), 3499-3511.

Dinkova-Kostova, A. T., Holtzclaw, W. D., Cole, R. N., Itoh, K., Wakabayashi, N., Katoh, Y., . . . Talalay, P. (2002). Direct evidence that sulfhydryl groups of Keap1 are the sensors regulating induction of phase 2 enzymes that protect against carcinogens and oxidants. *Proc Natl Acad Sci U S A, 99*(18), 11908-11913. doi:10.1073/pnas.172398899

Dinkova-Kostova, A. T., Liby, K. T., Stephenson, K. K., Holtzclaw, W. D., Gao, X., Suh, N., . . . Talalay, P. (2005). Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. *Proc Natl Acad Sci U S A, 102*(12), 4584-4589. doi:10.1073/pnas.0500815102

Dinkova-Kostova, A. T., Massiah, M. A., Bozak, R. E., Hicks, R. J., & Talalay, P. (2001). Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups. *Proc Natl Acad Sci U S A, 98*(6), 3404-3409. doi:10.1073/pnas.051632198

Dinkova-Kostova, A. T., & Talalay, P. (2010). NAD(P)H:quinone acceptor oxidoreductase 1 (NQO1), a multifunctional antioxidant enzyme and exceptionally versatile cytoprotector. *Arch Biochem Biophys, 501*(1), 116-123. doi:10.1016/j.abb.2010.03.019

Docquier, A., Augereau, P., Lapierre, M., Harmand, P. O., Badia, E., Annicotte, J. S., . . . Cavailles, V. (2012). The RIP140 gene is a transcriptional target of E2F1. *PLoS One, 7*(5), e35839. doi:10.1371/journal.pone.0035839

Docquier, A., Harmand, P. O., Fritsch, S., Chanrion, M., Darbon, J. M., & Cavailles, V. (2010). The transcriptional coregulator RIP140 represses E2F1 activity and discriminates breast cancer subtypes. *Clin Cancer Res, 16*(11), 2959-2970. doi:10.1158/1078-0432.CCR-09-3153

Douglas, P. M., & Dillin, A. (2010). Protein homeostasis and aging in neurodegeneration. *J Cell Biol, 190*(5), 719-729. doi:10.1083/jcb.201005144

Dubey, P., Raymond, G. V., Moser, A. B., Kharkar, S., Bezman, L., & Moser, H. W. (2005). Adrenal insufficiency in asymptomatic adrenoleukodystrophy patients identified by very long-chain fatty acid screening. *J Pediatr, 146*(4), 528-532. doi:10.1016/j.jpeds.2004.10.067

Duclot, F., Lapierre, M., Fritsch, S., White, R., Parker, M. G., Maurice, T., & Cavailles, V. (2012). Cognitive impairments in adult mice with constitutive inactivation of RIP140 gene expression. *Genes Brain Behav, 11*(1), 69-78. doi:10.1111/j.1601-183X.2011.00731.x

Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., . . . Greenberg, M. E. (1997). Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science, 275*(5300), 661-665.

Duka, T., Duka, V., Joyce, J. N., & Sidhu, A. (2009). Alpha-Synuclein contributes to GSK-3beta-catalyzed Tau phosphorylation in Parkinson's disease models. *Faseb J, 23*(9), 2820-2830. doi:10.1096/fj.08-120410

Dumont, M., Stack, C., Elipenahli, C., Jainuddin, S., Launay, N., Gerges, M., . . . Beal, M. F. (2014). PGC-1alpha overexpression exacerbates beta-amyloid and tau deposition in a transgenic mouse model of Alzheimer's disease. *Faseb J, 28*(4), 1745-1755. doi:10.1096/fj.13-236331

Dumser, M., Bauer, J., Lassmann, H., Berger, J., & Forss-Petter, S. (2007). Lack of adrenoleukodystrophy protein enhances oligodendrocyte disturbance and microglia activation in mice with combined Abcd1/Mag deficiency. *Acta Neuropathol, 114*(6), 573-586. doi:10.1007/s00401-007-0288-4

Dupuis, L., Oudart, H., Rene, F., Gonzalez de Aguilar, J. L., & Loeffler, J. P. (2004). Evidence for defective energy homeostasis in amyotrophic lateral sclerosis: benefit of a high-energy diet in a transgenic mouse model. *Proc Natl Acad Sci U S A, 101*(30), 11159-11164. doi:10.1073/pnas.0402026101

Echtay, K. S., Roussel, D., St-Pierre, J., Jekabsons, M. B., Cadenas, S., Stuart, J. A., . . . Brand, M. D. (2002). Superoxide activates mitochondrial uncoupling proteins. *Nature, 415*(6867), 96-99. doi:10.1038/415096a

Eglitis, M. A., & Mezey, E. (1997). Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci U S A, 94*(8), 4080-4085.

Eichler, F. S., Ren, J. Q., Cossoy, M., Rietsch, A. M., Nagpal, S., Moser, A. B., . . . Ransohoff, R. M. (2008). Is microglial apoptosis an early pathogenic change in cerebral X-linked adrenoleukodystrophy? *Ann Neurol, 63*(6), 729-742. doi:10.1002/ana.21391

Ellrichmann, G., Petrasch-Parwez, E., Lee, D. H., Reick, C., Arning, L., Saft, C., . . . Linker, R. A. (2011). Efficacy of fumaric acid esters in the R6/2 and YAC128 models of Huntington's disease. *PLoS One, 6*(1), e16172. doi:10.1371/journal.pone.0016172

Engelen, M., Barbier, M., Dijkstra, I. M., Schur, R., de Bie, R. M., Verhamme, C., . . . Kemp, S. (2014). X-linked adrenoleukodystrophy in women: a cross-sectional cohort study. *Brain, 137*(Pt 3), 693-706. doi:10.1093/brain/awt361

Engelen, M., Ofman, R., Dijkgraaf, M. G., Hijzen, M., van der Wardt, L. A., van Geel, B. M., . . . Kemp, S. (2010). Lovastatin in X-linked adrenoleukodystrophy. *N Engl J Med, 362*(3), 276-277. doi:10.1056/NEJMc0907735

Engelen, M., Schackmann, M. J., Ofman, R., Sanders, R. J., Dijkstra, I. M., Houten, S. M., . . . Kemp, S. (2012a). Bezafibrate lowers very long-chain fatty acids in X-linked adrenoleukodystrophy fibroblasts by inhibiting fatty acid elongation. *J Inherit Metab Dis, 35*(6), 1137-1145. doi:10.1007/s10545-012-9471-4

Engelen, M., Tran, L., Ofman, R., Brennecke, J., Moser, A. B., Dijkstra, I. M., . . . Kemp, S. (2012b). Bezafibrate for X-linked adrenoleukodystrophy. *PLoS One, 7*(7), e41013. doi:10.1371/journal.pone.0041013

Esterbauer, H., Schaur, R. J., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med, 11*(1), 81-128.

Fanconi, A., Prader, A., Isler, W., Luethy, F., & Siebenmann, R. (1963). Addison's Disease With Cerebral Sclerosis in Childhood. A Hereditary Syndrome Transmitted Through Chromosome X? *Helv Paediatr Acta, 18*, 480-501.

Fancy, S. P., Baranzini, S. E., Zhao, C., Yuk, D. I., Irvine, K. A., Kaing, S., . . . Rowitch, D. H. (2009). Dysregulation of the Wnt pathway inhibits timely myelination and remyelination in the mammalian CNS. *Genes Dev, 23*(13), 1571-1585. doi:10.1101/gad.1806309

Fang, E. F., Scheibye-Knudsen, M., Brace, L. E., Kassahun, H., SenGupta, T., Nilsen, H., . . . Bohr, V. A. (2014). Defective mitophagy in XPA via PARP-1 hyperactivation and NAD(+)/SIRT1 reduction. *Cell, 157*(4), 882-896. doi:10.1016/j.cell.2014.03.026

Farr, S. A., Ripley, J. L., Sultana, R., Zhang, Z., Niehoff, M. L., Platt, T. L., . . . Butterfield, D. A. (2014). Antisense oligonucleotide against GSK-3beta in brain of SAMP8 mice improves learning and memory and decreases oxidative stress: Involvement of transcription factor Nrf2 and implications for Alzheimer disease. *Free Radic Biol Med, 67*, 387-395. doi:10.1016/j.freeradbiomed.2013.11.014

Favreau, L. V., & Pickett, C. B. (1991). Transcriptional regulation of the rat NAD(P)H:quinone reductase gene. Identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. *J Biol Chem, 266*(7), 4556-4561.

Feige, J. N., & Auwerx, J. (2007). Transcriptional coregulators in the control of energy homeostasis. *Trends Cell Biol, 17*(6), 292-301. doi:10.1016/j.tcb.2007.04.001

Feng, X., Krogh, K. A., Wu, C. Y., Lin, Y. W., Tsai, H. C., Thayer, S. A., & Wei, L. N. (2014). Receptor-interacting protein 140 attenuates endoplasmic reticulum stress in neurons and protects against cell death. *Nat Commun, 5*, 4487. doi:10.1038/ncomms5487

Feng, X., Lin, Y. L., & Wei, L. N. (2015). Behavioral stress reduces RIP140 expression in astrocyte and increases brain lipid accumulation. *Brain Behav Immun, 46*, 270-279. doi:10.1016/j.bbi.2015.02.008

Ferdinandusse, S., Jimenez-Sanchez, G., Koster, J., Denis, S., Van Roermund, C. W., Silva-Zolezzi, I., . . . Valle, D. (2015). A novel bile acid biosynthesis defect due to a deficiency of peroxisomal ABCD3. *Hum Mol Genet, 24*(2), 361-370. doi:10.1093/hmg/ddu448

Ferger, A. I., Campanelli, L., Reimer, V., Muth, K. N., Merdian, I., Ludolph, A. C., & Witting, A. (2010). Effects of mitochondrial dysfunction on the immunological properties of microglia. *J Neuroinflammation, 7*, 45. doi:10.1186/1742-2094-7-45

Fernandes, A. P., & Holmgren, A. (2004). Glutaredoxins: glutathione-dependent redox enzymes with functions far beyond a simple thioredoxin backup system. *Antioxid Redox Signal, 6*(1), 63-74. doi:10.1089/152308604771978354

Ferrer, I., Barrachina, M., & Puig, B. (2002). Glycogen synthase kinase-3 is associated with neuronal and glial hyperphosphorylated tau deposits in Alzheimer's disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration. *Acta Neuropathol, 104*(6), 583-591. doi:10.1007/s00401-002-0587-8

Ferrer, I., Kapfhammer, J. P., Hindelang, C., Kemp, S., Troffer-Charlier, N., Broccoli, V., . . . Pujol, A. (2005). Inactivation of the peroxisomal ABCD2 transporter in the mouse leads to late-onset ataxia involving mitochondria, Golgi and endoplasmic reticulum damage. *Hum Mol Genet, 14*(23), 3565-3577. doi:10.1093/hmg/ddi384

Flaisher-Grinberg, S., Tsai, H. C., Feng, X., & Wei, L. N. (2014). Emotional regulatory function of receptor interacting protein 140 revealed in the ventromedial hypothalamus. *Brain Behav Immun, 40*, 226-234. doi:10.1016/j.bbi.2014.03.019

Flavigny, E., Sanhaj, A., Aubourg, P., & Cartier, N. (1999). Retroviral-mediated adrenoleukodystrophy-related gene transfer corrects very long chain fatty acid metabolism in adrenoleukodystrophy fibroblasts: implications for therapy. *FEBS Lett, 448*(2-3), 261-264.

Flohe, L., Toppo, S., Cozza, G., & Ursini, F. (2011). A comparison of thiol peroxidase mechanisms. *Antioxid Redox Signal, 15*(3), 763-780. doi:10.1089/ars.2010.3397

Folch, J., Junyent, F., Verdaguer, E., Auladell, C., Pizarro, J. G., Beas-Zarate, C., . . . Camins, A. (2012). Role of cell cycle re-entry in neurons: a common apoptotic mechanism of neuronal cell death. *Neurotox Res, 22*(3), 195-207. doi:10.1007/s12640-011-9277-4

Forman, H. J., Davies, K. J., & Ursini, F. (2014). How do nutritional antioxidants really work: nucleophilic tone and para-hormesis versus free radical scavenging in vivo. *Free Radic Biol Med, 66*, 24-35. doi:10.1016/j.freeradbiomed.2013.05.045

Forss-Petter, S., Werner, H., Berger, J., Lassmann, H., Molzer, B., Schwab, M. H., . . . Nave, K. A. (1997). Targeted inactivation of the X-linked adrenoleukodystrophy gene in mice. *J Neurosci Res, 50*(5), 829-843. doi:10.1002/(SICI)1097-4547(19971201)50:5<829::AID-JNR19>3.0.CO;2-W

Fouquet, F., Zhou, J. M., Ralston, E., Murray, K., Troalen, F., Magal, E., . . . Aubourg, P. (1997). Expression of the adrenoleukodystrophy protein in the human and mouse central nervous system. *Neurobiol Dis, 3*(4), 271-285. doi:10.1006/nbdi.1997.0127

Fourcade, S., Lopez-Erauskin, J., Galino, J., Duval, C., Naudi, A., Jove, M., . . . Pujol, A. (2008). Early oxidative damage underlying neurodegeneration in X-adrenoleukodystrophy. *Hum Mol Genet, 17*(12), 1762-1773. doi:10.1093/hmg/ddn085

Fourcade, S., Ruiz, M., Guilera, C., Hahnen, E., Brichta, L., Naudi, A., . . . Pujol, A. (2010). Valproic acid induces antioxidant effects in X-linked adrenoleukodystrophy. *Hum Mol Genet, 19*(10), 2005-2014. doi:10.1093/hmg/ddq082

Fourcade, S., Savary, S., Albet, S., Gauthe, D., Gondcaille, C., Pineau, T., . . . Bugaut, M. (2001). Fibrate induction of the adrenoleukodystrophy-related gene (ABCD2): promoter analysis and role of the peroxisome proliferator-activated receptor PPARalpha. *Eur J Biochem, 268*(12), 3490-3500.

Fox, R. J., Miller, D. H., Phillips, J. T., Hutchinson, M., Havrdova, E., Kita, M., . . . Investigators, C. S. (2012). Placebo-controlled phase 3 study of oral BG-12 or glatiramer in multiple sclerosis. *N Engl J Med, 367*(12), 1087-1097. doi:10.1056/NEJMoa1206328

Franco-Iborra, S., Vila, M., & Perier, C. (2016). The Parkinson Disease Mitochondrial Hypothesis: Where Are We at? *Neuroscientist, 22*(3), 266-277. doi:10.1177/1073858415574600

Freyberg, Z., Ferrando, S. J., & Javitch, J. A. (2010). Roles of the Akt/GSK-3 and Wnt signaling pathways in schizophrenia and antipsychotic drug action. *Am J Psychiatry, 167*(4), 388-396. doi:10.1176/appi.ajp.2009.08121873

Fridovich, I. (1995). Superoxide radical and superoxide dismutases. *Annu Rev Biochem, 64*, 97-112. doi:10.1146/annurev.bi.64.070195.000525

Fritah, A., Steel, J. H., Parker, N., Nikolopoulou, E., Christian, M., Carling, D., & Parker, M. G. (2012). Absence of RIP140 reveals a pathway regulating glut4-

dependent glucose uptake in oxidative skeletal muscle through UCP1-mediated activation of AMPK. *PLoS One, 7*(2), e32520. doi:10.1371/journal.pone.0032520

Funfschilling, U., Supplie, L. M., Mahad, D., Boretius, S., Saab, A. S., Edgar, J., . . . Nave, K. A. (2012). Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. *Nature, 485*(7399), 517-521. doi:10.1038/nature11007

Gabaldon, T., Snel, B., van Zimmeren, F., Hemrika, W., Tabak, H., & Huynen, M. A. (2006). Origin and evolution of the peroxisomal proteome. *Biol Direct, 1*, 8. doi:10.1186/1745-6150-1-8

Galea, E., Launay, N., Portero-Otin, M., Ruiz, M., Pamplona, R., Aubourg, P., . . . Pujol, A. (2012). Oxidative stress underlying axonal degeneration in adrenoleukodystrophy: a paradigm for multifactorial neurodegenerative diseases? *Biochim Biophys Acta, 1822*(9), 1475-1488. doi:10.1016/j.bbadis.2012.02.005

Galino, J., Ruiz, M., Fourcade, S., Schluter, A., Lopez-Erauskin, J., Guilera, C., . . . Pujol, A. (2011). Oxidative damage compromises energy metabolism in the axonal degeneration mouse model of X-adrenoleukodystrophy. *Antioxid Redox Signal, 15*(8), 2095-2107. doi:10.1089/ars.2010.3877

Gardiner, K. (2006). Transcriptional dysregulation in Down syndrome: predictions for altered protein complex stoichiometries and post-translational modifications, and consequences for learning/behavior genes ELK, CREB, and the estrogen and glucocorticoid receptors. *Behav Genet, 36*(3), 439-453. doi:10.1007/s10519-006-9051-1

Geillon, F., Gondcaille, C., Charbonnier, S., Van Roermund, C. W., Lopez, T. E., Dias, A. M., . . . Savary, S. (2014). Structure-function analysis of peroxisomal ATP-binding cassette transporters using chimeric dimers. *J Biol Chem, 289*(35), 24511-24520. doi:10.1074/jbc.M114.575506

Geillon, F., Gondcaille, C., Raas, Q., Dias, A. M., Pecqueur, D., Truntzer, C., . . . Trompier, D. (2017). Peroxisomal ATP-binding cassette transporters form mainly tetramers. *J Biol Chem*. doi:10.1074/jbc.M116.772806

Genin, E. C., Geillon, F., Gondcaille, C., Athias, A., Gambert, P., Trompier, D., & Savary, S. (2011). Substrate specificity overlap and interaction between adrenoleukodystrophy protein (ALDP/ABCD1) and adrenoleukodystrophy-related protein (ALDRP/ABCD2). *J Biol Chem, 286*(10), 8075-8084. doi:10.1074/jbc.M110.211912

Gilg, A. G., Singh, A. K., & Singh, I. (2000). Inducible nitric oxide synthase in the central nervous system of patients with X-adrenoleukodystrophy. *J Neuropathol Exp Neurol, 59*(12), 1063-1069.

Ginhoux, F., Greter, M., Leboeuf, M., Nandi, S., See, P., Gokhan, S., . . . Merad, M. (2010). Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science, 330*(6005), 841-845. doi:10.1126/science.1194637

Giralt, A., & Villarroya, F. (2012). SIRT3, a pivotal actor in mitochondrial functions: metabolism, cell death and aging. *Biochem J, 444*(1), 1-10. doi:10.1042/BJ20120030

Gnaiger, E. (2001). Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. *Respir Physiol, 128*(3), 277-297.

Gold, R., Kappos, L., Arnold, D. L., Bar-Or, A., Giovannoni, G., Selmaj, K., . . . Investigators, D. S. (2012). Placebo-controlled phase 3 study of oral BG-12 for

relapsing multiple sclerosis. *N Engl J Med, 367*(12), 1098-1107. doi:10.1056/NEJMoa1114287

Goldfischer, S., Moore, C. L., Johnson, A. B., Spiro, A. J., Valsamis, M. P., Wisniewski, H. K., . . . Gartner, L. M. (1973). Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. *Science, 182*(4107), 62-64.

Gong, B., Pan, Y., Vempati, P., Zhao, W., Knable, L., Ho, L., . . . Pasinetti, G. M. (2013). Nicotinamide riboside restores cognition through an upregulation of proliferator-activated receptor-gamma coactivator 1alpha regulated beta-secretase 1 degradation and mitochondrial gene expression in Alzheimer's mouse models. *Neurobiol Aging, 34*(6), 1581-1588. doi:10.1016/j.neurobiolaging.2012.12.005

Gopal, S., Mikulskis, A., Gold, R., Fox, R. J., Dawson, K. T., & Amaravadi, L. (2017). Evidence of activation of the Nrf2 pathway in multiple sclerosis patients treated with delayed-release dimethyl fumarate in the Phase 3 DEFINE and CONFIRM studies. *Mult Scler*, 1352458517690617. doi:10.1177/1352458517690617

Gozzelino, R., Jeney, V., & Soares, M. P. (2010). Mechanisms of cell protection by heme oxygenase-1. *Annu Rev Pharmacol Toxicol, 50*, 323-354. doi:10.1146/annurev.pharmtox.010909.105600

Gray, M. W., Burger, G., & Lang, B. F. (1999). Mitochondrial evolution. *Science, 283*(5407), 1476-1481.

Green, D. R., Galluzzi, L., & Kroemer, G. (2011). Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science, 333*(6046), 1109-1112. doi:10.1126/science.1201940

Grek, C. L., Zhang, J., Manevich, Y., Townsend, D. M., & Tew, K. D. (2013). Causes and consequences of cysteine S-glutathionylation. *J Biol Chem, 288*(37), 26497-26504. doi:10.1074/jbc.R113.461368

Griffin, D. E., Moser, H. W., Mendoza, Q., Moench, T. R., O'Toole, S., & Moser, A. B. (1985). Identification of the inflammatory cells in the central nervous system of patients with adrenoleukodystrophy. *Ann Neurol, 18*(6), 660-664. doi:10.1002/ana.410180606

Griffin, J. W., Goren, E., Schaumburg, H., Engel, W. K., & Loriaux, L. (1977). Adrenomyeloneuropathy: a probable variant of adrenoleukodystrophy. I. Clinical and endocrinologic aspects. *Neurology, 27*(12), 1107-1113.

Gross, C. C., Schulte-Mecklenbeck, A., Klinsing, S., Posevitz-Fejfar, A., Wiendl, H., & Klotz, L. (2016). Dimethyl fumarate treatment alters circulating T helper cell subsets in multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm, 3*(1), e183. doi:10.1212/NXI.0000000000000183

Guimaraes, C. P., Domingues, P., Aubourg, P., Fouquet, F., Pujol, A., Jimenez-Sanchez, G., . . . Azevedo, J. E. (2004). Mouse liver PMP70 and ALDP: homomeric interactions prevail in vivo. *Biochim Biophys Acta, 1689*(3), 235-243. doi:10.1016/j.bbadis.2004.04.001

Guo, X., Snider, W. D., & Chen, B. (2016). GSK3beta regulates AKT-induced central nervous system axon regeneration via an eIF2Bepsilon-dependent, mTORC1-independent pathway. *Elife, 5*, e11903. doi:10.7554/eLife.11903

Gupta, P., Ho, P. C., Huq, M. D., Khan, A. A., Tsai, N. P., & Wei, L. N. (2008). PKCepsilon stimulated arginine methylation of RIP140 for its nuclear-cytoplasmic export in adipocyte differentiation. *PLoS One, 3*(7), e2658. doi:10.1371/journal.pone.0002658

Gupta, P., Huq, M. D., Khan, S. A., Tsai, N. P., & Wei, L. N. (2005). Regulation of co-repressive activity of and HDAC recruitment to RIP140 by site-specific

phosphorylation. *Mol Cell Proteomics, 4*(11), 1776-1784. doi:10.1074/mcp.M500236-MCP200

Haider, L., Fischer, M. T., Frischer, J. M., Bauer, J., Hoftberger, R., Botond, G., . . . Lassmann, H. (2011). Oxidative damage in multiple sclerosis lesions. *Brain, 134*(Pt 7), 1914-1924. doi:10.1093/brain/awr128

Hajra, A. K., & Bishop, J. E. (1982). Glycerolipid biosynthesis in peroxisomes via the acyl dihydroxyacetone phosphate pathway. *Ann N Y Acad Sci, 386*, 170-182.

Hall, C. N., Klein-Flugge, M. C., Howarth, C., & Attwell, D. (2012). Oxidative phosphorylation, not glycolysis, powers presynaptic and postsynaptic mechanisms underlying brain information processing. *J Neurosci, 32*(26), 8940-8951. doi:10.1523/JNEUROSCI.0026-12.2012

Hallberg, M., Morganstein, D. L., Kiskinis, E., Shah, K., Kralli, A., Dilworth, S. M., . . . Christian, M. (2008). A functional interaction between RIP140 and PGC-1alpha regulates the expression of the lipid droplet protein CIDEA. *Mol Cell Biol, 28*(22), 6785-6795. doi:10.1128/MCB.00504-08

Halliwell, B. (2000). The antioxidant paradox. *Lancet, 355*(9210), 1179-1180. doi:10.1016/S0140-6736(00)02075-4

Halliwell, B. (2001). Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging, 18*(9), 685-716.

Halliwell, B. (2006). Oxidative stress and neurodegeneration: where are we now? *J Neurochem, 97*(6), 1634-1658. doi:10.1111/j.1471-4159.2006.03907.x

Halliwell, B. (2007). Biochemistry of oxidative stress. *Biochem Soc Trans, 35*(Pt 5), 1147-1150. doi:10.1042/BST0351147

Halliwell, B. (2011). Free radicals and antioxidants - quo vadis? *Trends Pharmacol Sci, 32*(3), 125-130. doi:10.1016/j.tips.2010.12.002

Halliwell, B. (2014). Cell culture, oxidative stress, and antioxidants: avoiding pitfalls. *Biomed J, 37*(3), 99-105. doi:10.4103/2319-4170.128725

Handschin, C., Chin, S., Li, P., Liu, F., Maratos-Flier, E., Lebrasseur, N. K., . . . Spiegelman, B. M. (2007). Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1alpha muscle-specific knock-out animals. *J Biol Chem, 282*(41), 30014-30021. doi:10.1074/jbc.M704817200

Hanger, D. P., Hughes, K., Woodgett, J. R., Brion, J. P., & Anderton, B. H. (1992). Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. *Neurosci Lett, 147*(1), 58-62.

Hara, T., Nakamura, K., Matsui, M., Yamamoto, A., Nakahara, Y., Suzuki-Migishima, R., . . . Mizushima, N. (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature, 441*(7095), 885-889. doi:10.1038/nature04724

Harman, D. (1956). Aging: a theory based on free radical and radiation chemistry. *J Gerontol, 11*(3), 298-300.

Hayakawa, K., Esposito, E., Wang, X., Terasaki, Y., Liu, Y., Xing, C., . . . Lo, E. H. (2016). Transfer of mitochondria from astrocytes to neurons after stroke. *Nature, 535*(7613), 551-555. doi:10.1038/nature18928

Hayes, J. D., Flanagan, J. U., & Jowsey, I. R. (2005). Glutathione transferases. *Annu Rev Pharmacol Toxicol, 45*, 51-88. doi:10.1146/annurev.pharmtox.45.120403.095857

Hayes, J. D., McMahon, M., Chowdhry, S., & Dinkova-Kostova, A. T. (2010). Cancer chemoprevention mechanisms mediated through the Keap1-Nrf2 pathway. *Antioxid Redox Signal, 13*(11), 1713-1748. doi:10.1089/ars.2010.3221

Hecht, D., & Zick, Y. (1992). Selective inhibition of protein tyrosine phosphatase activities by H2O2 and vanadate in vitro. *Biochem Biophys Res Commun, 188*(2), 773-779.

Heinzer, A. K., Watkins, P. A., Lu, J. F., Kemp, S., Moser, A. B., Li, Y. Y., . . . Smith, K. D. (2003). A very long-chain acyl-CoA synthetase-deficient mouse and its relevance to X-linked adrenoleukodystrophy. *Hum Mol Genet, 12*(10), 1145-1154.

Herbst, E. A., Bonen, A., & Holloway, G. P. (2015). Changes in nuclear receptor corepressor RIP140 do not influence mitochondrial content in the cortex. *Appl Physiol Nutr Metab, 40*(10), 1086-1088. doi:10.1139/apnm-2015-0192

Herrup, K., & Yang, Y. (2007). Cell cycle regulation in the postmitotic neuron: oxymoron or new biology? *Nat Rev Neurosci, 8*(5), 368-378. doi:10.1038/nrn2124

Herzog, B., Hallberg, M., Seth, A., Woods, A., White, R., & Parker, M. G. (2007). The nuclear receptor cofactor, receptor-interacting protein 140, is required for the regulation of hepatic lipid and glucose metabolism by liver X receptor. *Mol Endocrinol, 21*(11), 2687-2697. doi:10.1210/me.2007-0213

Hettema, E. H., van Roermund, C. W., Distel, B., van den Berg, M., Vilela, C., Rodrigues-Pousada, C., . . . Tabak, H. F. (1996). The ABC transporter proteins Pat1 and Pat2 are required for import of long-chain fatty acids into peroxisomes of Saccharomyces cerevisiae. *Embo J, 15*(15), 3813-3822.

Ho, J. K., Moser, H., Kishimoto, Y., & Hamilton, J. A. (1995). Interactions of a very long chain fatty acid with model membranes and serum albumin. Implications for the pathogenesis of adrenoleukodystrophy. *J Clin Invest, 96*(3), 1455-1463. doi:10.1172/JCI118182

Ho, P. C., Chang, K. C., Chuang, Y. S., & Wei, L. N. (2011). Cholesterol regulation of receptor-interacting protein 140 via microRNA-33 in inflammatory cytokine production. *Faseb J, 25*(5), 1758-1766. doi:10.1096/fj.10-179267

Ho, P. C., Lin, Y. W., Tsui, Y. C., Gupta, P., & Wei, L. N. (2009). A negative regulatory pathway of GLUT4 trafficking in adipocyte: new function of RIP140 in the cytoplasm via AS160. *Cell Metab, 10*(6), 516-523. doi:10.1016/j.cmet.2009.09.012

Ho, P. C., Tsui, Y. C., Feng, X., Greaves, D. R., & Wei, L. N. (2012). NF-kappaB-mediated degradation of the coactivator RIP140 regulates inflammatory responses and contributes to endotoxin tolerance. *Nat Immunol, 13*(4), 379-386. doi:10.1038/ni.2238

Hoeflich, K. P., Luo, J., Rubie, E. A., Tsao, M. S., Jin, O., & Woodgett, J. R. (2000). Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. *Nature, 406*(6791), 86-90. doi:10.1038/35017574

Hoffmann, C., Dietrich, M., Herrmann, A. K., Schacht, T., Albrecht, P., & Methner, A. (2017). Dimethyl Fumarate Induces Glutathione Recycling by Upregulation of Glutathione Reductase. *Oxid Med Cell Longev, 2017*, 6093903. doi:10.1155/2017/6093903

Hoftberger, R., Kunze, M., Weinhofer, I., Aboul-Enein, F., Voigtlander, T., Oezen, I., . . . Berger, J. (2007). Distribution and cellular localization of adrenoleukodystrophy protein in human tissues: implications for X-linked adrenoleukodystrophy. *Neurobiol Dis, 28*(2), 165-174. doi:10.1016/j.nbd.2007.07.007

Holmstrom, K. M., Baird, L., Zhang, Y., Hargreaves, I., Chalasani, A., Land, J. M., . . . Abramov, A. Y. (2013). Nrf2 impacts cellular bioenergetics by controlling

substrate availability for mitochondrial respiration. *Biol Open, 2*(8), 761-770. doi:10.1242/bio.20134853

Holmstrom, K. M., & Finkel, T. (2014). Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nat Rev Mol Cell Biol, 15*(6), 411-421. doi:10.1038/nrm3801

Holtz, W. A., Turetzky, J. M., Jong, Y. J., & O'Malley, K. L. (2006). Oxidative stress-triggered unfolded protein response is upstream of intrinsic cell death evoked by parkinsonian mimetics. *J Neurochem, 99*(1), 54-69. doi:10.1111/j.1471-4159.2006.04025.x

Holzerova, E., Danhauser, K., Haack, T. B., Kremer, L. S., Melcher, M., Ingold, I., . . . Distelmaier, F. (2016). Human thioredoxin 2 deficiency impairs mitochondrial redox homeostasis and causes early-onset neurodegeneration. *Brain, 139*(Pt 2), 346-354. doi:10.1093/brain/awv350

Holzinger, A., Kammerer, S., Berger, J., & Roscher, A. A. (1997). cDNA cloning and mRNA expression of the human adrenoleukodystrophy related protein (ALDRP), a peroxisomal ABC transporter. *Biochem Biophys Res Commun, 239*(1), 261-264. doi:10.1006/bbrc.1997.7391

Holzinger, A., Mayerhofer, P., Berger, J., Lichtner, P., Kammerer, S., & Roscher, A. A. (1999). Full length cDNA cloning, promoter sequence, and genomic organization of the human adrenoleukodystrophy related (ALDR) gene functionally redundant to the gene responsible for X-linked adrenoleukodystrophy. *Biochem Biophys Res Commun, 258*(2), 436-442. doi:10.1006/bbrc.1999.0535

Hoozemans, J. J., van Haastert, E. S., Eikelenboom, P., de Vos, R. A., Rozemuller, J. M., & Scheper, W. (2007). Activation of the unfolded protein response in Parkinson's disease. *Biochem Biophys Res Commun, 354*(3), 707-711. doi:10.1016/j.bbrc.2007.01.043

Hoozemans, J. J., Veerhuis, R., Van Haastert, E. S., Rozemuller, J. M., Baas, F., Eikelenboom, P., & Scheper, W. (2005). The unfolded protein response is activated in Alzheimer's disease. *Acta Neuropathol, 110*(2), 165-172. doi:10.1007/s00401-005-1038-0

Hou, N. S., Gutschmidt, A., Choi, D. Y., Pather, K., Shi, X., Watts, J. L., . . . Taubert, S. (2014). Activation of the endoplasmic reticulum unfolded protein response by lipid disequilibrium without disturbed proteostasis in vivo. *Proc Natl Acad Sci U S A, 111*(22), E2271-2280. doi:10.1073/pnas.1318262111

Huang, J., Viswakarma, N., Yu, S., Jia, Y., Bai, L., Vluggens, A., . . . Reddy, J. K. (2011). Progressive endoplasmic reticulum stress contributes to hepatocarcinogenesis in fatty acyl-CoA oxidase 1-deficient mice. *Am J Pathol, 179*(2), 703-713. doi:10.1016/j.ajpath.2011.04.030

Huang, S., Wang, Y., Gan, X., Fang, D., Zhong, C., Wu, L., . . . Yan, S. S. (2015). Drp1-mediated mitochondrial abnormalities link to synaptic injury in diabetes model. *Diabetes, 64*(5), 1728-1742. doi:10.2337/db14-0758

Hubbard, W. C., Moser, A. B., Liu, A. C., Jones, R. O., Steinberg, S. J., Lorey, F., . . . Raymond, G. V. (2009). Newborn screening for X-linked adrenoleukodystrophy (X-ALD): validation of a combined liquid chromatography-tandem mass spectrometric (LC-MS/MS) method. *Mol Genet Metab, 97*(3), 212-220. doi:10.1016/j.ymgme.2009.03.010

Hubbs, A. F., Benkovic, S. A., Miller, D. B., O'Callaghan, J. P., Battelli, L., Schwegler-Berry, D., & Ma, Q. (2007). Vacuolar leukoencephalopathy with widespread

astrogliosis in mice lacking transcription factor Nrf2. *Am J Pathol, 170*(6), 2068-2076. doi:10.2353/ajpath.2007.060898

Hughes, K., Nikolakaki, E., Plyte, S. E., Totty, N. F., & Woodgett, J. R. (1993). Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. *Embo J, 12*(2), 803-808.

Huq, M. D., Gupta, P., Tsai, N. P., White, R., Parker, M. G., & Wei, L. N. (2006). Suppression of receptor interacting protein 140 repressive activity by protein arginine methylation. *Embo J, 25*(21), 5094-5104. doi:10.1038/sj.emboj.7601389

Huq, M. D., & Wei, L. N. (2005). Post-translational modification of nuclear co-repressor receptor-interacting protein 140 by acetylation. *Mol Cell Proteomics, 4*(7), 975-983. doi:10.1074/mcp.M500015-MCP200

Hwang, C., Sinskey, A. J., & Lodish, H. F. (1992). Oxidized redox state of glutathione in the endoplasmic reticulum. *Science, 257*(5076), 1496-1502.

Hyun, D. H., Lee, M., Halliwell, B., & Jenner, P. (2003). Proteasomal inhibition causes the formation of protein aggregates containing a wide range of proteins, including nitrated proteins. *J Neurochem, 86*(2), 363-373.

Igarashi, M., Schaumburg, H. H., Powers, J., Kishmoto, Y., Kolodny, E., & Suzuki, K. (1976). Fatty acid abnormality in adrenoleukodystrophy. *J Neurochem, 26*(4), 851-860.

Iida, K., Itoh, K., Kumagai, Y., Oyasu, R., Hattori, K., Kawai, K., . . . Yamamoto, M. (2004). Nrf2 is essential for the chemopreventive efficacy of oltipraz against urinary bladder carcinogenesis. *Cancer Res, 64*(18), 6424-6431. doi:10.1158/0008-5472.CAN-04-1906

Ilieva, E. V., Ayala, V., Jove, M., Dalfo, E., Cacabelos, D., Povedano, M., . . . Portero-Otin, M. (2007). Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. *Brain, 130*(Pt 12), 3111-3123. doi:10.1093/brain/awm190

Imai, S., Armstrong, C. M., Kaeberlein, M., & Guarente, L. (2000). Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature, 403*(6771), 795-800. doi:10.1038/35001622

Innamorato, N. G., Rojo, A. I., Garcia-Yague, A. J., Yamamoto, M., de Ceballos, M. L., & Cuadrado, A. (2008). The Transcription Factor Nrf2 Is a Therapeutic Target against Brain Inflammation. *The Journal of Immunology, 181*(1), 680-689. doi:10.4049/jimmunol.181.1.680

International Multiple Sclerosis Genetics, C., Wellcome Trust Case Control, C., Sawcer, S., Hellenthal, G., Pirinen, M., Spencer, C. C., . . . Compston, A. (2011). Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature, 476*(7359), 214-219. doi:10.1038/nature10251

Ito, M., Blumberg, B. M., Mock, D. J., Goodman, A. D., Moser, A. B., Moser, H. W., . . . Powers, J. M. (2001). Potential environmental and host participants in the early white matter lesion of adreno-leukodystrophy: morphologic evidence for CD8 cytotoxic T cells, cytolysis of oligodendrocytes, and CD1-mediated lipid antigen presentation. *J Neuropathol Exp Neurol, 60*(10), 1004-1019.

Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., . . . Nabeshima, Y. (1997). An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochem Biophys Res Commun, 236*(2), 313-322. doi:10.1006/bbrc.1997.6943

Itoh, K., Igarashi, K., Hayashi, N., Nishizawa, M., & Yamamoto, M. (1995). Cloning and characterization of a novel erythroid cell-derived CNC family transcription

factor heterodimerizing with the small Maf family proteins. *Mol Cell Biol, 15*(8), 4184-4193.

Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T., Igarashi, K., Engel, J. D., & Yamamoto, M. (1999). Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. *Genes Dev., 13*(1), 76-86. doi:10.1101/gad.13.1.76

Izzo, A., Manco, R., Bonfiglio, F., Cali, G., De Cristofaro, T., Patergnani, S., . . . Nitsch, L. (2014). NRIP1/RIP140 siRNA-mediated attenuation counteracts mitochondrial dysfunction in Down syndrome. *Hum Mol Genet, 23*(16), 4406-4419. doi:10.1093/hmg/ddu157

Jakel, R. J., Townsend, J. A., Kraft, A. D., & Johnson, J. A. (2007). Nrf2-mediated protection against 6-hydroxydopamine. *Brain Res, 1144*, 192-201. doi:10.1016/j.brainres.2007.01.131

Jang, J., Park, S., Jin Hur, H., Cho, H. J., Hwang, I., Pyo Kang, Y., . . . Kim, D. W. (2016). 25-hydroxycholesterol contributes to cerebral inflammation of X-linked adrenoleukodystrophy through activation of the NLRP3 inflammasome. *Nat Commun, 7*, 13129. doi:10.1038/ncomms13129

Jang, Y. C., Lustgarten, M. S., Liu, Y., Muller, F. L., Bhattacharya, A., Liang, H., . . . Van Remmen, H. (2010). Increased superoxide in vivo accelerates age-associated muscle atrophy through mitochondrial dysfunction and neuromuscular junction degeneration. *Faseb J, 24*(5), 1376-1390. doi:10.1096/fj.09-146308

Jensen, P. K. (1966). Antimycin-insensitive oxidation of succinate and reduced nicotinamide-adenine dinucleotide in electron-transport particles. I. pH dependency and hydrogen peroxide formation. *Biochim Biophys Acta, 122*(2), 157-166.

Jeong, H., Cohen, D. E., Cui, L., Supinski, A., Savas, J. N., Mazzulli, J. R., . . . Krainc, D. (2011). Sirt1 mediates neuroprotection from mutant huntingtin by activation of the TORC1 and CREB transcriptional pathway. *Nat Med, 18*(1), 159-165. doi:10.1038/nm.2559

Jiang, M., Wang, J., Fu, J., Du, L., Jeong, H., West, T., . . . Duan, W. (2011). Neuroprotective role of Sirt1 in mammalian models of Huntington's disease through activation of multiple Sirt1 targets. *Nat Med, 18*(1), 153-158. doi:10.1038/nm.2558

Jing, X., Shi, H., Zhang, C., Ren, M., Han, M., Wei, X., . . . Lou, H. (2015). Dimethyl fumarate attenuates 6-OHDA-induced neurotoxicity in SH-SY5Y cells and in animal model of Parkinson's disease by enhancing Nrf2 activity. *Neuroscience, 286*, 131-140. doi:10.1016/j.neuroscience.2014.11.047

Johnson, D. A., Amirahmadi, S., Ward, C., Fabry, Z., & Johnson, J. A. (2010). The absence of the pro-antioxidant transcription factor Nrf2 exacerbates experimental autoimmune encephalomyelitis. *Toxicol Sci, 114*(2), 237-246. doi:10.1093/toxsci/kfp274

Johnson, D. A., & Johnson, J. A. (2015). Nrf2--a therapeutic target for the treatment of neurodegenerative diseases. *Free Radic Biol Med, 88*(Pt B), 253-267. doi:10.1016/j.freeradbiomed.2015.07.147

Johri, A., & Beal, M. F. (2012a). Mitochondrial dysfunction in neurodegenerative diseases. *J Pharmacol Exp Ther, 342*(3), 619-630. doi:10.1124/jpet.112.192138

Johri, A., Calingasan, N. Y., Hennessey, T. M., Sharma, A., Yang, L., Wille, E., . . . Beal, M. F. (2012b). Pharmacologic activation of mitochondrial biogenesis exerts widespread beneficial effects in a transgenic mouse model of

Huntington's disease. *Hum Mol Genet, 21*(5), 1124-1137. doi:10.1093/hmg/ddr541

Joyeux, A., Cavailles, V., Balaguer, P., & Nicolas, J. C. (1997). RIP 140 enhances nuclear receptor-dependent transcription in vivo in yeast. *Mol Endocrinol, 11*(2), 193-202. doi:10.1210/mend.11.2.9884

Junn, E., Han, S. H., Im, J. Y., Yang, Y., Cho, E. W., Um, H. D., . . . Choi, I. (2000). Vitamin D3 up-regulated protein 1 mediates oxidative stress via suppressing the thioredoxin function. *J Immunol, 164*(12), 6287-6295.

Kaidanovich-Beilin, O., Lipina, T. V., Takao, K., van Eede, M., Hattori, S., Laliberte, C., . . . Woodgett, J. R. (2009). Abnormalities in brain structure and behavior in GSK-3alpha mutant mice. *Mol Brain, 2*, 35. doi:10.1186/1756-6606-2-35

Kaidery, N. A., Banerjee, R., Yang, L., Smirnova, N. A., Hushpulian, D. M., Liby, K. T., . . . Thomas, B. (2013). Targeting Nrf2-mediated gene transcription by extremely potent synthetic triterpenoids attenuate dopaminergic neurotoxicity in the MPTP mouse model of Parkinson's disease. *Antioxid Redox Signal, 18*(2), 139-157. doi:10.1089/ars.2011.4491

Kamat, C. D., Gadal, S., Mhatre, M., Williamson, K. S., Pye, Q. N., & Hensley, K. (2008). Antioxidants in central nervous system diseases: preclinical promise and translational challenges. *J Alzheimers Dis, 15*(3), 473-493.

Kamijo, K., Taketani, S., Yokota, S., Osumi, T., & Hashimoto, T. (1990). The 70-kDa peroxisomal membrane protein is a member of the Mdr (P-glycoprotein)-related ATP-binding protein superfamily. *J Biol Chem, 265*(8), 4534-4540.

Kang, K. W., Choi, S. H., & Kim, S. G. (2002). Peroxynitrite activates NF-E2-related factor 2/antioxidant response element through the pathway of phosphatidylinositol 3-kinase: the role of nitric oxide synthase in rat glutathione S-transferase A2 induction. *Nitric Oxide, 7*(4), 244-253.

Kang, K. W., Ryu, J. H., & Kim, S. G. (2000). The essential role of phosphatidylinositol 3-kinase and of p38 mitogen-activated protein kinase activation in the antioxidant response element-mediated rGSTA2 induction by decreased glutathione in H4IIE hepatoma cells. *Mol Pharmacol, 58*(5), 1017-1025.

Kanninen, K., Heikkinen, R., Malm, T., Rolova, T., Kuhmonen, S., Leinonen, H., . . . Koistinaho, J. (2009). Intrahippocampal injection of a lentiviral vector expressing Nrf2 improves spatial learning in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A, 106*(38), 16505-16510. doi:10.1073/pnas.0908397106

Kanninen, K., Malm, T. M., Jyrkkanen, H. K., Goldsteins, G., Keksa-Goldsteine, V., Tanila, H., . . . Koistinaho, J. (2008). Nuclear factor erythroid 2-related factor 2 protects against beta amyloid. *Mol Cell Neurosci, 39*(3), 302-313. doi:10.1016/j.mcn.2008.07.010

Kapeta, S., Chondrogianni, N., & Gonos, E. S. (2010). Nuclear erythroid factor 2-mediated proteasome activation delays senescence in human fibroblasts. *J Biol Chem, 285*(11), 8171-8184. doi:10.1074/jbc.M109.031575

Kappos, L., Gold, R., Miller, D. H., Macmanus, D. G., Havrdova, E., Limmroth, V., . . . Investigators, B. G. P. I. S. (2008). Efficacy and safety of oral fumarate in patients with relapsing-remitting multiple sclerosis: a multicentre, randomised, double-blind, placebo-controlled phase IIb study. *Lancet, 372*(9648), 1463-1472. doi:10.1016/S0140-6736(08)61619-0

Kashiwayama, Y., Seki, M., Yasui, A., Murasaki, Y., Morita, M., Yamashita, Y., . . . Imanaka, T. (2009). 70-kDa peroxisomal membrane protein related protein (P70R/ABCD4) localizes to endoplasmic reticulum not peroxisomes, and NH2-

terminal hydrophobic property determines the subcellular localization of ABC subfamily D proteins. *Exp Cell Res, 315*(2), 190-205. doi:10.1016/j.yexcr.2008.10.031

Katsouri, L., Blondrath, K., & Sastre, M. (2012). Peroxisome proliferator-activated receptor-gamma cofactors in neurodegeneration. *IUBMB Life, 64*(12), 958-964. doi:10.1002/iub.1097

Katsouri, L., Lim, Y. M., Blondrath, K., Eleftheriadou, I., Lombardero, L., Birch, A. M., . . . Sastre, M. (2016). PPARgamma-coactivator-1alpha gene transfer reduces neuronal loss and amyloid-beta generation by reducing beta-secretase in an Alzheimer's disease model. *Proc Natl Acad Sci U S A, 113*(43), 12292-12297. doi:10.1073/pnas.1606171113

Katsuoka, F., Motohashi, H., Ishii, T., Aburatani, H., Engel, J. D., & Yamamoto, M. (2005). Genetic evidence that small maf proteins are essential for the activation of antioxidant response element-dependent genes. *Mol Cell Biol, 25*(18), 8044-8051. doi:10.1128/MCB.25.18.8044-8051.2005

Keller, G. A., Warner, T. G., Steimer, K. S., & Hallewell, R. A. (1991). Cu,Zn superoxide dismutase is a peroxisomal enzyme in human fibroblasts and hepatoma cells. *Proc Natl Acad Sci U S A, 88*(16), 7381-7385.

Keller, J. N., Hanni, K. B., & Markesbery, W. R. (2000). Impaired proteasome function in Alzheimer's disease. *J Neurochem, 75*(1), 436-439.

Kemp, S., Berger, J., & Aubourg, P. (2012). X-linked adrenoleukodystrophy: clinical, metabolic, genetic and pathophysiological aspects. *Biochim Biophys Acta, 1822*(9), 1465-1474. doi:10.1016/j.bbadis.2012.03.012

Kemp, S., Ligtenberg, M. J., van Geel, B. M., Barth, P. G., Wolterman, R. A., Schoute, F., . . . Bolhuis, P. A. (1994). Identification of a two base pair deletion in five unrelated families with adrenoleukodystrophy: a possible hot spot for mutations. *Biochem Biophys Res Commun, 202*(2), 647-653.

Kemp, S., Pujol, A., Waterham, H. R., van Geel, B. M., Boehm, C. D., Raymond, G. V., . . . Moser, H. W. (2001). ABCD1 mutations and the X-linked adrenoleukodystrophy mutation database: role in diagnosis and clinical correlations. *Hum Mutat, 18*(6), 499-515. doi:10.1002/humu.1227

Kemp, S., Valianpour, F., Denis, S., Ofman, R., Sanders, R. J., Mooyer, P., . . . Wanders, R. J. (2005). Elongation of very long-chain fatty acids is enhanced in X-linked adrenoleukodystrophy. *Mol Genet Metab, 84*(2), 144-151. doi:10.1016/j.ymgme.2004.09.015

Kemp, S., Wei, H. M., Lu, J. F., Braiterman, L. T., McGuinness, M. C., Moser, A. B., . . . Smith, K. D. (1998). Gene redundancy and pharmacological gene therapy: implications for X-linked adrenoleukodystrophy. *Nat Med, 4*(11), 1261-1268. doi:10.1038/3242

Kemper, A. R., Brosco, J., Comeau, A. M., Green, N. S., Grosse, S. D., Jones, E., . . . Tanksley, S. (2017). Newborn screening for X-linked adrenoleukodystrophy: evidence summary and advisory committee recommendation. *Genet Med, 19*(1), 121-126. doi:10.1038/gim.2016.68

Khan, M., Pahan, K., Singh, A. K., & Singh, I. (1998). Cytokine-induced accumulation of very long-chain fatty acids in rat C6 glial cells: implication for X-adrenoleukodystrophy. *J Neurochem, 71*(1), 78-87.

Khurana, V., Lu, Y., Steinhilb, M. L., Oldham, S., Shulman, J. M., & Feany, M. B. (2006). TOR-mediated cell-cycle activation causes neurodegeneration in a Drosophila tauopathy model. *Curr Biol, 16*(3), 230-241. doi:10.1016/j.cub.2005.12.042

Kim, D., Nguyen, M. D., Dobbin, M. M., Fischer, A., Sananbenesi, F., Rodgers, J. T., . . . Tsai, L. H. (2007). SIRT1 deacetylase protects against neurodegeneration in models for Alzheimer's disease and amyotrophic lateral sclerosis. *Embo J, 26*(13), 3169-3179. doi:10.1038/sj.emboj.7601758

Kim, W. Y., Wang, X., Wu, Y., Doble, B. W., Patel, S., Woodgett, J. R., & Snider, W. D. (2009). GSK-3 is a master regulator of neural progenitor homeostasis. *Nat Neurosci, 12*(11), 1390-1397. doi:10.1038/nn.2408

Kitada, T., Asakawa, S., Hattori, N., Matsumine, H., Yamamura, Y., Minoshima, S., . . . Shimizu, N. (1998). Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. *Nature, 392*(6676), 605-608. doi:10.1038/33416

Klein, J. A., & Ackerman, S. L. (2003). Oxidative stress, cell cycle, and neurodegeneration. *J Clin Invest, 111*(6), 785-793. doi:10.1172/JCI18182

Klein, P. S., & Melton, D. A. (1996). A molecular mechanism for the effect of lithium on development. *Proc Natl Acad Sci U S A, 93*(16), 8455-8459.

Knazek, R. A., Rizzo, W. B., Schulman, J. D., & Dave, J. R. (1983). Membrane microviscosity is increased in the erythrocytes of patients with adrenoleukodystrophy and adrenomyeloneuropathy. *J Clin Invest, 72*(1), 245-248.

Kobayashi, A., Kang, M. I., Okawa, H., Ohtsuji, M., Zenke, Y., Chiba, T., . . . Yamamoto, M. (2004). Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol Cell Biol, 24*(16), 7130-7139. doi:10.1128/MCB.24.16.7130-7139.2004

Kobayashi, K., Tomiki, H., Inaba, Y., Ichikawa, M., Kim, B. S., & Koh, C. S. (2015). Dimethyl fumarate suppresses Theiler's murine encephalomyelitis virus-induced demyelinating disease by modifying the Nrf2-Keap1 pathway. *Int Immunol, 27*(7), 333-344. doi:10.1093/intimm/dxv006

Kobayashi, T., Shinnoh, N., Kondo, A., & Yamada, T. (1997). Adrenoleukodystrophy protein-deficient mice represent abnormality of very long chain fatty acid metabolism. *Biochem.Biophys.Res.Commun., 232*(3), 631-636.

Koch, A., Thiemann, M., Grabenbauer, M., Yoon, Y., McNiven, M. A., & Schrader, M. (2003). Dynamin-like protein 1 is involved in peroxisomal fission. *J Biol Chem, 278*(10), 8597-8605. doi:10.1074/jbc.M211761200

Kojo, S. (2012). Oxygen is the key factor associated with the difference between in vivo and in vitro effects of antioxidants. *Proc Natl Acad Sci U S A, 109*(30), E2028; author reply E2029. doi:10.1073/pnas.1205916109

Komatsu, M., Kurokawa, H., Waguri, S., Taguchi, K., Kobayashi, A., Ichimura, Y., . . . Yamamoto, M. (2010). The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol, 12*(3), 213-223. doi:10.1038/ncb2021

Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., . . . Tanaka, K. (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature, 441*(7095), 880-884. doi:10.1038/nature04723

Korenke, G. C., Christen, H. J., Kruse, B., Hunneman, D. H., & Hanefeld, F. (1997). Progression of X-linked adrenoleukodystrophy under interferon-beta therapy. *J Inherit Metab Dis, 20*(1), 59-66.

Korenke, G. C., Fuchs, S., Krasemann, E., Doerr, H. G., Wilichowski, E., Hunneman, D. H., & Hanefeld, F. (1996). Cerebral adrenoleukodystrophy (ALD) in only one of monozygotic twins with an identical ALD genotype. *Ann Neurol, 40*(2), 254-257. doi:10.1002/ana.410400221

Korhonen, J. A., Pham, X. H., Pellegrini, M., & Falkenberg, M. (2004). Reconstitution of a minimal mtDNA replisome in vitro. *Embo J, 23*(12), 2423-2429. doi:10.1038/sj.emboj.7600257

Kovacs, W. J., Charles, K. N., Walter, K. M., Shackelford, J. E., Wikander, T. M., Richards, M. J., . . . Faust, P. L. (2012). Peroxisome deficiency-induced ER stress and SREBP-2 pathway activation in the liver of newborn PEX2 knock-out mice. *Biochim Biophys Acta, 1821*(6), 895-907. doi:10.1016/j.bbalip.2012.02.011

Kraft, A. D., Johnson, D. A., & Johnson, J. A. (2004). Nuclear factor E2-related factor 2-dependent antioxidant response element activation by tert-butylhydroquinone and sulforaphane occurring preferentially in astrocytes conditions neurons against oxidative insult. *J Neurosci, 24*(5), 1101-1112. doi:10.1523/JNEUROSCI.3817-03.2004

Krassioukov, A. V., Ackery, A., Schwartz, G., Adamchik, Y., Liu, Y., & Fehlings, M. G. (2002). An in vitro model of neurotrauma in organotypic spinal cord cultures from adult mice. *Brain Res Brain Res Protoc, 10*(2), 60-68.

Kruska, N., Schonfeld, P., Pujol, A., & Reiser, G. (2015). Astrocytes and mitochondria from adrenoleukodystrophy protein (ABCD1)-deficient mice reveal that the adrenoleukodystrophy-associated very long-chain fatty acids target several cellular energy-dependent functions. *Biochim Biophys Acta, 1852*(5), 925-936. doi:10.1016/j.bbadis.2015.01.005

Kryscio, R. J., Abner, E. L., Caban-Holt, A., Lovell, M., Goodman, P., Darke, A. K., . . . Schmitt, F. A. (2017). Association of Antioxidant Supplement Use and Dementia in the Prevention of Alzheimer's Disease by Vitamin E and Selenium Trial (PREADViSE). *JAMA Neurol*. doi:10.1001/jamaneurol.2016.5778

Kwak, M. K., Wakabayashi, N., Itoh, K., Motohashi, H., Yamamoto, M., & Kensler, T. W. (2003). Modulation of gene expression by cancer chemopreventive dithiolethiones through the Keap1-Nrf2 pathway. Identification of novel gene clusters for cell survival. *J Biol Chem, 278*(10), 8135-8145. doi:10.1074/jbc.M211898200

Lalonde, R., & Strazielle, C. (2011). Brain regions and genes affecting limb-clasping responses. *Brain Res Rev, 67*(1-2), 252-259. doi:10.1016/j.brainresrev.2011.02.005

Landi, L., Fiorentini, D., Galli, M. C., Segura-Aguilar, J., & Beyer, R. E. (1997). DT-Diaphorase maintains the reduced state of ubiquinones in lipid vesicles thereby promoting their antioxidant function. *Free Radic Biol Med, 22*(1-2), 329-335.

Lannuzel, A., Aubourg, P., & Tardieu, M. (1998). Excessive production of tumour necrosis factor alpha by peripheral blood mononuclear cells in X-linked adrenoleukodystrophy. *Eur J Paediatr Neurol, 2*(1), 27-32.

Larsson, N. G., Wang, J., Wilhelmsson, H., Oldfors, A., Rustin, P., Lewandoski, M., . . . Clayton, D. A. (1998). Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nat Genet, 18*(3), 231-236. doi:10.1038/ng0398-231

Lastres-Becker, I., Garcia-Yague, A. J., Scannevin, R. H., Casarejos, M. J., Kugler, S., Rabano, A., & Cuadrado, A. (2016). Repurposing the NRF2 Activator Dimethyl Fumarate as Therapy Against Synucleinopathy in Parkinson's Disease. *Antioxid Redox Signal, 25*(2), 61-77. doi:10.1089/ars.2015.6549

Lastres-Becker, I., Ulusoy, A., Innamorato, N. G., Sahin, G., Rabano, A., Kirik, D., & Cuadrado, A. (2012). alpha-Synuclein expression and Nrf2 deficiency cooperate to aggravate protein aggregation, neuronal death and inflammation in early-stage

Parkinson's disease. *Hum Mol Genet, 21*(14), 3173-3192. doi:10.1093/hmg/dds143

Lau, A., Tian, W., Whitman, S. A., & Zhang, D. D. (2013). The predicted molecular weight of Nrf2: it is what it is not. *Antioxid Redox Signal, 18*(1), 91-93. doi:10.1089/ars.2012.4754

Launay, N., Aguado, C., Fourcade, S., Ruiz, M., Grau, L., Riera, J., . . . Pujol, A. (2015). Autophagy induction halts axonal degeneration in a mouse model of X-adrenoleukodystrophy. *Acta Neuropathol, 129*(3), 399-415. doi:10.1007/s00401-014-1378-8

Launay, N., Ruiz, M., Fourcade, S., Schluter, A., Guilera, C., Ferrer, I., . . . Pujol, A. (2013). Oxidative stress regulates the ubiquitin-proteasome system and immunoproteasome functioning in a mouse model of X-adrenoleukodystrophy. *Brain, 136*(Pt 3), 891-904. doi:10.1093/brain/aws370

Launay, N., Ruiz, M., Grau, L., Ortega, F. J., Ilieva, E. V., Martinez, J. J., . . . Fourcade, S. (2017). Tauroursodeoxycholic bile acid arrests axonal degeneration by inhibiting the unfolded protein response in X-linked adrenoleukodystrophy. *Acta Neuropathol, 133*(2), 283-301. doi:10.1007/s00401-016-1655-9

Lazarow, P. B., & De Duve, C. (1976). A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc Natl Acad Sci U S A, 73*(6), 2043-2046.

Lazo, O., Contreras, M., Hashmi, M., Stanley, W., Irazu, C., & Singh, I. (1988). Peroxisomal lignoceroyl-CoA ligase deficiency in childhood adrenoleukodystrophy and adrenomyeloneuropathy. *Proc Natl Acad Sci U S A, 85*(20), 7647-7651.

Lebovitz, R. M., Zhang, H., Vogel, H., Cartwright, J., Jr., Dionne, L., Lu, N., . . . Matzuk, M. M. (1996). Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc Natl Acad Sci U S A, 93*(18), 9782-9787.

Lee, J. H., Yu, W. H., Kumar, A., Lee, S., Mohan, P. S., Peterhoff, C. M., . . . Nixon, R. A. (2010). Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. *Cell, 141*(7), 1146-1158. doi:10.1016/j.cell.2010.05.008

Lee, J. M., Calkins, M. J., Chan, K., Kan, Y. W., & Johnson, J. A. (2003). Identification of the NF-E2-related factor-2-dependent genes conferring protection against oxidative stress in primary cortical astrocytes using oligonucleotide microarray analysis. *J Biol Chem, 278*(14), 12029-12038. doi:10.1074/jbc.M211558200

Lee, Y. S., Kennedy, W. D., & Yin, Y. W. (2009). Structural insight into processive human mitochondrial DNA synthesis and disease-related polymerase mutations. *Cell, 139*(2), 312-324. doi:10.1016/j.cell.2009.07.050

Lehr, H. A., Vajkoczy, P., Menger, M. D., & Arfors, K. E. (1999). Do vitamin E supplements in diets for laboratory animals jeopardize findings in animal models of disease? *Free Radic Biol Med, 26*(3-4), 472-481.

Leonardsson, G., Steel, J. H., Christian, M., Pocock, V., Milligan, S., Bell, J., . . . Parker, M. G. (2004). Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc Natl Acad Sci U S A, 101*(22), 8437-8442. doi:10.1073/pnas.0401013101

Leone, T. C., Lehman, J. J., Finck, B. N., Schaeffer, P. J., Wende, A. R., Boudina, S., . . . Kelly, D. P. (2005). PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis. *PLoS Biol, 3*(4), e101. doi:10.1371/journal.pbio.0030101

Li, P., Fan, W., Xu, J., Lu, M., Yamamoto, H., Auwerx, J., . . . Olefsky, J. M. (2011). Adipocyte NCoR knockout decreases PPARgamma phosphorylation and enhances PPARgamma activity and insulin sensitivity. *Cell, 147*(4), 815-826. doi:10.1016/j.cell.2011.09.050

Li, R., Bianchet, M. A., Talalay, P., & Amzel, L. M. (1995). The three-dimensional structure of NAD(P)H:quinone reductase, a flavoprotein involved in cancer chemoprotection and chemotherapy: mechanism of the two-electron reduction. *Proc Natl Acad Sci U S A, 92*(19), 8846-8850.

Li, X., Zhang, D., Hannink, M., & Beamer, L. J. (2004). Crystal structure of the Kelch domain of human Keap1. *J Biol Chem, 279*(52), 54750-54758. doi:10.1074/jbc.M410073200

Li, Y. C., & Gao, W. J. (2011). GSK-3beta activity and hyperdopamine-dependent behaviors. *Neurosci Biobehav Rev, 35*(3), 645-654. doi:10.1016/j.neubiorev.2010.08.001

Licht-Mayer, S., Wimmer, I., Traffehn, S., Metz, I., Bruck, W., Bauer, J., . . . Lassmann, H. (2015). Cell type-specific Nrf2 expression in multiple sclerosis lesions. *Acta Neuropathol, 130*(2), 263-277. doi:10.1007/s00401-015-1452-x

Licht-Murava, A., Paz, R., Vaks, L., Avrahami, L., Plotkin, B., Eisenstein, M., & Eldar-Finkelman, H. (2016). A unique type of GSK-3 inhibitor brings new opportunities to the clinic. *Sci Signal, 9*(454), ra110. doi:10.1126/scisignal.aah7102

Lillig, C. H., Berndt, C., & Holmgren, A. (2008). Glutaredoxin systems. *Biochim Biophys Acta, 1780*(11), 1304-1317. doi:10.1016/j.bbagen.2008.06.003

Lim, A. C., & Qi, R. Z. (2003). Cyclin-dependent kinases in neural development and degeneration. *J Alzheimers Dis, 5*(4), 329-335.

Lin, J., Puigserver, P., Donovan, J., Tarr, P., & Spiegelman, B. M. (2002). Peroxisome proliferator-activated receptor gamma coactivator 1beta (PGC-1beta), a novel PGC-1-related transcription coactivator associated with host cell factor. *J Biol Chem, 277*(3), 1645-1648. doi:10.1074/jbc.C100631200

Lin, J., Wu, P. H., Tarr, P. T., Lindenberg, K. S., St-Pierre, J., Zhang, C. Y., . . . Spiegelman, B. M. (2004). Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell, 119*(1), 121-135. doi:10.1016/j.cell.2004.09.013

Lin, M. T., & Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature, 443*(7113), 787-795. doi:10.1038/nature05292

Lin, S. X., Lisi, L., Dello Russo, C., Polak, P. E., Sharp, A., Weinberg, G., . . . Feinstein, D. L. (2011). The anti-inflammatory effects of dimethyl fumarate in astrocytes involve glutathione and haem oxygenase-1. *ASN Neuro, 3*(2). doi:10.1042/AN20100033

Lin, W., & Popko, B. (2009). Endoplasmic reticulum stress in disorders of myelinating cells. *Nat Neurosci, 12*(4), 379-385. doi:10.1038/nn.2273

Lin, Y. W., Lee, B., Liu, P. S., & Wei, L. N. (2016). Receptor-Interacting Protein 140 Orchestrates the Dynamics of Macrophage M1/M2 Polarization. *J Innate Immun, 8*(1), 97-107. doi:10.1159/000433539

Lindholm, D., Wootz, H., & Korhonen, L. (2006). ER stress and neurodegenerative diseases. *Cell Death Differ, 13*(3), 385-392. doi:10.1038/sj.cdd.4401778

Linker, R. A., & Haghikia, A. (2016). Dimethyl fumarate in multiple sclerosis: latest developments, evidence and place in therapy. *Ther Adv Chronic Dis, 7*(4), 198-207. doi:10.1177/2040622316653307

Linker, R. A., Lee, D. H., Ryan, S., van Dam, A. M., Conrad, R., Bista, P., . . . Gold, R. (2011). Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain, 134*(Pt 3), 678-692. doi:10.1093/brain/awq386

Linnebank, M., Kemp, S., Wanders, R. J., Kleijer, W. J., van der Sterre, M. L., Gartner, J., . . . Wullner, U. (2006). Methionine metabolism and phenotypic variability in X-linked adrenoleukodystrophy. *Neurology, 66*(3), 442-443. doi:10.1212/01.wnl.0000196491.42058.6f

Litjens, N. H., Rademaker, M., Ravensbergen, B., Rea, D., van der Plas, M. J., Thio, B., . . . Nibbering, P. H. (2004). Monomethylfumarate affects polarization of monocyte-derived dendritic cells resulting in down-regulated Th1 lymphocyte responses. *Eur J Immunol, 34*(2), 565-575. doi:10.1002/eji.200324174

Liu, K. J., Arron, J. R., Stankunas, K., Crabtree, G. R., & Longaker, M. T. (2007). Chemical rescue of cleft palate and midline defects in conditional GSK-3beta mice. *Nature, 446*(7131), 79-82. doi:10.1038/nature05557

Liu, L. X., Janvier, K., Berteaux-Lecellier, V., Cartier, N., Benarous, R., & Aubourg, P. (1999). Homo- and heterodimerization of peroxisomal ATP-binding cassette half-transporters. *J Biol Chem, 274*(46), 32738-32743.

Liu, P. S., Lin, Y. W., Burton, F. H., & Wei, L. N. (2015). Injecting engineered anti-inflammatory macrophages therapeutically induces white adipose tissue browning and improves diet-induced insulin resistance. *Adipocyte, 4*(2), 123-128. doi:10.4161/21623945.2014.981438

Liu, P. S., Lin, Y. W., Lee, B., McCrady-Spitzer, S. K., Levine, J. A., & Wei, L. N. (2014). Reducing RIP140 expression in macrophage alters ATM infiltration, facilitates white adipose tissue browning, and prevents high-fat diet-induced insulin resistance. *Diabetes, 63*(12), 4021-4031. doi:10.2337/db14-0619

Llorens-Martin, M., Jurado, J., Hernandez, F., & Avila, J. (2014). GSK-3beta, a pivotal kinase in Alzheimer disease. *Front Mol Neurosci, 7*, 46. doi:10.3389/fnmol.2014.00046

Loes, D. J., Fatemi, A., Melhem, E. R., Gupte, N., Bezman, L., Moser, H. W., & Raymond, G. V. (2003). Analysis of MRI patterns aids prediction of progression in X-linked adrenoleukodystrophy. *Neurology, 61*(3), 369-374.

Loes, D. J., Hite, S., Moser, H., Stillman, A. E., Shapiro, E., Lockman, L., . . . Krivit, W. (1994). Adrenoleukodystrophy: a scoring method for brain MR observations. *AJNR Am J Neuroradiol, 15*(9), 1761-1766.

Loewe, R., Holnthoner, W., Groger, M., Pillinger, M., Gruber, F., Mechtcheriakova, D., . . . Petzelbauer, P. (2002). Dimethylfumarate inhibits TNF-induced nuclear entry of NF-kappa B/p65 in human endothelial cells. *J Immunol, 168*(9), 4781-4787.

Lombard-Platet, G., Savary, S., Sarde, C. O., Mandel, J. L., & Chimini, G. (1996). A close relative of the adrenoleukodystrophy (ALD) gene codes for a peroxisomal protein with a specific expression pattern. *Proc Natl Acad Sci U S A, 93*(3), 1265-1269.

Longbrake, E. E., & Cross, A. H. (2015). Dimethyl fumarate associated lymphopenia in clinical practice. *Mult Scler, 21*(6), 796-797. doi:10.1177/1352458514559299

Longbrake, E. E., Ramsbottom, M. J., Cantoni, C., Ghezzi, L., Cross, A. H., & Piccio, L. (2016). Dimethyl fumarate selectively reduces memory T cells in multiple sclerosis patients. *Mult Scler, 22*(8), 1061-1070. doi:10.1177/1352458515608961

Lopez-Erauskin, J., Fourcade, S., Galino, J., Ruiz, M., Schluter, A., Naudi, A., . . . Pujol, A. (2011). Antioxidants halt axonal degeneration in a mouse model of X-adrenoleukodystrophy. *Ann Neurol, 70*(1), 84-92. doi:10.1002/ana.22363

Lopez-Erauskin, J., Galino, J., Bianchi, P., Fourcade, S., Andreu, A. L., Ferrer, I., . . . Pujol, A. (2012). Oxidative stress modulates mitochondrial failure and cyclophilin D function in X-linked adrenoleukodystrophy. *Brain, 135*(Pt 12), 3584-3598. doi:10.1093/brain/aws292

Lopez-Erauskin, J., Galino, J., Ruiz, M., Cuezva, J. M., Fabregat, I., Cacabelos, D., . . . Pujol, A. (2013). Impaired mitochondrial oxidative phosphorylation in the peroxisomal disease X-linked adrenoleukodystrophy. *Hum Mol Genet, 22*(16), 3296-3305. doi:10.1093/hmg/ddt186

Lopez, N., Tormo, C., De Blas, I., Llinares, I., & Alom, J. (2013). Oxidative stress in Alzheimer's disease and mild cognitive impairment with high sensitivity and specificity. *J Alzheimers Dis, 33*(3), 823-829. doi:10.3233/JAD-2012-121528

Lovestone, S., Boada, M., Dubois, B., Hull, M., Rinne, J. O., Huppertz, H. J., . . . investigators, A. (2015). A phase II trial of tideglusib in Alzheimer's disease. *J Alzheimers Dis, 45*(1), 75-88. doi:10.3233/JAD-141959

Lu, J. F., Lawler, A. M., Watkins, P. A., Powers, J. M., Moser, A. B., Moser, H. W., & Smith, K. D. (1997). A mouse model for X-linked adrenoleukodystrophy. *Proc Natl Acad Sci U S A, 94*(17), 9366-9371.

Lu, S. C. (2013). Glutathione synthesis. *Biochim Biophys Acta, 1830*(5), 3143-3153. doi:10.1016/j.bbagen.2012.09.008

Ludtmann, M. H., Angelova, P. R., Zhang, Y., Abramov, A. Y., & Dinkova-Kostova, A. T. (2014). Nrf2 affects the efficiency of mitochondrial fatty acid oxidation. *Biochem J, 457*(3), 415-424. doi:10.1042/BJ20130863

Luthman, M., & Holmgren, A. (1982). Rat liver thioredoxin and thioredoxin reductase: purification and characterization. *Biochemistry, 21*(26), 6628-6633.

Ma, Q. (2013). Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol, 53*, 401-426. doi:10.1146/annurev-pharmtox-011112-140320

MacAulay, K., Doble, B. W., Patel, S., Hansotia, T., Sinclair, E. M., Drucker, D. J., . . . Woodgett, J. R. (2007). Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. *Cell Metab, 6*(4), 329-337. doi:10.1016/j.cmet.2007.08.013

MacManus, D. G., Miller, D. H., Kappos, L., Gold, R., Havrdova, E., Limmroth, V., . . . Dawson, K. (2011). BG-12 reduces evolution of new enhancing lesions to T1-hypointense lesions in patients with multiple sclerosis. *J Neurol, 258*(3), 449-456. doi:10.1007/s00415-010-5777-z

Magni, G., Amici, A., Emanuelli, M., Orsomando, G., Raffaelli, N., & Ruggieri, S. (2004). Enzymology of NAD+ homeostasis in man. *Cell Mol Life Sci, 61*(1), 19-34. doi:10.1007/s00018-003-3161-1

Magrane, J., & Manfredi, G. (2009). Mitochondrial function, morphology, and axonal transport in amyotrophic lateral sclerosis. *Antioxid Redox Signal, 11*(7), 1615-1626. doi:10.1089/ARS.2009.2604

Mahmood, A., Raymond, G. V., Dubey, P., Peters, C., & Moser, H. W. (2007). Survival analysis of haematopoietic cell transplantation for childhood cerebral X-linked adrenoleukodystrophy: a comparison study. *Lancet Neurol, 6*(8), 687-692. doi:10.1016/S1474-4422(07)70177-1

Mahmood, D. F., Abderrazak, A., El Hadri, K., Simmet, T., & Rouis, M. (2013). The thioredoxin system as a therapeutic target in human health and disease. *Antioxid Redox Signal, 19*(11), 1266-1303. doi:10.1089/ars.2012.4757

Maier, E. M., Mayerhofer, P. U., Asheuer, M., Kohler, W., Rothe, M., Muntau, A. C., . . . Berger, J. (2008). X-linked adrenoleukodystrophy phenotype is independent of ABCD2 genotype. *Biochem Biophys Res Commun, 377*(1), 176-180. doi:10.1016/j.bbrc.2008.09.092

Mandelkow, E. M., Drewes, G., Biernat, J., Gustke, N., Van Lint, J., Vandenheede, J. R., & Mandelkow, E. (1992). Glycogen synthase kinase-3 and the Alzheimer-like state of microtubule-associated protein tau. *FEBS Lett, 314*(3), 315-321.

Manevich, Y., Feinstein, S. I., & Fisher, A. B. (2004). Activation of the antioxidant enzyme 1-CYS peroxiredoxin requires glutathionylation mediated by heterodimerization with pi GST. *Proc Natl Acad Sci U S A, 101*(11), 3780-3785. doi:10.1073/pnas.0400181101

Martin, D., Rojo, A. I., Salinas, M., Diaz, R., Gallardo, G., Alam, J., . . . Cuadrado, A. (2004). Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol. *J Biol Chem, 279*(10), 8919-8929. doi:10.1074/jbc.M309660200

Martin, D., Salinas, M., Lopez-Valdaliso, R., Serrano, E., Recuero, M., & Cuadrado, A. (2001). Effect of the Alzheimer amyloid fragment Abeta(25-35) on Akt/PKB kinase and survival of PC12 cells. *J Neurochem, 78*(5), 1000-1008.

Mastroeni, R., Bensadoun, J. C., Charvin, D., Aebischer, P., Pujol, A., & Raoul, C. (2009). Insulin-like growth factor-1 and neurotrophin-3 gene therapy prevents motor decline in an X-linked adrenoleukodystrophy mouse model. *Ann Neurol, 66*(1), 117-122. doi:10.1002/ana.21677

Matsukawa, T., Asheuer, M., Takahashi, Y., Goto, J., Suzuki, Y., Shimozawa, N., . . . Tsuji, S. (2011). Identification of novel SNPs of ABCD1, ABCD2, ABCD3, and ABCD4 genes in patients with X-linked adrenoleukodystrophy (ALD) based on comprehensive resequencing and association studies with ALD phenotypes. *Neurogenetics, 12*(1), 41-50. doi:10.1007/s10048-010-0253-6

Matus, S., Glimcher, L. H., & Hetz, C. (2011). Protein folding stress in neurodegenerative diseases: a glimpse into the ER. *Curr Opin Cell Biol, 23*(2), 239-252. doi:10.1016/j.ceb.2011.01.003

McBride, H. M., Neuspiel, M., & Wasiak, S. (2006). Mitochondria: more than just a powerhouse. *Curr Biol, 16*(14), R551-560. doi:10.1016/j.cub.2006.06.054

McGuinness, M. C., Lu, J. F., Zhang, H. P., Dong, G. X., Heinzer, A. K., Watkins, P. A., . . . Smith, K. D. (2003). Role of ALDP (ABCD1) and mitochondria in X-linked adrenoleukodystrophy. *Mol Cell Biol, 23*(2), 744-753.

McGuinness, M. C., Powers, J. M., Bias, W. B., Schmeckpeper, B. J., Segal, A. H., Gowda, V. C., . . . Smith, K. D. (1997). Human leukocyte antigens and cytokine expression in cerebral inflammatory demyelinative lesions of X-linked adrenoleukodystrophy and multiple sclerosis. *J Neuroimmunol, 75*(1-2), 174-182.

McGuinness, M. C., Zhang, H. P., & Smith, K. D. (2001). Evaluation of pharmacological induction of fatty acid beta-oxidation in X-linked adrenoleukodystrophy. *Mol Genet Metab, 74*(1-2), 256-263.

McMahon, M., Itoh, K., Yamamoto, M., Chanas, S. A., Henderson, C. J., McLellan, L. I., . . . Hayes, J. D. (2001). The Cap'n'Collar basic leucine zipper transcription factor Nrf2 (NF-E2 p45-related factor 2) controls both constitutive and inducible expression of intestinal detoxification and glutathione biosynthetic enzymes. *Cancer Res., 61*(8), 3299-3307.

McMahon, M., Thomas, N., Itoh, K., Yamamoto, M., & Hayes, J. D. (2004). Redox-regulated turnover of Nrf2 is determined by at least two separate protein domains, the redox-sensitive Neh2 degron and the redox-insensitive Neh6 degron. *J Biol Chem, 279*(30), 31556-31567. doi:10.1074/jbc.M403061200

McMahon, M., Thomas, N., Itoh, K., Yamamoto, M., & Hayes, J. D. (2006). Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism: a two-site interaction model for the Nrf2-Keap1 complex. *J Biol Chem, 281*(34), 24756-24768. doi:10.1074/jbc.M601119200

McNaught, K. S., & Jenner, P. (2001). Proteasomal function is impaired in substantia nigra in Parkinson's disease. *Neurosci Lett, 297*(3), 191-194.

Medina, M., & Avila, J. (2014). New insights into the role of glycogen synthase kinase-3 in Alzheimer's disease. *Expert Opin Ther Targets, 18*(1), 69-77. doi:10.1517/14728222.2013.843670

Menegon, S., Columbano, A., & Giordano, S. (2016). The Dual Roles of NRF2 in Cancer. *Trends Mol Med, 22*(7), 578-593. doi:10.1016/j.molmed.2016.05.002

Mihaylova, M. M., & Shaw, R. J. (2011). The AMPK signalling pathway coordinates cell growth, autophagy and metabolism. *Nat Cell Biol, 13*(9), 1016-1023. doi:10.1038/ncb2329

Milenkovic, D., Matic, S., Kuhl, I., Ruzzenente, B., Freyer, C., Jemt, E., . . . Larsson, N. G. (2013). TWINKLE is an essential mitochondrial helicase required for synthesis of nascent D-loop strands and complete mtDNA replication. *Hum Mol Genet, 22*(10), 1983-1993. doi:10.1093/hmg/ddt051

Miller, W. P., Rothman, S. M., Nascene, D., Kivisto, T., DeFor, T. E., Ziegler, R. S., . . . Orchard, P. J. (2011). Outcomes after allogeneic hematopoietic cell transplantation for childhood cerebral adrenoleukodystrophy: the largest single-institution cohort report. *Blood, 118*(7), 1971-1978. doi:10.1182/blood-2011-01-329235

Min, K. T., & Benzer, S. (1999). Preventing neurodegeneration in the Drosophila mutant bubblegum. *Science, 284*(5422), 1985-1988.

Mishra, H. K., Prots, I., Havlicek, S., Kohl, Z., Perez-Branguli, F., Boerstler, T., . . . Winner, B. (2016). GSK3beta-dependent dysregulation of neurodevelopment in SPG11-patient iPSC model. *Ann Neurol, 79*(5), 826-840. doi:10.1002/ana.24633

Mitsumoto, H., Santella, R. M., Liu, X., Bogdanov, M., Zipprich, J., Wu, H. C., . . . Factor-Litvak, P. (2008). Oxidative stress biomarkers in sporadic ALS. *Amyotroph Lateral Scler, 9*(3), 177-183. doi:10.1080/17482960801933942

Miura, S., Tomitsuka, E., Kamei, Y., Yamazaki, T., Kai, Y., Tamura, M., . . . Ezaki, O. (2006). Overexpression of peroxisome proliferator-activated receptor gamma co-activator-1alpha leads to muscle atrophy with depletion of ATP. *Am J Pathol, 169*(4), 1129-1139.

Mobasher, M. A., Gonzalez-Rodriguez, A., Santamaria, B., Ramos, S., Martin, M. A., Goya, L., . . . Valverde, A. M. (2013). Protein tyrosine phosphatase 1B modulates GSK3beta/Nrf2 and IGFIR signaling pathways in acetaminophen-induced hepatotoxicity. *Cell Death Dis, 4*, e626. doi:10.1038/cddis.2013.150

Mochel, F., & Haller, R. G. (2011). Energy deficit in Huntington disease: why it matters. *J Clin Invest, 121*(2), 493-499. doi:10.1172/JCI45691

Moi, P., Chan, K., Asunis, I., Cao, A., & Kan, Y. W. (1994). Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc. Natl. Acad. Sci. U. S. A., 91*(21), 9926-9930.

Morales Pantoja, I. E., Hu, C. L., Perrone-Bizzozero, N. I., Zheng, J., & Bizzozero, O. A. (2016). Nrf2-dysregulation correlates with reduced synthesis and low glutathione levels in experimental autoimmune encephalomyelitis. *J Neurochem, 139*(4), 640-650. doi:10.1111/jnc.13837

Morato, L., Bertini, E., Verrigni, D., Ardissone, A., Ruiz, M., Ferrer, I., . . . Pujol, A. (2014). Mitochondrial dysfunction in central nervous system white matter disorders. *Glia, 62*(11), 1878-1894. doi:10.1002/glia.22670

Morato, L., Galino, J., Ruiz, M., Calingasan, N. Y., Starkov, A. A., Dumont, M., . . . Pujol, A. (2013). Pioglitazone halts axonal degeneration in a mouse model of X-linked adrenoleukodystrophy. *Brain, 136*(Pt 8), 2432-2443. doi:10.1093/brain/awt143

Morato, L., Ruiz, M., Boada, J., Calingasan, N. Y., Galino, J., Guilera, C., . . . Pujol, A. (2015). Activation of sirtuin 1 as therapy for the peroxisomal disease adrenoleukodystrophy. *Cell Death Differ, 22*(11), 1742-1753. doi:10.1038/cdd.2015.20

Morel, F., Rauch, C., Petit, E., Piton, A., Theret, N., Coles, B., & Guillouzo, A. (2004). Gene and protein characterization of the human glutathione S-transferase kappa and evidence for a peroxisomal localization. *J Biol Chem, 279*(16), 16246-16253. doi:10.1074/jbc.M313357200

Moser, A. B., Borel, J., Odone, A., Naidu, S., Cornblath, D., Sanders, D. B., & Moser, H. W. (1987). A new dietary therapy for adrenoleukodystrophy: biochemical and preliminary clinical results in 36 patients. *Ann Neurol, 21*(3), 240-249. doi:10.1002/ana.410210305

Moser, A. B., Kreiter, N., Bezman, L., Lu, S., Raymond, G. V., Naidu, S., & Moser, H. W. (1999a). Plasma very long chain fatty acids in 3,000 peroxisome disease patients and 29,000 controls. *Ann Neurol, 45*(1), 100-110.

Moser, A. B., & Moser, H. W. (1999b). The prenatal diagnosis of X-linked adrenoleukodystrophy. *Prenat Diagn, 19*(1), 46-48.

Moser, H. W., Loes, D. J., Melhem, E. R., Raymond, G. V., Bezman, L., Cox, C. S., & Lu, S. E. (2000). X-Linked adrenoleukodystrophy: overview and prognosis as a function of age and brain magnetic resonance imaging abnormality. A study involving 372 patients. *Neuropediatrics, 31*(5), 227-239. doi:10.1055/s-2000-9236

Moser, H. W., Mahmood, A., & Raymond, G. V. (2007). X-linked adrenoleukodystrophy. *Nat Clin Pract Neurol, 3*(3), 140-151. doi:10.1038/ncpneuro0421

Moser, H. W., Moser, A. B., Frayer, K. K., Chen, W., Schulman, J. D., O'Neill, B. P., & Kishimoto, Y. (1981). Adrenoleukodystrophy: increased plasma content of saturated very long chain fatty acids. *Neurology, 31*(10), 1241-1249.

Moser, H. W., Moser, A. B., Kawamura, N., Murphy, J., Suzuki, K., Schaumburg, H., & Kishimoto, Y. (1980). Adrenoleukodystrophy: elevated C26 fatty acid in cultured skin fibroblasts. *Ann Neurol, 7*(6), 542-549. doi:10.1002/ana.410070607

Moser, H. W., Moser, A. B., Smith, K. D., Bergin, A., Borel, J., Shankroff, J., . . . et al. (1992). Adrenoleukodystrophy: phenotypic variability and implications for therapy. *J Inherit Metab Dis, 15*(4), 645-664.

Moser, H. W., Raymond, G. V., Lu, S. E., Muenz, L. R., Moser, A. B., Xu, J., . . . Odone, A. (2005). Follow-up of 89 asymptomatic patients with adrenoleukodystrophy treated with Lorenzo's oil. *Arch Neurol, 62*(7), 1073-1080. doi:10.1001/archneur.62.7.1073

Moser, H. W., Smith, K. D., Watkins, P. A., Powers, J., & Moser, A. B. (2001). X-linked adrenoleukodystrophy. In C. R. Scriver (Ed.), *The Metabolic and Molecular Bases of Inherited disease* (8th ed., Vol. II, pp. 3257-3301). New-York: McGraw-Hill.

Mosser, J., Douar, A. M., Sarde, C. O., Kioschis, P., Feil, R., Moser, H., . . . Aubourg, P. (1993). Putative X-linked adrenoleukodystrophy gene shares unexpected homology with ABC transporters. *Nature, 361*(6414), 726-730. doi:10.1038/361726a0

Mosser, J., Lutz, Y., Stoeckel, M. E., Sarde, C. O., Kretz, C., Douar, A. M., . . . Mandel, J. L. (1994). The gene responsible for adrenoleukodystrophy encodes a peroxisomal membrane protein. *Hum Mol Genet, 3*(2), 265-271. doi:10.1093/hmg/3.2.265

Motonaga, K., Itoh, M., Hirayama, A., Hirano, S., Becker, L. E., Goto, Y., & Takashima, S. (2001). Up-regulation of E2F-1 in Down's syndrome brain exhibiting neuropathological features of Alzheimer-type dementia. *Brain Res, 905*(1-2), 250-253.

Mudo, G., Makela, J., Di Liberto, V., Tselykh, T. V., Olivieri, M., Piepponen, P., . . . Lindholm, D. (2012). Transgenic expression and activation of PGC-1alpha protect dopaminergic neurons in the MPTP mouse model of Parkinson's disease. *Cell Mol Life Sci, 69*(7), 1153-1165. doi:10.1007/s00018-011-0850-z

Muffat, J., Li, Y., Yuan, B., Mitalipova, M., Omer, A., Corcoran, S., . . . Jaenisch, R. (2016). Efficient derivation of microglia-like cells from human pluripotent stem cells. *Nat Med, 22*(11), 1358-1367. doi:10.1038/nm.4189

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochem J, 417*(1), 1-13. doi:10.1042/BJ20081386

Naidu, S., Bresnan, M. J., Griffin, D., O'Toole, S., & Moser, H. W. (1988). Childhood adrenoleukodystrophy. Failure of intensive immunosuppression to arrest neurologic progression. *Arch Neurol, 45*(8), 846-848.

Nassif, M., Matus, S., Castillo, K., & Hetz, C. (2010). Amyotrophic lateral sclerosis pathogenesis: a journey through the secretory pathway. *Antioxid Redox Signal, 13*(12), 1955-1989. doi:10.1089/ars.2009.2991

Nautiyal, J., Christian, M., & Parker, M. G. (2013). Distinct functions for RIP140 in development, inflammation, and metabolism. *Trends Endocrinol Metab, 24*(9), 451-459. doi:10.1016/j.tem.2013.05.001

Netik, A., Forss-Petter, S., Holzinger, A., Molzer, B., Unterrainer, G., & Berger, J. (1999). Adrenoleukodystrophy-related protein can compensate functionally for adrenoleukodystrophy protein deficiency (X-ALD): implications for therapy. *Hum Mol Genet, 8*(5), 907-913.

Neupert, W., & Herrmann, J. M. (2007). Translocation of proteins into mitochondria. *Annu Rev Biochem, 76*, 723-749. doi:10.1146/annurev.biochem.76.052705.163409

Neymotin, A., Calingasan, N. Y., Wille, E., Naseri, N., Petri, S., Damiano, M., . . . Kiaei, M. (2011). Neuroprotective effect of Nrf2/ARE activators, CDDO ethylamide and CDDO trifluoroethylamide, in a mouse model of amyotrophic lateral sclerosis. *Free Radic Biol Med, 51*(1), 88-96.

Nibbering, P. H., Thio, B., Zomerdijk, T. P., Bezemer, A. C., Beijersbergen, R. L., & van Furth, R. (1993). Effects of monomethylfumarate on human granulocytes. *J Invest Dermatol, 101*(1), 37-42.

Nieuwkamp, D. J., Murk, J. L., van Oosten, B. W., Cremers, C. H., Killestein, J., Viveen, M. C., . . . Consortium, P. M. L. i. D. M. P. (2015). PML in a patient

without severe lymphocytopenia receiving dimethyl fumarate. *N Engl J Med, 372*(15), 1474-1476. doi:10.1056/NEJMc1413724

Nioi, P., McMahon, M., Itoh, K., Yamamoto, M., & Hayes, J. D. (2003). Identification of a novel Nrf2-regulated antioxidant response element (ARE) in the mouse NAD(P)H:quinone oxidoreductase 1 gene: reassessment of the ARE consensus sequence. *Biochem J, 374*(Pt 2), 337-348. doi:10.1042/BJ20030754

Norambuena, A., Wallrabe, H., McMahon, L., Silva, A., Swanson, E., Khan, S. S., . . . Bloom, G. S. (2017). mTOR and neuronal cell cycle reentry: How impaired brain insulin signaling promotes Alzheimer's disease. *Alzheimers Dement, 13*(2), 152-167. doi:10.1016/j.jalz.2016.08.015

Nunnari, J., & Suomalainen, A. (2012). Mitochondria: in sickness and in health. *Cell, 148*(6), 1145-1159. doi:10.1016/j.cell.2012.02.035

O'Brien, W. T., Harper, A. D., Jove, F., Woodgett, J. R., Maretto, S., Piccolo, S., & Klein, P. S. (2004). Glycogen synthase kinase-3beta haploinsufficiency mimics the behavioral and molecular effects of lithium. *J Neurosci, 24*(30), 6791-6798. doi:10.1523/JNEUROSCI.4753-03.2004

Oezen, I., Rossmanith, W., Forss-Petter, S., Kemp, S., Voigtlander, T., Moser-Thier, K., . . . Berger, J. (2005). Accumulation of very long-chain fatty acids does not affect mitochondrial function in adrenoleukodystrophy protein deficiency. *Hum Mol Genet, 14*(9), 1127-1137. doi:10.1093/hmg/ddi125

Ofman, R., Dijkstra, I. M., van Roermund, C. W., Burger, N., Turkenburg, M., van Cruchten, A., . . . Kemp, S. (2010). The role of ELOVL1 in very long-chain fatty acid homeostasis and X-linked adrenoleukodystrophy. *EMBO Mol Med, 2*(3), 90-97. doi:10.1002/emmm.201000061

Ogura, T., Tong, K. I., Mio, K., Maruyama, Y., Kurokawa, H., Sato, C., & Yamamoto, M. (2010). Keap1 is a forked-stem dimer structure with two large spheres enclosing the intervening, double glycine repeat, and C-terminal domains. *Proc Natl Acad Sci U S A, 107*(7), 2842-2847. doi:10.1073/pnas.0914036107

Oyake, T., Itoh, K., Motohashi, H., Hayashi, N., Hoshino, H., Nishizawa, M., . . . Igarashi, K. (1996). Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site. *Mol Cell Biol, 16*(11), 6083-6095.

Pajares, M., Jimenez-Moreno, N., Garcia-Yague, A. J., Escoll, M., de Ceballos, M. L., Van Leuven, F., . . . Cuadrado, A. (2016). Transcription factor NFE2L2/NRF2 is a regulator of macroautophagy genes. *Autophagy, 12*(10), 1902-1916. doi:10.1080/15548627.2016.1208889

Pan, Y., Schroeder, E. A., Ocampo, A., Barrientos, A., & Shadel, G. S. (2011). Regulation of yeast chronological life span by TORC1 via adaptive mitochondrial ROS signaling. *Cell Metab, 13*(6), 668-678. doi:10.1016/j.cmet.2011.03.018

Pandey, G. N., Dwivedi, Y., Rizavi, H. S., Teppen, T., Gaszner, G. L., Roberts, R. C., & Conley, R. R. (2009). GSK-3beta gene expression in human postmortem brain: regional distribution, effects of age and suicide. *Neurochem Res, 34*(2), 274-285. doi:10.1007/s11064-008-9770-1

Park, J., Choi, H., Min, J. S., Park, S. J., Kim, J. H., Park, H. J., . . . Lee, D. S. (2013). Mitochondrial dynamics modulate the expression of pro-inflammatory mediators in microglial cells. *J Neurochem, 127*(2), 221-232. doi:10.1111/jnc.12361

Parodi, B., Rossi, S., Morando, S., Cordano, C., Bragoni, A., Motta, C., . . . Uccelli, A. (2015). Fumarates modulate microglia activation through a novel HCAR2

signaling pathway and rescue synaptic dysregulation in inflamed CNS. *Acta Neuropathol, 130*(2), 279-295. doi:10.1007/s00401-015-1422-3

Parola, M., Leonarduzzi, G., Biasi, F., Albano, E., Biocca, M. E., Poli, G., & Dianzani, M. U. (1992). Vitamin E dietary supplementation protects against carbon tetrachloride-induced chronic liver damage and cirrhosis. *Hepatology, 16*(4), 1014-1021.

Paupe, V., Dassa, E. P., Goncalves, S., Auchere, F., Lonn, M., Holmgren, A., & Rustin, P. (2009). Impaired nuclear Nrf2 translocation undermines the oxidative stress response in Friedreich ataxia. *PLoS One, 4*(1), e4253. doi:10.1371/journal.pone.0004253

Peeters, A., Shinde, A. B., Dirkx, R., Smet, J., De Bock, K., Espeel, M., . . . Baes, M. (2015). Mitochondria in peroxisome-deficient hepatocytes exhibit impaired respiration, depleted DNA, and PGC-1alpha independent proliferation. *Biochim Biophys Acta, 1853*(2), 285-298. doi:10.1016/j.bbamcr.2014.11.017

Peineau, S., Bradley, C., Taghibiglou, C., Doherty, A., Bortolotto, Z. A., Wang, Y. T., & Collingridge, G. L. (2008). The role of GSK-3 in synaptic plasticity. *Br J Pharmacol, 153 Suppl 1*, S428-437. doi:10.1038/bjp.2008.2

Pekkari, K., & Holmgren, A. (2004). Truncated thioredoxin: physiological functions and mechanism. *Antioxid Redox Signal, 6*(1), 53-61. doi:10.1089/152308604771978345

Peng, H., Li, H., Sheehy, A., Cullen, P., Allaire, N., & Scannevin, R. H. (2016). Dimethyl fumarate alters microglia phenotype and protects neurons against proinflammatory toxic microenvironments. *J Neuroimmunol, 299*, 35-44. doi:10.1016/j.jneuroim.2016.08.006

Perez-Costas, E., Gandy, J. C., Melendez-Ferro, M., Roberts, R. C., & Bijur, G. N. (2010). Light and electron microscopy study of glycogen synthase kinase-3beta in the mouse brain. *PLoS One, 5*(1), e8911. doi:10.1371/journal.pone.0008911

Perissi, V., Jepsen, K., Glass, C. K., & Rosenfeld, M. G. (2010). Deconstructing repression: evolving models of co-repressor action. *Nat Rev Genet, 11*(2), 109-123. doi:10.1038/nrg2736

Petersen, R. C., Thomas, R. G., Grundman, M., Bennett, D., Doody, R., Ferris, S., . . . Alzheimer's Disease Cooperative Study, G. (2005). Vitamin E and donepezil for the treatment of mild cognitive impairment. *N Engl J Med, 352*(23), 2379-2388. doi:10.1056/NEJMoa050151

Petrillo, S., Piemonte, F., Pastore, A., Tozzi, G., Aiello, C., Pujol, A., . . . Bertini, E. (2013). Glutathione imbalance in patients with X-linked adrenoleukodystrophy. *Mol Genet Metab, 109*(4), 366-370. doi:10.1016/j.ymgme.2013.05.009

Phillips, J. T., Selmaj, K., Gold, R., Fox, R. J., Havrdova, E., Giovannoni, G., . . . Meltzer, L. (2015). Clinical Significance of Gastrointestinal and Flushing Events in Patients with Multiple Sclerosis Treated with Delayed-Release Dimethyl Fumarate. *Int J MS Care, 17*(5), 236-243. doi:10.7224/1537-2073.2014-069

Picard, F., Gehin, M., Annicotte, J., Rocchi, S., Champy, M. F., O'Malley, B. W., . . . Auwerx, J. (2002). SRC-1 and TIF2 control energy balance between white and brown adipose tissues. *Cell, 111*(7), 931-941.

Piccoli, C., Izzo, A., Scrima, R., Bonfiglio, F., Manco, R., Negri, R., . . . Capitanio, N. (2013). Chronic pro-oxidative state and mitochondrial dysfunctions are more pronounced in fibroblasts from Down syndrome foeti with congenital heart defects. *Hum Mol Genet, 22*(6), 1218-1232. doi:10.1093/hmg/dds529

Pickering, A. M., Linder, R. A., Zhang, H., Forman, H. J., & Davies, K. J. (2012). Nrf2-dependent induction of proteasome and Pa28alphabeta regulator are required for

adaptation to oxidative stress. *J Biol Chem, 287*(13), 10021-10031. doi:10.1074/jbc.M111.277145

Pirinen, E., Canto, C., Jo, Y. S., Morato, L., Zhang, H., Menzies, K. J., . . . Auwerx, J. (2014). Pharmacological Inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. *Cell Metab, 19*(6), 1034-1041. doi:10.1016/j.cmet.2014.04.002

Pitha-Rowe, I., Liby, K., Royce, D., & Sporn, M. (2009). Synthetic triterpenoids attenuate cytotoxic retinal injury: cross-talk between Nrf2 and PI3K/AKT signaling through inhibition of the lipid phosphatase PTEN. *Invest Ophthalmol Vis Sci, 50*(11), 5339-5347. doi:10.1167/iovs.09-3648

Powelka, A. M., Seth, A., Virbasius, J. V., Kiskinis, E., Nicoloro, S. M., Guilherme, A., . . . Czech, M. P. (2006). Suppression of oxidative metabolism and mitochondrial biogenesis by the transcriptional corepressor RIP140 in mouse adipocytes. *J Clin Invest, 116*(1), 125-136. doi:10.1172/JCI26040

Powers, J. M., DeCiero, D. P., Cox, C., Richfield, E. K., Ito, M., Moser, A. B., & Moser, H. W. (2001). The dorsal root ganglia in adrenomyeloneuropathy: neuronal atrophy and abnormal mitochondria. *J Neuropathol Exp Neurol, 60*(5), 493-501.

Powers, J. M., Liu, Y., Moser, A. B., & Moser, H. W. (1992). The inflammatory myelinopathy of adreno-leukodystrophy: cells, effector molecules, and pathogenetic implications. *J Neuropathol Exp Neurol, 51*(6), 630-643.

Powers, J. M., Pei, Z., Heinzer, A. K., Deering, R., Moser, A. B., Moser, H. W., . . . Smith, K. D. (2005). Adreno-leukodystrophy: oxidative stress of mice and men. *J Neuropathol Exp Neurol, 64*(12), 1067-1079. doi:10.1097/01.jnen.0000190064.28559.a4

Powers, J. M., & Schaumberg, H. H. (1974). Adreno-leukodystrophy. Similar ultrastructural changes in adrenal cortical and Schwann cells. *Arch Neurol, 30*(5), 406-408.

Prickaerts, J., Moechars, D., Cryns, K., Lenaerts, I., van Craenendonck, H., Goris, I., . . . Steckler, T. (2006). Transgenic mice overexpressing glycogen synthase kinase 3beta: a putative model of hyperactivity and mania. *J Neurosci, 26*(35), 9022-9029. doi:10.1523/JNEUROSCI.5216-05.2006

Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., & Spiegelman, B. M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell, 92*(6), 829-839. doi:10.1016/S0092-8674(00)81410-5

Pujol, A. (2016). Novel Therapeutic Targets and Drug Candidates for Modifying Disease Progression in Adrenoleukodystrophy. *Endocr Dev, 30*, 147-160. doi:10.1159/000439340

Pujol, A., Ferrer, I., Camps, C., Metzger, E., Hindelang, C., Callizot, N., . . . Mandel, J. L. (2004). Functional overlap between ABCD1 (ALD) and ABCD2 (ALDR) transporters: a therapeutic target for X-adrenoleukodystrophy. *Hum Mol Genet, 13*(23), 2997-3006. doi:10.1093/hmg/ddh323

Pujol, A., Hindelang, C., Callizot, N., Bartsch, U., Schachner, M., & Mandel, J. L. (2002). Late onset neurological phenotype of the X-ALD gene inactivation in mice: a mouse model for adrenomyeloneuropathy. *Hum Mol Genet, 11*(5), 499-505. doi:10.1093/hmg/11.5.499

Qin, W., Haroutunian, V., Katsel, P., Cardozo, C. P., Ho, L., Buxbaum, J. D., & Pasinetti, G. M. (2009). PGC-1alpha expression decreases in the Alzheimer disease brain as a function of dementia. *Arch Neurol, 66*(3), 352-361. doi:10.1001/archneurol.2008.588

Rachakonda, G., Sekhar, K. R., Jowhar, D., Samson, P. C., Wikswo, J. P., Beauchamp, R. D., . . . Freeman, M. L. (2010). Increased cell migration and plasticity in Nrf2-deficient cancer cell lines. *Oncogene, 29*(25), 3703-3714. doi:10.1038/onc.2010.118

Rada, P., Rojo, A. I., Chowdhry, S., McMahon, M., Hayes, J. D., & Cuadrado, A. (2011). SCF/{beta}-TrCP promotes glycogen synthase kinase 3-dependent degradation of the Nrf2 transcription factor in a Keap1-independent manner. *Mol Cell Biol, 31*(6), 1121-1133. doi:10.1128/MCB.01204-10

Rada, P., Rojo, A. I., Evrard-Todeschi, N., Innamorato, N. G., Cotte, A., Jaworski, T., . . . Cuadrado, A. (2012). Structural and functional characterization of Nrf2 degradation by the glycogen synthase kinase 3/beta-TrCP axis. *Mol Cell Biol, 32*(17), 3486-3499. doi:10.1128/MCB.00180-12

Ramsey, C. P., Glass, C. A., Montgomery, M. B., Lindl, K. A., Ritson, G. P., Chia, L. A., . . . Jordan-Sciutto, K. L. (2007). Expression of Nrf2 in neurodegenerative diseases. *J Neuropathol Exp Neurol, 66*(1), 75-85. doi:10.1097/nen.0b013e31802d6da9

Ranganathan, S., & Bowser, R. (2003). Alterations in G(1) to S phase cell-cycle regulators during amyotrophic lateral sclerosis. *Am J Pathol, 162*(3), 823-835. doi:10.1016/S0002-9440(10)63879-5

Rasmussen, M., Moser, A. B., Borel, J., Khangoora, S., & Moser, H. W. (1994). Brain, liver, and adipose tissue erucic and very long chain fatty acid levels in adrenoleukodystrophy patients treated with glyceryl trierucate and trioleate oils (Lorenzo's oil). *Neurochem Res, 19*(8), 1073-1082.

Ravikumar, B., Duden, R., & Rubinsztein, D. C. (2002). Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. *Hum Mol Genet, 11*(9), 1107-1117.

Raymond, G. V., Seidman, R., Monteith, T. S., Kolodny, E., Sathe, S., Mahmood, A., & Powers, J. M. (2010). Head trauma can initiate the onset of adreno-leukodystrophy. *J Neurol Sci, 290*(1-2), 70-74. doi:10.1016/j.jns.2009.11.005

Rhee, S. G., Chae, H. Z., & Kim, K. (2005). Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic Biol Med, 38*(12), 1543-1552. doi:10.1016/j.freeradbiomed.2005.02.026

Rhein, V., Song, X., Wiesner, A., Ittner, L. M., Baysang, G., Meier, F., . . . Eckert, A. (2009). Amyloid-beta and tau synergistically impair the oxidative phosphorylation system in triple transgenic Alzheimer's disease mice. *Proc Natl Acad Sci U S A, 106*(47), 20057-20062. doi:10.1073/pnas.0905529106

Rhodin, J. (1954). *Correlation of Ultrastructural Organization and Function in Normal and Experimentally Changed Proximal Convoluted Tubule Cells of the Mouse Kidney: An Electron Microscopic Study Including an Experimental Analysis of the Conditions for Fixation of the Renal Tissue for High Resolution Electron Microscopy.* (PhD Thesis), Karolinska Universitet, Sweden.

Rice, A. C., Keeney, P. M., Algarzae, N. K., Ladd, A. C., Thomas, R. R., & Bennett, J. P., Jr. (2014). Mitochondrial DNA copy numbers in pyramidal neurons are decreased and mitochondrial biogenesis transcriptome signaling is disrupted in Alzheimer's disease hippocampi. *J Alzheimers Dis, 40*(2), 319-330. doi:10.3233/JAD-131715

Richter, C., Park, J. W., & Ames, B. N. (1988). Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci U S A, 85*(17), 6465-6467.

Ring, D. B., Johnson, K. W., Henriksen, E. J., Nuss, J. M., Goff, D., Kinnick, T. R., . . . Harrison, S. D. (2003). Selective glycogen synthase kinase 3 inhibitors potentiate insulin activation of glucose transport and utilization in vitro and in vivo. *Diabetes, 52*(3), 588-595. doi:10.2337/diabetes.52.3.588

Ristow, M., Zarse, K., Oberbach, A., Kloting, N., Birringer, M., Kiehntopf, M., . . . Bluher, M. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A, 106*(21), 8665-8670. doi:10.1073/pnas.0903485106

Rojo, A. I., Innamorato, N. G., Martin-Moreno, A. M., De Ceballos, M. L., Yamamoto, M., & Cuadrado, A. (2010). Nrf2 regulates microglial dynamics and neuroinflammation in experimental Parkinson's disease. *Glia, 58*(5), 588-598. doi:10.1002/glia.20947

Rojo, A. I., Rada, P., Egea, J., Rosa, A. O., Lopez, M. G., & Cuadrado, A. (2008a). Functional interference between glycogen synthase kinase-3 beta and the transcription factor Nrf2 in protection against kainate-induced hippocampal cell death. *Mol Cell Neurosci, 39*(1), 125-132. doi:10.1016/j.mcn.2008.06.007

Rojo, A. I., Rada, P., Mendiola, M., Ortega-Molina, A., Wojdyla, K., Rogowska-Wrzesinska, A., . . . Cuadrado, A. (2014). The PTEN/NRF2 axis promotes human carcinogenesis. *Antioxid Redox Signal, 21*(18), 2498-2514. doi:10.1089/ars.2014.5843

Rojo, A. I., Sagarra, M. R., & Cuadrado, A. (2008b). GSK-3beta down-regulates the transcription factor Nrf2 after oxidant damage: relevance to exposure of neuronal cells to oxidative stress. *J Neurochem, 105*(1), 192-202. doi:10.1111/j.1471-4159.2007.05124.x

Ron, D., & Walter, P. (2007). Signal integration in the endoplasmic reticulum unfolded protein response. *Nat Rev Mol Cell Biol, 8*(7), 519-529. doi:10.1038/nrm2199

Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., . . . et al. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature, 362*(6415), 59-62. doi:10.1038/362059a0

Rosenkranz, T., Novas, M., & Terborg, C. (2015). PML in a patient with lymphocytopenia treated with dimethyl fumarate. *N Engl J Med, 372*(15), 1476-1478. doi:10.1056/NEJMc1415408

Ruiz, M., Jove, M., Schluter, A., Casasnovas, C., Villarroya, F., Guilera, C., . . . Pujol, A. (2015). Altered glycolipid and glycerophospholipid signaling drive inflammatory cascades in adrenomyeloneuropathy. *Hum Mol Genet, 24*(24), 6861-6876. doi:10.1093/hmg/ddv375

Rushmore, T. H., Morton, M. R., & Pickett, C. B. (1991). The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *J Biol Chem, 266*(18), 11632-11639.

Rushmore, T. H., & Pickett, C. B. (1990). Transcriptional regulation of the rat glutathione S-transferase Ya subunit gene. Characterization of a xenobiotic-responsive element controlling inducible expression by phenolic antioxidants. *J Biol Chem, 265*(24), 14648-14653.

Sabens Liedhegner, E. A., Gao, X. H., & Mieyal, J. J. (2012). Mechanisms of altered redox regulation in neurodegenerative diseases--focus on S--glutathionylation. *Antioxid Redox Signal, 16*(6), 543-566. doi:10.1089/ars.2011.4119

Safiulina, D., Kaasik, A., Seppet, E., Peet, N., Zharkovsky, A., & Seppet, E. (2004). Method for in situ detection of the mitochondrial function in neurons. *J Neurosci Methods, 137*(1), 87-95. doi:10.1016/j.jneumeth.2004.02.027

Sakurai, H., Chiba, H., Miyoshi, H., Sugita, T., & Toriumi, W. (1999). IkappaB kinases phosphorylate NF-kappaB p65 subunit on serine 536 in the transactivation domain. *J Biol Chem, 274*(43), 30353-30356. doi:10.1074/jbc.274.43.30353

Salazar, M., Rojo, A. I., Velasco, D., de Sagarra, R. M., & Cuadrado, A. (2006). Glycogen synthase kinase-3beta inhibits the xenobiotic and antioxidant cell response by direct phosphorylation and nuclear exclusion of the transcription factor Nrf2. *J Biol Chem, 281*(21), 14841-14851. doi:10.1074/jbc.M513737200

Salinas, M., Diaz, R., Abraham, N. G., Ruiz de Galarreta, C. M., & Cuadrado, A. (2003). Nerve growth factor protects against 6-hydroxydopamine-induced oxidative stress by increasing expression of heme oxygenase-1 in a phosphatidylinositol 3-kinase-dependent manner. *J Biol Chem, 278*(16), 13898-13904. doi:10.1074/jbc.M209164200

Salinas, M., Martin, D., Alvarez, A., & Cuadrado, A. (2001). Akt1/PKBalpha protects PC12 cells against the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium and reduces the levels of oxygen-free radicals. *Mol Cell Neurosci, 17*(1), 67-77. doi:10.1006/mcne.2000.0921

Sanders, R. J., Ofman, R., Duran, M., Kemp, S., & Wanders, R. J. (2006). Omega-oxidation of very long-chain fatty acids in human liver microsomes. Implications for X-linked adrenoleukodystrophy. *J Biol Chem, 281*(19), 13180-13187. doi:10.1074/jbc.M513481200

Sarlette, A., Krampfl, K., Grothe, C., Neuhoff, N., Dengler, R., & Petri, S. (2008). Nuclear erythroid 2-related factor 2-antioxidative response element signaling pathway in motor cortex and spinal cord in amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol, 67*(11), 1055-1062. doi:10.1097/NEN.0b013e31818b4906

Saxena, S., Cabuy, E., & Caroni, P. (2009). A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. *Nat Neurosci, 12*(5), 627-636. doi:10.1038/nn.2297

Saxton, R. A., & Sabatini, D. M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. *Cell, 168*(6), 960-976. doi:10.1016/j.cell.2017.02.004

Scannevin, R. H., Chollate, S., Jung, M. Y., Shackett, M., Patel, H., Bista, P., . . . Rhodes, K. J. (2012). Fumarates promote cytoprotection of central nervous system cells against oxidative stress via the nuclear factor (erythroid-derived 2)-like 2 pathway. *J Pharmacol Exp Ther, 341*(1), 274-284. doi:10.1124/jpet.111.190132

Scarpulla, R. C. (2006). Nuclear control of respiratory gene expression in mammalian cells. *J Cell Biochem, 97*(4), 673-683. doi:10.1002/jcb.20743

Scarpulla, R. C. (2011). Metabolic control of mitochondrial biogenesis through the PGC-1 family regulatory network. *Biochim Biophys Acta, 1813*(7), 1269-1278. doi:10.1016/j.bbamcr.2010.09.019

Scarpulla, R. C., Vega, R. B., & Kelly, D. P. (2011). Transcriptional integration of mitochondrial biogenesis. *Trends Endocrinol Metab, 23*(9), 459-466.

Schapira, A. H., Olanow, C. W., Greenamyre, J. T., & Bezard, E. (2014). Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: future therapeutic perspectives. *Lancet, 384*(9942), 545-555. doi:10.1016/S0140-6736(14)61010-2

Schaumburg, H. H., Powers, J. M., Raine, C. S., Suzuki, K., & Richardson, E. P., Jr. (1975). Adrenoleukodystrophy. A clinical and pathological study of 17 cases. *Arch Neurol, 32*(9), 577-591.

Schilder, P. (1924). Die Encephalitis periaxialis diffusa. *Arch Psychiatr. Nervenkr., 71*, 327–356.

Schilling, S., Goelz, S., Linker, R., Luehder, F., & Gold, R. (2006). Fumaric acid esters are effective in chronic experimental autoimmune encephalomyelitis and suppress macrophage infiltration. *Clin Exp Immunol, 145*(1), 101-107. doi:10.1111/j.1365-2249.2006.03094.x

Schimrigk, S., Brune, N., Hellwig, K., Lukas, C., Bellenberg, B., Rieks, M., . . . Przuntek, H. (2006). Oral fumaric acid esters for the treatment of active multiple sclerosis: an open-label, baseline-controlled pilot study. *Eur J Neurol, 13*(6), 604-610. doi:10.1111/j.1468-1331.2006.01292.x

Schipper, H. M., Bennett, D. A., Liberman, A., Bienias, J. L., Schneider, J. A., Kelly, J., & Arvanitakis, Z. (2006). Glial heme oxygenase-1 expression in Alzheimer disease and mild cognitive impairment. *Neurobiol Aging, 27*(2), 252-261. doi:10.1016/j.neurobiolaging.2005.01.016

Schipper, H. M., Liberman, A., & Stopa, E. G. (1998). Neural heme oxygenase-1 expression in idiopathic Parkinson's disease. *Exp Neurol, 150*(1), 60-68. doi:10.1006/exnr.1997.6752

Schluter, A., Espinosa, L., Fourcade, S., Galino, J., Lopez, E., Ilieva, E., . . . Pujol, A. (2012). Functional genomic analysis unravels a metabolic-inflammatory interplay in adrenoleukodystrophy. *Hum Mol Genet, 21*(5), 1062-1077. doi:10.1093/hmg/ddr536

Schluter, A., Fourcade, S., Ripp, R., Mandel, J. L., Poch, O., & Pujol, A. (2006). The evolutionary origin of peroxisomes: an ER-peroxisome connection. *Mol Biol Evol, 23*(4), 838-845. doi:10.1093/molbev/msj103

Schluter, A., Real-Chicharro, A., Gabaldon, T., Sanchez-Jimenez, F., & Pujol, A. (2010). PeroxisomeDB 2.0: an integrative view of the global peroxisomal metabolome. *Nucleic Acids Res, 38*(Database issue), D800-805. doi:10.1093/nar/gkp935

Schmidt, H. H., Stocker, R., Vollbracht, C., Paulsen, G., Riley, D., Daiber, A., & Cuadrado, A. (2015). Antioxidants in Translational Medicine. *Antioxid Redox Signal, 23*(14), 1130-1143. doi:10.1089/ars.2015.6393

Schmidt, S., Marrosu, G. M., Kolsch, H., Haase, C. G., Ferenczik, S., Sokolowski, P., . . . Klockgether, T. (2003). Genetic variations and humoral immune responses to myelin oligodendroglia glycoprotein in adult phenotypes of X-linked adrenoleukodystrophy. *J Neuroimmunol, 135*(1-2), 148-153.

Schoenfeld, R., Wong, A., Silva, J., Li, M., Itoh, A., Horiuchi, M., . . . Cortopassi, G. (2010). Oligodendroglial differentiation induces mitochondrial genes and inhibition of mitochondrial function represses oligodendroglial differentiation. *Mitochondrion, 10*(2), 143-150. doi:10.1016/j.mito.2009.12.141

Schrader, M., Costello, J., Godinho, L. F., & Islinger, M. (2015). Peroxisome-mitochondria interplay and disease. *J Inherit Metab Dis, 38*(4), 681-702. doi:10.1007/s10545-015-9819-7

Schrader, M., & Fahimi, H. D. (2004). Mammalian peroxisomes and reactive oxygen species. *Histochem Cell Biol, 122*(4), 383-393. doi:10.1007/s00418-004-0673-1

Schreiber, S. N., Emter, R., Hock, M. B., Knutti, D., Cardenas, J., Podvinec, M., . . . Kralli, A. (2004). The estrogen-related receptor alpha (ERRalpha) functions in PPARgamma coactivator 1alpha (PGC-1alpha)-induced mitochondrial biogenesis. *Proc Natl Acad Sci U S A, 101*(17), 6472-6477. doi:10.1073/pnas.0308686101

Schroder, J. M., Mayer, M., & Weis, J. (1996). Mitochondrial abnormalities and intrafamilial variability of sural nerve biopsy findings in adrenomyeloneuropathy. *Acta Neuropathol, 92*(1), 64-69.

Schulz, G. E., Schirmer, R. H., Sachsenheimer, W., & Pai, E. F. (1978). The structure of the flavoenzyme glutathione reductase. *Nature, 273*(5658), 120-124.

Schweckendiek, W. (1959). [Treatment of psoriasis vulgaris]. *Med Monatsschr, 13*(2), 103-104.

Selkoe, D. J. (2003). Folding proteins in fatal ways. *Nature, 426*(6968), 900-904. doi:10.1038/nature02264

Semmler, A., Bao, X., Cao, G., Kohler, W., Weller, M., Aubourg, P., & Linnebank, M. (2009). Genetic variants of methionine metabolism and X-ALD phenotype generation: results of a new study sample. *J Neurol, 256*(8), 1277-1280. doi:10.1007/s00415-009-5114-6

Sesso, H. D., Buring, J. E., Christen, W. G., Kurth, T., Belanger, C., MacFadyen, J., . . . Gaziano, J. M. (2008). Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA, 300*(18), 2123-2133. doi:10.1001/jama.2008.600

Seth, A., Steel, J. H., Nichol, D., Pocock, V., Kumaran, M. K., Fritah, A., . . . Parker, M. (2007). The transcriptional corepressor RIP140 regulates oxidative metabolism in skeletal muscle. *Cell Metab, 6*(3), 236-245. doi:10.1016/j.cmet.2007.08.004

Shah, N., & Singh, I. (2017). MicroRNA Profiling Identifies miR-196a as Differentially Expressed in Childhood Adrenoleukodystrophy and Adult Adrenomyeloneuropathy. *Mol Neurobiol, 54*(2), 1392-1403. doi:10.1007/s12035-016-9746-0

Shan, Y., Schoenfeld, R. A., Hayashi, G., Napoli, E., Akiyama, T., Iodi Carstens, M., . . . Cortopassi, G. A. (2013). Frataxin deficiency leads to defects in expression of antioxidants and Nrf2 expression in dorsal root ganglia of the Friedreich's ataxia YG8R mouse model. *Antioxid Redox Signal, 19*(13), 1481-1493. doi:10.1089/ars.2012.4537

Shani, N., Jimenez-Sanchez, G., Steel, G., Dean, M., & Valle, D. (1997). Identification of a fourth half ABC transporter in the human peroxisomal membrane. *Hum Mol Genet, 6*(11), 1925-1931.

Sheng, B., Wang, X., Su, B., Lee, H. G., Casadesus, G., Perry, G., & Zhu, X. (2012). Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease. *J Neurochem, 120*(3), 419-429. doi:10.1111/j.1471-4159.2011.07581.x

Shih, A. Y., Imbeault, S., Barakauskas, V., Erb, H., Jiang, L., Li, P., & Murphy, T. H. (2005). Induction of the Nrf2-driven antioxidant response confers neuroprotection during mitochondrial stress in vivo. *J Biol Chem, 280*(24), 22925-22936. doi:10.1074/jbc.M414635200

Shin, J. H., Ko, H. S., Kang, H., Lee, Y., Lee, Y. I., Pletinkova, O., . . . Dawson, T. M. (2011). PARIS (ZNF746) repression of PGC-1alpha contributes to neurodegeneration in Parkinson's disease. *Cell, 144*(5), 689-702. doi:10.1016/j.cell.2011.02.010

Shin, S., Wolgamott, L., Yu, Y., Blenis, J., & Yoon, S. O. (2011). Glycogen synthase kinase (GSK)-3 promotes p70 ribosomal protein S6 kinase (p70S6K) activity and cell proliferation. *Proc Natl Acad Sci U S A, 108*(47), E1204-1213. doi:10.1073/pnas.1110195108

Shinnoh, N., Yamada, T., Yoshimura, T., Furuya, H., Yoshida, Y., Suzuki, Y., . . . Kobayashi, T. (1995). Adrenoleukodystrophy: the restoration of peroxisomal

beta-oxidation by transfection of normal cDNA. *Biochem Biophys Res Commun, 210*(3), 830-836. doi:10.1006/bbrc.1995.1733

Shuaib, A., Lees, K. R., Lyden, P., Grotta, J., Davalos, A., Davis, S. M., . . . Investigators, S. I. T. (2007). NXY-059 for the treatment of acute ischemic stroke. *N Engl J Med, 357*(6), 562-571. doi:10.1056/NEJMoa070240

Siegel, D., Bolton, E. M., Burr, J. A., Liebler, D. C., & Ross, D. (1997). The reduction of alpha-tocopherolquinone by human NAD(P)H: quinone oxidoreductase: the role of alpha-tocopherolhydroquinone as a cellular antioxidant. *Mol Pharmacol, 52*(2), 300-305.

Siemerling, E., & Creutzfeldt, H. G. (1923). Bronzekrankheit und sklerosierende encephalomyelitis. *Arch. Psychiatr Nervenkr., 68*, 217-244.

Simpson, E. P., Henry, Y. K., Henkel, J. S., Smith, R. G., & Appel, S. H. (2004). Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden. *Neurology, 62*(10), 1758-1765.

Singh, A. K., Dhaunsi, G. S., Gupta, M. P., Orak, J. K., Asayama, K., & Singh, I. (1994). Demonstration of glutathione peroxidase in rat liver peroxisomes and its intraorganellar distribution. *Arch Biochem Biophys, 315*(2), 331-338. doi:10.1006/abbi.1994.1508

Singh, A. K., Dobashi, K., Gupta, M. P., Asayama, K., Singh, I., & Orak, J. K. (1999). Manganese superoxide dismutase in rat liver peroxisomes: biochemical and immunochemical evidence. *Mol Cell Biochem, 197*(1-2), 7-12.

Singh, I., Khan, M., Key, L., & Pai, S. (1998a). Lovastatin for X-linked adrenoleukodystrophy. *N Engl J Med, 339*(10), 702-703. doi:10.1056/NEJM199809033391012

Singh, I., Moser, A. E., Goldfischer, S., & Moser, H. W. (1984a). Lignoceric acid is oxidized in the peroxisome: implications for the Zellweger cerebro-hepato-renal syndrome and adrenoleukodystrophy. *Proc Natl Acad Sci U S A, 81*(13), 4203-4207.

Singh, I., Moser, A. E., Moser, H. W., & Kishimoto, Y. (1984b). Adrenoleukodystrophy: impaired oxidation of very long chain fatty acids in white blood cells, cultured skin fibroblasts, and amniocytes. *Pediatr Res, 18*(3), 286-290. doi:10.1203/00006450-198403000-00016

Singh, I., Pahan, K., & Khan, M. (1998b). Lovastatin and sodium phenylacetate normalize the levels of very long chain fatty acids in skin fibroblasts of X-adrenoleukodystrophy. *FEBS Lett, 426*(3), 342-346.

Singh, I., & Pujol, A. (2010). Pathomechanisms underlying X-adrenoleukodystrophy: a three-hit hypothesis. *Brain Pathol, 20*(4), 838-844. doi:10.1111/j.1750-3639.2010.00392.x

Singh, J., & Giri, S. (2014). Loss of AMP-activated protein kinase in X-linked adrenoleukodystrophy patient-derived fibroblasts and lymphocytes. *Biochem Biophys Res Commun, 445*(1), 126-131. doi:10.1016/j.bbrc.2014.01.126

Singh, J., Olle, B., Suhail, H., Felicella, M. M., & Giri, S. (2016). Metformin-induced mitochondrial function and ABCD2 up-regulation in X-linked adrenoleukodystrophy involves AMP-activated protein kinase. *J Neurochem, 138*(1), 86-100. doi:10.1111/jnc.13562

Sivachenko, A., Gordon, H. B., Kimball, S. S., Gavin, E. J., Bonkowsky, J. L., & Letsou, A. (2016). Neurodegeneration in a Drosophila model of adrenoleukodystrophy: the roles of the Bubblegum and Double bubble acyl-CoA synthetases. *Dis Model Mech, 9*(4), 377-387. doi:10.1242/dmm.022244

Smirnova, E., Shurland, D. L., Ryazantsev, S. N., & van der Bliek, A. M. (1998). A human dynamin-related protein controls the distribution of mitochondria. *J Cell Biol, 143*(2), 351-358.

Smith, J. J., & Aitchison, J. D. (2013). Peroxisomes take shape. *Nat Rev Mol Cell Biol, 14*(12), 803-817. doi:10.1038/nrm3700

Spencer, C. M., Crabtree-Hartman, E. C., Lehmann-Horn, K., Cree, B. A., & Zamvil, S. S. (2015). Reduction of CD8(+) T lymphocytes in multiple sclerosis patients treated with dimethyl fumarate. *Neurol Neuroimmunol Neuroinflamm, 2*(3), e76. doi:10.1212/NXI.0000000000000076

St-Pierre, J., Drori, S., Uldry, M., Silvaggi, J. M., Rhee, J., Jager, S., . . . Spiegelman, B. M. (2006). Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators. *Cell, 127*(2), 397-408. doi:10.1016/j.cell.2006.09.024

Stack, C., Ho, D., Wille, E., Calingasan, N. Y., Williams, C., Liby, K., . . . Beal, M. F. (2010). Triterpenoids CDDO-ethyl amide and CDDO-trifluoroethyl amide improve the behavioral phenotype and brain pathology in a transgenic mouse model of Huntington's disease. *Free Radic Biol Med, 49*(2), 147-158. doi:10.1016/j.freeradbiomed.2010.03.017

Stadtman, E. R., & Levine, R. L. (2000). Protein oxidation. *Ann N Y Acad Sci, 899*, 191-208.

Stambolic, V., Ruel, L., & Woodgett, J. R. (1996). Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Curr Biol, 6*(12), 1664-1668.

Stambolic, V., & Woodgett, J. R. (1994). Mitogen inactivation of glycogen synthase kinase-3 beta in intact cells via serine 9 phosphorylation. *Biochem J, 303 (Pt 3)*, 701-704.

Stolz, D. B., Zamora, R., Vodovotz, Y., Loughran, P. A., Billiar, T. R., Kim, Y. M., . . . Watkins, S. C. (2002). Peroxisomal localization of inducible nitric oxide synthase in hepatocytes. *Hepatology, 36*(1), 81-93. doi:10.1053/jhep.2002.33716

Stoof, T. J., Flier, J., Sampat, S., Nieboer, C., Tensen, C. P., & Boorsma, D. M. (2001). The antipsoriatic drug dimethylfumarate strongly suppresses chemokine production in human keratinocytes and peripheral blood mononuclear cells. *Br J Dermatol, 144*(6), 1114-1120.

Sugiura, A., Mattie, S., Prudent, J., & McBride, H. M. (2017). Newly born peroxisomes are a hybrid of mitochondrial and ER-derived pre-peroxisomes. *Nature, 542*(7640), 251-254. doi:10.1038/nature21375

Supplie, L. M., Duking, T., Campbell, G., Diaz, F., Moraes, C. T., Gotz, M., . . . Nave, K. A. (2017). Respiration-Deficient Astrocytes Survive As Glycolytic Cells In Vivo. *J Neurosci, 37*(16), 4231-4242. doi:10.1523/JNEUROSCI.0756-16.2017

Sutherland, C., Leighton, I. A., & Cohen, P. (1993). Inactivation of glycogen synthase kinase-3 beta by phosphorylation: new kinase connections in insulin and growth-factor signalling. *Biochem J, 296 (Pt 1)*, 15-19.

Suzuki, K., Bose, P., Leong-Quong, R. Y., Fujita, D. J., & Riabowol, K. (2010). REAP: A two minute cell fractionation method. *BMC Res Notes, 3*, 294. doi:10.1186/1756-0500-3-294

Swerdlow, R. H., Burns, J. M., & Khan, S. M. (2010). The Alzheimer's disease mitochondrial cascade hypothesis. *J Alzheimers Dis, 20 Suppl 2*, S265-279. doi:10.3233/JAD-2010-100339

Sykiotis, G. P., & Bohmann, D. (2008). Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in Drosophila. *Dev Cell, 14*(1), 76-85. doi:10.1016/j.devcel.2007.12.002

Szepanowski, F., Donaldson, D. M., Hartung, H. P., Mausberg, A. K., Kleinschnitz, C., Kieseier, B. C., & Stettner, M. (2017). Dimethyl fumarate accelerates peripheral nerve regeneration via activation of the anti-inflammatory and cytoprotective Nrf2/HO-1 signaling pathway. *Acta Neuropathol*. doi:10.1007/s00401-017-1676-z

Taanman, J. W. (1999). The mitochondrial genome: structure, transcription, translation and replication. *Biochim Biophys Acta, 1410*(2), 103-123.

Talalay, P., & Dinkova-Kostova, A. T. (2004). Role of nicotinamide quinone oxidoreductase 1 (NQO1) in protection against toxicity of electrophiles and reactive oxygen intermediates. *Methods Enzymol, 382*, 355-364. doi:10.1016/S0076-6879(04)82019-6

Tanji, K., Maruyama, A., Odagiri, S., Mori, F., Itoh, K., Kakita, A., . . . Wakabayashi, K. (2013). Keap1 is localized in neuronal and glial cytoplasmic inclusions in various neurodegenerative diseases. *J Neuropathol Exp Neurol, 72*(1), 18-28. doi:10.1097/NEN.0b013e31827b5713

Tebay, L. E., Robertson, H., Durant, S. T., Vitale, S. R., Penning, T. M., Dinkova-Kostova, A. T., & Hayes, J. D. (2015). Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease. *Free Radic Biol Med, 88*(Pt B), 108-146. doi:10.1016/j.freeradbiomed.2015.06.021

ter Haar, E., Coll, J. T., Austen, D. A., Hsiao, H. M., Swenson, L., & Jain, J. (2001). Structure of GSK3beta reveals a primed phosphorylation mechanism. *Nat Struct Biol, 8*(7), 593-596. doi:10.1038/89624

Thau, N., Knippenberg, S., Korner, S., Rath, K. J., Dengler, R., & Petri, S. (2012). Decreased mRNA expression of PGC-1alpha and PGC-1alpha-regulated factors in the SOD1G93A ALS mouse model and in human sporadic ALS. *J Neuropathol Exp Neurol, 71*(12), 1064-1074. doi:10.1097/NEN.0b013e318275df4b

Thimmulappa, R. K., Mai, K. H., Srisuma, S., Kensler, T. W., Yamamoto, M., & Biswal, S. (2002). Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. *Cancer Res, 62*(18), 5196-5203.

Toki, T., Itoh, J., Kitazawa, J., Arai, K., Hatakeyama, K., Akasaka, J., . . . Ito, E. (1997). Human small Maf proteins form heterodimers with CNC family transcription factors and recognize the NF-E2 motif. *Oncogene, 14*(16), 1901-1910. doi:10.1038/sj.onc.1201024

Tolosa, E., Litvan, I., Hoglinger, G. U., Burn, D., Lees, A., Andres, M. V., . . . Investigators, T. (2014). A phase 2 trial of the GSK-3 inhibitor tideglusib in progressive supranuclear palsy. *Mov Disord, 29*(4), 470-478. doi:10.1002/mds.25824

Tong, K. I., Katoh, Y., Kusunoki, H., Itoh, K., Tanaka, T., & Yamamoto, M. (2006). Keap1 recruits Neh2 through binding to ETGE and DLG motifs: characterization of the two-site molecular recognition model. *Mol Cell Biol, 26*(8), 2887-2900. doi:10.1128/MCB.26.8.2887-2900.2006

Tremblay, M. E., Stevens, B., Sierra, A., Wake, H., Bessis, A., & Nimmerjahn, A. (2011). The role of microglia in the healthy brain. *J Neurosci, 31*(45), 16064-16069. doi:10.1523/JNEUROSCI.4158-11.2011

Treumer, F., Zhu, K., Glaser, R., & Mrowietz, U. (2003). Dimethylfumarate is a potent inducer of apoptosis in human T cells. *J Invest Dermatol, 121*(6), 1383-1388. doi:10.1111/j.1523-1747.2003.12605.x

Troffer-Charlier, N., Doerflinger, N., Metzger, E., Fouquet, F., Mandel, J. L., & Aubourg, P. (1998). Mirror expression of adrenoleukodystrophy and adrenoleukodystrophy related genes in mouse tissues and human cell lines. *Eur J Cell Biol, 75*(3), 254-264. doi:10.1016/S0171-9335(98)80121-0

Tsunemi, T., Ashe, T. D., Morrison, B. E., Soriano, K. R., Au, J., Roque, R. A., . . . La Spada, A. R. (2012). PGC-1alpha rescues Huntington's disease proteotoxicity by preventing oxidative stress and promoting TFEB function. *Sci Transl Med, 4*(142), 142ra197. doi:10.1126/scitranslmed.3003799

Tullet, J. M., Hertweck, M., An, J. H., Baker, J., Hwang, J. Y., Liu, S., . . . Blackwell, T. K. (2008). Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. *Cell, 132*(6), 1025-1038. doi:10.1016/j.cell.2008.01.030

Turk, B. R., Theisen, B. E., Nemeth, C. L., Marx, J. S., Shi, X., Rosen, M., . . . Fatemi, A. (2017). Antioxidant Capacity and Superoxide Dismutase Activity in Adrenoleukodystrophy. *JAMA Neurol*. doi:10.1001/jamaneurol.2016.5715

Uehara, T., Nakamura, T., Yao, D., Shi, Z. Q., Gu, Z., Ma, Y., . . . Lipton, S. A. (2006). S-nitrosylated protein-disulphide isomerase links protein misfolding to neurodegeneration. *Nature, 441*(7092), 513-517. doi:10.1038/nature04782

Uto, T., Contreras, M. A., Gilg, A. G., & Singh, I. (2008). Oxidative imbalance in nonstimulated X-adrenoleukodystrophy-derived lymphoblasts. *Dev Neurosci, 30*(6), 410-418. doi:10.1159/000191212

van der Vlies, D., Makkinje, M., Jansens, A., Braakman, I., Verkleij, A. J., Wirtz, K. W., & Post, J. A. (2003). Oxidation of ER resident proteins upon oxidative stress: effects of altering cellular redox/antioxidant status and implications for protein maturation. *Antioxid Redox Signal, 5*(4), 381-387. doi:10.1089/152308603768295113

van der Zand, A., Gent, J., Braakman, I., & Tabak, H. F. (2012). Biochemically distinct vesicles from the endoplasmic reticulum fuse to form peroxisomes. *Cell, 149*(2), 397-409. doi:10.1016/j.cell.2012.01.054

van Engen, C. E., Ofman, R., Dijkstra, I. M., van Goethem, T. J., Verheij, E., Varin, J., . . . Barbier, M. (2016). CYP4F2 affects phenotypic outcome in adrenoleukodystrophy by modulating the clearance of very long-chain fatty acids. *Biochim Biophys Acta, 1862*(10), 1861-1870. doi:10.1016/j.bbadis.2016.07.006

van Geel, B. M., Assies, J., Haverkort, E. B., Koelman, J. H., Verbeeten, B., Jr., Wanders, R. J., & Barth, P. G. (1999). Progression of abnormalities in adrenomyeloneuropathy and neurologically asymptomatic X-linked adrenoleukodystrophy despite treatment with "Lorenzo's oil". *J Neurol Neurosurg Psychiatry, 67*(3), 290-299.

van Geel, B. M., Bezman, L., Loes, D. J., Moser, H. W., & Raymond, G. V. (2001). Evolution of phenotypes in adult male patients with X-linked adrenoleukodystrophy. *Ann Neurol, 49*(2), 186-194.

van Geel, B. M., Poll-The, B. T., Verrips, A., Boelens, J. J., Kemp, S., & Engelen, M. (2015). Hematopoietic cell transplantation does not prevent myelopathy in X-linked adrenoleukodystrophy: a retrospective study. *J Inherit Metab Dis, 38*(2), 359-361. doi:10.1007/s10545-014-9797-1

van Horssen, J., Drexhage, J. A., Flor, T., Gerritsen, W., van der Valk, P., & de Vries, H. E. (2010). Nrf2 and DJ1 are consistently upregulated in inflammatory multiple sclerosis lesions. *Free Radic Biol Med, 49*(8), 1283-1289. doi:10.1016/j.freeradbiomed.2010.07.013

van Muiswinkel, F. L., de Vos, R. A., Bol, J. G., Andringa, G., Jansen Steur, E. N., Ross, D., . . . Drukarch, B. (2004). Expression of NAD(P)H:quinone oxidoreductase in the normal and Parkinsonian substantia nigra. *Neurobiol Aging, 25*(9), 1253-1262. doi:10.1016/j.neurobiolaging.2003.12.010

van Roermund, C. W., Ijlst, L., Wagemans, T., Wanders, R. J., & Waterham, H. R. (2014). A role for the human peroxisomal half-transporter ABCD3 in the oxidation of dicarboxylic acids. *Biochim Biophys Acta, 1841*(4), 563-568. doi:10.1016/j.bbalip.2013.12.001

van Roermund, C. W., Visser, W. F., Ijlst, L., van Cruchten, A., Boek, M., Kulik, W., . . . Wanders, R. J. (2008). The human peroxisomal ABC half transporter ALDP functions as a homodimer and accepts acyl-CoA esters. *Faseb J, 22*(12), 4201-4208. doi:10.1096/fj.08-110866

Vargas, C. R., Wajner, M., Sirtori, L. R., Goulart, L., Chiochetta, M., Coelho, D., . . . Mello, C. F. (2004). Evidence that oxidative stress is increased in patients with X-linked adrenoleukodystrophy. *Biochim Biophys Acta, 1688*(1), 26-32. doi:10.1016/j.bbadis.2003.10.004

Vaziri, H., Dessain, S. K., Ng Eaton, E., Imai, S. I., Frye, R. A., Pandita, T. K., . . . Weinberg, R. A. (2001). hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell, 107*(2), 149-159.

Verdin, E. (2015). NAD(+) in aging, metabolism, and neurodegeneration. *Science, 350*(6265), 1208-1213. doi:10.1126/science.aac4854

Vidal, R., Caballero, B., Couve, A., & Hetz, C. (2011). Converging pathways in the occurrence of endoplasmic reticulum (ER) stress in Huntington's disease. *Curr Mol Med, 11*(1), 1-12.

Vo, N., Fjeld, C., & Goodman, R. H. (2001). Acetylation of nuclear hormone receptor-interacting protein RIP140 regulates binding of the transcriptional corepressor CtBP. *Mol Cell Biol, 21*(18), 6181-6188.

von Otter, M., Landgren, S., Nilsson, S., Celojevic, D., Bergstrom, P., Hakansson, A., . . . Zetterberg, H. (2010a). Association of Nrf2-encoding NFE2L2 haplotypes with Parkinson's disease. *Bmc Medical Genetics, 11*. doi:10.1186/1471-2350-11-36

von Otter, M., Landgren, S., Nilsson, S., Zetterberg, M., Celojevic, D., Bergstrom, P., . . . Zetterberg, H. (2010b). Nrf2-encoding NFE2L2 haplotypes influence disease progression but not risk in Alzheimer's disease and age-related cataract. *Mechanisms of Ageing and Development, 131*(2), 105-110. doi:10.1016/j.mad.2009.12.007

Wakabayashi, N., Itoh, K., Wakabayashi, J., Motohashi, H., Noda, S., Takahashi, S., . . . Yamamoto, M. (2003). Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. *Nat Genet, 35*(3), 238-245. doi:10.1038/ng1248

Wanders, R. J. (2003). Defects in mitochondrial and peroxisomal fatty acid oxidation *Advances in Molecular and Cell Biology* (Vol. Volume 33, pp. 295-317): Elsevier.

Wanders, R. J., van Roermund, C. W., van Wijland, M. J., Nijenhuis, A. A., Tromp, A., Schutgens, R. B., . . . et al. (1987). X-linked adrenoleukodystrophy: defective peroxisomal oxidation of very long chain fatty acids but not of very long chain fatty acyl-CoA esters. *Clin Chim Acta, 165*(2-3), 321-329.

Wanders, R. J., van Roermund, C. W., van Wijland, M. J., Schutgens, R. B., van den Bosch, H., Schram, A. W., & Tager, J. M. (1988). Direct demonstration that the deficient oxidation of very long chain fatty acids in X-linked adrenoleukodystrophy is due to an impaired ability of peroxisomes to activate very long chain fatty acids. *Biochem Biophys Res Commun, 153*(2), 618-624.

Wanders, R. J., & Waterham, H. R. (2006). Biochemistry of mammalian peroxisomes revisited. *Annu Rev Biochem, 75*, 295-332. doi:10.1146/annurev.biochem.74.082803.133329

Wang, H., Brown, J., & Martin, M. (2011). Glycogen synthase kinase 3: a point of convergence for the host inflammatory response. *Cytokine, 53*(2), 130-140. doi:10.1016/j.cyto.2010.10.009

Wang, Q., Li, W. X., Dai, S. X., Guo, Y. C., Han, F. F., Zheng, J. J., . . . Huang, J. F. (2017). Meta-Analysis of Parkinson's Disease and Alzheimer's Disease Revealed Commonly Impaired Pathways and Dysregulation of NRF2-Dependent Genes. *J Alzheimers Dis, 56*(4), 1525-1539. doi:10.3233/jad-161032

Wang, Y., Busin, R., Reeves, C., Bezman, L., Raymond, G., Toomer, C. J., . . . Steinberg, S. (2011). X-linked adrenoleukodystrophy: ABCD1 de novo mutations and mosaicism. *Mol Genet Metab, 104*(1-2), 160-166. doi:10.1016/j.ymgme.2011.05.016

Wang, Y., Yang, J., & Yi, J. (2012). Redox sensing by proteins: oxidative modifications on cysteines and the consequent events. *Antioxid Redox Signal, 16*(7), 649-657. doi:10.1089/ars.2011.4313

Wang, Y. X., Santa-Cruz, K., DeCarli, C., & Johnson, J. A. (2000). NAD(P)H : quinone oxidoreductase activity is increased in hippocampal pyramidal neurons of patients with Alzheimer's disease. *Neurobiology of Aging, 21*(4), 525-531. doi:10.1016/s0197-4580(00)00114-7

Ward, R. J., Dexter, D. T., & Crichton, R. R. (2012). Chelating agents for neurodegenerative diseases. *Curr Med Chem, 19*(17), 2760-2772.

Waterham, H. R., Ferdinandusse, S., & Wanders, R. J. (2016). Human disorders of peroxisome metabolism and biogenesis. *Biochim Biophys Acta, 1863*(5), 922-933. doi:10.1016/j.bbamcr.2015.11.015

Wei, L. N., Farooqui, M., & Hu, X. (2001). Ligand-dependent formation of retinoid receptors, receptor-interacting protein 140 (RIP140), and histone deacetylase complex is mediated by a novel receptor-interacting motif of RIP140. *J Biol Chem, 276*(19), 16107-16112. doi:10.1074/jbc.M010185200

Wei, L. N., Hu, X., Chandra, D., Seto, E., & Farooqui, M. (2000). Receptor-interacting protein 140 directly recruits histone deacetylases for gene silencing. *J Biol Chem, 275*(52), 40782-40787. doi:10.1074/jbc.M004821200

Weller, M., Liedtke, W., Petersen, D., Opitz, H., & Poremba, M. (1992). Very-late-onset adrenoleukodystrophy: possible precipitation of demyelination by cerebral contusion. *Neurology, 42*(2), 367-370.

Welsh, G. I., & Proud, C. G. (1993). Glycogen synthase kinase-3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. *Biochem J, 294 (Pt 3)*, 625-629.

Weydt, P., Pineda, V. V., Torrence, A. E., Libby, R. T., Satterfield, T. F., Lazarowski, E. R., . . . La Spada, A. R. (2006). Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. *Cell Metab, 4*(5), 349-362. doi:10.1016/j.cmet.2006.10.004

Whitaker, R. M., Corum, D., Beeson, C. C., & Schnellmann, R. G. (2016). Mitochondrial Biogenesis as a Pharmacological Target: A New Approach to Acute and Chronic Diseases. *Annu Rev Pharmacol Toxicol, 56*, 229-249. doi:10.1146/annurev-pharmtox-010715-103155

Whitcomb, R. W., Linehan, W. M., & Knazek, R. A. (1988). Effects of long-chain, saturated fatty acids on membrane microviscosity and adrenocorticotropin responsiveness of human adrenocortical cells in vitro. *J Clin Invest, 81*(1), 185-188. doi:10.1172/JCI113292

White, K. A., Yore, M. M., Deng, D., & Spinella, M. J. (2005). Limiting effects of RIP140 in estrogen signaling: potential mediation of anti-estrogenic effects of retinoic acid. *J Biol Chem, 280*(9), 7829-7835. doi:10.1074/jbc.M412707200

White, R., Leonardsson, G., Rosewell, I., Ann Jacobs, M., Milligan, S., & Parker, M. (2000). The nuclear receptor co-repressor nrip1 (RIP140) is essential for female fertility. *Nat Med, 6*(12), 1368-1374. doi:10.1038/82183

Wiesinger, C., Eichler, F. S., & Berger, J. (2015). The genetic landscape of X-linked adrenoleukodystrophy: inheritance, mutations, modifier genes, and diagnosis. *Appl Clin Genet, 8*, 109-121. doi:10.2147/TACG.S49590

Wiesinger, C., Kunze, M., Regelsberger, G., Forss-Petter, S., & Berger, J. (2013). Impaired very long-chain acyl-CoA beta-oxidation in human X-linked adrenoleukodystrophy fibroblasts is a direct consequence of ABCD1 transporter dysfunction. *J Biol Chem, 288*(26), 19269-19279. doi:10.1074/jbc.M112.445445

Wilkinson, K. D. (2000). Ubiquitination and deubiquitination: targeting of proteins for degradation by the proteasome. *Semin Cell Dev Biol, 11*(3), 141-148. doi:10.1006/scdb.2000.0164

Wilms, H., Sievers, J., Rickert, U., Rostami-Yazdi, M., Mrowietz, U., & Lucius, R. (2010). Dimethylfumarate inhibits microglial and astrocytic inflammation by suppressing the synthesis of nitric oxide, IL-1beta, TNF-alpha and IL-6 in an in-vitro model of brain inflammation. *J Neuroinflammation, 7*, 30. doi:10.1186/1742-2094-7-30

Winterbourn, C. C. (1993). Superoxide as an intracellular radical sink. *Free Radic Biol Med, 14*(1), 85-90.

Witte, M. E., Mahad, D. J., Lassmann, H., & van Horssen, J. (2014). Mitochondrial dysfunction contributes to neurodegeneration in multiple sclerosis. *Trends Mol Med, 20*(3), 179-187. doi:10.1016/j.molmed.2013.11.007

Witte, M. E., Nijland, P. G., Drexhage, J. A., Gerritsen, W., Geerts, D., van Het Hof, B., . . . van Horssen, J. (2013). Reduced expression of PGC-1alpha partly underlies mitochondrial changes and correlates with neuronal loss in multiple sclerosis cortex. *Acta Neuropathol, 125*(2), 231-243. doi:10.1007/s00401-012-1052-y

Woodgett, J. R. (1990). Molecular cloning and expression of glycogen synthase kinase-3/factor A. *Embo J, 9*(8), 2431-2438.

Xue, J., Zhao, H., Shang, G., Zou, R., Dai, Z., Zhou, D., . . . Xu, Y. (2013). RIP140 is associated with subclinical inflammation in type 2 diabetic patients. *Exp Clin Endocrinol Diabetes, 121*(1), 37-42. doi:10.1055/s-0032-1323683

Yamamoto, H., Williams, E. G., Mouchiroud, L., Canto, C., Fan, W., Downes, M., . . . Auwerx, J. (2011). NCoR1 is a conserved physiological modulator of muscle mass and oxidative function. *Cell, 147*(4), 827-839. doi:10.1016/j.cell.2011.10.017

Yamamoto, T., Suzuki, T., Kobayashi, A., Wakabayashi, J., Maher, J., Motohashi, H., & Yamamoto, M. (2008). Physiological significance of reactive cysteine

residues of Keap1 in determining Nrf2 activity. *Mol Cell Biol, 28*(8), 2758-2770. doi:10.1128/MCB.01704-07

Yamashita, H., Avraham, S., Jiang, S., London, R., Van Veldhoven, P. P., Subramani, S., . . . Avraham, H. (1999). Characterization of human and murine PMP20 peroxisomal proteins that exhibit antioxidant activity in vitro. *J Biol Chem, 274*(42), 29897-29904.

Yan, J., Liu, X. H., Han, M. Z., Wang, Y. M., Sun, X. L., Yu, N., . . . Chen, Z. Y. (2015). Blockage of GSK3beta-mediated Drp1 phosphorylation provides neuroprotection in neuronal and mouse models of Alzheimer's disease. *Neurobiol Aging, 36*(1), 211-227. doi:10.1016/j.neurobiolaging.2014.08.005

Yang, L., Calingasan, N. Y., Thomas, B., Chaturvedi, R. K., Kiaei, M., Wille, E. J., . . . Beal, M. F. (2009). Neuroprotective effects of the triterpenoid, CDDO methyl amide, a potent inducer of Nrf2-mediated transcription. *PLoS One, 4*(6), e5757. doi:10.1371/journal.pone.0005757

Yang, W., & Hekimi, S. (2010). A mitochondrial superoxide signal triggers increased longevity in Caenorhabditis elegans. *PLoS Biol, 8*(12), e1000556. doi:10.1371/journal.pbio.1000556

Yao, H. B., Shaw, P. C., Wong, C. C., & Wan, D. C. (2002). Expression of glycogen synthase kinase-3 isoforms in mouse tissues and their transcription in the brain. *J Chem Neuroanat, 23*(4), 291-297.

Ylikallio, E., & Suomalainen, A. (2012). Mechanisms of mitochondrial diseases. *Ann Med, 44*(1), 41-59. doi:10.3109/07853890.2011.598547

Yoh, K., Itoh, K., Enomoto, A., Hirayama, A., Yamaguchi, N., Kobayashi, M., . . . Takahashi, S. (2001). Nrf2-deficient female mice develop lupus-like autoimmune nephritis. *Kidney Int, 60*(4), 1343-1353. doi:10.1046/j.1523-1755.2001.00939.x

Yoo, M. H., Gu, X., Xu, X. M., Kim, J. Y., Carlson, B. A., Patterson, A. D., . . . Hatfield, D. L. (2010). Delineating the role of glutathione peroxidase 4 in protecting cells against lipid hydroperoxide damage and in Alzheimer's disease. *Antioxid Redox Signal, 12*(7), 819-827. doi:10.1089/ars.2009.2891

Yoo, S. E., Chen, L., Na, R., Liu, Y., Rios, C., Van Remmen, H., . . . Ran, Q. (2012). Gpx4 ablation in adult mice results in a lethal phenotype accompanied by neuronal loss in brain. *Free Radic Biol Med, 52*(9), 1820-1827. doi:10.1016/j.freeradbiomed.2012.02.043

You, J., Yue, Z., Chen, S., Chen, Y., Lu, X., Zhang, X., . . . Liu, P. (2017). Receptor-interacting Protein 140 represses Sirtuin 3 to facilitate hypertrophy, mitochondrial dysfunction and energy metabolic dysfunction in cardiomyocytes. *Acta Physiol (Oxf), 220*(1), 58-71. doi:10.1111/apha.12800

Yuan, R., Meng, Q., Nautiyal, J., Flurkey, K., Tsaih, S. W., Krier, R., . . . Paigen, B. (2012). Genetic coregulation of age of female sexual maturation and lifespan through circulating IGF1 among inbred mouse strains. *Proc Natl Acad Sci U S A, 109*(21), 8224-8229. doi:10.1073/pnas.1121113109

Zarrouk, A., Vejux, A., Nury, T., El Hajj, H. I., Haddad, M., Cherkaoui-Malki, M., . . . Lizard, G. (2012). Induction of mitochondrial changes associated with oxidative stress on very long chain fatty acids (C22:0, C24:0, or C26:0)-treated human neuronal cells (SK-NB-E). *Oxid Med Cell Longev, 2012*, 623257. doi:10.1155/2012/623257

Zenkov, N. K., Menshchikova, E. B., & Tkachev, V. O. (2013). Keap1/Nrf2/ARE redox-sensitive signaling system as a pharmacological target. *Biochemistry (Mosc), 78*(1), 19-36. doi:10.1134/S0006297913010033

Zhang, D. D., & Hannink, M. (2003). Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. *Mol Cell Biol, 23*(22), 8137-8151. doi:10.1128/mcb.23.22.8137-8151.2003

Zhang, D. D., Lo, S. C., Cross, J. V., Templeton, D. J., & Hannink, M. (2004). Keap1 is a redox-regulated substrate adaptor protein for a Cul3-dependent ubiquitin ligase complex. *Mol Cell Biol, 24*(24), 10941-10953. doi:10.1128/MCB.24.24.10941-10953.2004

Zhang, Y., & Martin, S. G. (2014). Redox proteins and radiotherapy. *Clin Oncol (R Coll Radiol), 26*(5), 289-300. doi:10.1016/j.clon.2014.02.003

Zhao, W., Varghese, M., Yemul, S., Pan, Y., Cheng, A., Marano, P., . . . Pasinetti, G. M. (2011). Peroxisome proliferator activator receptor gamma coactivator-1alpha (PGC-1alpha) improves motor performance and survival in a mouse model of amyotrophic lateral sclerosis. *Mol Neurodegener, 6*(1), 51. doi:10.1186/1750-1326-6-51

Zheng, B., Liao, Z., Locascio, J. J., Lesniak, K. A., Roderick, S. S., Watt, M. L., . . . Global, P. D. G. E. C. (2010). PGC-1alpha, a potential therapeutic target for early intervention in Parkinson's disease. *Sci Transl Med, 2*(52), 52ra73. doi:10.1126/scitranslmed.3001059

Zilliacus, J., Holter, E., Wakui, H., Tazawa, H., Treuter, E., & Gustafsson, J. A. (2001). Regulation of glucocorticoid receptor activity by 14--3-3-dependent intracellular relocalization of the corepressor RIP140. *Mol Endocrinol, 15*(4), 501-511. doi:10.1210/mend.15.4.0624

Zschiedrich, I., Hardeland, U., Krones-Herzig, A., Berriel Diaz, M., Vegiopoulos, A., Muggenburg, J., . . . Herzig, S. (2008). Coactivator function of RIP140 for NFkappaB/RelA-dependent cytokine gene expression. *Blood, 112*(2), 264-276. doi:10.1182/blood-2007-11-121699