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## Childhood-onset movement disorders

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# Chapter 1

Introduction to the thesis



# INTRODUCTION

Movement disorders comprise a heterogeneous group of neurological disorders of varying aetiologies in which normal movement is either hampered (hypokinetic movement disorders) or mixed with involuntary, excessive movements (hyperkinetic movement disorders). Some movement disorders are the consequence of neurodegeneration, such as Parkinson's disease; dysfunction rather than degeneration appears to underlie others, as in the case of essential tremor. Furthermore, movement disorders can occur as a consequence of a genetic condition, sometimes mediated by neurodegeneration (e.g. in Huntington's disease), but not necessarily so (e.g. paroxysmal kinesigenic dyskinesia). The diversity of these disorders, united only by neurological impairment of normal movement as part of their symptomatology, is vast: not only in the cardinal type of movement disorder(s) caused by a given disease, but also in terms of disease course, prognosis, heritability, extracerebral symptoms and treatment possibilities. This diversity is particularly abundant in movement disorders manifesting in childhood, where the differential diagnosis comprises conditions of genetic, degenerative, metabolic, toxic, developmental, (post)infectious and idiopathic aetiology. Clinical phenotyping of these disorders can be quite challenging and is often complemented by imaging and laboratory studies in order to arrive at a diagnosis.

Genetic delineation of paediatric movement disorders is rapidly evolving, benefiting from advances made in the field of genetics, in particular next generation sequencing techniques such as whole-exome sequencing (WES). Whereas classification used to rely on symptomatology and/or imaging and laboratory findings, the number of diseases now primarily diagnosed by demonstration of a genetic abnormality is increasing<sup>1</sup>. This molecular and aetiological classification results in genetically stratified patient categories, which may have varying clinical phenotypes, but which could theoretically facilitate more specific and cause-related scientific research. In addition, homogenous patient groups classified according to their molecular defects could enhance the future implementation of targeted therapies. However, as a consequence of this strict demarcation and classification based on molecular defects, clinical research into genetic childhood-onset movement disorders is often hampered by the fact that this usually concerns small patient cohorts, making it difficult to answer clinically relevant questions about a particular patient category.

A solution to this problem of answering clinical questions in small sized cohorts of patients with rare disorders is found in the use of model organisms. By the virtue of their aetiology, genetic movement disorders are often readily translated to a laboratory model, thus limiting the exploratory phase of research that needs to be done in a patient cohort. Examples of model organism research include unbiased testing of large compound screens (e.g. drugs approved for other diseases), which could lead to unexpected and novel therapeutics, as well as studying the pathophysiology of diseases; in other words, the disease process itself with the crucial cellular events involved in the cascade leading to the observed phenotypes, as well as potential interventions. Both these approaches in model systems could yield important information that can be translated to patients, avoiding exploratory research in the patients themselves and enabling a preselection of valuable and promising approaches. In addition to limiting the amount of research in patients and shifting from exploring to validating, model organisms often offer superior tools



to study genes, proteins, metabolites and drugs in disease states.

A myriad of different models is available, extending from acellular systems to complete higher organisms. Naturally, the choice of model organism depends on the research questions to be answered. Fundamental questions can often be answered in a test tube (*in vitro*) or in a unicellular organism such as *Saccharomyces cerevisiae*: examples of such questions include the identification of binding partners of a given protein (often done by two-hybrid setups) or the relative enzymatic activity of a mutant protein compared to its wildtype counterpart. Questions that more specifically target a tissue or an organ often involve more complex models, such as nematodes (*Caenorhabditis elegans*), insects (*Drosophila melanogaster*) or rodents (*Mus musculus*, *Rattus norvegicus*).

### ***Drosophila* as a model organism for neurological disease**

The fruit fly, *Drosophila melanogaster*, has been used to study neurological phenomena for several decades and has proven its value in the field of neuroscience by virtue of its reasonably complex nervous system, its capability to recapitulate several key features of neurological disease such as neurodegeneration and epilepsy, and the relative ease with which the organism can be manipulated and studied<sup>2-4</sup>. The fruit fly has a life cycle of around 12 days, which enables fast assembly of a desired genotype by means of crossing at relatively low costs. All these properties have motivated researchers to employ the fruit fly in research into, amongst others, Parkinson's disease<sup>5</sup>, polyglutamine diseases such as Huntington's disease and spinocerebellar ataxias<sup>6,7</sup>, dystonia<sup>8</sup> and epilepsy<sup>9,10</sup>. Given its importance for the interpretation of this thesis, the merits of *Drosophila* in research of both neurodegeneration and epilepsy disorders will be discussed separately in the following paragraphs.

The history of neurodegeneration research in *Drosophila* starts with behavioural studies. Indeed, one of the first neurodegenerative mutants described, *drop dead*<sup>1</sup>, was isolated on the basis of its erratic movement upon aging<sup>11</sup> and was revealed to have a brain "shot full of holes". After *drop dead*, many other genes were found to cause neurodegenerative phenotypes with brain vacuolisation, behavioural abnormalities and shortened lifespan in *Drosophila*, and over half of these are related to orthologues associated with neurodegeneration in mouse or human<sup>2</sup>. In addition to *drop dead*, Benzer also described mutants with abnormal responses to various stimuli<sup>11</sup>. About the mutant *easily shocked* (*eas*), he writes "When subjected to a mechanical jolt, the mutant displays a syndrome not unlike an epileptic seizure: the fly takes a few faltering steps, falls on its back, flails its legs and wings wildly, and coils its abdomen under. [...] The fly then goes into a coma, lasting some minutes, after which it revives and walks around as if nothing had happened."<sup>11</sup>. Since *eas*, more mutants have been found to exhibit abnormal seizure-like behaviour in response to stimuli. These *Drosophila* mutants include *slamdance* (*sda*, a mutant allele of *julius seizure*<sup>9</sup>), *bang senseless* (*bss*, a mutant allele of *paralytic*<sup>12</sup>) and *technical knockout* (*tko*<sup>13</sup>). Their phenotype is characterised by a lowered resistance to either a mechanical or electrical precipitating stimulus: bang sensitivity, which describes seizure-

\* Genes discovered in *Drosophila* are customarily named after the phenotype observed in the respective mutant. This can lead to confusion, since, for example, the *white* gene is in fact responsible for the normal red colour of the eye: in *white*'s **absence**, the eyes are white. Also, some of the genetic nomenclature is filled with humorous names such as *cheap date* (hypersensitive to alcohol), *Cleopatra* (lethal together with *Asp*), *hamlet* (affects 2B cells) and *tinman* (has no heart).



like behaviour and paralysis upon a mechanical stimulus, and a decreased electrophysiological seizure threshold. This threshold is determined by the voltage which, when administered in a high-frequency pulse directly to the brain, is sufficient to evoke seizure-like firing over the giant fiber pathway as recorded at a peripheral muscle recording<sup>14</sup>. In line with this seizure behaviour representing *bona fide* epileptic phenomena, seizure phenotypes were shown to be amenable by clinically used anticonvulsants<sup>15–17</sup>. A third seizure provocation paradigm, with epileptic activity as precipitated by a thermal stimulus, was validated more recently by the fly model for genetic epilepsy with febrile seizures plus (GEFS+) caused by mutations in *SCN1A*<sup>18</sup>. These heat-induced seizures were also electrophysiologically documented and aggravated by administration of GABA<sub>A</sub>R-antagonist picrotoxin, a known chemoconvulsant<sup>18</sup>. Moving one step further, downstream targets which can be targeted by novel anticonvulsants are beginning to emerge<sup>10</sup>.

In this thesis, the power of *Drosophila melanogaster* as a tool to study the pathogenesis of neurological disease is exploited in the context of two different genetic childhood-onset movement disorders, **pantothenate kinase-associated neurodegeneration (PKAN)**, the most frequently occurring subtype of Neurodegeneration with Brain Iron Accumulation (NBIA); and **North Sea progressive myoclonus epilepsy (NS-PME)**, one of the genetically delineated subtypes of progressive myoclonic epilepsy (PME). The remainder of this introduction will elaborate on these two diseases by placing them in their respective clinical and cell biological context.

### **Pantothenate kinase-associated neurodegeneration (PKAN) and Neurodegeneration with Brain Iron Accumulation (NBIA)**

Pantothenate kinase-associated neurodegeneration (PKAN) is a rare, devastating and relentlessly progressive childhood-onset neurodegenerative disease featuring movement disorders, most often dystonia (involuntary movements leading to abnormal posturing and/or writhing) and choreoathetosis<sup>19</sup>. All these symptoms worsen as the disease progresses, leading to loss of ambulation and independence: eventually, patients often succumb to complications such as pneumonia<sup>20</sup>. Neurodegeneration takes place almost exclusively in the globus pallidus, which exhibits prominent iron deposition<sup>19</sup>. Because of this particular finding, PKAN is one of the diseases commonly referred to as Neurodegeneration with Brain Iron Accumulation (NBIA), a group of disorders formerly associated with the eponym of Hallervorden and Spatz. These German neuropathologists, whose names have fallen into disfavour as a consequence of their unethical wartime activities, described a familial neurodegenerative disease featuring extrapyramidal symptoms and iron accumulation in the globus pallidus as early as 1922<sup>21</sup>. Since then, reports of “Hallervorden-Spatz syndrome” have steadily found their way into the medical literature, reaching this syndromic diagnosis via clinical symptomatology and autopsy results. The introduction of MR imaging enabled this diagnosis to be made during a patient’s lifetime: brain iron deposition can be detected as a hypointensity in T2-weighted MRI-sequences as the iron (in particular the ferric Fe<sup>3+</sup>) facilitates the relaxation of protons in neighbouring water molecules<sup>22</sup>. Starting from 2001, identification of the underlying genetic defect became possible<sup>23</sup>: as a consequence, the “Hallervorden-Spatz syndrome” became NBIA, a constellation of diseases of various genetic aetiologies united by the presence of



excessive brain iron in specific though distinct brain areas, with PKAN as its most common subtype<sup>19</sup>. At the moment of writing, 10 different NBIA genes are known, all giving rise to a specific NBIA subtype with distinct symptomatology<sup>24</sup>.

Retrospectively, this renders it difficult to utilise the data collected in the Hallervorden-Spatz era, since the underlying genetic causes of reported cases may differ: indeed, the different NBIA-subtypes are now considered to be separate entities rather than variants of the same disease<sup>24</sup>. This can be justified not only by the differences in symptomatology, but also in age of onset, rate of symptom progression, regional distribution of iron accumulation, mode of inheritance, extracerebral manifestations and neuropathological features. To stress this latter point: in case reports of Hallervorden-Spatz syndrome, various aggregates such as Lewy bodies and neurofibrillary tangles were reported<sup>25-27</sup>. Nevertheless, a careful analysis of genetically identified patients showed that PKAN does not feature any Lewy body pathology<sup>28,29</sup> whereas synuclein accumulation is abundantly present in PLAN<sup>30</sup>. Neurofibrillary tangles, absent in PKAN<sup>29</sup>, were observed in BPAN<sup>31</sup>, a different NBIA subtype with a different genetic cause. This distinction is of utmost importance, as it limits the potential of translating scientific findings in PKAN to other, more common neurodegenerative diseases such as Parkinson's disease or Alzheimer's disease. In addition, it implies that, although they share some clinical features, the pathophysiology of these subtypes diverges at the molecular level, justifying the strict separation between the NBIA subtypes based on the genes involved.

The biosynthesis of coenzyme A starting from pantothenate comprises five enzymatic steps, the first of which is catalysed by PANK. The final two steps are carried out by a bifunctional enzyme (PPAT-DPCK or CoASY) in humans. Disease associations are noted.

### **Pantothenate kinase-associated neurodegeneration and Coenzyme A**

The discovery of mutations in PANK2 as the cause of PKAN kick-started research into the disease's underlying pathophysiological aberrations. PANK2 belongs to the family of pantothenate kinases, along with PANK1, PANK3 and PANK4. Of these four, only PANK2 resides in the mitochondrion<sup>32</sup>. Pantothenate kinase functions in the biosynthesis of coenzyme A (CoA), an indispensable cofactor in many metabolic reactions<sup>33</sup>: indeed, it has been estimated that CoA partakes in approximately 9% of all reactions in the cell<sup>34</sup>. The substrate for PANK is pantothenate (vitamin B<sub>5</sub>), which is absorbed from the diet and converted into phosphopantothenate<sup>33</sup>: the subsequent action of phosphopantetheoylcysteine synthetase (PPCS), phosphopantetheoylcysteine decarboxylase (PPCDC), phosphopantetheine adenyl transferase (PPAT) and dephosphocoenzyme A kinase (DPCK) leads to the production of CoA (Figure 1). In humans, the final two steps are carried out by a bifunctional PPAT-DPCK enzyme named CoA synthase (CoASY); interestingly, another NBIA subtype is associated with a defect in this mitochondrial enzyme<sup>35</sup>. This NBIA, referred to as CoPAN in reference to PKAN, is similar to PKAN in many respects, also featuring iron accumulation and neurodegeneration in the globus pallidus. Although alterations in CoA levels in PKAN patient samples have (surprisingly) never been reported, decreased biosynthesis of CoA appears to be a feasible pathophysiological mechanism, explaining why deficiencies of both PANK2 and CoASY lead to the same neurodegenerative phenotype. However, given the ubiquitous nature of CoA and its

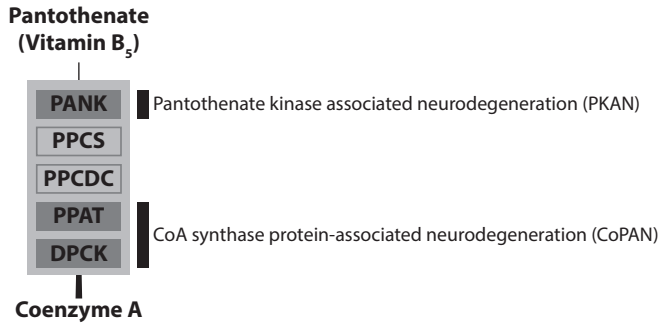


Figure 1 | **Biosynthesis of coenzyme A from pantothenate.**

The biosynthesis of coenzyme A starting from pantothenate comprises five enzymatic steps, the first of which is catalysed by PANK. The final two steps are carried out by a bifunctional enzyme (PPAT-DPCK or CoASY) in humans. Disease associations are noted.

involvement in numerous different processes, it seems counterintuitive that a CoA deficiency would lead to such localised (neuro)pathology: indeed, the fallout of a global CoA deprivation would affect many enzymes that rely on a CoA-species, the deficiencies of which alone already cause extracerebral or more widespread pathology (table). Nevertheless, a fruit fly model of the disease caused by hypomorphic pantothenate kinase (*fumble*, abbreviated *fbI*) can be rescued by pantethine, a possible precursor to CoA biosynthetic intermediate 4-phosphopantetheine<sup>36</sup>. Although the *PANK2*<sup>-/-</sup> mouse model disappointingly features no neurodegeneration under normal conditions<sup>37</sup>, a neurological phenotype can be provoked by either pantothenic acid deprivation<sup>38</sup> or a ketogenic diet<sup>39</sup>; this latter phenotype is rescued when pantethine is administered<sup>39</sup>. Finally, levels of CoA-species have been measured in skin fibroblasts of CoPAN patients; despite the fact that the biosynthetic capacity of the terminal CoA biosynthesis enzymes in patient cells was shown to be reduced to 20% of controls<sup>35</sup>, the free and total CoA levels were not different from control cells<sup>35</sup>. However, acetyl-CoA levels were found to be decreased, a difference which reached significance in one patient and failed to do so in the second<sup>35</sup>. These findings are in concordance with a more specific, but nevertheless CoA-related, pathophysiological substrate for PKAN.

### **Pantothenate kinase-associated neurodegeneration beyond Coenzyme A**

More downstream consequences have remained elusive, despite the extensive exploitation of model organisms. Currently, PKAN is modelled in fruit flies<sup>36,40</sup>, zebrafish<sup>41</sup>, mice<sup>37,39</sup>, patient fibroblasts<sup>42,43</sup> and neurons derived from PKAN patient induced pluripotent stem cells (iPSC)<sup>44</sup>. In addition, information is available concerning metabolites in peripheral blood of patients<sup>45</sup>, as well as patients suffering from intoxications with the PANK inhibitor hopantenate (HoPan)<sup>46,47</sup>. Briefly, theories about the pathways implicated in PKAN will be discussed, together with data from models addressing these pathways. A summary is provided in Figure 2.



## Mitochondrial dysfunction

Consistent with the mitochondrial localisation of PANK2, mitochondrial dysfunction has been a prime suspect in the pathophysiology of PKAN. In addition, CoA is a cofactor in many mitochondrial reactions, including the tricarboxylic acid cycle (TCA, or citric acid cycle) and fatty acid  $\beta$ -oxidation. As a consequence, mitochondrial function has been well-studied in the context of PKAN. Indeed, in many PKAN models, mitochondrial dysfunction is noted: exponents of this include decreased mitochondrial membrane potential<sup>39,44,48</sup>, a decreased oxygen consumption rate<sup>42,49</sup> and morphological abnormalities of the mitochondria<sup>36,50</sup>. In *Drosophila* and mice, these phenotypes are reversed upon administration of pantethine<sup>36,39,44</sup>, implying that these are direct consequences of a CoA-related pathology.

In a metabolic screen of peripheral blood of 14 PKAN patients, lactate levels, but not pyruvate levels, were significantly elevated compared to controls matched for age and sex<sup>45</sup>. Similarly, massive lactic acidosis has been reported in patients with hyperammonaemia and encephalopathy secondary to hopantenate intoxication<sup>46,47,51,52</sup>. Contrary to the metabolic findings in PKAN patients, hopantenate intoxication also features elevated pyruvate levels<sup>51,52</sup>. Lactic acidosis has been observed upon administration of hopantenate to dogs<sup>53</sup> and was prevented by co-administration of pantothenic acid<sup>53</sup>; however, HoPan-fed mice do not feature lactic acidosis, instead demonstrating hypoglycaemia unresponsive to pyruvate administration<sup>54</sup>. The biological differences underlying the discrepancy between these findings is unknown.

## Fatty acid metabolism

Fatty acid metabolism has also been implicated in the pathophysiology of PKAN. Given the involvement of CoA and acyl-CoA in the handling of these metabolites, lipids have been studied in some detail in PKAN models.

Although the PANK2<sup>-/-</sup> mice show no neurological phenotype under normal conditions<sup>37</sup>, a ketogenic diet (composed of 79,2% fat and 8% protein) provokes neuropathological abnormalities in both the central nervous system and the peripheral nervous system<sup>39</sup>. Levels of bile acids (derived from cholesterol), various sterols, fatty acids and triacylglycerides were found to be lower in PKAN patient blood samples compared to controls<sup>45</sup>. In patients treated with hopantenate,  $\gamma$ -hydroxy fatty acids were found in the urine, possibly suggesting impaired beta-oxidation<sup>46</sup>. However, the metabolic profile in the urine of these patients was distinct from that observed in patients suffering from defects in  $\beta$ -oxidation, such as medium chain acyl-CoA dehydrogenase deficiency (MCADD), long chain acyl-CoA dehydrogenase deficiency (LCAD) or multiple acyl-CoA dehydrogenase deficiency (MAD)<sup>52</sup>. This suggests that, at least in massive interference with most likely hepatic Coenzyme A metabolism, fatty acid metabolism is affected, but the mechanism remains unclear and does not resemble more well-known  $\beta$ -oxidation defects.






## Iron accumulation

A third possibility is a causal role for the iron accumulation in the pathophysiology of PKAN. Interestingly, none of the organisms referred to previously reproduces the iron accumulation observed in patients. In patient fibroblasts and iPSC neurons, formation of iron-sulfur clusters (ISCs) appears impaired since the





activities, but not the levels, of enzymes depending on these ISCs are decreased<sup>43,44</sup>. The other pathway of generating biologically active iron intermediates is the production of heme: heme levels were also found to be decreased in cellular models of PKAN<sup>43,44</sup>. However, ferrochelatase, one of the heme biosynthesis enzymes, itself depends on an ISC, and ISC depletion leads to secondary heme synthesis defects<sup>55</sup>. Therefore, in these models, impairment of ISC biosynthesis appears to be a major pathway explaining deregulation of iron utilisation. The cause of this ISC biosynthesis defect in cell models of PKAN is still unknown. However, certain enzymes need one or more ISCs to be functional, such as aconitase, lipoic acid synthase and NADH dehydrogenase: phenotypes and biochemical derangements secondary to dysfunction of these enzymes form a possible pathophysiological mechanism. Evidence for a possible role of ISCs in neuropathology comes from Friedreich's ataxia, where deficiency of the ISC-chaperone frataxin leads to neurodegeneration and iron accumulation.

	 <b>Fruit fly</b> ( <i>Drosophila melanogaster</i> )	 <b>Mouse</b> ( <i>Mus musculus</i> )	 <b>PKAN patient fibroblasts</b>	 <b>PKAN patient IPSC neurons</b>	 <b>PKAN patient peripheral blood sample</b>
<b>Specifications of model</b>	Disruptions in dPANK/ <i>fumble</i> or treatment with hopantenate (HoPan, PANK inhibitor)	Disruption in mPANK2/PANK2 (with or without ketogenic diet)	Fibroblasts obtained from PKAN patients (mutations conform)	Neurons from IPS cells derived from PKAN patients (mutations conform)	Peripheral blood from PKAN patients (with or without overnight fast)
<b>CoA levels</b>	Decreased	Not reported	Not reported*	Not reported	Not reported
<b>Mitochondria</b>	<ul style="list-style-type: none"> <li>Abnormal mitochondrial morphology</li> <li>Reduced mitochondrial membrane potential</li> <li>Increase in ROS</li> </ul>	<ul style="list-style-type: none"> <li>Abnormal mitochondrial morphology</li> <li>Reduced mitochondrial membrane potential</li> <li>Decreased OCR</li> </ul>	<ul style="list-style-type: none"> <li>Increase in ROS</li> <li>Decreased ATP</li> <li>Reduced mitochondrial membrane potential</li> </ul>	<ul style="list-style-type: none"> <li>Abnormal mitochondrial morphology</li> <li>Reduced mitochondrial membrane potential</li> <li>Increase in ROS</li> <li>Decreased OCR</li> </ul>	<ul style="list-style-type: none"> <li>Increase in lactate</li> </ul>
<b>Fatty acids / lipids</b>	<ul style="list-style-type: none"> <li>Reduced TAG levels</li> <li>Reduced phospholipids: PS, PC, PE</li> </ul>	Not reported	<ul style="list-style-type: none"> <li>Reduced lanosterol, lathosterol, palmitic acid and oleic acid levels</li> </ul>	Not reported	<ul style="list-style-type: none"> <li>Reduced TAG levels</li> <li>Reduced phospholipids: LPC, PC, SM</li> <li>Reduced lanosterol and lathosterol levels</li> <li>Reduced bile acid levels</li> </ul>
<b>Iron pathology</b>	Not reported	Not reported	<ul style="list-style-type: none"> <li>Reduced activity (but not levels) of Fe-S cluster containing enzymes</li> <li>Reduced heme levels</li> </ul>	<ul style="list-style-type: none"> <li>Reduced activity (but not levels) of Fe-S cluster containing enzymes</li> <li>Reduced heme levels (in NPCs)</li> </ul>	Not observed

\* In CoPan fibroblasts, no decrease in free or total CoA

Figure 2 | Pathophysiological elements of PKAN and their counterparts in various PKAN models

As discussed in the main text, different types of cellular pathology are hypothesised to underlie PKAN, some of which are recapitulated by model organisms.

(IPS induced pluripotent stem, ROS reactive oxygen species, OCR oxygen consumption rate, TAG triacylglycerides, PS phosphatidylserine, PC phosphatidylcholine, PE phosphatidylethanolamine, LPC lysophosphatidylcholine, SM sphingomyelin, NPC neural progenitor cells)



The spatiotemporal relationship between the iron accumulation in the CNS and neurodegeneration have led some to consider the iron accumulation as the cause of the neurodegeneration. This can be rationalised by the observation that the formation of reactive oxygen species (ROS) is accelerated in the presence of ferric iron via the Fenton reaction<sup>56</sup>. More rationale follows from the treatment of Wilson's disease (hepatolenticular degeneration), a disorder caused by mutations interfering with copper metabolism. The use of chelating agents to counteract the copper accumulation in the basal ganglia of these patients improves their extrapyramidal symptoms and prevents further degeneration<sup>57</sup>. This has led to the hypothesis that metal accumulation may be the culprit in PKAN, and that iron chelating therapy may prove beneficial. A particular iron chelator, deferiprone, is able to cross the blood-brain barrier and has been shown to reduce brain iron levels in patients with PKAN<sup>58</sup>. Whether the sequestration of this iron also affects the clinical course of the disease is the subject of a large and ongoing trial, the results of which have not yet been published.

### Hypoxia

More recently, hypoxia has been suggested as a pathophysiological element in PKAN<sup>59</sup>. The damage observed in the globus pallidus in PKAN resembles ischaemic lesions in this same region. The globus pallidus has a high metabolic demand owing to its role as a tonic inhibitor projecting on the thalamus, making it vulnerable to metabolic insults and energy deficits. This symptomatology is recapitulated by intoxications that lead to cellular hypoxia, such as carbon monoxide and cyanide: survivors often feature damage localised to the globus pallidus<sup>60,61</sup>. Therefore, it has been proposed that hypoxia, either real or mis-sensed, plays a role in the pathophysiology of PKAN. Phenomenologically, hypoxia shares many features with mitochondrial dysfunction, since mitochondria are responsible for carrying out the oxidative metabolism that is inhibited or impossible under hypoxic conditions. Lactic acidosis, for example, is found both in hypoxia and in mitochondrial failure. Hypoxia induces gene expression of numerous genes among which is pyruvate dehydrogenase kinase, which in turn leads to inhibition of the pyruvate dehydrogenase complex, the gatekeeper of oxidative mitochondrial metabolism: not surprisingly, pyruvate dehydrogenase complex deficiency mimics cellular hypoxia, much like intoxications with cyanide or carbon monoxide do. A molecular connection between hypoxia and impaired CoA production has, however, not been characterized.

### Treatment

The scarcity of knowledge about the pathophysiology of PKAN has hampered the development of targeted treatments for PKAN patients. Currently, treatment consists of antidystonic medication (trihexyphenidyl, baclofen, gabapentin or local injections with botulinum toxin A) and treatment of concomitant symptoms. Deep brain stimulation has been shown to improve symptoms and quality of life in PKAN<sup>62</sup>. Unfortunately, no disease-modifying treatment is available. Despite the beneficial effect of pantethine in models of PKAN, the compound has not been used in clinical applications, mostly because of its chemical lability in biological samples<sup>63</sup>. Considering the beneficial effect of pantethine in multiple models of PKAN, the search for derivatives or substitutes that provide the same biological effect with a



more favourable pharmacological profile may provide a therapeutic strategy. Recently, it was suggested that excess CoA may exert deleterious effects in muscle<sup>64</sup>, possibly limiting the use of therapeutics aimed at increasing CoA levels in the brain. With this in mind, more research into downstream targets involved in PKAN pathophysiology may yield novel targets for treatment, which may be combined with CoA-based therapies and, depending on the results of the deferiprone trial, iron chelating therapy.

The remainder of this introduction is dedicated to North Sea Progressive Myoclonus Epilepsy (NS-PME), a disease which, like PKAN, is caused by a known genetic defect and leads to a progressive childhood-onset movement disorder.

### **North Sea Progressive Myoclonus Epilepsy and the Progressive myoclonus epilepsies (PMEs)**

The progressive myoclonus epilepsies (PMEs) are a genetically heterogeneous group of disorders characterised by myoclonus (brief involuntary muscle jerks) and epilepsy, both of which become more severe in the course of the disease (progressive)<sup>65</sup>. Although initially recognized by Ramsay-Hunt in 1922 as *dysynergia cerebellaris myoclonica*<sup>66</sup>, the eponymous Ramsay Hunt syndrome was deemed insufficiently specific by the consensus statement of Marseille<sup>67</sup>, as it covered not only the progressive myoclonic epilepsies but also the progressive myoclonus ataxias (PMAs), which are classically associated with cerebellar dysfunction (ataxia) and infrequent seizures. However, it was recognised that many cases of PME also feature ataxia<sup>68</sup>, and therefore, considerable overlap exists between these clinical entities representing the two ends of the PMA-PME spectrum<sup>67,69</sup>.

As with NBIA, the advances in genetics have enabled the discovery of various genetic defects underlying PME. Among these subtypes, the presence or absence of cognitive decline is a useful distinguishing feature. Major subtypes of PME featuring dementia include Lafora body disease and the neuronal ceroid lipofuscinoses<sup>70</sup>. The archetype representing PME subtypes which leave cognitive function unscathed is Unverricht-Lundborg disease (ULD), or “Baltic myoclonus”, associated with mutations in cystatin B (CSTB)<sup>70</sup>. After the discovery of CSTB mutations, cases of PME without cognitive decline lacking mutations in CSTB were considered “ULD-like” and genetic alterations in SCARB2<sup>71–73</sup> and PRICKLE1<sup>74</sup> were found to cause PME in some of these families .

A novel genetic cause for ULD-like PME was found in 2011, when a mutation in Golgi SNAP receptor complex member 2 (GOSR2) was identified in five families suffering from a particular subtype of PME<sup>75</sup>. The symptoms were remarkably homogeneous with early ataxia (around 2 years of age), followed by myoclonus later in childhood and epilepsy becoming more prominent during adolescence<sup>76–78</sup>. Other distinctive features are areflexia, scoliosis and elevated serum creatine kinase levels<sup>77,78</sup>. Interestingly, nearly all patients identified thus far homozygously carry the same mutation in GOSR2, suggesting a founder effect. Most families with this type of PME identified so far originate from countries bordering the North Sea and for this reason the disease was dubbed “North Sea progressive myoclonus epilepsy” (NS-PME)<sup>77</sup>. Due to this founder effect and the location around the North Sea, NS-PME is relatively common in the Netherlands, particularly in the north, where a cohort of 5 patients was reported soon after the discovery of the GOSR2 mutation<sup>78</sup>. Neuropathology is only available for a single case of NS-



PME, demonstrating mild atrophy without gross abnormalities<sup>75</sup>. Alzheimer type II gliosis was noted in the basal ganglia region. In the cerebellar vermis, there was gliosis and minor loss of Purkinje cells, but no focal neuronal degeneration elsewhere in the brain<sup>75</sup>. This apparent lack of neurodegeneration is in stark contrast with ULD, where Purkinje cell loss in the cerebellum is prominent, thus characterising ULD as a neurodegenerative disorder<sup>79–81</sup>.

Currently, treatment for NS-PME is symptomatic, and typically involves a combination of anticonvulsants to control the seizures and (often to a lesser extent) myoclonus. Care must be taken in the choice for anticonvulsants, as some are known to exacerbate myoclonus<sup>70</sup>.

Fundamental knowledge about the consequences of mutations in *GOSR2* is scarce. The mutation causative of NS-PME was initially reported to cause failure of the protein to localise to the *cis*-Golgi in patient fibroblasts<sup>75</sup>, however, this was later disproved<sup>82</sup>. The mutation does not interfere with *GOSR2* expression levels or its native interaction with binding partner ARF1<sup>75</sup>. The knockout of yeast *GOSR2* orthologue *bos1* could be complemented by wildtype *bos1*, but not by *bos1* carrying the patient mutation, demonstrating the nature of the mutation in *GOSR2* to be a loss of function<sup>75</sup>. This was later reinforced by liposome studies showing a reduced SNARE fusion rate for yeast Bos1 carrying the patient mutation compared to wildtype Bos1<sup>82</sup>.

Recently, *Drosophila* has been used to provide a model for NS-PME by interfering genetically with *GOSR2*-orthologue *membrin*<sup>82</sup>. Ubiquitous overexpression of mutant *membrin* in a *membrin*-deficient background caused mostly pharate or early adult lethality, with morphological and electrophysiological abnormalities in the nervous system observed in late larval stages<sup>82</sup>. Nevertheless, it is still unknown which cell type confers these changes, and which processes derail on the cellular level to cause the human disease.

At first glance, PKAN and NS-PME are two very different diseases, with a different underlying genetic defect and a different clinical symptomatology. However, it is the challenge of studying them that unites NS-PME and PKAN. For both diseases patient populations are small, making it difficult to conduct intervention studies; also, affected tissue is unattainable during life. This makes model organisms of utmost importance to gain insights that can be subsequently translated to the clinic. This thesis explores the use of *Drosophila* for this purpose.



# AIM AND OUTLINE OF THE THESIS

In this thesis, *Drosophila melanogaster* was used to gain insight in both clinically relevant and fundamental processes underlying genetic childhood-onset movement disorders PKAN and NS-PME.

To demonstrate that *Drosophila* is able to provide answers to biologically and clinically relevant questions in the field of neurodegeneration, we reviewed the existing literature concerning this subject in **Chapter 2**. Several techniques that were used in the later chapters of the thesis are highlighted in this chapter as well.

The thesis then focuses on PKAN, first with a chemical approach aimed to transform pantethine into a viable therapeutic: this involved the development of derivatives that may feature more favourable pharmacokinetic and pharmacodynamic properties. In **Chapter 3** we described the chemical synthesis and characterisation of TBTP-pantetheine, a derivative of pantethine designed to demonstrate enhanced pharmacological properties in order to overcome obstacles that hamper the use of the lead compound, most notably serum instability and its low lipophilicity.

Taking advantage of the ease with which genetic and metabolic alterations can be studied in *Drosophila*, we investigated the biosynthesis of CoA in **Chapter 4**, where we showed that *de novo* biosynthesis of CoA can occur in the absence of *dPANK/fbl* by providing the downstream substrate 4'-phosphopantetheine, thus providing a means for CoA replacement therapy in PKAN that does not rely on pantetheine.

In order to delineate a possible comprehensive pathophysiological mechanism for PKAN and related disorders, we used the versatility of *Drosophila* in **Chapter 5** to provide evidence for a CoA-dependent pathway converging on pyruvate dehydrogenase. In addition to possibly providing novel therapeutic targets, this pathway may also be able to explain disruption of ISC metabolism and as such, iron accumulation.

In **Chapter 6**, the focus is changed to NS-PME, where we created a novel *Drosophila* model for this disease by RNAi-mediated knockdown of *membrin*, the *Drosophila* orthologue of *GOSR2*, in order to study which cell types are involved in disease pathophysiology.

**Chapter 7** offers a general discussion of the thesis, in which the different chapters are evaluated separately as well as in conjunction with each other. At the end, it ventures into future perspectives in the fields of PKAN and NS-PME.



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