The synthesis of minds and molecules leads to potential therapy for pantothenate kinase-associated neurodegeneration

Pantothenate kinase-associated neurodegeneration (PKAN) is an autosomal recessive disorder caused by mutations in the mitochondrial enzyme pantothenate kinase 2 (PANK2). In this issue of *Brain* Brunetti *et al.* (2014) report the use of a ketogenic diet in a mouse knock-out of PANK2 to more accurately model the human disease and further show that feeding with pantethine may be a possible treatment strategy for PKAN in humans.

The history of our understanding of PKAN is fascinating, but has grim beginnings. In 1922, Julius Hallervorden and Hugo Spatz, two eminent neuropathologists, described the clinical condition that subsequently bore their name, as well as the neuropathological hallmark of PKAN: iron deposition in the globus pallidus and substantia nigra (zona reticularis). However, in the following years both actively participated in Nazi euthanasia programmes while working at the Kaiser Wilhelm Institute for Brain Research (Zeidman and Pandey, 2012). Although these shameful activities were reported shortly after the end of World War II the medical community was not widely aware of them until 1992, when concerted attempts were made to replace the eponym Hallervorden-Spatz syndrome with a more suitable and respectful alternative (Shevell, 2012).

More recent work on PKAN has been elegant and impressive, particularly studies from the laboratory of Susan Hayflick, where the gene was located to chromosome 20p (Taylor *et al*., 1996) and the term ‘neurodegeneration with brain iron accumulation type 1’ (NBIA1) was proposed. When mutations were identified in *PANK2* the term PKAN was suggested instead (Zhou *et al*., 2003) and this was quickly followed by comprehensive paper delineating the clinical, radiological and imaging findings in a series of genetically characterized patients (Hayflick *et al*., 2003). PKAN turns out to be one of a family of NBIA s and has led to an emergence of studies aimed at elucidating the molecular pathogenesis of brain iron accumulation (Rouault, 2013).

The clinical features of PKAN include childhood-onset dystonia, dysarthria, rigidity and choreoathetosis, and up to one-third of patients also have clinical or electro-retinographic evidence of a retinopathy. A small, but significant, subset of patients have a later onset with developmental delay, psychiatric problems, speech defects, freezing episodes and spasticity. The iron deposition in the basal ganglia results in the characteristic and highly descriptive ‘eye-of-the-tiger’ sign found on MRI.

Despite brain iron accumulation being one of the central features of the condition, it remains one of the most poorly understood and what is known about the pathogenesis of the disorder incorporates information gleaned from the pre-molecular era of classical biochemistry. That story starts with the discovery of coenzyme A (by Fritz Lipmann in 1953) and the role of its thiolester, acetyl CoA, in the citric acid cycle [also known as the tricarboxylic acid (TCA) cycle or Krebs cycle discovered by Hans Krebs].

Cellular energy is derived from food in the form of fats, carbohydrate and protein. Acetyl CoA is the key intermediate component in the complex biochemical pathways leading from food intake to the provision of cellular energy in the form of ATP (see simplified pathways shown in Fig. 1). In the brain, under normal circumstances, glucose is the obligatory energy substrate and it is oxidized after entering the citric acid cycle via pyruvate. In other tissues, fatty acids are the major energy substrates and are delivered into the mitochondria by the carnitine shuttle system where they are metabolized for ATP production through β oxidation.

However, on sustained fasting or a very low carbohydrate diet, the balance of liver metabolism switches to gluconeogenesis and...
fatty acid biosynthesis is suppressed. Less malonyl CoA is produced and this relieves inhibition of the uptake of fatty acids via the carnitine shuttle. The acetyl CoA generated by this increased β oxidation is diverted into the formation of ketone bodies which are released from the liver (which cannot use them) to extrahepatic tissues (including the brain) sparing glucose utilization and limiting the need to generate glucogenic precursors from muscle. During normal metabolism this cellular system is finely tuned to accommodate changes in dietary intake and energy requirements of different tissues.

Through this central role in cellular biochemical pathways, acetyl CoA is critical for the synthesis of fatty acids (used in numerous pathways including energy storage, phospholipid membrane formation, and signalling pathways), the synthesis of complex lipids including cholesterol, bile salts, sex hormones, aldosterone, and cortisol, and the production of the neurotransmitter acetylcholine. Acetyl CoA is also important for the acetylation of histones, which allows the formation of open chromatin and promotes gene transcription (Siudeja et al., 2011).

Coenzyme A is synthesized in a five step reaction from vitamin B5 (pantothenic acid or pantothenate), cysteine and ATP (Fig. 2), an essential dietary nutrient in animals as it cannot be synthesized de novo. The first and rate-limiting catalytic step of CoA biosynthesis is phosphorylation of pantothenate to 4'-phosphopantothenate by pantothenate kinase (Fig. 1). In the cytosol this reaction is catalysed by PANK1 and 3 and in the mitochondria by PANK2. The subsequent enzymatic steps are all thought to occur in the cytosol and lead to the formation of CoA, the direct precursor of acetyl CoA (formed in the cytosol from citrate exiting the TCA cycle and within the mitochondria from pyruvate). Given the central role of acetyl CoA in cellular metabolism, it is not surprising that its formation by PANK2 activity is very tightly controlled and hints at why genetic mutations have such a devastating clinical effect.

In PANK2 deficiency the delicate balance between fatty acid oxidation/synthesis and the control of glucose entry into the TCA cycle as outlined above is drastically altered. Mitochondrial levels of CoA and its products are reduced, leading to an elevation of lactate (as a result of reduced entry of pyruvate into the TCA cycle), reductions in fatty acid synthesis leading to a deficiency of membrane lipids such as sphingomyelin, and reductions in steroid synthesis leading to low levels of cholesterol, its derivatives and bile salts (Leoni et al., 2012). There are also abnormalities of ATP generation and structural mitochondrial changes with disrupted cristae. Despite low CoA levels in mitochondria there is not widespread CoA deficiency (as in some other genetic conditions)
possibly because PANK1 and PANK3 remain active in the cytosol and are able to partially compensate for the disruption of mitochondrial metabolism.

Animal models have been crucial in understanding PANK2 deficiency, but they have not successfully recapitulated all the features found in human disease. A *Pank2/C0/C0* knockout mouse showed retinal degeneration, growth abnormalities and azoospermia (Kuo et al., 2005), but did not have motor abnormalities until challenged with a pantothenate-deficient diet (Kuo et al., 2007), whereas a double knockout of *Pank1* and *Pank2* in mice caused severe hypoglycaemia, hyperketonemia and early death (Garcia et al., 2012). A hypomorphic Drosophila model of PANK deficiency did not reveal iron accumulation but did show some of the key biochemical features of PANK2 deficiency including reductions of mitochondrial acetyl CoA and evidence of neurodegeneration (Wu et al., 2009). In a key follow-up report Rana et al. (2010) showed that the abnormalities in these Drosophila were partially rescued by feeding with pantethine.

Brunetti and colleagues have extended this work further by stressing *Pank2/C0/C0* mice with a ketogenic diet. This diet has received much publicity for its use in resistant epilepsy, but in the context of PANK2 deficiency the use of a high fat, low carbohydrate diet aggravates the already perturbed cellular metabolism. In the liver, there is impaired β oxidation and ketone body formation as the result of low CoA levels and in the brain there is limited glucose availability and reduced availability of ketones. The only other energy source is muscle, which may explain the weight loss. The mechanisms leading to mitochondrial damage are still not clear but may include oxidative stress from free radicals as a result of the CoA deficiency. The *Pank2/C0/C0* mouse stressed with the ketogenic diet has a severe phenotype, which more accurately (although still imperfectly) resembles the human disorder, and includes a variety of neurological features such as locomotor abnormalities and mitochondrial abnormalities at the structural and biochemical level.

Having produced a more severe model, the team fed the *Pank2/C0/C0* mice with pantethine. The rationale for the use of pantethine is based on experiments in the 1950’s on the enzymatic conversion of pantothenic acid to CoA (referenced in Rana et al., 2010), showing that pantethine and pantetheine (synthetic derivatives of pantothenate) can be converted to 4’-phosphopantetheine (Fig. 1), and thus bypass the block we now know to be caused by mutations in PANK2. Pantethine ameliorates many of the features in the *Pank2/C0/C0* mice fed the ketogenic diet, but just as importantly it is safe and well tolerated.

So where do we go with PKAN research? Despite major efforts using mouse and fly models, brain iron accumulation found in the human condition has not been replicated even in the presence of the ketogenic diet suggesting that strategies other than mouse models will be required to understand this feature. In addition, the finding of PANK2 in the nucleus (Alfonso-Pecchio et al., 2012) supports the notion that it is involved in histone acetylation, via acetyl CoA (Fig. 1 and Pandey et al., 2013) and therefore may be a disorder of transcriptional regulation, a theory that unites a number of neurodegenerative disorders and in which histone deacetylase inhibition is being investigated as a potential therapy (Chuang et al., 2009).

Brunetti et al. (2014) pave the way for a new era in PKAN research with the potential for development of clinical trials with the use of novel biomarkers. They show how we can combine sophisticated modern molecular biology with an (almost) forgotten Golden Age of classical biochemistry to develop a potential treatment for a complex neurodegenerative disorder. Many other neurological conditions may yet benefit from this approach.

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References


