

## ● REVIEW

# Precision medicine in pantothenate kinase-associated neurodegeneration

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**Funding:** This work was supported by FIS PI16/00786 grant, Instituto de Salud Carlos III, Spain and Fondo Europeo de Desarrollo Regional (FEDER-Unión Europea), Proyectos de Investigación de Excelencia de la Junta de Andalucía CTS-5725, and by AEPMI (Asociación de Enfermos de Patología Mitocondrial) and ENACH (Asociación de Enfermos de Neurodegeneración con Acumulación Cerebral de Hierro) (to JASA).

## Abstract

Neurodegeneration with brain iron accumulation is a broad term that describes a heterogeneous group of progressive and invalidating neurologic disorders in which iron deposits in certain brain areas, mainly the basal ganglia. The predominant clinical symptoms include spasticity, progressive dystonia, Parkinson's disease-like symptoms, neuropsychiatric alterations, and retinal degeneration. Among the neurodegeneration with brain iron accumulation disorders, the most frequent subtype is pantothenate kinase-associated neurodegeneration (PKAN) caused by defects in the gene encoding the enzyme pantothenate kinase 2 (PANK2) which catalyzed the first reaction of the coenzyme A biosynthesis pathway. Currently there is no effective treatment to prevent the inexorable course of these disorders. The aim of this review is to open up a discussion on the utility of using cellular models derived from patients as a valuable tool for the development of precision medicine in PKAN. Recently, we have described that dermal fibroblasts obtained from PKAN patients can manifest the main pathological changes of the disease such as intracellular iron accumulation accompanied by large amounts of lipofuscin granules, mitochondrial dysfunction and a pronounced increase of markers of oxidative stress. In addition, PKAN fibroblasts showed a morphological senescence-like phenotype. Interestingly, pantothenate supplementation, the substrate of the PANK2 enzyme, corrected all pathophysiological alterations in responder PKAN fibroblasts with low/residual PANK2 enzyme expression. However, pantothenate treatment had no favourable effect on PKAN fibroblasts harbouring mutations associated with the expression of a truncated/incomplete protein. The correction of pathological alterations by pantothenate in individual mutations was also verified in induced neurons obtained by direct reprogramming of PKAN fibroblasts. Our observations indicate that pantothenate supplementation can increase/stabilize the expression levels of PANK2 in specific mutations. Fibroblasts and induced neurons derived from patients can provide a useful tool for recognizing PKAN patients who can respond to pantothenate treatment. The presence of low but significant PANK2 expression which can be increased in particular mutations gives valuable information which can support the treatment with high dose of pantothenate. The evaluation of personalized treatments *in vitro* of fibroblasts and neuronal cells derived from PKAN patients with a wide range of pharmacological options currently available, and monitoring its effect on the pathophysiological changes, can help for a better therapeutic strategy. In addition, these cell models will be also useful for testing the efficacy of new therapeutic options developed in the future.

**Key Words:** neurodegeneration with brain iron accumulation; pantothenate kinase-associated neurodegeneration; pantothenate kinase 2; pantothenate; induced neurons; precision medicine; induced neuron; fibroblast

## Introduction

Neurodegeneration with brain iron accumulation (NBIA) encompasses a group of uncommon neurodegenerative disorders which manifest dystonia, rigidity, choreoathetosis, retinal degeneration or optic atrophy, neuropsychiatric abnormalities and early death. Anatomopathological features include iron deposition in the basal ganglia and to a lesser degree in substantia nigra and adjacent areas, and the prominent presence of widespread axonal dilations, termed spheroids bodies, in the central nervous system, corresponding to damaged neurons (Gregory et al., 2009). Currently, more than twelve genes with their associated mutations are

recognized to be the cause of the different clinical entities of NBIA (Di Meo and Tiranti, 2018). However, near 20% of the patients are still genetically unidentified.

Despite extensive research efforts in the development of new treatments, there is still no successful treatment for stopping the progression of neurodegeneration in NBIA. Thus, alternative therapeutic research strategies are needed.

Mutations in the pantothenate kinase 2 (PANK2) gene, that codes for an essential enzyme in coenzyme A (CoA) biosynthesis, are the most common form of NBIA; it accounts for almost the 50% of cases (Levi and Finazzi, 2014). The phenotypic spectrum of pantothenate kinase-associated

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doi: 10.4103/1673-5374.251203

Received: November 28, 2018

Accepted: January 2, 2019

neurodegeneration (PKAN) includes classic PKAN and atypical PKAN. Classic PKAN is characterized by starting in early childhood (before 6 years of age in 88% of cases) of progressive dystonia, rigidity, dysarthria, choreoathetosis, and pigmentary retinal degeneration. Atypical PKAN is characterized by later onset (age > 10 years), marked speech defects, psychiatric disturbances, and a slower progression of disease (Hayflick et al., 2003, 2006).

The pantothenate kinase gene family includes PANK1a, PANK1b, PANK2 and PANK 3, but only PANK2 mutations are responsible for causing PKAN. The enzyme PANK2 catalyzes the reaction of (R)-pantothenate into (R)-4'-phosphopantothenate using ATP, and it is tightly regulated by feedback inhibition by CoA and CoA thioesters. The defective PANK2 enzyme is predicted to impair the biosynthesis of CoA, resulting in a myriad of metabolic alterations including deficient energy production, impaired biosynthesis and replacement of phospholipids in cell membranes, and increased oxidative stress (Gregory and Hayflick, 2005; Kurian and Hayflick, 2013). Particularly, low CoA levels are the cause of reduced neuronal protection, which means that sensitivity to oxidative damage is enhanced in affected brain regions. Taking into account that brain itself is an organ very susceptible to oxidative stress caused by reactive oxygen species (ROS), the damage can be extensive. ROS production increases due to iron accumulation and, as a consequence, enhances the cellular susceptibility of suffer harmful effects such as lipid peroxidation, cell membranes damage and apoptosis. Moreover, mutant PANK2 neurons derived from knockout mice have an altered mitochondrial membrane potential and deficient mitochondrial respiration which can contribute to ROS generation (Brunetti et al., 2012). However, the precise etiology and pathomechanisms of PKAN are still largely unknown.

Although great efforts have been for the modelling of the disease, they have produced incomplete phenotypic alterations such as brain iron accumulation and movement disorder, possibly because the localization of PANK2 in mitochondria has only been reported in humans and primates. In mice, the PANK2 homolog protein has been detected in the cytosol (Leonardi et al., 2007). In contrast, other researchers have reported a mitochondrial localization although a mouse PANK2 mitochondrial targeting sequence has not been identified (Johnson et al., 2004; Brunetti et al., 2012); a PANK2 knockout mouse showed reduced growth, retinal degeneration and male infertility, but no movement disorders or signs of iron accumulation in the brain were observed (Kuo et al., 2005). However, a deficient diet in pantothenic acid caused movement alterations in the knockout mice but iron deposition in the basal ganglia was not detected (Kuo et al., 2007). Moreover, other model organisms, such as *Drosophila*, have only a single PANK isoform (fumble) (Afshar et al., 2001) and its suppression caused alterations during the development of the central nervous system (Bosveld et al., 2008) that could be rescued by pantothenic acid supplementation (Rana et al., 2010), but no neuronal iron accumulation was detected. In the Zebrafish model,

morpholino-mediated PANK2 down-regulation causes abnormal development of central nervous system, particularly in the telencephalon, and vascular structures (Zizioli et al., 2016).

Given the absence of appropriate animal models for PKAN research, patient-derived cells can serve as an alternative approach to disease modelling for basic investigation and evaluation of the efficacy of potential therapeutics. The rationale of using skin fibroblasts derived from patients is based on the fact that they can be easily obtained from small skin biopsies, and that they can be expanded by traditional cell culture techniques and share with other research laboratories for further studies. In addition, particular fibroblast cell lines derived from patients harbouring genetic mutations are available from several cell repositories. All these characteristics and properties make possible that a great variety of experiments can be performed using fibroblast cell lines from a large number of patients. Biochemical and cellular studies of dermal fibroblasts from patients with neurological diseases have provided a lot of useful information on the pathomechanisms of these disorders (Connolly, 1998). The justification for this strategy is based on the hypothesis that, although neurological genetic diseases are primarily located within the central nervous system, fibroblasts in tissue culture derived from neurologic patients harbor the particular mutation (even after several subcultures) and can reproduce many of the alterations observed within the central nervous system. However, fibroblasts are not fully representative of neuronal dysfunction given the unique characteristics of these cells. For this reason new tools have been added to the methodology used in the generation of cellular models from neurologically affected patients.

The recent generation technology of induced pluripotent stem cells (iPSCs) from somatic cells of donors with known genetic diseases and its subsequent neuronal differentiation allows disease modelling and the examination of the underlying molecular mechanisms in the most affected cells in neurological disorders (Colman and Dreesen, 2009). In this way, the iPSCs and neuronal differentiation offer the opportunity to establish *in vitro* models of neurodegenerative diseases such as PKAN. However, this approach has several disadvantages since the technique is complex, expensive and time consuming (Dolmetsch and Geschwind, 2011).

Recently, using different combinations of lineage-specific transcription factors it is possible to directly convert somatic cells across different germ layers. For example, fibroblasts can be directly converted into neuronal cells without transiting through the pluripotent state (Ladewig et al., 2013). The first successful direct conversion of murine fibroblasts into induced neurons (iNs) was achieved in 2010 when Wernig and colleagues identified a combination of three proneural factors (achaete-scute complex-like 1 (Ascl1), brain-2 (Brn2) and myelin transcription factor 1 like (Myt1l)), which were able to convert murine embryonic and postnatal fibroblasts into functional neurons *in vitro* (Vierbuchen et al., 2010). About one year later, this approach was translated to human fibroblasts, using the additional factor NeuroD1 to obtain

iNs (Pang et al., 2011).

Over the next years, new tools and approaches have been found to improve efficiency of neuronal conversion. For example, a combination of proneural genes expression with micro RNAs such as miR-9/9\* or miR-124 resulted in functional neurons from human fibroblasts (Yoo et al., 2011). Recently, it was shown that combinations of small molecules and proneural growth factors, as well as silencing of reprogramming barriers such as the repressor element-1 silencing transcription factor complex, can improve conversion efficiency up to more than 100% (Ladewig et al., 2012; Pfisterer et al., 2016; Drouin-Ouellet et al., 2017), solving one of the main problems of direct reprogramming.

Direct reprogramming has several advantages in comparison with the generation of iPSCs-derived neurons, such as the relative simplicity and short time requirements (Ladewig et al., 2013). In addition, iNs, unlike iPSCs, maintain the ageing (Mertens et al., 2015) and the epigenetic marks of the donor (Horvath, 2013; Huh et al., 2016), making them excellent candidates for modelling neuronal pathophysiology in age-related disorders. Moreover, unlike human iPSCs that are susceptible to form tumors following transplantation (Miura et al., 2009), it has been demonstrated that iNs obtained through *in vivo* reprogramming do not undergo tumorigenic processes, indicating that in the future they could be a promising tool for cellular therapy, for example, by performing direct reprogramming *in vivo* of endogenous murine astrocytes or transplanted human cells (Torper et al., 2013). Therefore, the generation of iNs by direct reprogramming from patient-derived fibroblasts suffering from PKAN and other NBIA subtypes holds enormous promise for understanding the pathogenesis of these disorders and for therapeutic approaches. In the future, direct reprogramming of fibroblasts into more specific cells such as GABAergic or dopamine neurons will be also more informative about the complex interrelationship among iron, neurotransmitters and other metabolites. The articles used in this review were retrieved by electronic search of the Medline database for literature describing pantothenate kinase associated neurodegeneration, NBIA, precision medicine in neurodegenerative diseases, direct reprogramming and lipofuscin.

### Cellular Models of Pantothenate Kinase-Associated Neurodegeneration

Previous studies have shown that alterations of iron metabolism and oxidative stress can be found in fibroblasts derived from PKAN patients (Campanella et al., 2012). Non-specific systemic cytological aberrations observed in NBIA cells include circulating lymphocytes with vacuoles and cytoplasmic inclusion bodies and bone marrow macrophages containing ceroid-lipofuscin granules, analogous to those detected in the storage disorder of neuronal ceroid lipofuscinosis (Swaiman et al., 1983). Lipofuscin accumulation can be caused by high levels of lipid peroxidation, a process triggered by iron (Defendini et al., 1973; Park et al., 1975). The study of these cytological anomalies may help to elucidate the fundamental pathophysiology in the NBIA.

On the other hand, neuronal cells differentiated from PKAN-derived iPSCs showed significant mitochondrial alterations, with changes in cristae structure and decreased mitochondrial membrane potential (Orellana et al., 2016). Importantly, patient-derived neurons also manifest profound alterations of mitochondrial function and electrophysiological behaviour, along with alteration of mitochondrial iron-dependent pathways, cytosolic iron homeostasis and increased oxidative stress.

Recently, our group has described that patient-derived fibroblasts harboring different PANK2 mutations manifest many of the physiopathological characteristics found in other cellular models of the disease such as intracellular iron and lipofuscin accumulation, increased ROS levels and mitochondrial dysfunction (Álvarez-Córdoba et al., 2018). Furthermore, iron accumulation was detected for the first time in iNs obtained by direct reprogramming.

However, iNs generated using this protocol of direct trans-differentiation have several disadvantages. For instance, maintaining iNs in culture is difficult and achieving a great number of iNs at 80–100 days post-infection is very challenging, restrictive and expensive, since cell death can be observed from 30 days post-infection. This fact may hinder electrophysiological characterization of iNs, since action potentials have been only observed at 80–100 days post-infection (Drouin-Ouellet et al., 2017). Moreover, iNs tend to form clusters during the reprogramming process hampering isolation of individual cells for further analysis.

### Iron/Lipofuscin in Pantothenate Kinase-Associated Neurodegeneration

Iron is an indispensable metal for cell survival due to its participation in a large number of iron-containing proteins and enzymes involved in different cellular pathways (Nunez et al., 2012). However, iron may participate in potentially dangerous free radical-generating reactions, and its accumulation may promote the generation of hydroxyl radical which induces lipid/protein oxidation and nucleic acid damage (Salvador et al., 2010; Lan et al., 2016). Commonly, Perls' Prussian blue staining of PKAN brain tissue shows an extensive deposition of iron surrounding blood vessels largely in the globus pallidus but not strictly respecting the limits of this structure (Kruer, 2013). Thus, iron accumulation can also be observed in the substantia nigra and other brain areas. However, both the subcellular location of iron deposits and the chemical form of iron deposition are not well-known. Various hypotheses have been suggested to explain the mechanisms of iron accumulation.

One of these hypotheses proposes that aberrant lipofuscin peroxidation to neuromelanin, the brain dark pigment which is structurally related to melanin, and a defective cysteine dioxygenase function lead to anomalous iron accumulation in the brain (Perry et al., 1985; Gregory and Hayflick, 2005). In this respect, up to 20 percent of the total iron is bound within neuromelanin in the central nervous system (Gerlach et al., 2003). Furthermore, neuromelanin binds iron in the ferric form rendering it redox-inactive and, as a consequence,

plays a physiological role in intraneuronal iron homeostasis (Gerlach et al., 2008). Increased tissue iron content found in PKAN may saturate iron-chelating sites on neuromelanin and promote an increased production of ROS. Thus, neuromelanin is suggested to play a dual role, both toxic and protective, depending on the cellular context (Zucca et al., 2018). The synthesis of neuromelanin is neuroprotective since it removes from the cytosol the reactive/toxic quinones that would otherwise induce neurotoxicity (Sulzer et al., 2000). Neuromelanin further plays a protective role by chelating potentially toxic metals, including Fe, and other metals (Bohic et al., 2008), drugs and organic toxicants (Karlsson and Lindquist, 2016). However, neuromelanin can play a toxic role when released by degenerating neurons; under these conditions, neuromelanin releases high amounts of metals and organic chemicals and may trigger cytotoxic effects and microglia activation, producing reactive and pro-inflammatory molecules that induce further neuronal death (Zhang et al., 2011).

Interestingly, iron content in the normal brain is particularly high in globus pallidus and substantia nigra, the most affected regions in PKAN. In turn, the product of PANK2 enzymatic activity is phosphopantothenate which afterwards is condensed with L-cysteine in the CoA biosynthesis pathway. Consequently, as phosphopantothenate biosynthesis is deficient in PKAN, L-cysteine can be accumulated (Perry et al., 1985). On the other hand, high L-cysteine levels can suffer fast auto-oxidation when iron is present, which results in free radical production and cytotoxicity (Yoon et al., 2000). Two other PANK2 substrates, N-pantothenoyl-cysteine and pantetheine, which contain cysteine residues are also predicted to accumulate and may also contribute to the pathophysiology of the disease (Gregory and Hayflick, 2005).

In the particular context of dopaminergic neurons the conjunction of dopamine, high levels of L-cysteine and iron in PKAN can be very detrimental. Dopamine is a highly reactive molecule, which is stabilized in acidic synaptic vesicles. However, free cytosolic dopamine can auto-oxidize, generating ROS, and lead to the formation of toxic quinones (Biosa et al., 2018). The interaction between iron and dopamine, producing neurotoxic intermediate or endproducts that are specific to this vulnerable brain region has been extensively examined; for a most detailed review see Hare and Double (2016). The two predominant mechanisms behind iron-mediated dopamine neurotoxicity involve the non-enzymatic catalytic production of o-quinones (Napolitano et al., 1995; Zhang et al., 2012), and as part of an intermediary iron-dopamine complex that precedes quinone formation (Pezzella et al., 1997).

In addition, previous studies have demonstrated that dopamine can be oxidized in the presence of L-cysteine, producing a complex mixture of cysteinyl conjugates of the chemical messenger. Then, these dopamine derivatives can be easily further oxidized to a number of dihydrobenzothiazines which cause the irreversible inhibition of mitochondrial complex I (Li and Dryhurst, 1997) triggering a sustained increase in ROS, DNA oxidation, and caspase 3 activation (Zhang and Dryhurst, 1994; Berman and Hastings, 1999).

Another possible explanation is that intracellular iron ac-

cumulation comes from neuronal apoptosis. In fact, it has been observed that ceramide-mediated apoptosis is due to an increased cellular iron uptake (Matsunaga et al., 2004). Alternatively, our findings support the hypothesis that iron can be accumulated by forming lipofuscin granules which have a high iron weight fraction (about 5%) and are significantly increased in PKAN fibroblasts (Álvarez-Córdoba et al., 2018).

Lipofuscin is a brown-yellow, electron-dense, autofluorescent material composed of highly oxidized aggregate of covalently cross-linked proteins and lipids (Double et al., 2008). Metals, including Fe, Cu, Zn, Al, Mn and Ca, may amount up to 2% of lipofuscin (Jolly et al., 1995). Lipofuscin is insoluble and is not degraded by lysosomal enzymes or the proteasomal system, which is responsible for the recognition and degradation of misfolded and oxidatively damaged proteins (Jung et al., 2007). Different cell types, such as neurons, hepatocytes and cardiomyocytes have been observed to accumulate lipofuscin granules which are associated with replicative and stress-induced cell senescence. Some authors suggest that mitochondria are involved in the formation of lipofuscin (Konig et al., 2017). In fact, it has been shown that isolated mitochondria can spontaneously form lipofuscin granules without any supplementary factors (Frolova et al., 2015). The accumulation of lipofuscin is considered one of the best-known biomarkers of aging (Brunk and Terman, 2002) and in our study we demonstrated that this accumulation is actually associated with the presence of senescent-related morphological alterations in PKAN cells (Álvarez-Córdoba et al., 2018). Several works have proposed that lipofuscin actively participates in the pathophysiological alterations of senescent cells. Accordingly, lipofuscin has been observed to inhibit the proteasome (Powell et al., 2005), the central protein complex for removal of damaged, oxidized or misfolded proteins of the cell. Proteasome activity is inhibited due to its binding to exposed hydrophobic amino acid coating lipofuscin granules (Höhn and Grune, 2013). Therefore, although the proteasome is bound to lipofuscin, it is ineffective in eliminating the oxidized proteins and consequently, the degradation of other substrates is reduced. Furthermore, lipofuscin is also able to reduce lysosomal function resulting in depressed autophagy (Kurz et al., 2008). The deleterious synergic effects of both alterations, proteasome and lysosome impairment, can strongly facilitate and promote lipofuscin formation (Jung et al., 2007).

Moreover, lipofuscin's ability to incorporate transition metals such as iron is one of its main cytotoxicity factors (Jolly et al., 1995). Iron is the most abundant transition metal in mammal cells, and its binding to lipofuscin results in a redox-active surface which is able to catalyze the Fenton's reaction. This characteristic makes lipofuscin to contribute to the formation of free radicals and oxidatively modified lipids, proteins and nucleic acids, which finally may induce cell death (Reeg and Grune, 2015). Cellular oxidative status has been previously studied in PKAN fibroblasts (Campanella et al., 2012); oxidative stress signals were detected in patient-derived cells, and also ROS production was increased after iron supplementation in these cells. Corroborating

these results, we found increased carbonylated protein levels and high mitochondrial lipid peroxidation in PANK2 mutant fibroblasts. Interestingly, these alterations were prevented by pantothenate treatment in responder cells (Álvarez-Córdoba et al., 2018). Lipofuscin accumulation in PKAN cells may result from increased oxidative stress and lipid peroxidation, a process induced by iron (Defendini et al., 1973; Park et al., 1975). Excessive accumulation of lipofuscin in NBIA has been also observed in different cell types such as retinal vessel pericytes, conjunctival fibroblasts and macrophages (Luckenbach et al., 1983).

Impaired iron metabolism in PKAN fibroblasts has been previously described (Santambrogio et al., 2015) and it has been attributed to the impairment of mitochondrial iron-sulphur cluster and heme biosynthesis pathways (Campanella et al., 2012; Orellana et al., 2016). In our work, we have demonstrated that both processes are indeed affected in PANK2 mutant fibroblasts. Additionally, pantothenate treatment partially corrected both alterations in responder cells (Álvarez-Córdoba et al., 2018). Iron-sulphur clusters are prosthetic groups of many cytosolic and mitochondrial enzymes such as respiratory complexes and aconitases (Lill et al., 2014). Therefore, iron-sulphur cluster deficiency may affect many biosynthetic pathways and lead to mitochondrial dysfunction. One consequence of this deficiency is the inefficient utilization of iron that may cause iron accumulation in mitochondria. In turn, iron accumulation may cause increased free radical production that provokes further cellular damage (Lu and Cortopassi, 2007). Furthermore, ineffective mitochondrial iron utilization may represent the signal that promotes the dysregulated and excessive iron import into the cells and, with time, leads to iron accumulation (Orellana et al., 2016). Thus, PANK2 downregulation by specific siRNA (small interfering RNA) in several human cell lines induced a marked decline in cell proliferation together with surprising signs of iron deficiency and increase of transferrin receptor expression levels and induction of ferroportin mRNA (Poli et al., 2010).

In agreement with these findings, our data support the hypothesis that impaired mitochondrial iron metabolism leads to cytosolic iron deficiency (reduced labile iron pool) and a vicious cycle of increased iron uptake due to increased expression of Fe<sup>2+</sup> transporters such as divalent metal transporter 1 and posterior sequestration in mitochondria and finally in lipofuscin granules (Huang et al., 2011). Curiously, both alterations, reduced free iron levels and increased expression levels of divalent metal transporter 1, were partially corrected by pantothenate treatment in responder fibroblasts (Álvarez-Córdoba et al., 2018).

Furthermore, the amount of aconitase, an iron-dependent enzyme, was also reduced, both in the cytosol and in the mitochondria (Poli et al., 2010; Orellana et al., 2016). Interestingly, cytosolic aconitase is a bifunctional protein that exerts its function as aconitase and/or modulating intracellular iron homeostasis depending on iron availability. When cellular iron levels are high, aconitase binds to a 4Fe-4S cluster and catalyzes the conversion of citrate to isocitrate (Volz, 2008).

This substrate is afterwards metabolized generating alpha-ketoglutarate and NADPH, an essential cofactor for many enzymatic reactions involved in lipid metabolism (Koh et al., 2004). Otherwise, when iron content is low, cytosolic aconitase suffers a conformation change and binds iron-responsive elements which regulates ferritin and transferrin receptor mRNA stability and translation rates (Volz, 2008). As a consequence, the translation of ferritin mRNA is disrupted while increasing the stability of transferrin receptor mRNA.

Given these potential important relationships with lipid synthesis and iron homeostasis, cytosolic aconitase alteration may have an essential role in PKAN physiopathology. Moreover, a paradoxical deficiency of free iron in PKAN may partially explain the defects in cytosolic and mitochondrial aconitase activity, a major iron sensor that is inactivated by low iron availability (Tong and Rouault, 2007).

### Strategy for Finding Alternative Treatments for Pantothenate Kinase-Associated Neurodegeneration

Our observations suggest that pantothenate is able to increase the expression levels of PANK2 in patient-derived fibroblasts harboring selected mutations (Álvarez-Córdoba et al., 2018). In addition, using iNs generated by direct reprogramming of mutant fibroblasts, the positive effect of pantothenate was also confirmed. These observations lead us to propose our screening model as a useful tool for identifying patients with PANK2 mutations with residual enzyme activity that respond *in vitro* to pantothenate supplementation. More importantly, the existence of residual enzyme expression which can be significantly enhanced in cells derived from some affected individuals raises the possibility of treatment using high dose of pantothenate. This hypothesis must be confirmed comparing both the effect of pantothenate in cell cultures and patient studies in controlled clinical trials. In addition, information about multiple types of the PANK2 gene mutations and their sensitivity to pantothenate supplementation will be necessary for paving the way towards personalized therapies in PKAN.

Furthermore, personalized screening strategies can facilitate the detection of more pharmacological chaperones able to stabilize the expression levels and activity of the mutant enzyme. Preliminary results of our group have identified several compounds which are indeed able to restore PANK2 expression levels and pathophysiological alterations in fibroblasts from PKAN patients.

A large number of mutations related to human diseases produce the destabilization of specific proteins. Interestingly, small molecules that work as pharmacological chaperones can rescue the activity of unstable proteins (Newton et al., 2011; Goldin et al., 2012; Andreotti et al., 2014). However, a concrete disorder will be suitable for therapy with pharmacological chaperones depending on its genotype (Andreotti et al., 2010). Confirming this assumption our results have shown that several mutations of PANK2, but not all, can be rescued by pantothenate (Álvarez-Córdoba et al., 2018).

Therefore, a strategy for identifying more pharmacological chaperones able to restore the activity of the mutant PANK2 enzyme both in fibroblasts and iNs can lead to potential therapeutic alternatives in selected patients. Following this approach, there are several cases of small drugs that are already approved which have been successfully repositioned as pharmacological chaperones for the treatment of rare disorders (Hay Mele et al., 2015): doxorubicin, an anti-cancer anthracycline, for Cystic fibrosis (Maitra and Hamilton, 2007), Diltiazem, an antihypertensive, for Gaucher disease (Rigat and Mahuran, 2009), Ambroxol, a mucolytic agent, for Fabry and Gaucher disease (Bendikov-Bar et al., 2013), Acetylcysteine, another mucolytic agent, for Pompe disease (Porto et al., 2012), Pyrimethamine, an anti-parasitic drug, for GM2 gangliosidosis (Maegawa et al., 2007), carbamazepine, a dibenzazepine, for hyperinsulinemic hypoglycemia (Martin et al., 2013) and Salicylate, a well-known anti-inflammatory agent, for pendred syndrome (Ishihara et al., 2010). Recently, an allosteric PANK activator that crosses the blood-brain barrier (PZ-2891) has been identified (Sharma et al., 2018). Interestingly, PZ-2891 therapy improved locomotor activity and life span of a knockout mouse model of brain CoA deficiency.

## Precision Medicine in Pantothenate Kinase-Associated Neurodegeneration

Precision medicine refers to the tailoring of medical treatment to the individual characteristics of each patient. The application of precision medicine to the treatment of neurodegenerative disorders such as PKAN appears to be highly promising in contrast to the traditional “one-drug-fits-all” approach (Strafella et al., 2018). In fact, neurodegenerative pathologies can present variable clinical features even in patients with the same disease or mutation who therefore are very unlikely to benefit of a single drug. Neurological diseases are promisingly suited models for precision medicine because of the rapidly expanding genetic knowledge base, phenotypic classification, the development of biomarkers and the potential modifying treatments (Tan et al., 2016).

In this context, the development of a precision medicine approach using fibroblasts and iNs derived from PKAN patients could represent an excellent possibility to identify optimal treatments.

In the last years, our knowledge of genetic bases of NBIA disorders including PKAN has improved enormously using diagnosis tools based on whole-exome sequencing and gene panels (Tello et al., 2018). The information about the precise genetic alteration together with the creation of collaborative networks among medical centers, research institutes and highly qualified specialists can be decisive for moving the precision medicine from the laboratory bench to the bedside in PKAN. To this purpose, the present “review” has been thought to discuss the main components which may be part of precise and personalized treatment programs applied to PKAN (Figure 1).

The approach of precision medicine is already success-

fully applied in different healthcare areas such as oncology, cardiology and nutrition, as well as for rare diseases (Schee Genannt Halfmann et al., 2017).

In neurodegenerative diseases, early precision medicine attempts have been most significant in Alzheimer’s disease. For example, anti-amyloid- $\beta$  monoclonal antibody therapy is now being tested in patients with known mutations causing Alzheimer’s disease with the aim to prevent neurodegeneration in genetically similar patients (the Dominantly Inherited Alzheimer Network (DIAN) Trial (ClinicalTrials.gov number NCT01760005).

Furthermore, apolipoprotein E genotyping has the potential to identify persons at risk for Alzheimer’s disease (Kim et al., 2009). Individuals with certain variations in this gene may be at higher risk than the general population for developing the condition, making them ideal candidates for earlier diagnosis, treatment and prevention strategies.

Today, Parkinson disease is treated as one condition, but research is pointing towards the presence of different subtypes, which could give opportunities to precision medicine approaches. Although dopamine replacement therapy or manipulation of brain dopaminergic pathways, and in particular levodopa has the ability to improve the motor symptoms of Parkinson disease, some motor complications still hamper the treatment strategies in Parkinson disease (Titova and Chaudhuri, 2017). However, the complex nature of Parkinson disease as well as Alzheimer’s disease coupled with clinical phenotypic heterogeneity presents major challenges for formulating successful personalized medicine.

## Conclusion

PKAN patients-derived fibroblasts and iNs can be useful cellular models for both understanding the underlying pathological mechanisms of the disease and the development of screening tools that allow the assessment of potential effective drugs in patients who suffer from PKAN.

Precision medicine in PKAN implies the development of therapies and treatment procedures best suited for an individual patient, taking into consideration both genetic and environmental factors. Thus, ‘omic’ studies (e.g., genomics, transcriptomics, proteomics and metabolomics) in combination with the evaluation of patient-derived cells response to several potential treatments will provide a more rational therapeutic approach for PKAN patients. For all these reasons, it is feasible to conclude that a personalized approach to treating PKAN will become more widely applicable in the future.

**Author contributions:** Manuscript drafting and finalizing: MÁC, MVP, IVG, SPC, JMSR, MTR, JAJ, ABVT, and JASA.

**Conflicts of interest:** None declared.

**Financial support:** This work was supported by FIS PI16/00786 grant, Instituto de Salud Carlos III, Spain and Fondo Europeo de Desarrollo Regional (FEDER-Unión Europea), Proyectos de Investigación de Excelencia de la Junta de Andalucía CTS-5725, and by AEPMI (Asociación de Enfermos de Patología Mitocondrial) and ENACH (Asociación de Enfermos de Neurodegeneración con Acumulación Cerebral de Hierro) (to JASA).

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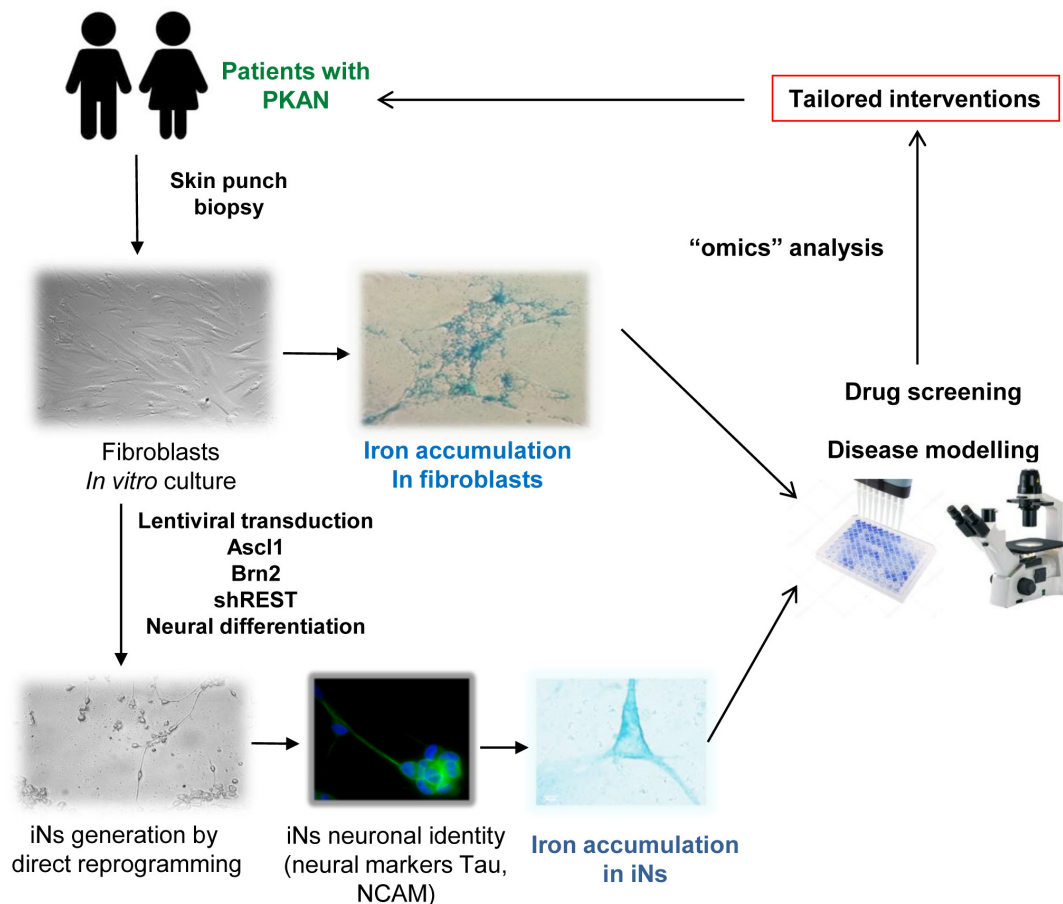
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**Figure 1 Disease modelling and drug screening strategy in PKAN.**

Fibroblasts and induced neurons derived from PKAN patients can be useful cellular models for detecting pathophysiological alterations and valuable tools for screening potential therapies. PKAN: Pantothenate kinase-associated neurodegeneration; *Ascl1*: achaete-scute complex-like 1; *Brn2*: brain-2; *shREST*: short hairpin RNA against the repressor element-1 silencing transcription factor complex; NCAM: neural cell adhesion molecule; iNs: induced neurons.

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P-Reviewer: Sun Y; C-Editors: Zhao M, Yu J; T-Editor: Liu XL