Finding a cure for the rare but severe KIF1A Associated Neurological Disorder, what we (need to) know.

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Abstract: In the last few years, neurological diseases have been among the leading causes of death worldwide. A severe but rare neurodegenerative disorder is KIF1A Associated Neurological Disorder (KAND). This disorder is only identified in a few hundred people worldwide, mainly because the symptoms and genetic mutation associated with this disease wildly vary between patients making it hard to diagnose. Symptoms of this disease include cognitive impairment, spastic paraplegia, and epilepsy, among many others. KAND is caused by a genetic mutation in the kinesin family member 1A (*KIF1A*) gene, which codes for a motor protein responsible for the active intracellular transport of synaptic vesicle precursors. Here, I review recent insights on KAND and the molecular mechanism behind different *KIF1A* variants.

Layman's abstract: Cells are the basic unit of life, in which biochemical reactions are well separated in space and time. Only through this separation can a precise biological function arise, which forms the basis of the specific function of our organs, such as our brain. The brain's cells, called neurons, have a remarkable architecture with very long compartments. For an adequate distribution of molecules to the different locations in the cell, specialized motor proteins are at play. One example of a motor protein is kinesin family member 1A (KIF1A), which is only found in neurons. This protein walks along roads that span the entire width of the cell, called microtubules. When KIF1A is not correctly functioning, diseases arise, showing the importance of KIF1A. One of these diseases is a severe but rare disorder called KIF1A Associated Neurological Disorder (KAND). This disorder is only identified in a few hundred people worldwide, mainly because the symptoms and underlying cause associated with this disease wildly vary between patients, making it hard to diagnose. Symptoms of this disease include intellectual disability, epilepsy, spasticity (stiffness) of the legs, and many others. KAND is caused by a mutation in the DNA coding for KIF1A. DNA is the material that carries the information on how proteins should be built. This mutation alters the code for KIF1A, which in many cases leads to less transport carried out by KIF1A. In the last few years, many different mutations have been found in the DNA coding for KIF1A in patients with KAND. However, what is not known yet is how the transport of molecules is actually disturbed by this KIF1A mutation.
Here, I will review what is already known about this DNA mutation. I will first explain which diseases are associated with this mutation, followed by an explanation of how the transport of molecules works. I will then explain more about KIF1A and its function, and what might be going wrong on the molecular level in patients with KAND. Lastly, I will elaborate on whether we can cure this disease.

Introduction:

Neurological diseases were found to be the second leading cause of death in the past few years. Ranking just below cardiovascular diseases, these diseases accounted for 9.4 million deaths worldwide in 2019[1]. The term neurological disease is used for every disease which affects the central or peripheral nervous system[2], with the most common diseases being strokes, Alzheimer's disease, Parkinson's disease, migraine, epilepsy, and multiple sclerosis[3]. In 2019, 87.2% of the deaths by neurological diseases were caused by

strokes and dementia alone[1]. Much research has been done into the most common neurological diseases, but there is still a lot to discover about the less common neurological diseases. One of those less common neurological diseases is KIF1A Associated Neurological Disorder (KAND). This disorder is only identified to a little over 350 people throughout the world. Diagnosis of KAND is difficult because the kinesin family member 1A (*KIF1A)* gene mutation causing this disease often occurs spontaneously and is non-inherited. Therefore, the actual number of people affected by KAND might be much higher. There is no cure for KAND yet, and the disease symptoms actually vary between patients. Symptoms of KAND include spasticity and neurological abnormalities, epilepsy, impaired vision, developmental delays, and issues with coordination, muscle tone, and speech[4].

KIF1A is a gene encoding the kinesin-like protein KIF1A. This protein is part of the kinesin family proteins (KIFs), which are microtubule-dependent motor proteins responsible for the transport of protein complexes, membrane vesicles and organelles, and mRNA[5]. The neuron-specific KIF1A motor protein is responsible for the axonal transport along microtubules (MTs) of synaptic vesicle precursors (SVP)[5,6]. In addition, KIF1A also transports postsynaptic proteins, which play a role in learning, memory, and synaptic plasticity[7-9].

A wide range of phenotypes in differing severity is associated with both dominant and recessive variants of *KIF1A*. Homozygous recessive mutations are often linked to hereditary sensory and autonomic neuropathy, a severe form of a neurodegenerative disorder, or hereditary spastic paraplegia (HSP). Heterozygous dominant variants are linked to a wide variety of phenotypes. These include intellectual disability, optic atrophy, axial hypotonia, spasticity, growth failure, and progressive cerebellar atrophy[10-12]. The many different variants of *KIF1A* and different phenotypes make it hard to find a cure. Even similar variants are not always associated with similar phenotypes among different patients[4]. Over the past few years, many variants of *KIF1A* have been discovered. Interestingly, not every variant associated with disease was found to be a loss of function variant. For example, the V8M mutation in humans was found to cause hyperactivation of motor activity and led to increased SVP transport[13].

The molecular mechanism of how *KIF1A* variants lead to diseases is poorly understood. In 2004, LoTurco et al. found that *Kif1a* RNAi leads to a decreased number of neurons[14]. This relationship was further investigated by Carabalona et al. (2016), who found that this can be explained by a shift in the asymmetric to symmetric brain stem cell ratio when *KIF1A* is knocked down by shRNA[15]. That intracellular active transport is affected by *KIF1A* variants was already known, but the underlying cause is not yet completely understood. Aguilera et al. (2021) contributed to our understanding of this underlying mechanism. They found that the human R169T mutation, which led to NESCAV syndrome in patients, reduced the ATPase activity and microtubule-binding of KIF1A[16]. This finding is consistent with the findings of Budaitis et al. (2021). Their research showed that the V8M and Y89D variants of *KIF1A* led to reduced velocities due to reduced ATPase activity and microtubule-binding, reduced landing rates, and decreased force generation[17].

Here, I review recently obtained knowledge about the *KIF1A* mutation and the related neurodegenerative diseases KAND. I address the neurodegenerative diseases associated with *KIF1A* variants, intracellular transport focusing on motor proteins and the deficits in this transport

on a molecular level of the most common *KIF1A* variants. Furthermore, I elaborate on future possibilities to cure the disease using drugs or genetics.

The diseases:

Three major diseases have been associated with variants in *KIF1A*.

KAND:

Pathogenic variants in *KIF1A* cause a group of progressive neurodegenerative diseases better known as KAND[18-29]. These variants can be inherited recessively or dominantly. However, the most severe phenotypes of KAND are associated with *de novo* variants. Approximately 115 disease-causing variants of *KIF1A* have been described in the literature to date[29]. Many of these variants are missense mutations located within the motor domain of the protein[18-29]. More than 30% of the patients identified with KAND have private variants, meaning that there are likely many more variants still to be identified. Apart from the diverging variants found in patients, KAND also encompasses a broad range of phenotypes. The symptoms can include neurodevelopmental delay, autism, intellectual disability, progressive spastic paraplegia, optic nerve atrophy, peripheral and autonomic neuropathy, cerebellar and cerebral atrophy, spasticity, and seizures[18-29].

HSP and NESCAV:

HSP is a name given to a group of neuropathological disorders mainly characterized by spasticity of the lower limbs and abnormal gait[30]. Children affected by this disease often experience a delay in walking. In 2011, Erlich et al. were the first to link a *KIF1A* variant to HSP. Using whole-exome sequencing, they found that a causative mutation in the motor domain of KIF1A was the cause of HSP in a single inbred Palestinian family[10].

Neurodegeneration and spasticity with or without cerebellar atrophy or cortical visual impairment (NESCAV syndrome) occur as a consequence of *de novo* missense variants of KIF1A located in the motor domain. Patients with NESCAV can experience intellectual disabilities, spastic paraparesis, peripheral neuropathy, language and motor delay, hyperreflexia, and hypotonia. In addition, MRI images of these patients can show brain and optic nerve atrophy[16]. Experiments performed in neuroblastoma cells showed that several *de novo* missense variants of *KIF1A* led to an accumulation of the motor protein in the cell body instead of the neurite tips[21,31].

Molecular mechanisms and the associated proteins:

To understand the molecular basis of these diseases, we need to understand the involved molecular mechanisms and contributing molecules. Because *KIF1A* encodes a motor protein, I will discuss intracellular transport and its players in the following.

Active intracellular transport:

Since eukaryotic cells are usually in the micrometer range, cell organelles are spatially distant. However, to create a complete metabolic chain, they need to be connected. This explains the need for the cytoskeleton in most eukaryotic cells[32]. Since the cytoplasm is rather crowded, diffusion alone is insufficient to transport molecules within the cell, creating the need for active intracellular transport. This active transport is mediated by molecular motor proteins, which walk along the filaments of the cytoskeleton and thereby transport various types of cargo from one site to another[33]. The importance of this active intracellular transport can be seen from the many different diseases, amongst them neuronal diseases, which arise when the intracellular transport system fails[33].

Cytoskeleton filaments:

The cytoskeleton carries out different functions within the cell. It generates forces, determines the cell's shape and spatial organization, and serves as the road where the molecular motor proteins walk along[32]. The cytoskeleton is composed of different polymers: microtubules, actin filaments, and intermediate filaments. Intermate filaments are the least well-understood of the three components. They are known to be an essential structural element of cells by absorbing mechanical stress and therefore providing the cell with mechanical integrity[34-35]. The intracellular transport is, however, restricted to actin filaments and microtubules[32].

Microtubules:

Microtubules have complex assembly and disassembly dynamics and are the stiffest cytoskeletal filaments. The length of microtubules can span distances in the same order as the cell diameter[32], and they are highly dynamic and alternate between growing and shrinking phases[36]. The binding and hydrolysis of GTP at the E-site (nucleotide exchangeable site) of β-tubulin is the cause of this non-equilibrium dynamic instability[36]. Apart from β-tubulin, microtubules also contain the globular protein α-tubulin. Together they form the subunit of microtubules, an α/β-tubulin heterodimer[32]. These dimers can spontaneously assemble into protofilaments. To form the actual microtubule, twelve till fourteen of these filaments assemble into a helical cylinder[37]. Because the filaments are built of heterodimers, the two ends of the microtubule are chemically different. This causes a local polarity which plays a vital role in intracellular transport since it determines the walking direction of the molecular motors. The end that exposes β-tubulin is by convention called the plus-end of the microtubule (Fig. 1)[32]. The plus-end is more dynamic than the minus-end and is known to grow faster. Growth of the microtubule occurs by adding a single tubulin dimer or small oligomers. Shrinking can also happen at both ends, especially when the ends are not anchored (Fig. 1)[32].

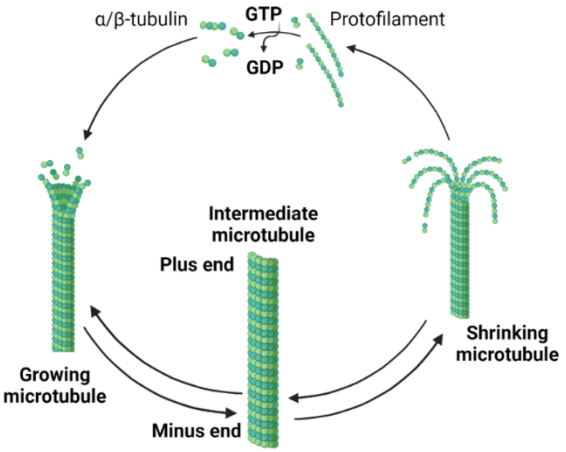

Fig. 1: Microtubule dynamics. Stable (intermediate) microtubules can either grow or shrink at both ends, but the plus end is more dynamic. Growing occurs when GTP-bound α/β-tubulin heterodimers add to the microtubule end. This requires energy, which is provided by the hydrolysis of the bound GTP. Shrinking occurs by the release of protofilaments or heterodimers from the microtubule. Image adapted from Appert-Rolland et al. (2015)[38].

Actin filaments:

Actin filaments are more flexible than microtubules. They are composed of two twisted protofilaments and form highly interconnected lattices. Actin filaments give the cell membrane its mechanical strength and shape. Just like microtubules, actin filaments can grow on the plus-end or disassemble from the minus-end if the end is not stabilized[32].

Molecular motors:

Motor proteins are molecules that can move along cytoskeletal filaments, such as actin filaments or microtubules. In order to do so, they require energy, which they derive from hydrolyzing ATP. The hydrolysis of ATP triggers a conformational change in the motor protein, resulting in a step along the filament[33].

There are three types of motor protein families: myosins, kinesins, and dyneins. Kinesins and dyneins use microtubules as tracks, whereas myosins move on actin fillaments[33].

Myosin superfamily:
Myosin II, the most famous myosin, is present in our muscles. A large number of these myosins complete a coordinated motion along the actin filaments, which leads to muscle contraction. Most myosins move towards the plus-end of actin filaments. Myosin VI is the only one that can move towards the minus-end (Fig. 2)[39]. Apart from their role in muscle contractions, a few myosins are also involved in intracellular transport, where they transport organelles and vesicles[40]. In the following, the focus will be on dynein and kinesin, the two most important motor protein families for microtubule-based transport.

Dynein superfamily:
With a molecular weight of 1.2 MDa, dyneins are the largest molecular motors[41]. The dynein family can be divided into two groups: cytoplasmic dyneins and axonemal dyneins. Cytoplasmic dyneins are the ones that play a role in intracellular transport. On the other hand, axonemal dyneins are involved in the motion of cilia or flagella[42]. Only the first group will be considered here. Cytoplasmic dynein is responsible for most long-range minus-end-directed transport along microtubules (Fig. 2). By using different multifunctional adaptors that can regulate dynein function, these dyneins can transport a wide variety of cargo[42]. The head of a dynein has a ring shape, from which a stalk stretches out that can bind to the microtubule. The cargo carried by dynein is attached to a long tail and points away from the microtubule. Dynein is attached to the microtubules when ATP is not bound and detaches from it when ATP does bind. This is a significant difference compared to kinesins, which are bound to microtubules when ATP is bound and released when ATP is hydrolyzed[32]. Interestingly, in some experiments, dynein was found to step forward and backward in the absence of nucleotides when it was under load[43].

Dynein and kinesin have overlapping binding sites. This causes them to block one another on encounters in bidirectional transport on microtubules. Unlike kinesin, dynein shows irregular stepping properties. Not only does their step size vary, but they can also step sideways. It is hypothesized that this is needed to bypass obstacles[44-45].

Kinesin superfamily:
The kinesin superfamily can be subdivided into several kinesin families[46]. All of them can exert force on microtubules and have similar motor domains[47]. However, their motion patterns vary between the different subfamilies. This can be explained by differences in the remaining structures of the molecule. The position of the motor domain in the molecule determines to which family a kinesin belongs[10]. Most kinesins (KIFs) move towards the plus-end of microtubules, when the motor domain is n-terminal. Some can move in the opposite direction when the motor domain is c-terminal (Fig. 2). Within the axon, KIFs are responsible for both fast and slow transport. Fast transport is needed for membranous organelles, while cytosolic and cytoskeletal proteins are transported slowly. Various cargo vesicles are handled by different KIFs, while the function of these KIFs sometimes seems redundant. KIF5, which is part of the kinesin 1 family and also identified as a slow transport motor, transports presynaptic membrane or active zone vesicles and amyloid precursor protein-containing vesicles in axons. KIF3 is responsible for the transport

of plasma membrane precursors. KIF1A and KIF1Bβ are responsible for the transport of synaptic vesicle precursors[48].

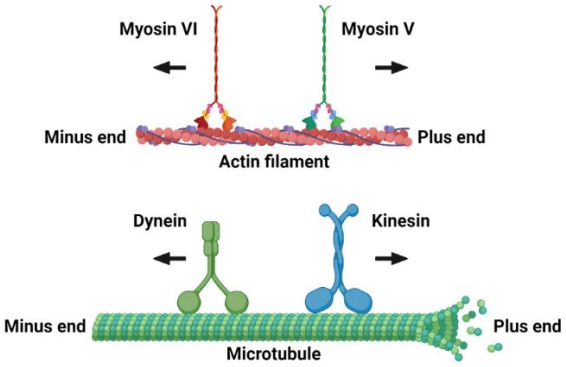

Fig. 2: The different molecular motor proteins. The only myosin which can walk to the minus end of actin filaments is myosin VI. All other myosins move towards the plus end of actin filaments. Dyneins and kinesins both walk along microtubules, however, dyneins walk towards the minus end, while most kinesins walk towards the plus end. Image adapted from Gross et al. (2007)[49].

The role of KIFs in physiological processes is not entirely understood yet, but their role in axonal transport, microtubule activity, ciliogenesis, intracellular trafficking, and cell-cycle progression and regulation is being increasingly studied. During early development and organogenesis, they have been found to play a pivotal role. Experiments that included functional disruption of *KIF* genes in mice often led to embryonic lethality. This is the case for *Kif3* and *Kif5,* for example[50-55]. KIF1A, KIF5, and KIF17 also have a fundamental role in neuronal function since they are responsible for the transport of transmitter receptors and synaptic vesicle precursors along axons and dendrites[56]. Their molecular motor activity is important for the higher brain function, which includes memory and learning. Environmental enrichment was even found to upregulate KIF1A in mouse hippocampi, revealing that KIF1A is indispensable for learning enhancement[57]. Impaired function of these KIFs can result in ocular motility disorders and peripheral neuropathies, as can be seen in patients with KAND[58-59].

<u>KIF1A and KIF1Bβ:</u>

KIF1A and KIF1Bβ are part of the kinesin 3 family and similar molecular motors. Both motors transport synaptic vesicle precursors[60-61]. This transport is needed since synaptic transmission propagates nerve impulses between neurons and their target cells. To become functionally mature by endocytosis at the synaptic plasma membrane, synaptic vesicle precursors need to be transported from cell bodies to the synapse[57,62]. A major difference between both motors is that KIF1A is a neuron-specific motor, whereas KIF1Bβ can be found in both neurons and glia[63].

From molecules to disease: the KIF1A mutation:

In 1991, Otsuka et al. found that a mutation in the *unc-104* (the *C. elegans* ortholog of *KIF1A*, caused decreased synaptic vesicle precursor transport in axons[64]. Knocking-out *Kif1a* in mice leads to lethality soon after birth, which is caused by massive neuronal and axonal cell body degeneration in the central nervous system. This was also accompanied by sensory and motor disturbances, evident in the hindlimbs. Vesicles were found to be clustering in the cell body of neurons, and the number of nerve terminals was decreased[65]. Knocking-out the *Kif1b* gene in mice gives a similar phenotype and also leads to lethality in the perinatal period. These knock-out mice also showed synaptic dysfunction and severe neuronal degeneration[61]. Heterozygous mutations save the mice from lethality but leave the mice with progressive peripheral neuropathy. These experiments show that, even though both motor proteins transport the same cargo, KIF1A and KIF1B cannot compensate for each other. Abnormally

low level of either one of them lead to lethality or a neuronal phenotype.

In 2010, Tsai et al. already established that *Kif1a* RNAi blocks basal interkinetic nuclear migration in brain neural stem cells and decreases the number of neurons[14]. Although they found different effects of *Kif1a* RNAi, the relationship between these effects remained unclear. To get more clarity on the specific role of KIF1A in diseases, Carabalona et al. (2016) continued this research and chose to use shRNA to knock down *Kif1A* in embryonic rat brain. They found that when interkinetic nuclear migration was blocked, cell nuclei accumulated, but cell cycle progression persisted. The ratio of asymmetric to symmetric brain stem cells did decrease and might explain the decrease in neurons found in their previous research since neurons in the subventricular zone and intermediate zone are generated from asymmetric brain stem cells. Neurons in the subventricular or intermediate zone have a multipolar morphology. After a while, these neurons take on a bipolar morphology and migrate to the cortical plate. *Kif1a* shRNA was found to almost entirely prevent this multipolar to bipolar transition, trapping the neurons in the subventricular and intermediate zone (Fig. 3). Even though these trapped neurons cannot progress, they were found to express the mature neuronal markers. This shows that the maturation process is not affected by *Kif1a* shRNA, purely the mechanisms coordinating morphogenesis[15].

As mentioned above, mutations in the KIF1A motor domain are associated with different neuronal diseases and symptoms. However, why different mutations cause these differences in the diseases remains unclear. Pennings et al. (2020) hypothesized that HSP is caused by mild transport defects, whereas NESCAVS would be caused by severe transport defects[28]. Pennings et al., however, only took loss-of-function mutations into account. In 2019, Chiba et al. were the first to find gain-of-function mutations in humans. They found that SPG with intellectual disabilities is mainly caused by loss-of-function mutations of *KIF1A.* Whereas in contrast, three human KIF1A mutations, namely KIF1A(V8M), KIF1A(A255V), and KIF1A(R350G), were found to be gain-of-function mutations. Patients with these mutations still had SPG but did not show signs of intellectual disabilities[6].

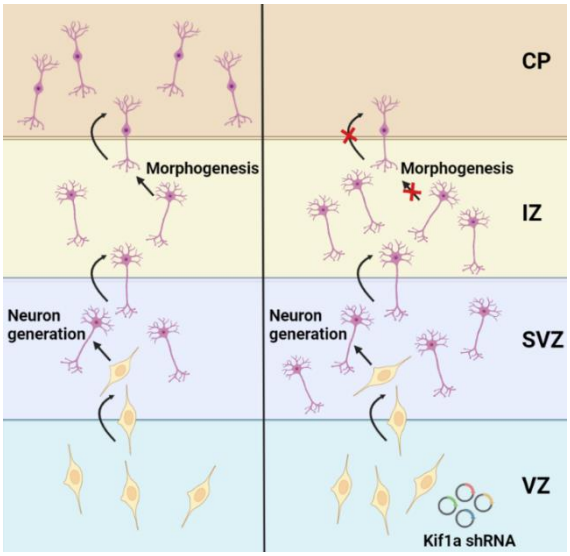

Fig. 3: *Kif1a* shRNA blocks morphogenesis in neurons. The ventricular zone (VZ) contains neural stem cells (yellow), which are also present in the subventricular zone (SVZ). Here, the neural stem cells will differentiate into multipolar neurons (purple) which can then migrate to the intermediate zone (IZ). In the IZ, morphogenesis will transform the multipolar neurons into bipolar neurons, after which they can migrate into the cortical plate (CP). In the presence of *Kif1a* shRNA, morphogenesis is blocked, trapping the multipolar neurons in the SVZ and IZ.

Although it is known that the *KIF1A* variants lead to defects in molecular transport and a decrease in the number of neurons, the exact mechanism is unknown. However, recent investigations began to shed light on these mechanisms. Aguilera et al. (2021) studied the underlying

mechanism and looked into patients with the R169T mutation who showed the typical signs of NESCAV syndrome. They found that this *de novo* missense mutation, located in the motor domain of KIF1A, decreases the ATPase activity, thereby decreasing protein motility and its ability to bind to microtubules[16].

In 2021, Budaitis et al. looked into the force-generating properties of KIF1A using optical tweezers. They found that kinesin 3 motors already detach from the microtubules under the low force of 3 pN, whereas kinesin 1 motors can withstand forces of 5-6 pN. This is not due to the strength of the motor-microtubule interaction since KIF1A has a higher microtubule affinity than kinesin 1 members. Although the KIF1A motors detach under low forces, they quickly reattach to the microtubules. This property was found to be linked to the positively charged loop present in kinesin 3 members. Replacement of this loop by the loop of kinesin 1 members led to a decrease in the amount of force-generating events.

They also investigated two KIF1A disease variants, namely V8M and Y89D. These mutations were chosen because they were expected to be in a location of the motor protein, which was associated with force generation and occurs in highly conserved areas of the kinesin superfamily. These mutations induced multiple impairments of motor function. These mutated motors showed reduced velocities due to reduced ATPase activity and microtubule-binding, reduced landing rates, and decreased force generation. However, the ability to quickly reattach to the microtubule was not affected. They also increased the number of motility events significantly, indicating that these mutations relieve autoinhibition. For V8M, this finding was consistent with the findings of Chiba et al. (2019). However, Chiba et al. proposed that this mutation causes hyperactivation of KIF1A. This was based on comparisons of V8M to the autoinhibited wildtype motor (this is the state of KIF1A in the absence of cargo), whereas in their research, they showed that V8M is actually inhibited in multiple motility properties when you compare it to the uninhibited wildtype motor[17].

How KIF1A mutations lead to disease is still unclear. However, from the previously mentioned research, it is possible to suggest that the problem lies within the ATPase activity and microtubule-binding abilities of KIF1A.

Discussion: Is there a cure in sight?

KAND is a severe but rare disease linked to different variants of *KIF1A*. The disease causes different symptoms, even if patients have the same *KIF1A* variant. Both recessive and dominant mutations can lead to this disease. However, the most severe phenotypes are found in patients with *de novo* variants[18-29].

The *KIF1A* gene encodes the KIF1A motor protein, a kinesin that transports synaptic vesicle precursors along microtubules. Even though there are many different kinesins, it is unlikely that another kinesin can compensate for the KIF1A mutation[10,46]. The only other kinesin that transports the same cargo is kinesin KIF1Bβ. Otsuka et al. (1991) showed that having abnormally low levels of one of them still leads to lethality or a neuronal phenotype. This means that KIF1Bβ does not compensate for the defects in transport caused by the KIF1A mutation. However, this was concluded from wildtype level KIF1Bβ[64]. It would be interesting to see whether overexpressing KIF1Bβ can overcome these defects in intracellular transport. Perhaps it will outcompete KIF1A for the synaptic vesicle precursor cargo in larger quantities.

Carabalona et al. found that *KIF1A* RNAi can block the basal interkinetic nuclear migration and decreases the number of neurons[14]. Using shRNA, they concluded that this was due to the disturbed multipolar to bipolar transition of brain neural stem cells, preventing these cells from progressing to the cortical plate. However, the cells did show all of the mature neuronal markers in the right order[15]. Future research should be done into the possibility of freeing these trapped neurons and whether or not this alleviates or cures symptoms of KAND.

Different variants of *KIF1A* can cause different symptoms and have differences on the molecular level. Several variants have been investigated over time, and one recurring conclusion was that some mutations led to decreases in the ATPase activity of KIF1A, thereby decreasing protein motility and its ability to bind to microtubules[14-17]. This suggests that restoring the ATPase activity in KIF1A variants might cure the disease. Possible solutions for this include using chaperones to correctly fold the misfolded protein or using small molecule drugs to bypass the mutated portions of the gene. Such a small molecule drug is used to treat spinal muscular atrophy, where the drug Evrysdi does not correct the mutated gene but bypasses the mutated portions leading to a fully functioning protein[66]. It remains to be investigated whether this is an option for KAND.

We are not there yet in terms of finding a cure for KAND. However, KIF1A and KAND have become more popular research topics in the past few years, partly due to the KIF1A.ORG community[4]. This community also raised the interest of Ovid Therapeutics, a company that specializes in rare diseases which especially focuses on rare neurological diseases. They are now looking into new medicines and therapies for KAND. A few of their strategies include: using RNAi or shRNA to degrade the defective maternal or paternal copy of the gene, disabling the mutant protein, replacing the defective gene, and editing errors in the mutant gene or finding. Replacing the defective gene is very challenging due to the relatively big size of the protein. CRISPR-cas9 might be a possibility in repairing the allele. However, this might trigger off-target side effects and therefore needs to be investigated extensively before it can be used to treat KAND in humans. One strategy to disable mutant KIF1A protein is by using aptamers. These aptamers can bind to specific mutant forms of KIF1A and disrupt the function of mutant protein[67]. Most of these possible treatment strategies have some downsides. KAND is a disease for which the mutation varies between patients. Some patients have homozygous, and others have heterozygous mutations. For patients with homozygous mutations, disabling the mutant gene or protein would leave them without functioning KIF1A, which will be lethal. Also, each of the approaches mentioned above would need to be adapted to the specific mutation of a patient. Finding a global therapeutic strategy that can be used for every patient regardless of their mutation would be ideal, but in order to find such an approach, we would need to fully understand the changes caused by *KIF1A* mutations on the molecular level. Therefore, I think the road to a cure for KAND should continue to focus on the molecular pathway first.

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