# Overarching pathomechanisms in inherited peripheral neuropathies, spastic paraplegias, and cerebellar ataxias

**TITLE**:

Overarching pathomechanisms in inherited peripheral neuropathies, spastic paraplegias and cerebellar ataxias

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**Key words**: neurogenetic disease; DNA and RNA processes; protein quality control; mitochondria and metabolism; ion channels; axonal transport

Met opmerkingen [LVdV1]: Up to six keywords; maybe we drop 'rare neurological disease' to keep one keyword per pathomechanistic theme

Met opmerkingen [JDW2]: Akkoord? Processes in plaats van metabolism

Met opmerkingen [VT3R2]: DNA en RNA zijn op zich al geen trefwoorden. Dus misschien gewoon beter dit weglaten.

Ofwel vervangen door:
Disease associated mutations

## Abstract

International consortia collaborating on the genetics of rare diseases have significantly boosted our understanding of inherited neurological disorders. Historical clinical classification boundaries were drawn between disorders with seemingly different etiologies, such as inherited peripheral neuropathies, spastic paraplegias, and cerebellar ataxias. These clinically defined borders are being challenged by the identification of mutations in genes displaying wide phenotypic spectra and by shared pathomechanistic themes, which are valuable indications for therapy development. We highlight common cellular alterations that underlie

this genetic landscape, including alteration of cytoskeleton, axonal transport, mitochondrial function and DNA repair response. Finally, we discuss venues for future research using the long axonopathies of the peripheral nervous system as a model to explore other neurogenetic disorders.

## Common grounds in rare neurological disorders

Rare inherited neurological diseases exhibit a profound level of complexity, both on a clinical and on a genetic level. These inherited disorders are currently classified based on their clinical presentation or genetic etiology. Yet, despite these classifications it has been pointed out that underlying neurobiological processes are shared amongst these neurological disorders. (REF: Beaudin, 2022; REF Synofzik, REF: Timmerman 2013, REF: Synofzik & Schule 2017) As a case in point, this review addresses the pathomechanistic themes underlying three seemingly distinct disorders, namely inherited peripheral neuropathies (IPN), hereditary spastic paraplegias (HSP) and spinocerebellar ataxias (SCA) by using the viewing-point of their known disease-genes (Figure 1). Furthermore, we explore the potential advantages of incorporating these overarching pathomechanisms into genetic research and the development of therapeutic interventions. We stress that in addition to shared genetic backdrop, pathomechanistic processes are broadly shared between IPN, HSP and SCA, although certain ~~pathomechanisms~~ disease mechanisms can be more prominent in one of the three disorders.

The IPN is recognized by varying degrees and combinations of mainly distal sensory and motor disturbances as well as autonomic symptoms. The HSP is defined by progressive lower limb spasticity and weakness leading to restraints in mobility and reduced quality of life. Finally, core phenotypical features of SCA are gait imbalance, upper limb discoordination, dysarthric speech and nystagmus. Although the neurodegeneration leading to these disorders is localized at distinct anatomical regions – the peripheral nervous system, pyramidal tracts and cerebellum, respectively - clinical overlap syndromes are well-described as well as overlapping themes in their causative genes and underlying ~~patho~~mechanisms of disease. The axonopathy spectrum includes HSP and IPN, where in both cases the long neuronal axons are affected, in either the central or peripheral nervous system respectively [1]. Secondly, the combination of HSP and SCA is well established as two closely interlinked disorders of motor systems, coined as spastic ataxias [2].

It is worth mentioning that these group of disorders, namely IPN, HSP and SCA, are rare in the general population and can be easily mistaken for other diseases. Many of their symptoms and signs can resemble those of more common acquired disorders. Also common disorders can occur in families and therefore could mislead clinicians to pursue genetic testing. Failure to

**Met opmerkingen [VT4]:** Ik zou af en toe toch eens 'disease mechanisms' gebruiken als ander woord, want anders wordt het een monotone herhaling van 'pathomechanisms' in deze paar zinnen. Ook verder heb ik getracht af en toe pathomechanism anders te formuleren.
Ook disease pathomechanisme is 2x hetzelfde zeggen, dus kan je vervangen door 'mechanism of disease' of 'disease mechanism' maar dan zonder de 'patho-'

**Met opmerkingen [JB5]:** even kijken naar line-spacing in heel het documenten ook consistentie van de titels qua opmaak

distinguish acquired from inherited disorders is a frequent cause of significant delay in diagnosis and even undesirable exposure of patients to treatments. Diagnostic algorithms can guide clinicians in complex cases. Nevertheless, a slowly progressive disease, a positive family history and worsening of symptoms despite treatment are red flags that should shift the diagnostic scope towards the realm of inherited disorders [3].

## Genetic discoveries defy disease boundaries

The advent of Next-Generation-Sequencing (NGS) technologies has enabled the discovery of many gene-disease relationships over the past decades. More than 350 genes are currently established to cause either IPN, HSP or SCA, with many of them also described to cause overlapping phenotypes. Traditionally and until quite recently, diagnostic testing of patients with a suspected rare inherited neurological disease would consist of screening hand-picked single genes based upon the phenotypical pattern of the patient, or alternatively, a multiplex PCR with several disease-associated genes. Such gene-panels are however very time and resource-consuming when employed to screen for more than 100 genes and therefore no longer routinely used.

With the increased affordability of NGS, whole-exome sequencing (WES)-based diagnostic gene panels have been widely adopted in clinical practice, surmounting the rising number of genes to be tested due to locus heterogeneity [4]. However, to keep pace with the expanding genetic knowledge in IPN, HSP and SCA, these panels require regular updates to ensure their accuracy. Of note, the increasing overlap between these gene-panels questions the practicality and indicates a growing area of common ground between these disorders on a genetic level (see Outstanding questions).

The discovery of ~~pathomechanisms~~ disease mechanisms has majorly been driven by “forward genetics”, in which the study of disease-~~causing~~ associated mutations in Mendelian disease has pinpointed crucial cellular processes. More and more, it is becoming clear that different mutations and or genes converge on large pathomechanistic themes. We discern six major pathomechanistic processes currently known to be at play in IPN, HSP or SCA (Figure 2). Although such a classification is practical and insightful, we do realize this division is always arbitrary to some extent.

**Met opmerkingen [MF6]:** I think "decades" might be more fitting here; this is a general statement (not one specific to IPN / HSP / SCA), and NGS has been around for a while, more than just a decade.

**Met opmerkingen [MF7]:** I think this sentence can be skipped, if that seems ok (or rephrased); the phrasing is a bit odd (the suitability is not just for the review - the ideal is to present a classification which hopefully would be useful more generally), and overall, the sentence feels a bit repetitive and not fully needed.

When evaluating the relationship between pathomechanistic processes and the three disease categories, it should be noted that no single process is unique to a disease and vice versa. Different themes can be distinguished, such as e.g. transmembrane channels & receptors that are rarely affected in HSP but are most certainly ~~an~~ overlapping ~~pathomechanistic theme~~ between IPN and SCA. Cytoskeletal and axonal transport proteins are more affected in IPN, which is not surprising given the length of motor and sensory neurons, although examples exist where mutations in these proteins lead to HSP and SCA. Below we will discuss prominent examples of different biological themes, highlighting major overarching ~~patho~~mechanistic processes and the overlap in underlying genetic causes.

## Nuclear proteins, DNA, RNA, transcription & translation

Post-mitotic neurons are specifically sensitive to the quality control and homeostasis of the nucleus, transcription, and translation machinery. It is therefore not surprising that genes involved in the integrity of the components of the central dogma of molecular biology (replication, transcription, translation) are implicated in neurodegenerative disease.

As a first step, integrity of DNA and the DNA damage response (DDR) has long been recognized as a common ~~patho~~mechanism of disease in SCA and IPN. Ataxia telangiectasia, caused by recessive loss-of-function mutations in *ATM,* is a well-established example of a double-strand DNA repair disorder. Loss of functional ATM in DDR results in progressive cerebellar ataxia with oculomotor apraxia, telangiectasias, predisposition to neoplasms, immunodeficiency and peripheral neuropathy [5]. Furthermore, recessive *APTX* mutations cause cerebellar ataxia often with peripheral neuropathy through aprataxin deficiency, resulting in a single-strand DNA repair disorder [6], [7]. Interestingly, both *APTX* and *ATM* related disorders can be suspected by screening for elevated levels of alpha-fetoprotein [8]. The *RFC1* gene has been discovered to cause the combination of cerebellar ataxia, neuro(no)pathy and vestibular areflexia or CANVAS syndrome [9]. Although disease ~~patho~~mechanisms are currently unresolved, *RFC1* encodes the Replication factor C protein, initially identified to be essential for DNA replication and repair [10].

**Met opmerkingen [VT8]:** Ik heb de herhaling hier vermeden.

**Met opmerkingen [VT9]:** Is herhaling: ofwel disease ofwel patho ...

On the RNA level, both *POLR3A* and *POLR3B*, subunits of the RNA polymerase III, can cause spastic ataxia, with involvement of a demyelinating neuropathy in the case of *POLR3B* [11], [12]. The RNA polymerase III complex synthesizes abundant short non-coding RNAs, thereby regulating transcription, RNA processing and protein translation. Although disease ~~patho~~mechanisms remain unclear, insights have been gained through a mouse model that showed that mature oligodendrocytes likely fail to produce a myelin sheath of normal thickness [13]. Missense, nonsense, frameshift, and splice variants have all been identified to cause a similar phenotype, and all likely lead to a loss-of-function. Recently, in another mouse model, a decrease in RNA polymerase III subunit expression was reported to lead to defects in oligodendrocyte development [14].

The final step of the central dogma is translation from RNA to protein. Mutations in seven different amino-acyl-tRNA synthetases (ARSs), enzymes that attach the appropriate amino-acid to its corresponding tRNA, cause varying forms of IPN. The exact ~~patho~~mechanism of how mutations in these ubiquitously expressed and crucial genes cause the neuropathy has long been elusive, with different models showing that loss-of-function alone is not sufficient to explain the ~~patho~~mechanism of disease [15], [16]. Recently, it was shown that GARS1-associated mutations causing Charcot-Marie-Tooth's disease (CMT) sequester $tRNA^{Gly}$, thereby depleting it for translation and causing ribosome dwelling at the Glycine codons. This effect could be counteracted in both *Drosophila* and mice by overexpression of tRNAGly, thereby opening the road to potential therapies [17], [18]. In parallel, recent work in *Drosophila* and human cell lines has shown that the postulated toxic gain-of-function of *YARS1* might be due to a non-canonical role in actin bundling, which was shown to also be a function of *GARS1*, *HARS1* and *DARS1*, possibly uncovering a common pathomechanism between the different ARSs [19].

## Endoplasmic reticulum, ~~golgi~~ Golgi apparatus and intracellular quality control & recycling mechanisms

Each protein must be translated, folded into a functional structure, and transported to its relevant subcellular location. If protein translation, folding or transport fail, quality control or recycling mechanisms are triggered. Proteins are translated at ribosomes on the surface of the Endoplasmic Reticulum (ER). The ER also functions as protein transportation system and is

**Met opmerkingen [VT10]:** idem

**Met opmerkingen [MF11]:** I might be missing something but I'm not sure this sentence is clear; is the intention here *mutations* in the genes encoding the ARSs? If so, this should be phrased more explicitly, I think. (Are the ARSs themselves causing IPN, as the current phrasing states?)

**Met opmerkingen [MF12]:** Again, I don't think it fully makes sense to say that the proteins cause pathology. It's *mutations* or *aberrant function* of the proteins that causes pathology. It would be good to rephrase this sentence as well.

**Met opmerkingen [VT13]:** Check in de ganse paper: Golgi is altijd met een hoofdletter G, want meneer Golgi heeft zijn naam gegeven aan dit organel. Idem voor Schwann cell.

active in lipid synthesis. ER morphology regulators, like atlastins and reticulons, control membrane fusion and curvature of the ER, and mutations in these genes are known to cause neurological disorders, suggesting a critical role of ER formation in neuronal activity and function [20], [21]. Mutations in *ATL1* causes HSP or HSAN, while *ATL3* also causes HSAN [22]–[24]. *REEP1* and *REEP2,* also shape the ER and mutations in both of them cause HSP [25], [26].

The two major cellular degradation pathways are the ubiquitin-proteasome system (UPS) and autophagy. There is significant crosstalk between the two, and they are implicated in rare neurological and multisystemic disorders. Proteins that are to be degraded by the UPS pathway are tagged by poly-ubiquitin chains through E3 ubiquitin ligases, that recruit E2 ubiquitin-conjugating enzymes and E1 ubiquitin-activating enzyme. Different E3s confer specificity to the ubiquitin substrate proteins. Multiple genes that encode E3 ubiquitin ligases are disease-associated: mutations in *RNF170* are associated with recessive HSP and IPN, mutations in *RNF216* cause recessive SCA, and mutations in *RNF220* cause hypomyelinating leukodystrophy with ataxia, deafness, liver dysfunction and cardiomyopathy [27]–[30]. Mutations in the *LRSAM1* gene, a universally expressed RING-type E3 enzyme, cause CMT, yet pathomechanistic insights remain scarce due to a lack of known ubiquitination targets [31]. Other examples include *TRIM2*, *STUB1*, *DCAF8*, *UBE3C*, *UCHL1* and *UBAP1* [32]–[37].

Autophagy, literally meaning ‘self-devouring’, degrades unnecessary or dysfunctional components through a lysosome-dependent mechanism. A multitude of genes are involved in the autophagic pathway. Although autophagy disorders vary in disease onset and severity, they often share common features such as spastic ataxia, intellectual disability, and distal neurogenic or myopathic weakness [38]. Well-known examples include bi-allelic loss-of-function variants in genes that encode subunits of the adaptor protein complex 4 (AP4B1, AP4M1, AP4E1, AP4S1) that cause complex forms of HSP in children [39]. The adaptor protein complex incorporates transmembrane proteins into vesicles and recruits the necessary proteins for vesicle budding and transport [40].

Lastly, heat shock proteins are produced in response to stressful conditions, such as heat, cold, UV light, wound healing, etc. Many heat shock proteins are molecular chaperones that ensure the correct folding of newly synthesized proteins or aid in the refolding of proteins damaged

by the stress conditions. Heat shock protein-related disorders include IPN, SCA and HSP. For example, mutations in *DNAJC5* cause ceroid lipofuscinosis [42], a complex form of SCA with epilepsy and cognitive decline, while mutations in *HSPB1* and *HSPB8* cause IPN with or without myopathic involvement [43]. Importantly, HDAC6 was found to be upregulated in mutant *HSPB1* mice. Small molecule HDAC6 inhibitors were shown to partially rescue the phenotype in these mutant mice, after which the inhibition of HDAC6 as a therapeutic target was extended to other neuropathies (REFs).

### Lipid metabolism, cell membrane and myelin sheath

Lipids are fundamental organic molecules that play crucial roles in neuronal cell populations: they serve as building blocks for structural components and organelles, and they can function as signaling molecules or be employed as energy substrates. Depending on the specific disrupted metabolic pathway, inherited lipid metabolism disorders show distinct clinical manifestations [46].

Myelin sheaths, a highly lipid-rich material, insulate neurons and ensure efficient propagation of action potentials. Inherited disorders of myelin are limited in genetic heterogeneity but represent major proportions of disease subtypes, such as *PMP22* and *MPZ* in CMT and to a lesser extent *PLP1* in X-linked HSP or Pelizaeus-Merzbacher disease. Interestingly, acquired myelin disorders such as sub-acute combined degeneration due to vitamin B12 deficiency can show overlap in the underlying ~~pathomechanism~~disease mechanism, and are therefore valuable targets for shared therapeutic development [47].

The *PLP1*-related diseases range from a life-threatening early onset leukodystrophy (conatal form of Pelizaeus-Merzbacher disease) towards a less severe childhood onset complex form of HSP, with a variable degree of intellectual disability and axonal peripheral neuropathy. The *PLP1* encodes proteolipid protein 1 which constitutes the major myelin protein in the central nervous system [48]. Most patients are affected by *PLP1* duplication mutations, and therapeutic development is directed towards reduction of expression levels [49]. Several commonalities can be observed between *PLP1*- and *PMP22*-related conditions. The *PMP22* gene encodes peripheral myelin protein 22 which is mainly expressed in Schwann cells where it contributes to myelin integrity and compaction. CMT1A, a demyelinating peripheral

neuropathy with varying inter- and intrafamilial severity, is caused by duplication of *PMP22* [50]. Therefore, *PLP1* and *PMP22* can be considered as two ends of inherited myelin related disorders, with overlap in ~~patho~~the mechanism of disease and therefore likely also in therapeutic approaches to be developed.

Biallelic mutations in *PNPLA6* represent the pleiotropic effects of alterations in glycerophospholipid metabolism which is a major lipid pathway. In *Drosophila*, disease-associated mutations result in a disruption of lipid levels due to a dysregulation in phospholipase activity [51]. The complex human phenotype consists of varying combinations of CNS and PNS involvement, chorioretinal dystrophy, hypogonadotropic hypogonadism and other hormonal disturbances and dystrophic changes such as hair anomalies. Interestingly, other genes implicated in glycerophospholipid metabolism such as *PLA2G6, PCYT1A, PCYT2, EPT1* show similar overlapping core features [46], [52].

Met opmerkingen [MF14]: The logic of the argumentation here wasn't great, given the earlier punctuation and the organization of the sentences. If the statement is about the pleiotropic effects, and this is followed by a colon, one is expecting the rest of the sentence to describe pleiotropic effects; but this is actually discussed only in a later sentence. It's also better to place 'drosophila' early in the sentence, otherwise the emphasis is on drosophila, instead of the on the content (in a way, the previous phrasing could imply that the findings are relevant *only* for drosophila). Please see whether the suggested changes seem fine.

Finally, biallelic variants in *CNTNAP1*, encoding Contactin-associated protein 1, are reported to cause hypomyelination of both CNS and PNS and commonly lead to infantile death. While this spans the whole CNS-PNS spectrum of myelin disorders it also bridges towards acquired demyelinating disorders, namely anti-contactin-1 (CNTN1) antibody-associated nodopathy [53], [54].

## Transmembrane channels, receptors and intercellular contact

Transmembrane channels and transporters can have a variety of different functions, but they share the overall role of transporting cargoes or compounds from one cellular compartment to another. Like transmembrane channels, membrane receptors are embedded within the plasma membrane. They activate specific processes through the binding of extracellular molecules. Impaired channels, transporters and receptors are associated with a multitude of neurological diseases including epilepsy, hemiplegic migraine, periodic palsies, ataxia, peripheral neuropathy, and other conditions, collectively referred to as 'channelopathies' [55]–[57].

The potassium channels *KCNA1*, *KCNA2*, *KCNC3* and *KCND3* all give rise to some form of SCA when mutated [58]–[62]. Mutations can be either dominant negative or lead to haploinsufficiency. Loss-of-function and missense variants in *KCNA1* underlie Episodic Ataxia 1

(EA1). *KCNA1* encodes the Kv1.1 voltage-gated potassium (Kv) channel, which dampens neuronal excitability. Studies have been performed to identify channel openers, which can reduce the excess neural excitability in disease state. A recent study, for example, evaluated botanical medicines used by Native Americans, including extracts from common nettle and Pacific ninebark root to treat locomotor ataxia. The authors describe that extracts from these plants are able to rescue ataxia-linked mutant potassium channel activity [63]. Similarly to mutations in potassium channels, mutations in low-voltage gated calcium channels such as *CACNA1G*, *CACNA2D* and *CACNA1A* lead to SCA, likely due to alterations in neuronal excitability [58], [64]. Mutations in the sodium channel *SCN2A* also lead to episodic ataxia [65]. Other sodium channels, like *SCN9A*, *SCN10A* and *SCN11A* are linked to forms of IPN [57], [66]. Due to the channels' intrinsic capacities to respond to changes in the (intra)cellular space or to cause such changes, most of the aforementioned disorders exhibit one or both of the more specific features associated with channelopathies. These features can include episodic and sometimes paroxysmal events, such as cerebellar ataxia, diplopia or migraine, and positive neurogenic symptoms such as neuropathic pain and hyperesthesia. Recognition of these symptoms can not only provide guidance in interpreting genomic variants but also valuable feedback in estimating therapy effectiveness. Drug trials targeting these calcium, potassium, and sodium channels offer proof-of-concept in several of these channelopathies.(REF: Paola Imbrici, 2016, review paper: Therapeutic Approaches to Genetic Ion Channelopathies and Perspectives in Drug Discovery)

Gap junctions are channel-forming structures contacting plasma membranes of neighboring cells, thereby allowing communication between different cell types [67]. Mutations in *GJB1*, encoding the gap junction protein connexin-32, lead to an X-linked form of demyelinating IPN by reducing the amount of connexin-32 available at the cell surface, both in human cell models as in mice models [68]. Similar to the symptoms seen in individuals with channelopathies, mutations in *GJB1* are reported to sometimes cause episodic weakness or transient encephalopathy with or without evidence of white matter disease. Mutations in connexin-31, encoded by *GJB3*, are causative of dominant demyelinating IPN with hearing loss [69]. On the other hand, recessive missense or loss-of-function mutations in *GJC2* (Connexin 47) cause Pelizaeus-Merzbacher-Like Disease or a late-onset complex form of HSP [70].

**Met opmerkingen [MF15]:** The word "nearly" is unclear here, I think. Consider rephrasing for more clarity.

**Met opmerkingen [MF16]:** Please revise for a better presentation. This sentence, unless I'm missing something, has no verb. Sentence should follow the basic structure of "Noun, verb, etc."

**Met opmerkingen [MF17]:** Same here.
Perhaps the intention was that these are the two features mentioned earlier? If so, the punctuation and sentence progression in this section does not work well - one needs to have a colon after "channelopathies" in the earlier sentence, skip the words Firstly/Secondly, and separate the two clauses describing each feature with a comma.

**Met opmerkingen [MF18]:** When it comes to 'therapeutic promise' sentences like here, TINS authors are asked please (i) to use more moderation in the phrasing; (ii) to provide supporting citations or to cite the clinical trials. For citing clinical trials, please see the Author Guidelines file shared early on; if citing clinical trials, please add a 'Resources' section ahead of teh references, where the clinical trial(s) webpage(s) will be added, and please follow carefully the other instructions in relation to citing Resource item.

**Met opmerkingen [MF19]:** Please rephrase for greater accuracy both in the wording and in the content. "Similar as" is incorrect in term of phrasing; *GJB1* is the gene, so it cannot show "episodic weakness; and as currently phrased, the comparison of the second part of the sentence to "channelopathies" doesn't make sense - I guess the intention was more around "Similar to the symptoms seen in some individuals with channelopathies", or something to that effect, but that's different from what's currently written.

## Axonal transport and cytoskeletal proteins

Axons contain tightly regulated cytoskeletal proteins, such as actin, microtubuli and neurofilaments along with motor proteins. These cytoskeletal proteins~~, that~~ maintain the cellular structure and ensure transport of transcripts, proteins and organelles within the neuron.

The α-II-spectrin, encoded by *SPTAN1,* plays a major role in the spectrin-actin axonal cytoskeleton through heterotetramerization with one of the β-spectrins (*SPTBN1, SPTBN2, SPTBN4, SPTBN5*) and interlinks actin rings, giving rise to the membrane-associated periodic skeleton (MPS). The MPS also displays a key scaffolding function, ensuring correct localization of ankyrins. Interestingly, mutations in all but one (*SPTBN5*) of the β-spectrins are reported to result in disorders similar to those caused by *SPTAN1* mutations, namely: developmental and epileptic encephalopathy (DEE), intellectual disability (ID), HSP, SCA and IPN [71]–[73].

Biallelic mutations in *AGTPBP1* cause childhood-onset cerebellar ataxia and progressive motor neuron(o)pathy and are therefore relevant in the differential diagnosis in non-5Q associated forms of spinal muscular atrophy. *AGTPBP1*, previously known as *CCP1*, encodes ATP/GTP-Binding Protein 1 which mediates tubulin posttranslational modifications through its deglutamylase activity. This disease association greatly impacted the field by highlighting the significance of dysregulation in the microtubule cytoskeleton [75].

*KIF1A*, encoding kinesin family member 1A, contributes to the anterograde axonal trafficking of cellular cargo. To date, 16 members of the kinesin superfamily have been identified as associated with neurological disorders. Herein, *KIF1A* and *KIF5A* show both extremes of the axonopathy spectrum (HSP-HMN), while *KIF1C* is associated with spastic ataxia with signs of PNS involvement. These so-called kinesinopathies underscore the importance of intact axonal transport in motor neurons [76]. The two other superfamilies of motor proteins, dyneins and myosins, show similar associations (REF: Berth, 2023: Disruption of axonal transport in neurodegeneration)

**Met opmerkingen [VT20]:** Zin is onduidelijk en te lang, daarom best 2 zinnen.

**Met opmerkingen [MF21]:** There is a mix-up here of gene- and protein-related statements: the content of the sentence is about the protein, but the sentence is built around the gene as the core noun. Please revise.

**Met opmerkingen [MF22]:** Please cite the relevant source at the end of this paper. If ref76 is relevant here, it could be cited, but without supporting citation, the sentence feels incomplete from the reader perspective. (Readers' cannot foresee whether the follow-up sentence will relate to the same study or not).

**Met opmerkingen [MF23]:** "show the value of axonal transport" is not a great way to put it. And the placing of ref76 was not optimal, given that the second part of the sentence is a general, interpretative statement.

## Metabolism and mitochondria

Inherited metabolic and mitochondrial disorders are known for their wide display of neurological and complex syndromes. Several of these disorders show overlap in their pathomechanisms, emphasizing the crosstalk between metabolism and mitochondria. The peripheral nervous system, pyramidal tracts and cerebellum are frequently involved in these neurometabolic and mitochondrial disorders[77], [78].

*MT-ATP6* encodes ATP6, a core subunit of ATP synthase, responsible for the generation of ATP as a final step in the oxidative phosphorylation pathway. It is one of the 13 proteins encoded by the mitochondrial DNA and is associated with a spectrum of diseases ranging from early-onset multisystemic neurodegeneration to adult-onset axonal form of CMT. Mutations in *MT-ATP6* affect the ATP synthase activity and cause full dependence on glycolysis [79], [80].
Mitochondrial proteases ensure that mitochondria can adapt to different metabolic demands and stress by reshaping the proteome. Functional m-AAA protease can be formed by homo-oligomers the AFG3-like protein 2 subunits or by hetero-oligomerization of paraplegin and AFG3-like protein 2. -Mutations in *AFG3L2* and *SPG7*, respectively encoding AFG3-like protein 2 and paraplegin, result in overlapping phenotypes when mutated, typically a combination of cerebellar ataxia and spasticity. Some patients show cognitive impairment, opthalmoparesis, ptosis and extrapyramidal symptoms. Loss-of-function leads to mitochondrial fragmentation and deficiencies in the axonal transport of mitochondria. Interestingly, non-mendelian inheritance is suggested in *SPG7*-associated disorders with *AFG3L2* as its main interactor [81]–[83].

Neurometabolic disorders can present as pure or complex multisystemic disorders, and their pathology reflects either toxic accumulation or a deficiency of metabolites. The discovery of biallelic *SORD* mutations causing a frequent type of hereditary motor neuropathy is a pertinent example. *SORD* encodes for sorbitol dehydrogenase which converts sorbitol into fructose and is well-known in the pathomechanism of diabetic neuropathy. Due to *SORD* mutations, toxic sorbitol levels accumulate, causing the CMT phenotype [84]. A clinical trial is currently ongoing to investigate compounds that reduce sorbitol levels (NCT05397665). A second example are recessive mutations in *MMACHC* which cause the combination of cerebellar ataxia and peripheral neuropathy due to a disturbed cobalamine metabolism (REF: Jordan P Lerner-Ellis,

**Met opmerkingen [VT24]:** Vertikaal Streepje ??? → referentie

nature 2006; DOI: 10.1038/ng1683. Acquired deficiencies of cobalamine can also lead to similar clinical presentations including subacute combined degeneration of the spinal cord and cerebellar ataxia [85].

Finally, recessive mutations in *SUCLA2* result in both mitochondrial and metabolic dysfunction through succinyl-CoA accumulation and increased lysine succinylation of proteins (REF: 86 herhalen). Patients show a severe syndromic presentation of IPN and encephalomyopathy, and can show typical signs of mitochondrial dysfunction, such as ptosis and ophtalmoplegia [86].

## Shared pathomechanisms in rare neurological disorders

The identification of novel mutations in rare neurological and neuromuscular diseases is in most cases a starting point for detailed studies of the causative gene and its protein function. Such studies have uncovered many vital biological pathways that directly or indirectly affect neurons. The strong focus of both clinicians and researchers on specific diseases has great scientific benefit although concentrating on a specific disease group or biological pathway does pose the risk of obscuring the broader ~~pathomechanistic~~ picture. Using three groups of disorders (HSP, IPN and SCA) we illustrate that these distinct clinical entities show extensive overlap on a genetic and ~~patho~~mechanistic level, that one could describe as locus and pathomechanistic pleiotropy. No pathomechanistic theme is unique to a single disease group, and many examples exist that would extend this paradigm even well beyond the three groups described above. The convergence of ~~patho~~disease mechanisms ~~across diseases~~ questions the research applicability of disease classifications that are mainly based on clinical features. A classification system with more emphasis on disease mechanisms could help shorten the time to final diagnosis and facilitate the design of ~~pathomechanism~~ focused therapies. These therapies have the potential to be applicable for more patients than the target population of single gene-, allele- or variant-based therapies (Figure 3)

## Genetic diagnoses require pathomechanistic insight

Whereas in the past genetic diagnoses would be made by screening hand-picked genes based on recognizable clinical patterns, nowadays a large fishing net can be cast employing next-generation diagnostic methodologies. Although no 'one-off' diagnostic test is readily available to screen for all types of variants such as single-nucleotide variants, small insertions and

**Met opmerkingen [MF25]:** Again, please cite the supporting reference(s) at this point. There is a specific argument here, so from the reader perspective there is expectation for a citation.

**Met opmerkingen [MF26]:** Please provide a supporting citation. If it's ref86, please move it to the end of the current sentence (or cite it twice).

**Met opmerkingen [VT27]:** Ik zou in één van de onderstaande paragrafen ergens pleiotropy vermelden, want nu komt dit enkel voor in de figuur en in 'highlights'.

**Met opmerkingen [VT28]:** Hier zou een zin over pleiotropy bij moeten omdat de lezer anders niet gaat weten waarom dit plots in de figuur 3 staat en in de highlights.

deletions, large structural variants, copy number variants (CNV) and repeat expansions, it is likely that in the near-future this will become the case with whole-genome sequencing technologies relying on long-read sequencing methodologies. Specific technologies each have their own limitations, yet the challenge of variant interpretation is shared among all and has become the current bottleneck. To avoid cumbersome and costly excursions in interpreting sequencing data, many currently apply a 'compromise' approach by using WES-based gene panels, including CNV analyses, in combination with specific repeat expansion analyses. This requires the referring clinician to have a well-established set of clinical patterns to go after the correct diagnostic path and order specific genetic analyses.

It is likely that soon different sorts of genetic testing, including long-read whole genome sequencing will become more cost-effective, leading to a situation where possibly one technology suffices to screen for all types of variants. Variant-interpretation will then take on an even-larger role, likely supported by artificial-intelligence driven algorithms to support decision-making and prioritizing variants. This is hoped to enable an unbiased view on the genetic variants underlying a certain disease. In the context of this approach, interpreting variants and their consequences in a ~~pathomechanism~~ disease mechanistic or biology-centered way of thinking will become crucial.

## Concluding remarks & future perspectives

It can be argued that the pace of genetic discovery in rare inherited neurological disorders surpassed the rate of advances in pathomechanistic understanding, increasing the gap between these two domains of knowledge. Starting from the elucidation of genetic variations that cause HSP, SCA and IPN, crucial and shared biological pathways have been revealed via extensive *in vitro* and *in vivo* research. In this review, we have shown through prominent examples that both disease and ~~patho~~mechanistic boundaries are to a certain extent arbitrary and great potential lies in approaching these diseases through a pathomechanistic-centered lens. Whole-genome sequencing coupled to interpretation of all variants in the 'Mendeliome', can ensure an unbiased view. Variant interpretation, however, presents a significant challenge in view of the wide array of genetic variants that emerge in this approach. Pathomechanistic understanding and corresponding disease classification bears the potential to aid in variant

**Met opmerkingen [MF29]:** I'm not sure the wording "will become a necessity" is optimal or fully accurate in this context. The same approach can be applied without a pathomechanism-centered approach, albeit perhaps with less effective outcomes. Perhaps "crucial", "pivotal","essential" or equivalent words can be used here.

**Met opmerkingen [MF30]:** The opening sentence of the Concluding Remarks is an important one, and I think could be phrased in a more polished manner.
./1 The word-pairing "rate [...] of understanding" isn't great. "Understanding" does not exactly have a rate; "rate" is more fitting in the context of discoveries/advances/findings.
./2 The overall statement, while generally correct, is an interpretative and somewhat personal and subjective synthesis of the state-of-the-field, so it can be nice to add some qualified such as "Arguably" or "It can be argued"
./3 I'm not sure the 'bed and bench' wording is ideal in the current context. Genetic insight can be seen as both a clinical tool and as basic-research knowledge. Ultimately, genetic diagnosis by itself is not a therapy (it isn't purely in the "bed" category), even if it can *inform* therapy. And somewhat similarly, mechanistic insights, while generally considered as basic-research knowledge, can inform therapy, as nicely discussed in the article. In short - I'm not sure the genetics/mechanisms gap is precisely equivalent to "bed and bench" gap in this case. Please see whether the suggested wording for instance may work.

interpretation and lead to cost-effective therapy development by involving larger patient groups.

## Declaration of interest

J.B. has received *ad hoc* consultancy compensation for activities with Sanofi, CSL Behring, Alnylam, Roche, Amylyx and ARGENX. J.B. submitted a patent on behalf of the University of Antwerp under European Patent Application No.20817176.9 entitled “Inhibitors of KDM5A for use in treatment of idiopathic inflammatory myopathies”.

V.T. submitted a patent on behalf of the University of Antwerp under patent application P184820EP00 entitled "A method for obtaining a neuromuscular organoid and use thereof"

The other authors declare no conflict of interest.

Met opmerkingen [MF31]: This should be either "submitted a patent" or "submitted patents"

**Met opmerkingen [VT32]:** Misschien beter gewoon kort samenvatten als:

J.B. and V.T. submitted patents on behalf of the University of Antwerp.

Ik denk dat dit de opmerking was van Moran Furman ...? Nu gaat het enkel over recente patenten.

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## Resources

[I] NCT05397665, this study is registered with ClinicalTrials.gov

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## Figure legends

Figure 1: **Anatomical- and cell biological-~~oriented~~ based representation per disease category in inherited neurological disorders.** In patients suffering from spinocerebellar ataxia (SCA), hereditary spastic paraplegia (HSP) or inherited peripheral neuropathy (IPN), the tissues being affected (left) include: the cerebellum (red), the pyramidal tract (purple) and the peripheral nervous system (yellow). As discussed in the main text, six main pathomechanistic themes (middle) represent the intrinsic common ground of these three categories of disorders, with each affecting specific compartments of neurons (right). These disease ~~patho~~mechanistic themes are partly shared across disorders, notwithstanding the disorders' marked differences in clinical presentation.

Figure 2: **Pathomechanistic themes underlying inherited peripheral neuropathies, hereditary spastic paraplegia and spinocerebellar ataxia.** Top panels: left panel, the central dogma in molecular biology with transcription, translation, and protein synthesis, as well as the DNA damage response; right panel, metabolism in the mitochondria in the form of the Krebs cycle. Second upper panel: the endoplasmic reticulum (ER) and the ~~golgi~~Golgi apparatus, with formation and morphology of the ER as main affected ~~pathomechanistic~~ disease mechanistic theme in some rare neurological ~~diseases~~disorders. Third row panels: left panel, transmembrane channels and gap junctions; right panel, myelin sheath surrounding neuronal cells and lipid metabolism. Lower panel: structural proteins including components of microtubules, the spectrin-actin cytoskeleton and axonal transport proteins.

Figure 3: **Pathomechanisms bridge genotype-phenotype associations.**
Top panel: Research focusing on associations between genotype and phenotype resulted in the recognition of locus heterogeneity (dotted circle 1) in which multiple loci can result in a similar phenotype and locus pleiotropy (dotted circle 2) in which a single locus can result in seemingly different phenotypes. Bottom panel: A focus on shared pathomechanisms can help clarify intrinsic connections between seemingly different disorders. This approach unveils how multiple genes can converge upon a single biological pathway, and conversely, how a singular crucial pathway can be implicated in the development of two or more distinct phenotypes. The latter phenomenon could be coined as "pathomechanism pleiotropy" related to "locus pleiotropy" (dotted circle 3). Enhanced focus on pathomechanisms and uniting cohorts of

**Met opmerkingen [JB33]:** misschien beter?

**Met opmerkingen [MF34]:** I think the heading should be somewhat more specific. From the previous heading, one could think that the figure relates to neurological disease as a whole (or even to diseases in general, not specifically neural disease). I suggested one possible adjustment, and feel free to modify differently if you wish.

**Met opmerkingen [MF35]:** The figure legend did not mention the right-hand side of the figure. Please see whether the suggested wording may work.

**Met opmerkingen [JB36]:** hier is het raar, elders heeft Vincent misschien wel hier en daar een punt - for the sake of variety...

**Met opmerkingen [VT37]:** Ook in de figuur moet golgi nog in hoofdletter staan: Golgi, zie je mail

**Met opmerkingen [VT38]:** Zie mijn comments in de file over 'highlights', maar in de conclusies staat 'pleiotropy' nergens vermeld.

patients with different neurogenetic disorders could increase effectiveness of diagnosis, scientific discovery and therapy design.