Nodes, paranodes and neuropathies

Abstract
Purpose of review
This review summarises recent evidence supporting the involvement of the specialized nodal and peri-nodal domains of myelinated axons in the pathology of acquired, inflammatory, peripheral neuropathies.

Recent findings
The identification of new target antigens in the inflammatory neuropathies heralds a revolution in diagnosis, and has already begun to inform increasingly targeted and individualised therapies. Rapid progress in our basic understanding of the highly specialised nodal regions of peripheral nerves serves to strengthen the links between their unique microstructural identities, functions and pathologies. In this context, the detection of auto-antibodies directed against nodal and peri-nodal targets is likely to be of increasing clinical importance. Anti-ganglioside antibodies have long been used in clinical practice as diagnostic serum biomarkers, and associate with specific clinical variants but not to the common forms of either acute or chronic demyelinating autoimmune neuropathy. It is now apparent that antibodies directed against several region-specific cell adhesion molecules (CAMs), including neurofascin (NF), contactin (CNTN) and contactin-associated protein (Caspr), can be linked to phenotypically distinct peripheral neuropathies. Importantly, the immunological characteristics of these antibodies facilitate the prediction of treatment responsiveness.

Summary
The search for the causes and mechanisms of inflammatory neuropathies has recently taken an exciting, new direction. The nodes of Ranvier, as well as the flanking paranodal and juxtaparanodal regions, have emerged as critical targets of the immuno-pathological processes underlying a sub-set of these conditions. These auto-antibodies are diagnostically useful, especially when they predict the response to specific treatments. Along with recently developed humanised neuropathy models, this provides both the impetus and means to identify further serological markers of these immune-driven, potentially reversible conditions. The increasing ability to identify distinct pathological subtypes of these disorders is likely to foster increasingly targeted and personalized therapeutic approaches.

Keywords
Node, paranode, juxtaparanode, inflammatory neuropathy

Introduction

Acquired peripheral neuropathies have a wide range of causes, including some that can result in devastating neurological deficits, including paralysis, sensory loss, autonomic disturbances, and respiratory failure. Guillain-Barré syndrome (GBS) and chronic inflammatory demyelinating polyneuropathy (CIDP) account for the majority of immune-mediated neuropathies. Within these broad groups, diverse subtypes are increasingly recognised. Because some of these have atypical features and respond poorly to standard therapies, their detection is of clear clinical importance.

Highly specialized regions of the peripheral nerve, in particular the node and paranode, are currently in the spotlight, as their dysfunction following antibody-mediated attack is thought to be critical to the pathogenesis of a significant proportion of these potentially treatable neuropathies. This review summarises the functional anatomy of the nodal region, and describes the emerging concept of peripheral "nodo-paranodopathies".

Organization and function of myelinated axon domains

During development of vertebrate peripheral nerves, Schwann cells myelinate axons that are larger than ~1 micron in diameter. Each myelin internode is flanked by nodes of Ranvier – the sites where axolemma is most exposed to the extracellular fluid, and where outward and inward currents of salutatory conduction take place. Complex axo-glial interactions shape the node, as well as the flanking paranodal and juxtaparanodal regions (fig

1). The axonal and glial components of each domain contain distinct molecular components. Our understanding of these molecules, their contribution to the molecular organization of myelinated axons, and the deleterious effects of their dysfunction, is rapidly expanding[1][2].

A

Node of Ranvier

Myelin

Axon

Paranode | Node | Paranode | Juxtaparanode | Internode

B

Node | Paranode | Juxtaparanode | Internode (Compact Myelin)

Schwann cell microvilli

Myelin

Periaxonal space

Axon

KEY

gliomedin | NF186/155 | CNTN1/2 | Caspr/2 | Gangliosides | 4.1B | MAG | PSD93/95

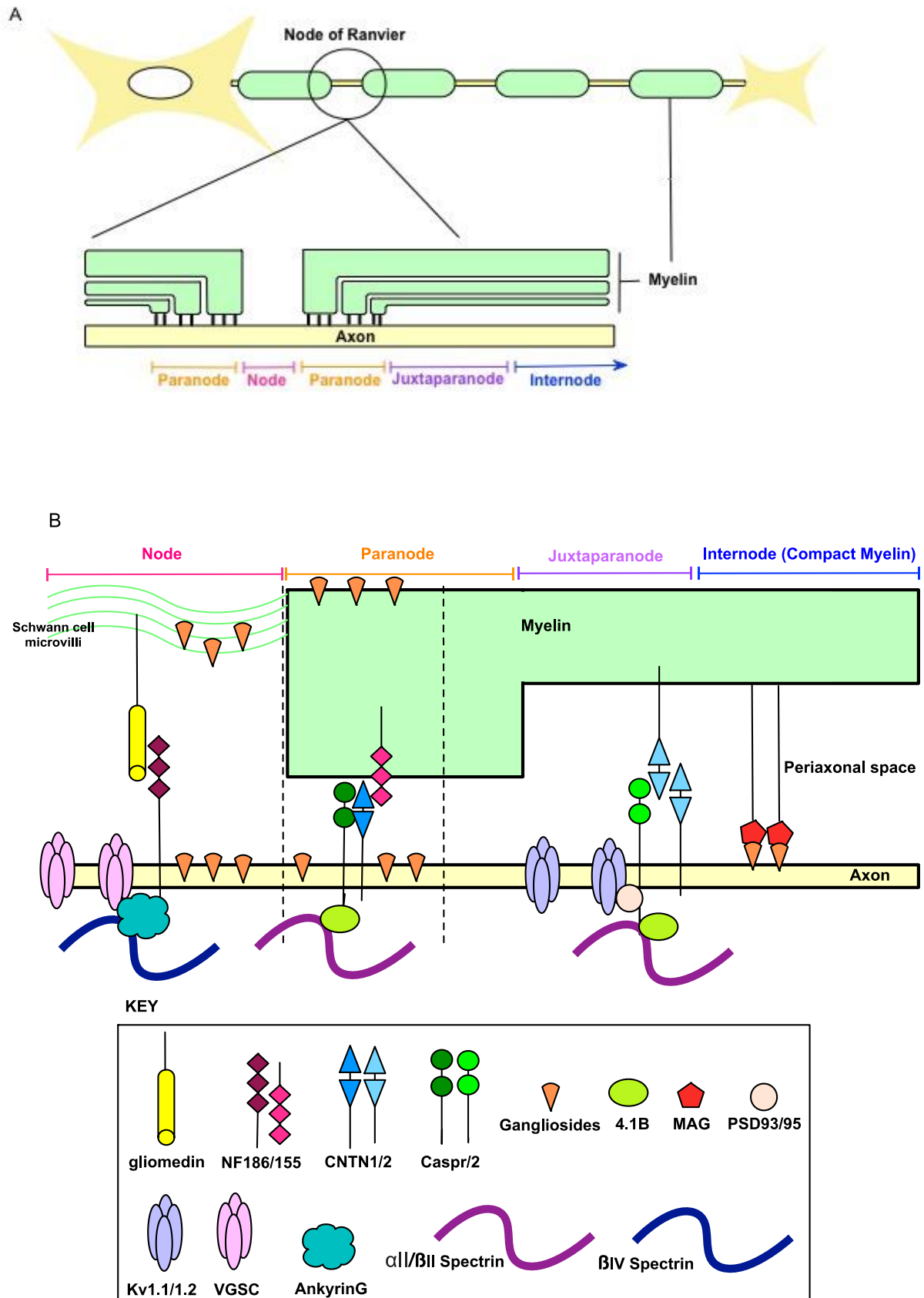Kv1.1/1.2 | VGSC | AnkyrinG | αII/βII Spectrin | βIV Spectrin

Figure 1.
(A) Overview of specialised nodal regions in a myelinated nerve fibre
(B) Molecular components of PNS nodes, paranodes and juxtaparanodes:
NF – neurofascin, Caspr – contactin associated protein, CNTN – contactin, VGSCs – voltage-gated sodium channels, VGKCs – voltage-gated potassium (K+) channels (Specifically Kv 1.1 and 1.2 at the juxtaparanode), MAG – myelin associated glycoprotein (found in non-compact myelin interacting with axonal molecules).
The axonal spectrin cytoskeleton protein mediate binding of Ankyrin G to NF186 and VGSCs at the node, 4.1B to the Caspr/CNTN complex at the paranode, and PSD93/95 with 4.1B to potassium channels at the juxtaparanode (Kv1.1/1.2).

Node (VGSC, Gliomedin, NF186, and NrCAM)

Peripheral (PNS) and central nervous system (CNS) nodes are formed by interactions between axons and glial cells, Schwann cells and oligodendrocytes/astrocytes, respectively. Nodal enrichment of voltage-gated Na^+ channels (VGSC) serves to generate inward current of the action potential, and the internodal compact myelin sheath reduces the internodal capacitance, enabling saltatory conduction. Nav1.2 predominates in developing nodes, later switching to Nav1.6 during myelination.

In the PNS, the nodal localization of VGSCs is governed by two types of axo-glial interactions. The first depends on a complicated set of molecular interactions that are depicted in Figure 1. Gliomedin - a CAM secreted by Schwann cell microvilli into the extracellular matrix - binds to neurofascin-186 (NF186) in the axolemma. NF186, in turn, recruits the nodal isoform of ankyrinG, which is the key adaptor protein that mediates the binding of NF186, NrCAM, and VGSCs to the nodal cytoskeletal protein βIV-spectrin.[2][3][4] The fact that NF186 null mice demonstrate disrupted axonal conduction highlights the role of NF186 in supporting the node.[5]

The second type of axoglial interaction results from the apposition of two, adjacent paranodes during development.[6] As internodes elongate during development, the paranodes act as fence, preventing the lateral diffusion of NF186, NrCAM, and VGSCs from under the myelin sheath, so that as adjacent internodes meet, the two opposing hemi-nodes form one node.

Paranode (NF155, Caspr, and CNTN)

The structural integrity, and thus function, of paranodes rely on septate-like junctions, which are unique structures comprised of neurofascin isoform 155 (NF155) on myelin loops, and heterodimers of contactin-1 (CNTN1) and contactin-associated protein (Caspr) on the axolemma (fig 1). Septate-like junctions are absent in mice that lack CNTN1, Caspr, or NF155, such that juxtaparanodal VGKCs are not excluded from the paranodal regions, allowing these K^+ channels to interfere with saltatory conduction. Nodes form normally in these mutant mice, but become altered as mice age; these changes, as well as axonal pathologies, result in severe neurological deficits.[7][8][9] Paranodal disruption, largely through juxtaparanodal VGKC invasion, has also been observed in CST-/- null mice, highlighting the essential role of sulfatide in stabilising the paranodal junctions.[10] Further, in the mice lacking gangliosides (GalNAcT-/-), Susuki *et al* demonstrated axolemmal detachment of paranodal myelin loops, resulting in a reduction in Caspr and NF155 staining in both PNS and CNS paranodes and, again, mislocalisation of juxtaparanodal potassium channels to the paranode.[11]

Juxtaparanode (VGKC, Caspr2, CNTN2)

At the juxtaparanode, a complex of Caspr2 (on the axon), contactin-2 (CNTN2, also known as transient axonal glycoprotein 1; TAG1, expressed on both the axonal and glial membranes[12]), PSD93/95, and 4.1B link the VGKCs to the axonal spectrin cytoskeleton.[13] These VGKCs are mostly comprised of Kv1.1 and Kv1.2 subtypes, and are involved in the repolarisation phase of action potentials.

<u>Overview of 'Nodo-Paranodopathies'</u>

Inflammatory neuropathies are phenotypically heterogeneous. With an evolving understanding of the underlying pathophysiology, newly defined subtypes, overlapping and evolving in their clinical, serological and electrophysiological features, are emerging.

Antibodies have been implicated in the pathogenesis of the inflammatory neuropathies for decades. Originally, as one might expect, traditional myelin antigens, such as myelin-associated glycoprotein (MAG) and myelin protein zero (P0), were considered to be the likely targets of auto-antibodies in demyelinating disorders.[14][15] Despite many years of study, however, there has been limited success in showing that such antibodies were widely present in, or pathogenically relevant to, the common types of inflammatory-demyelinating neuropathy.[16][17] Attention thus turned to the nodes of Ranvier and surrounding regions. Initial hints to the likely pathological importance of the node came from histological studies of GBS autopsy material[18], later supported by electrophysiology and experimental evidence of nodal targeting by neuropathy-associated antibodies. In the past 5 years, it has become apparent that physiological and/or structural damage to these regions may, at least in part, be responsible for the varied but defined clinical phenotypes in what were previously considered demyelinating or axonal disorders.

Anti-ganglioside antibodies were the first serum antibodies to be linked with GBS.[19] There is a particularly strong association with Miller Fisher syndrome (MFS) subtype, in which antibodies against the GQ1b ganglioside are detected in 95-99% of cases.[20] Although testing for these antibodies is utilised in clinical practice, the diagnosis of GBS continues to principally depend on the recognition of its clinical and electrophysiological features. More recently, tissue-based assays have been used to identify autoantibodies targeting the node and paranode in up to 30% of patients with immune-mediated neuropathies, including CIDP and the acute, inflammatory demyelinating polyneuropathy (AIDP) form of GBS.[21] Subsequent studies, largely based on transfected cell-based assays, have implicated NF155, NF186, gliomedin, CNTN1 and Caspr as the specific targets for a proportion of these antibodies. Others are undoubtedly still to be identified.

These developments are prompting a revised classification scheme, which recognises not only new electrophysiological categories but also incorporates these serological biomarkers. It is hoped that these revisions will more accurately reflect shared mechanisms of injury, disease course, and potential treatment strategies. To this end, the term "nodo-paranodopathy", first coined by Uncini and Susuki[22], has been adopted to represent neuropathies of any aetiology which are recognized to involve dysfunction of the node or paranode (Table 1). Pathological involvement of this region was at first largely inferred from electrophysiological studies. Although serological and pathological studies have strengthened this paradigm, the utility and validity of the terminology remain to be fully established. In particular, the potentially bi-directional relationship between nodal/parnodal disruption and demyelination, and the accuracy of a term that implies exclusively nodal pathological localisation in the presence of antibodies directed against more ubiquitously expressed antigens (notably gangliosides), endure as unresolved issues.

Localisation	Antigen	Associated neuropathy	Notable Phenotypic Features
NODE	**Gangliosides**	AMAN (**GM1**a/b, GD1a, GalNac-GD1a) AMSAN (GM1, GM1b, GD1a) MMN (**IgM GM1**) CANOMAD/sensory-ataxic (GD1b and/or other disialosyl gangliosides)	Anti-GM1 Abs are linked with - RCF - AMAN (IgG) and MMN (IgM)
	Gliomedin	EAN (AIDP), MMN	NF186 and gliomedin Abs are associated with an animal model of AIDP, and are found in MMN patients
	NF186	**AMAN**, AIDP, CIDP, CCPD	
PARANODE	**Gangliosides**	MFS (**GQ1b**, GT1a) PCB (**GT1a**, GQ1b)	Anti-GQ1b Abs suggest good treatment response/prognosis
	NF155	CCPD, AIDP, **CIDP**	- younger onset CIDP - particularly raised CSF protein levels - distal, motor predominant - ataxia and tremor - good response to Rituximab but not IVIg
	CNTN1	AIDP, **CIDP**	- older age - aggressive disease onset - motor predominant - early axonal loss - poor IVIg responsiveness
	Caspr	CIDP	Caspr Abs share similar features to NF155/CNTN1 Ab cohort, and associated with specifically with neuropathic pain
JUXTAPARANODE	**CNTN2/TAG1**	None specific	SNPs in TAG-1 gene may favour IVIg responsiveness in CIDP
	Caspr2	**None specific, but clear association with acquired neuromyotonia**	**Case studies link Caspr2 Abs to:** **Neuropathic pain** **Favourable response to IVIg in GBS**

Table 1: Conditions encompassed by "nodo-paranodopathies", their associated clinical features and antigen targets (strongest associations emboldened).
Variants of GBS: PCB - pharyngeal cervical brachial; AIDP - acute inflammatory demyelinating polyneuropathy; MFS, AMAN – acute motor axonal neuropathy; MMN – multifocal motor neuropathy; CANOMAD – chronic ataxic neuropathy, ophthalmoplegia, monoclonal IgM paraprotein, cold agglutinins disialosyl antibodies; RCF – reversible conduction failure; EAN – experimental autoimmune neuritis; CCPD – combined central and peripheral demyelination; SNP – single nucleotide polymorphism; gangliosides: GQ1b, GT1a, GM1a/b, GD1a; ganglioside complex: GalNac-GD1a.

Electrophysiological features

Traditionally, GBS has been classified as "demyelinating" or "axonal" mostly on the basis of electrophysiological studies (fig 2).[23] Conduction block (CB) or conduction velocity slowing were taken to indicate demyelination, with axonal degeneration characterised by reduced compound muscle action potential (CMAP) amplitudes, and associated with poor prognosis.

This view has been revised in light of new electrophysiological and experimental data that demonstrate "reversible conduction failure" (RCF) of motor nerves, which can rapidly recover, in an increasing number of cases of "axonal" GBS.[24] RCF (sometimes termed "axonal CB") is characterised by reversible conduction slowing or block, mimicking demyelination, but without temporal dispersion. It can result from disorders that disrupt the nodal axolemma, i.e. "nodo-paranodopathies", and has been suggested to be the neurophysiological correlate of antibody-mediated attack at the node or paranode.[24][25][26] Thus, initial neurophysiology showing CB could represent either nodo-paranodopathy with RCF or demyelination. Currently accepted electro-diagnostic criteria, which consider CB as a purely demyelinating phenomenon, likely underestimate the proportion of axonal GBS variants, particularly when neurophysiological studies are preformed early in the disease course [25][27]. It has therefore been proposed that individual patients should undergo serial studies to reduce misclassification.

As an alternative to serial neurophysiological studies, criteria have been proposed to optimize the reliability of a single study, thereby increasing the diagnostic sensitivity of demyelination as well as differentiating axonal subtypes within the first week.[25] Specifically, retrospective analysis of nerve conduction studies (NCS) in a large cohort of GBS patients, comparing the use of existing and modified criteria, revealed a significant shift in classification from AIDP (72% to 56%) and equivocal (10% to 8%) to axonal GBS (18% to 35%), which was further sub-divided into AMAN and AMSAN. These numbers closely resemble the diagnostic shift seen in patients who undergo serial NCS[27], although they have yet to be validated with cohorts using serial studies.

Based on these updated findings, a new study found that by retrospectively applying modified "early RCF criteria" and "de/remyelination criteria" in GBS patients who underwent serial NCS, it was possible to define two distinct groups of patients.[28] However, limitations, including inadequate specificity of electrophysiological cut-off values to categorise all patients, and the lack of clinical and prognostic correlation, should motivate subsequent studies to validate and strengthen these conclusions.

Patterns of electrophysiological abnormalities associated with antibody-mediated attack at the node and paranode have been described[24], and include either or a combination of axonal loss with universally reduced CMAP amplitudes (axonal degeneration), and acute motor CB with or without RCF. Of course, CB could be a manifestation of demyelination, and indeed the distinction between types of CB may be aided by the presence or absence of certain antibodies.[28][29] In GBS, far fewer electrophysiological features of demyelination, in conjunction with rapid resolution of reduced CMAPs and conduction slowing, were observed in IgG anti-GM1 antibody positive, compared to seronegative, patients.[29] Here, the CB was considered secondary to antibody-mediated loss of VGSCs, reducing conduction at the node, and would account for the rapid recovery observed in some AMAN patients. More generally, mechanisms leading to "axonal CB" are likely to be broad, and include i) myelin detachment at the paranode ii) nodal lengthening (which might both be considered limited forms of demyelination) iii) disruption of nodal VGSCs and generation of the action potential, and iv) disorganised polarisation leading to an "inexcitable" axolemma (fig 2.D). [30]

Ultimately, distinguishing transitory CB from axons irreversibly committed to degeneration has clear implications for the reversibility of peripheral nerve injury. The factors influencing whether axolemmal block will spontaneously resolve, transition to axonal degeneration, or indeed whether it can progress to segmental demyelination, are unknown and the subject of ongoing research.[30] In the context of spinal cord contusion, recent investigations support the concept of transiently damaged myelinated axons existing in a "metastable state", in which they maintain the capacity for spontaneous recovery, but beyond which permanent damage would occur.[31] It has been demonstrated in vivo that this sub-lethal state is characterised by intra-axonal accumulation of calcium, and that the application of a timely intervention, in this case sealing of calcium-permeable plasma-membrane pores, can favourably alter axonal fate. With an expanding understanding of factors determining progression of CB to axonal degeneration in peripheral neurons, it should follow that similar principles could be applied.

Pathological features

As eluded to previously, the molecular architecture of the nodal regions reflects their function to initiate, maintain and control conduction, and depends on specialized axo-glial interactions. Pathological studies of molecular organisation of peripheral nerves could provide insights into how the nodal region is affected in patients with GBS and CIDP, but are infrequently performed due to the invasive nature of nerve biopsy. Skin biopsies, however, can give a morphological assessment of myelinated nerve fibres, and has provided evidence of significant nodal disruption, particularly in patients with CIDP.[32] Specifically, in both skin and nerve[33] biopsies, elongation of the nodes, and localization of Caspr seem to be reliable diagnostic markers to differentiate between segmental demyelination and nodal/axonal pathology. Further, using VGSCs and Caspr as markers of the node and paranode, respectively, Cifuentes-Diaz *et al* were able to demonstrate altered structure at the node and paranode in peroneal nerve biopsies of 12 CIDP patients[33], though this was not recapitulated in the dermal nerve fibres.

In axonal neuropathies, such as AMAN, several factors observed in human and rabbit models also point toward pathological involvement of the node.[34],[35] In the rabbit model of AMAN, Susuki *et al* described a specific mechanism of nodal disruption in peripheral motor nerves, whereby anti-GM1 antibodies incur complement-mediated disruption of sodium channel clusters in association with MAC pore formation in the acute phase. Contributing to this, the axonal cytoskeleton and Schwann cell microvilli, and crucially their axo-glial interactions, are thought to be disrupted, further destabilising sodium channel clusters.[34] In addition, nodal lengthening and paranodal myelin detachment were noted, again affecting depolarization and manifesting electrophysiologically as CB.[30] These findings have important therapeutic implications, as this process has been shown to be reversed by the addition of complement inhibitors.[26]
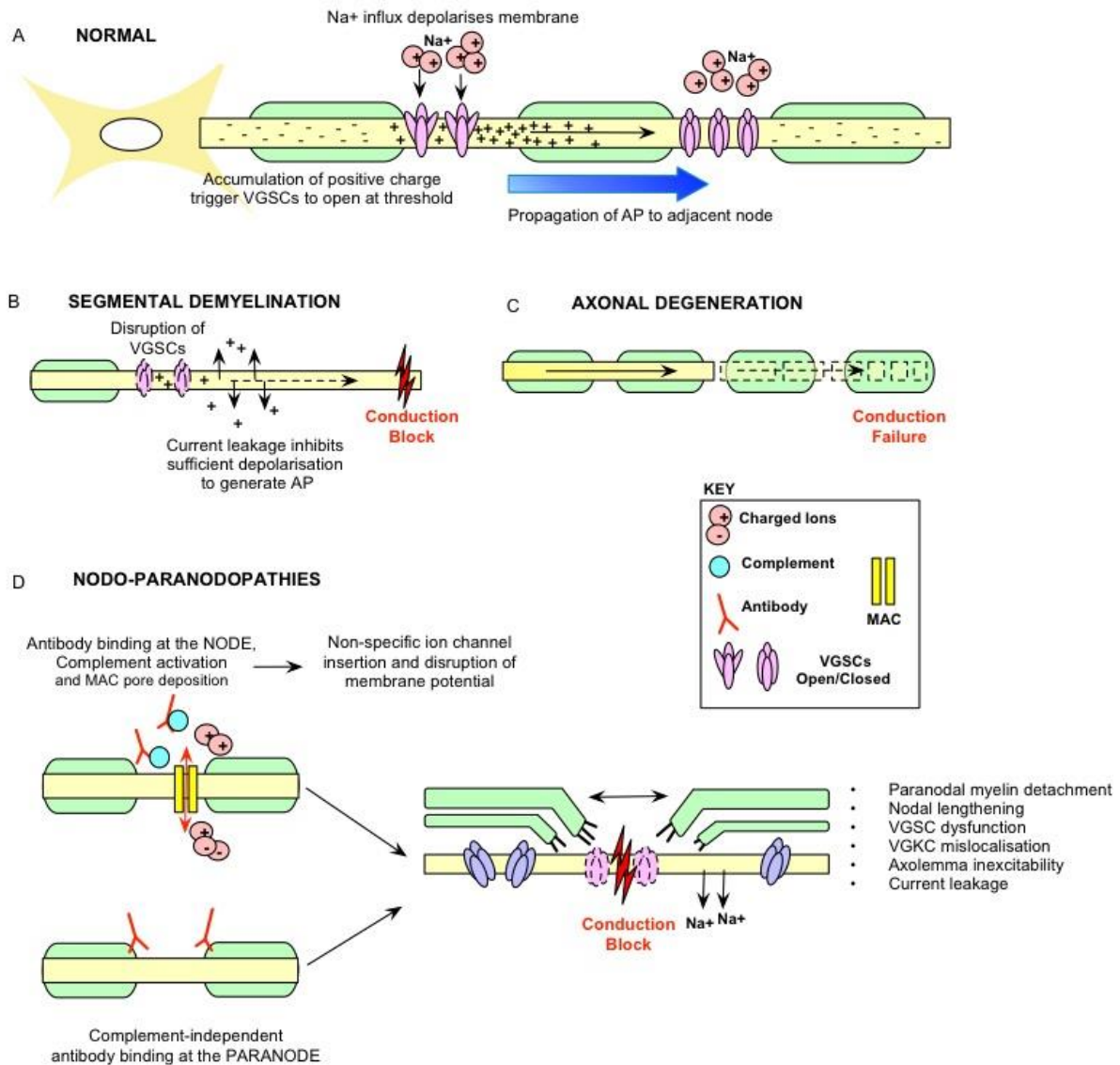
Figure 2. Pathological representation of saltatory conduction in a myelinated axon in normal and pathological states

(A) Accumulating positive charge causes sodium channels to open at a threshold, allowing an influx of sodium to depolarise the membrane and propagate the action potential to the adjacent node, repeating the cycle

(B) Demyelination leads to disruption of VGSCs and current leakage, impairing depolarisation at the nodal membrane, resulting in conduction slowing and block

(C) Axonal degeneration causing slowly reversible or irreversible conduction failure

(D) Antibody mediated attack at the node, activating the immunological cascade, and paranode, occurring independently of complement, both result in detachment of terminal myelin loops, and disruption of the ion channels and membrane potential. This impairs propagation of the AP.

MAC - Membrane attack complex

NORMAL

D   P

NORMAL CONDUCTION

Rapidly Reversible

NODO-PARANODOPATHY

D

P

RCF with CB

Nodo-Paranodopathy

Acute motor **CB** without excessive TD
(Could represent **RCF** or progression to axonal
degeneration and possibly demyelination)

AXONAL DEGENERATION
Irreversible / Slowly reversible

D   P

AXONAL LOSS (CONDUCTION FAILURE)

DEMYELINATION
Reversible

D

P (CB)

P (CB+TD)

Demyelintion with CB +/- TD

Axonal Degeneration

• **Reduced CMAP amplitude**
• **CV and DMLs normal or near normal**
  (remaining normally myelinated axons conduct
  normally but may be some disproportionate loss of fastest
  conducting fibres)

Demyelination

• **Slowed CV**
• **Conduction Block** and/or **TD**
• **Prolonged DMLs**

Figure 3. Suspected evolution of conduction deficits in Nodo-paranodopathies (Electrophysiological characteristics and appearances in accompanying boxes)
CB is defined as a reduction in CMAP amplitude on proximal (P) versus distal (D) nerve stimulation. This has been shown to rapidly revert to normal conduction ('RCF') in some patients, manifesting as rapid clinical improvement and return of the proximal CMAP amplitude to normal. Alternatively, this state could progress to axonal degeneration, revealed by an additional reduction in distal CMAP amplitudes. Whether paranodal demyelination or other nodal injury can act as a precursor to classical/segmental demyelination remains to be determined. When such demyelination does occur, reversion to normal conduction can be achieved through remyelination, although over a longer time-frame than seen with RCF, and typically in the presence of temporal dispersion (representing differing degrees of myelination and thus conduction velocities between individual nerve fibres). Alternatively, chronically demyelinated axons may subsequently degenerate.

CB – Conduction Block, **CV -** Conduction Velocity, **DMLs -** Distal Motor Latencies, **CMAP -** Compound Muscle Action Potential, **TD -** Temporal Dispersion

Neuropathies and the node

The node of Ranvier and paranode have emerged as targets for immune mediated pathology in GBS, CIDP, and MMN.[36] Gangliosides, glycosphingolipids containing sialic acid, are concentrated at the node and paranode and exposed axonal membranes at nerve terminals, and antibodies directed against these molecules are commonly associated with "axonal" forms of GBS.[37] In contrast, nodal adhesion molecules, in particular NF155, NF186, gliomedin, and contactin, are targeted in some neuropathies traditionally considered "demyelinating", such as CIDP, where their dysfunction leads to VGSC alteration and conduction defects.[21]

Anti-ganglioside antibodies

Since their original detection in chronic paraproteinaemic neuropathy in the 1980s[38], antibodies against gangliosides[39][19], have been identified in a diverse variety of inflammatory neuropathies. Of note, some of these have been historically considered axonal, and others demyelinating. There is now evidence to suggest that this apparent dichotomy is resolved by reconsidering these conditions as nodopathies.

Gangliosides are widely distributed throughout cell membranes in both the peripheral and central nervous systems. The relative abundance of the different types varies across motor and sensory nerves[40], and between individual cranial nerves.[41] Anti-ganglioside antibodies are reported to target the node (GM1[42], GD1a[26], GD1b/disialosyl [52}) or paranode (GQ1b[43]). However, these binding patterns do not always correlate with the distribution of the antigen revealed by other methodological means [44], and may be further influenced by variations in the fine specificity of different anti-ganglioside antibodies.

IgG antibodies to GM1, a widely expressed ganglioside at the nodal axolemma and on Schwann cell microvilli, are most frequently associated with AMAN[42] and MMN[45], serving as a pathophysiological link between these acute and chronic immune neuropathies. The mechanism by which anti-GM1 antibodies produce purely motor phenotypes is not well understood, however, as GM1 itself is known to be present in similar abundance in both motor and sensory nerves.[40] The fact that the ability of some anti-GM1 antibodies to access their target antigen is influenced by other *cis* interacting gangliosides within cell membranes[46] may be relevant. In a recent study, however, Harschnitz *et al* confirmed that hiPSC-derived motor and sensory neurons both express GM1, and demonstrated targeting of each cell type by IgM GM1 antibodies. Although such antibodies activate the classical complement pathway in both settings, motor nerves prove far more susceptible to subsequent axonal injury, for as yet unclear reasons.[47] Another line of evidence demonstrates that anti-ganglioside antibodies are rapidly internalised at certain sites, and that this internalisation attenuates complement mediated injury.[48][49] Region variations in this protective mechanism, or its localised failure, might also provide an explanation for the focality of antibody mediated injury in the context of a more widely expressed target antigen.

Acute and chronic ataxic neuropathies have been grouped by their association with antibodies directed against disialosyl gangliosides (which contain 2 sialic acid residues). In these conditions, a clear clinical-serological-pathological phenotype has emerged, characterised by sensory ataxia with relatively preserved motor function, anti-disialosyl ganglioside antibodies, and common pathophysiological effects centred on the node.[50]

Akin to the situation with GM1, above, anti-GD1b antibodies are found in both acute and chronic ataxic neuropathies, with IgG and IgM isotype predominance, respectively. In acute ataxic neuropathies, all of which could be considered part of the same clinical spectrum[51], anti-GD1b IgG antibodies have been linked to acute sensory and ataxic neuropathy (ASAN), considered a GBS variant.[52] These antibodies may cross react with GQ1b/GT1a, targets more commonly associated with MFS[20], ataxic GBS[53], and the pharyngeal-cervical-brachial variant of GBS. GD1b reactivity seems to be specific for Romberg-positive sensory ataxia, in contrast to anti-GQ1b antibodies, which more closely correlate with the Romberg-negative, "cerebellar-like" ataxia seen clinically in MFS or ataxic GBS.[51] The latter pathology is considered secondary to involvement of muscle spindle afferent fibres to the spinocerebellar system[50], rather than pathology of the cerebellum itself. Crucially, the presence of GQ1b antibodies correlates with prompt and complete recovery, both clinically and electrophysiologically. In the absence of demyelinating features, this rapid reversibility is felt to be indicative of immune-mediated RCF, localizing the pathology to the node / paranode[42] and/or the nerve terminal. Evidence of ganglioside antibody and complement-mediated nodal alteration in GBS rabbit models[22], corroborated in one ataxic GBS anti-GQ1b antibody positive patient where antibodies to GQ1b and GD1b specifically stained the nodes of sensory fibres[54], support this theory and suggest a final common pathway involving nodal VGSC and axo-glial junction disruption causing RCF. Unchecked, this would be expected to eventually lead to irreversible axonal degeneration.[55]

IgM class anti-disialosyl antibodies associated with chronic ataxic neuropathies such as CANOMAD (chronic ataxic neuropathy, ophthalmoplegia, IgM paraprotein, cold agglutinins and disialosyl antibodies), have also been shown to target the nodal axolemma, most recently in a myelinating culture system using human induced pluripotent stem cell (hiPSC)-derived sensory neurons. Interestingly, such reactivity could subsequently lead to either axonal degeneration or demyelination, determined in this model by the availability or otherwise of a source of complement.[56] This is consistent with previous pathological studies, which have revealed a mixture

of axonal and demyelinating features (reviewed in [50]), and suggests that initial conduction block may rapidly reverse, or be a prelude to either axonal degeneration or "classical" demyelination (fig 3). The pathological outcome is likely to be influenced by the duration of antibody exposure as well as the activity of the complement system.

Nodal protein antibodies

Experimental allergic neuritis (EAN) is presented as a model of demyelinating GBS/AIDP. In a paradigm using peripheral myelin as an immunogen, however, pathological effects were attributable to the disruption of nodal adhesion molecules and paranodal demyelination. Specifically, antibodies against NF186 and gliomedin resulted in dispersion of VGSCs and altered conduction, although, again, regions of segmental demyelination were also observed.[57] IgG antibodies with these specificities were also detected in the sera 62% of MMN patients, in whom they were speculatively linked with motor nerve conduction block. Again, as these CAMs are found at all nodes [58], the mechanism by which they cause the motor specific and localised injury required to produce isolated motor conduction block remain to be determined.

Recently, Devaux *et al* observed IgG antibodies targeting the nodal regions of rodent teased-nerve fibres in 44% of AIDP, 42% AMAN, and 30% CIDP patients, with specificity for the CAMs at the axo-glial interface. Antibodies against NF186 showed a significant correlation with AMAN, whereas antibodies reactive to the glial protein gliomedin were more commonly found in AIDP patients. The fact that specific antigen targets were not detected in over half of these patients indicates there are more to be found. Interestingly, all AMAN sera with antibodies against nodal adhesion molecules (NF186, gliomedin, or contactin), also contained anti-GM1 IgG antibodies, raising the possibility that pathology in some GBS patients may be driven by more than one antibody.

The downstream processes that occur after nodal-antibody binding seem to involve both humoral and cellular components. In a passive transfer model of anti-gliomedin mediated inflammatory neuropathy, early nodal disruption and paranodal demyelination was associated with IgG deposition and complement activation, whereas later demyelination was associated with prominent T-cell and macrophage infiltration, with little to no IgG deposition. Of further note, passive transfer of anti-gliomedin IgG to P2 immunised EAN rats substantially increased the frequency of demyelination without a similar effect on axonal degeneration.[59]

Non-immune nodopathies

Peripheral nodopathies can also be induced by toxic, ischaemic, nutritional, and genetic mechanisms. These are worth mentioning to highlight the importance of the node in non-immune-mediated disease processes. As many as 70% of critically ill patients develop neuropathies, and rat models mimicking sepsis reveal reduced nodal VGSC activity and axonal depolarization, again causing reversible CB.[60] There is currently no recognised treatment for critical illness neuropathy, but a future ability to detect the early stages of this disease process could pave the way for disease modifying therapies aimed at preventing subsequent axonal degeneration. Acute and chronic ischaemic neuropathies, often a result of a systemic vasculitis, have demonstrated reversible CB, presumably arising via an impact upon the energy dependent nodal ion channel pumps. However, this is thought more likely to lead to axonal degeneration than not.[30]

Neuropathies and the paranode

Axo-glial adhesion molecules at the paranode, anchoring the terminal myelin loops to the axon, are the target of antibodies in some neuropathies. Most notably, around 10% of CIDP patients have a distinct phenotype-serotype association with IgG antibodies against CNTN1 or NF155.[61][62][63] Evidence of their specificity and pathogenicity continues to accumulate. [64]

Anti-NF155 antibodies

Neurofascin has been proposed as a candidate nodal antigen in inflammatory demyelinating diseases of both the central[65] and peripheral nervous systems[21]. IgG4 antibodies against NF155 were originally detected in small cohorts of patients with inflammatory neuropathies, sharing some similar features but with varying response to treatment. Ng *et al* detected anti-NF155 IgG1 and/or IgG3 antibodies at low frequency in AIDP (3/65) and CIDP (5/119) patients.[66] This was followed by the detection of a similar proportion of IgG4 anti-NF155 antibodies in a Spanish CIDP cohort.[67] Overall this antibody has been found in between 4-18% of CIDP patients.[67][68][63] It is now apparent that the presence of anti-NF155 antibodies, specifically of the IgG4

subtype, is associated with a clinically homogenous CIDP phenotype, specifically being more aggressive, distal, motor predominant, and associated with ataxia, tremor, and good response to Rituximab but not IVIg.[69][63][67][62][64][70] Further defining the features of anti-NF155 antibody positive CIDP patients, Ogata *et al* noted a younger age at onset, with a significantly higher CSF protein, potentially reflecting prominent spinal root involvement.[68] This typical phenotype may not apply to all patients, however, with previous reports of anti-NF155 positive patients also displaying CNS demyelination and good response to IVIg.[65]

Pathological studies suggest that these antibodies cause destabilization of the transverse bands (also known as septate-like junctions), which link the paranodal myelin loops to the axon, with subsequent conduction slowing, likely secondary to nodal widening and paranodal demyelination.[71] Expanding on these findings, Koike *et al* noted that the macrophage-mediated demyelinating process thought to characterise CIDP was not apparent in nerve biopsies from 10 predominantly anti-NF155 antibody-positive CIDP patients, indicating an alternative pathogenic mechanism.[72] The fact that IgG4 subclass antibodies do not effectively fix complement suggests that anti-NF155 antibodies are likely to act by blocking interactions with the Caspr/CNTN1 complex. The pathological importance of these antibodies is further supported by the observation that IgG4 depletion with Rituximab correlates with clinical recovery in CIDP.[69]

Anti-CNTN1 antibodies

Anti-CNTN1 IgG4 antibodies are detected in a lower proportion of CIDP patients, who again share a relatively uniform clinical phenotype, characterised by older age, aggressive disease onset, motor predominance, early axonal loss and poor IVIg responsiveness.[73] They potentially induce their pathological effects via a comparable mechanism to that proposed for NF155 antibodies. They have been associated with specific paranodal structural alterations in myelinated axons[74], and block axo-glial interactions mediated by the Caspr-CNTN1-NF155 complex, without binding Fc receptors or triggering complement activation.[75] This may be of relevance to the IVIg resistance observed in anti-CNTN1 positive patients. Interestingly, fine alterations or mutations in the CNTN1-specific N-linked carbohydrate residues, which are essential for glycoprotein recognition, may not cause gross structural abnormalities but can influence the selectivity of CNTN1 binding, potentially disturbing the paranodal complex and inducing demyelination.[75] Of note, a homozygous mutation in the CNTN1 gene, thought to significantly reduce its expression at the neuromuscular junction, was detected in 4 neonates from a consanguineous family, with a lethal congenital myopathy syndrome.[76]

Anti-Caspr antibodies

Caspr has only recently been highlighted as a candidate target antigen in a study using serum from a cohort of 57 patients with GBS or CIDP, one from each group showing binding of Caspr at the paranodes of rodent teased fibres.[77] Neuropathic pain was a prominent feature in the anti-Caspr group, which was not described in patients with other anti-paranodal antibodies. The resolution of pain and functional recovery followed Rituximab treatment in the CIDP patient. In addition, the observed pathological features were not those of a small fibre neuropathy, the implication being that neuropathic pain was a direct consequence of paranodal antibody binding. This is not only important because it implicates the paranode as a primary site of pathology in the inflammatory neuropathies, but because i) it has shown binding at the paranode in a GBS patient and ii) it demonstrates the involvement of another culprit antibody subclass, IgG3, in the anti-Caspr GBS patient. Combined with a previous study displaying a IgG3-predominant immunoreactivity in patients with acute, or GBS-like onset CIDP[74], this implies a conversion of IgG3 and complement-mediated pathological mechanisms in the acute, to IgG4 and complement-independent mechanisms in the chronic phase of these disorders. Clearly this has implications for future therapeutic approaches to these specific antibody-mediated conditions. Interestingly, nerve conduction studies in the anti-Caspr positive CIDP patient showed evidence of temporal dispersion, thought to be an unequivocal indicator of demyelination/remyelination, yet nerve biopsy revealed axonal degeneration without demyelination, but with IgG deposition at the paranodes. This further highlights the difficulties in defining disease phenotypes using a single diagnostic modality, and suggests that the pathology in nodo-parnodopathies might not be as well circumscribed as initially envisaged.

One of the currently unanswered questions about these newly appreciated disorders is why antibodies directed against targets present at the paranodes of all myelinated nerves should produce specific patterns of injury. Furthermore, if anti-NF155, anti-CNTN1, and anti-Caspr antibodies all exert their pathological effects by disrupting the same axo-glial complex, why do their associated disorders have distinct clinical features? It may

be that subtle regional and/or fibre type differences exist in the conformation and accessibility of paranodal adhesion molecules, although this has not yet been demonstrated.

Other/genetic paranodopathies

Dismantling of the paranode has also been observed in the context of inherited neuropathies, namely Charcot-Marie-Tooth types (CMT) 1A and 1C, and congenital hypomyelinating neuropathy (CHN), a rare neonatal syndrome characterized by hypotonia and weakness. In 3 patients with CHN, mutations in CNTNAP1, the gene encoding Caspr, were described in association with pathophysiological alterations to the paranode. Here, loss of the transverse bands resulted in paranodal myelin loop detachment and reduced conduction velocities.[78] In mouse models mimicking CMT1A[79] and 1C[80], paranodal structural changes, including loss of transverse bands and peeling back of myelin loops in the former, or myelin infolding in the latter, are shown to affect conduction and likely contribute to both the demyelination and axonal degeneration seen in these conditions.

Finally, although currently reported at low frequency, mutations in the genes encoding nodal/paranodal CAMs are emerging, and beginning to provide useful insights into their functions in humans. Mutation of the gene encoding gliomedin (GLDN) at the node, and Caspr (CNTNAP1) at the paranode, have been linked to a handful of patients with the recessively inherited lethal congenital contractural syndromes 11 and 7 (LCCS), respectively, the most severe type of widespread joint contractures in neonates.[81][82] Interestingly, electron microscopy of the sciatic nerves from affected patients demonstrated significant nodal lengthening, and a reduction of myelinated nerve fibres. In LCCS7 this also corresponded with a marked reduction in motor nerve conduction velocities.

Neuropathies and the Juxtaparanode

Antigenic targets at the juxtaparanode are also now emerging, but their associated peripheral neuropathies are less well characterized. Normal function here depends on the stability of the VGKC-complex, in which VGKCs co-localise with CNTN2 and Caspr2 in myelinated peripheral neurons. VGKC-complex autoimmunity, originally linked to neuromyotonia, has become increasingly recognised in association with a broad spectrum of central more than peripheral nervous system phenotypes, at least some manifesting as a result of neuronal hyperexcitability.[83] Pathogenic IgG antibodies have now been shown to bind associated proteins such as LGI1 and Caspr2, rather than the ion channels themselves.[84][85][86] The mechanisms of injury postulated include reduction of VGKC density, with impairment of repolarization and neuronal hyperexcitability.[87] Mutations in specific potassium channel genes, in particular Kv1.1 (KCNA1), are likewise known result in a syndrome characterised by episodic ataxia and myokymia.[88][89][90]

Anti-Caspr2 antibodies

The strong expression of Caspr2 at the juxtaparanode, and its role in clustering VGKCs in myelinated neurons, make it a credible candidate target antigen in peripheral neuropathy. Caspr2 antibodies have been detected in large cohorts of patients with neurological disease, specifically manifesting with a combination of peripheral nerve hyperexcitability (e.g. cramps and fasciculations), either in isolation or as part of a disorder of acquired neuromyotonia (Isaacs' syndrome), as well as insomnia, limbic encephalitis, seizures, and dysautonomia.[86] Why Caspr2 antibodies selectively target the peripheral nerves in some cases, given the Caspr2 antigen is found in both the CNS and PNS, remains unclear.

More recently, Caspr2-IgG has been linked to neuropathic pain.[86][87] There is limited data to associate Caspr2 antibodies with a distinct neuropathy phenotype, and no *in vivo* pathogenicity studies to date. However, up to 17% of Caspr2 seropositive patients have evidence of a sensorimotor polyneuropathy.[83] One patient was reported to present with an aggressive and treatment-unresponsive axonal GBS, in the presence of lung adenocarcinoma.[91] In contrast, in two Caspr2-IgG positive paediatric cases of GBS[92], and in a small cohort of older male patients mainly presenting with a full complement of hyperexcitable symptoms and Caspr2 antibodies, treatment with IVIg induced complete recovery over 3-6 months.[93]

In one patient with seizures, a painful peripheral neuropathy and neuromyotonia, a homozygous mutation in the gene encoding Caspr2 (CNTNAP2) was detected, but it's pathogenicity was not confirmed in knockout mice, implying there may be crucial involvement of associated, or perhaps unidentified proteins.[86] Subsequent

genetic studies have identified CNTNAP2 mutations in patients with complex epilepsy syndromes. Specifically, these were present in 13 individuals with cortical dysplasia-focal epilepsy syndrome (CDFES)[94], characterised by treatment resistant epilepsy, gross motor deficits, mental retardation, and reduced or absent deep tendon reflexes, and in 3 patients with Pitt-Hopkins-Like syndrome 1[95], manifesting with seizures, severe congenital anomalies and mental retardation.

CNTN2

Although CNTN2/TAG-1 has not been implicated as an antigenic target in the pathogenesis of peripheral neuropathies, it is worth noting that genetic association studies have revealed the presence of specific single nucleotide polymorphism (SNP) in the TAG-1 gene significantly associates with IVIg responsiveness in Japanese CIDP patients.[96],[97] A further study of 24 Chinese, predominantly treatment-responsive, CIDP patients, however, did not find evidence of this [98], although differences in methodology between studies limit our ability to make firm conclusions.

Conclusion

Our pathophysiological understanding of the specialised nodal regions and their associated axo-glial proteins is growing, and is amenable to intriguing hypotheses related to their role in the pathogenesis of immune-mediated attack on the peripheral myelinated axon. Recently, antibodies directed against a number of key adhesion molecules have been implicated, by way of their presence in high titres, in both acute and chronic inflammatory neuropathies. Such developments have revolutionised the distinction between axonal and demyelinating peripheral neuropathies, and are beginning to outline new disease classifications based on seropositivity, enhanced electrophysiological classification, and identification of the presumptive underlying pathological targets and mechanisms. These classification schemes not only offer the promise of improved diagnosis and prognostication, but perhaps more importantly, should herald the use of targeted therapies directed against specifically determined disease processes. The challenge now is to ascertain the optimum methods for antibody detection, and for establishing the critical pathological mechanisms at play in individual patients.

CLINICAL PRACTICE POINTS – Antibody testing

Ganglioside antibodies
- IgG class GQ1b antibodies help identify complex ataxic/brainstem syndromes as inflammatory, providing diagnostic reassurance and support for the use of immunomodulatory therapy
- Testing for other anti-ganglioside antibodies in GBS patients helps differentiate pathological subtypes but does not add to prognostication, and, as yet, does not support the use of non-standard therapies

Paranodal protein antibodies
- Antibodies against the nodal proteins NF155, CNTN1 and Caspr can aid the diagnosis of inflammatory neuropathies with atypical features
- Consider testing for
  - NF155 antibodies in the context of neuropathies with young age of onset, motor predominance, ataxia, tremor or additional CNS involvement
  - CNTN1 antibodies in neuropathies with aggressive onset or motor predominance
  - Caspr antibodies in patients with rapidly progressive, motor predominant neuropathies and/or prominent neuropathic pain
- In patients with any IgG4 subclass paranodal-protein antibody, IVIg is often ineffective and rituximab should be considered

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