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MINI REVIEW

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Mechanistic insights into the pathogenesis of microtubule-targeting agent-induced peripheral neuropathy from pharmacogenetic and functional studies

Katherina C. Chua[1,2] | **Nura El-Haj**[2] | **Josefina Priotti**[2] | **Deanna L. Kroetz**[2,3]

[1]Pharmaceutical Sciences and Pharmacogenomics Graduate Program, University of California San Francisco, San Francisco, California, USA

[2]Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, California, USA

[3]Institute for Human Genetics, University of California San Francisco, San Francisco, California, USA

Correspondence
Deanna L. Kroetz, Department of Bioengineering and Therapeutic Sciences, University of California San Francisco, San Francisco, CA 94143-2911, USA.
Email: deanna.kroetz@ucsf.edu

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Abstract

Chemotherapy-induced peripheral neuropathy (CIPN) is a common dose-limiting toxicity that affects 30%–40% of patients undergoing cancer treatment. Although multiple mechanisms of chemotherapy-induced neurotoxicity have been described in preclinical models, these have not been translated into widely effective strategies for the prevention or treatment of CIPN. Predictive biomarkers to inform therapeutic approaches are also lacking. Recent studies have examined genetic risk factors associated with CIPN susceptibility. This review provides an overview of the clinical and pathologic features of CIPN and summarizes efforts to identify target pathways through genetic and functional studies. Structurally and mechanistically diverse chemotherapeutics are associated with CIPN; however, the current review is focused on microtubule-targeting agents since these are the focus of most pharmacogenetic association and functional studies of CIPN. Genome-wide pharmacogenetic association studies are useful tools to identify not only causative genes and genetic variants but also genetic networks implicated in drug response or toxicity and have been increasingly applied to investigations of CIPN. Induced pluripotent stem cell-derived models of human sensory neurons are especially useful to understand the mechanistic significance of genomic findings. Combined genetic and functional genomic efforts to understand CIPN hold great promise for developing therapeutic approaches for its prevention and treatment.

KEYWORDS
chemotherapy-induced peripheral neuropathy, genome-wide association studies, induced pluripotent stem cells, microtubule-targeting agents, sensory neurons

1 | INTRODUCTION

Scientific advances have led to improved therapeutic responses in cancer care and the development of cellular and targeted therapies. According to the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) programme, age-adjusted death rates for any cancer diagnosis have been steadily declining over the last four decades (199 deaths per 100,000 persons in 1975 to 149 deaths per 100,000 persons in 2018).[1] While cytotoxic antineoplastic treatments have contributed to decreasing mortality rates, these therapies also present

with their own acute and long-term toxicities. Chemotherapy agents target and eliminate rapidly dividing cells such as tumour cells. However, they can also affect dividing and non-dividing cells in healthy tissues and lead to serious adverse toxicities during or post-treatment with significant impacts on patients' quality of life. Thus, therapeutic clinical benefit can be offset by the need for dose adjustment and drug discontinuation in the face of severe toxicities, traditionally graded according to the National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE) scale. Current research efforts are focused on establishing strategies to mitigate and prevent cancer treatment-related toxicities. Pharmacogenomic association studies are one approach to identify candidate genes and pathways that might be targeted for the treatment and prevention of therapy-related toxicities.

2 | CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

The focus of this review is on the application of pharmacogenomic and functional validation approaches to the study of chemotherapy-induced peripheral neuropathy (CIPN), a common dose-limiting toxicity. Specifically, we will focus on microtubule-targeting agents (MTAs) since they have been the most widely studied in pharmacogenetic investigations. Taxanes (e.g., paclitaxel and docetaxel), vinca alkaloids (e.g., vincristine and vinblastine), epothilones (i.e., ixabepilone) and eribulin all target microtubules for their chemotherapeutic effects and are associated with varying incidence of peripheral neuropathy (Table 1).[3,9,10] In general, the newer MTAs, ixabepilone and eribulin, have lower incidence of peripheral neuropathy than the vinca alkaloids and taxanes (Table 1). One exception is the taxane docetaxel which is the least neurotoxic MTA.[4] Within a given drug class, incidence rates also vary widely. For example, docetaxel and the liposomal formulation of paclitaxel (nab-paclitaxel) are less neurotoxic than paclitaxel.[3,4,6,7] The onset and severity of symptoms are drug-specific but, in general, dependent on the frequency of dose, route of administration, and cumulative dose. Taxanes have shown acute neuropathic pain as early as the first cycle, which is not normally observed with other MTAs.[11]

2.1 | Clinical characteristics

It is important to recognize the clinical characteristics of CIPN to understand its functionally limiting effects and need for the development of toxicity mitigating approaches. CIPN encompasses both sensory and motor peripheral neuropathy. Sensory neuropathy is a predominant presentation that manifests in the hands and feet in a 'glove and stocking' distribution, as chemotherapy-induced nerve damage first occurs in the longest axons in distal nerves. Motor neuropathy is less common and usually occurs after sensory neuropathy. Patient experience of CIPN is variable and challenges our ability to characterize the severity of this toxicity. Typically described sensory neuropathic symptoms include numbness and/or tingling, alterations in tactile sensations, thermal hypersensitivity, and/or burning/painful sensations.[9,12] Patients with CIPN may exhibit hyporeflexia, reduced sensory perception to external touch and vibration, and reduced proprioception.[13] Furthermore, CIPN symptoms can present acutely, persist for years or even progress post-therapy.[3,14] In severe cases, sensorimotor deficits may be irreversible.[15] Severe CIPN symptoms can interfere with activities of daily living such as writing, dressing and walking, significantly limiting quality of life.[16] In addition, CIPN has significant economic implications; survivors with CIPN incur higher healthcare costs and higher rates of loss of employment secondary to debilitating symptoms.[9] The clinical picture is further complicated by individual variability linked to risk factors such as age, renal function, exposure history (including prior treatment with neurotoxic modalities and high dose

TABLE 1 Reported incidence rates and threshold cumulative doses for microtubule-targeting agent-induced peripheral neuropathy

Drug class	Chemotherapy agent	Onset cumulative dose	Incidence (%)
Vinca alkaloids	Vincristine	>4–20 mg^2	20–60[3,4]
	Vinorelbine	—	33[5]
Taxanes	Paclitaxel	≥800–1980 mg/m^2 [3]	59–93[3]
	Nab-paclitaxel	>260 mg/m^2 [6]	10–51[6,7]
	Docetaxel	>400–600 mg/m^2 [2]	6–10[4]
Epothilone	Ixabepilone	40 mg/m^2 [8]	15–24[8]
Halichondrin	Eribulin mesylate	≥10 mg/m^2 [5]	27–35[5]

Note: '—' means not known.

regimens), pre-existing neuropathy (either diabetic or inherited neuropathy), as well as comorbidities that predispose to neuropathies such as alcohol intake and diabetes.[3,14] Nevertheless, these risk factors do not completely explain the interindividual variation in CIPN.[17]

2.2 | Current treatment modalities of CIPN

Various individualized therapeutic modalities to address CIPN have been explored, largely based on our understanding of this neurotoxicity from preclinical models. The 2014 American Society of Clinical Oncology (ASCO) clinical practice guideline, updated in 2020, highlights potential preventative and therapeutic strategies for CIPN.[17,18] Investigated therapeutic modalities include chemo-protectants (amifostine, recombinant human leukaemia inhibitory factor and nimodipine), anticonvulsants (carbamazepine, oxcarbazepine, lamotrigine, gabapentin and pregabalin), antidepressants (nortriptyline, amitriptyline, venlafaxine and duloxetine), and various dietary supplements (calcium and magnesium, vitamin E, glutathione, acetylcysteine, glutamate/glutamine, omega-3 fatty acids, goshajinkigan, retinoic acid and diethyldithiocarbamate). The expert opinion of clinical and translational scientists compiled in the ASCO guidelines provided no strong recommendation for a therapeutic to prevent this neurotoxicity. However, a moderate recommendation was proposed for the use of duloxetine for the treatment of CIPN.[17] Despite the lack of strong supporting data from randomized clinical trials, other agents are still used in the management of CIPN, most notably gabapentin and pregabalin.[19] Often, chemotherapy is either dose-reduced or discontinued to address CIPN and avoid its progression.[20] This emphasizes a clinical challenge, balancing maximal expected therapeutic benefit to achieve best possible survival outcomes, while also considering the potential morbidity of therapy.

2.3 | Pathogenesis of CIPN

Extensive investigations in rodent models and in primary cultures of dorsal root ganglion neurons support our current understanding of the mechanistic basis of chemotherapy-induced sensory neuron toxicity. Several recent reviews have described these findings in detail, and a summary is provided in Table 2.[35,36] Validated mechanisms for MTAs include sensory neuron axonal degeneration leading to loss of intra-epidermal nerve endings,[37,38] mitochondrial vacuolation and defects in mitochondrial transport resulting in the production of reactive oxidative species,[24,39,40] alteration of neuronal ion channels implicated in the excitability of peripheral nerves,[41,42] and neuroinflammation.[32,33] These mechanisms have been targeted for therapeutic intervention and in some cases have been validated with serum biomarkers in clinical studies (Table 2).

Collectively, the literature highlights the biological complexity that underlies CIPN and underscores a need to utilize novel approaches to investigate this toxicity. Pharmacogenomic studies are likely to advance the field of research surrounding CIPN by facilitating the identification of predictive biomarkers and additional actionable targets. Furthermore, human reverse translational studies and novel *in vitro* models using human sensory neurons should serve as investigational models to mimic the human phenotype and provide a more adequate cellular representation of toxicity to further our understanding of underlying mechanisms of CIPN.

3 | GENETIC ASSOCIATION STUDIES PROVIDE CLUES TO THE MOLECULAR MECHANISMS UNDERLYING MTA-INDUCED PERIPHERAL NEUROPATHY

Genetic risk factors (e.g., single nucleotide polymorphisms [SNPs]) have been identified as predictive biomarkers of individual treatment decisions.[43] Currently, no genetic association has proven of clinical utility to predict risk of CIPN, but these efforts have revealed exciting insights into its pathophysiology. Human genetic association studies are useful tools to identify genetic networks implicated in toxicity or response to drugs and have been increasingly applied to study CIPN. These approaches have provided novel information regarding potential biological processes contributing to the pathophysiological mechanisms underlying MTA-induced neuropathy with the hope of translating this understanding into improved and novel preventive and therapeutic strategies. The fact that MTA-induced peripheral neuropathy is dose-dependent led to an initial focus on candidate genes implicated in pharmacokinetics. Genome-wide association studies (GWAS) have enabled an expansion from biased candidate gene studies and have provided a broader insight into which pathways are linked to this toxicity.

3.1 | Candidate gene studies

Candidate gene studies on MTA-induced peripheral neuropathy largely focused on SNPs in metabolizing enzymes (*CYP2C8* and *CYP3A4/5*) and transporters

TABLE 2 Major mechanisms of MTA-induced neurotoxicity, tested therapeutic strategies and predictive biomarkers identified in preclinical studies

Neurotoxic targets	Mechanism of neurotoxicity	Therapeutic strategies	Predictive biomarkers
Epidermis	Axonal degeneration of sensory neurons prevents distal growth and reinnervation of the epidermis as it turns over	Target-specific strategies (*in vitro* and *in vivo* rat models) • Neuropeptide modulation and neurotrophin delivery to restore epidermal nerve fibres; currently limited by lack of adequate delivery systems[21,22] • Lipoprotein receptor-related protein receptors (LRP)-mediated chemoattraction to promote axonal guidance[23]Non-specific strategies *in vivo* murine models: • exchange protein activated by cAMP (Epac) inhibition[24] • histone deacetylase 6 (HDAC6) inhibition[25]	Measurement of intra-epidermal nerve fibre (IENF) density[23,24,26] Measurement of neurofilament light chain (NFL) in cerebrospinal fluid and plasma as a marker of axonal degeneration[27]
Mitochondria	Mitochondrial vacuolation and dysfunction; increased production of oxidative reactive species (ROS) and oxidative stress; impairment in ATP production	Inhibition of ROS production or stimulation of degradation (antimycin A), mimic SOD (MMP9 mAb), restoration of ATP and NAD^+ (acetyl-L-carnitine), attenuation of peroxidation (duloxetine)[28,29]	MMP9
Calcium homeostasis	Changes in intracellular and extracellular calcium concentrations influence activation of voltage gated ion channels, membrane excitability, neurotransmitter release, and gene expression of neuronal cells	Modulation of Neuronal Calcium Sensor-1 (NCS-1) protein and its associated calcium regulation pathway in *in vitro* and murine models: use of lithium, TRPV pain receptor inhibitors (e.g., HTC-067047), calcium chelators (e.g., BAPTA), and use of calpastatin an endogenous calpain inhibitor[30]	Lack of *in vitro* and *in vivo* data to support use of a biomarker specific to calcium homeostasis in the context of neuropathy
Neuroinflammation	Oxidative stress and recruitment of inflammatory and immune mediators leads to damage to intracellular biomolecules, resulting in demyelination and disruption of the cytoskeleton of peripheral nerves and sensitization of signal transduction processes[31]	In vivo rodent models: Antibodies against cytokines CX3CL1 or CX3CR1 and IL-6, inhibition or antagonism of cytokines or cytokine receptors (Etanercept [TNF-α inhibitor], IL-1ra [IL-1 receptor antagonist] and S504393 [CCR2 antagonist]), IL-10 gene therapy[32–34]	CX3CR1 TNF-α IL-1 MCP-1/CCL2 IL-6

(*ABCB1* and *ABCC2*).[44–48] *ABCB1* variants and their role in taxane-induced neuropathy were investigated in early candidate gene studies and were replicated in some, but not all, validation cohorts.[44,49] A less functional *ABCB1* variant is consistent with a higher risk of developing neuropathy, since *ABCB1*-encoded P-glycoprotein effluxes toxic substances out of the peripheral nervous system. Analyses of genes implicated in vincristine pharmacokinetics have shown associations of *ABCC2* variants with increased neurotoxicity.[50] Other studies revealed that patients harbouring *CYP2C8* and *CYP3A4* polymorphisms had increased susceptibility to severe neuropathy.[47,51] This finding is also consistent with dose-dependent taxane toxicity and has been replicated in other studies.[52,53] CYP3A5 is the major catalyst of vincristine metabolism and individuals carrying *CYP3A5* variants associated with high expression are less likely to suffer severe neuropathy induced by vincristine.[54–56] Increased rates of vincristine metabolism in CYP3A5 high expressors are consistent with lower systemic and therefore sensory neuron exposure to this neurotoxin.

Candidate gene studies have also examined variants in genes related to drug targets. Polymorphisms disrupting biological pathways related to tau, actin, and microtubule dynamics may alter taxane effects in sensory neurons and cause neurodegeneration. Genetic variants in *MAPT*, *TUBB2A* and *GSK3β* associated with an increase in sensitivity to taxane-induced neuropathy support this hypothesis.[57,58] Genes encoding proteins regulating actin/microtubule cytoskeleton interactions (i.e., *ACTG1* and *CAPG*) were further highlighted in a study on vincristine-induced neuropathy.[59] In general, these candidate gene associations have not been widely replicated in independent populations and require further investigation.[60]

3.2 | Genome-wide association studies

Although candidate genes studies have provided fundamental insights on CIPN, genome-wide approaches revealed novel associations in an unbiased manner, investigating both direct and indirect genetic effects on MTA-induced peripheral neuropathy. Table 3 summarizes up-to-date findings from GWAS on MTA-induced neuropathy. Although the first candidate gene association studies hypothesized that neurotoxicity could be related to overall drug exposure, GWAS suggest that genes involved in nerve repair play a more important role than those implicated in pharmacokinetics or pharmacodynamics. The first GWAS on the onset and severity of paclitaxel-induced neuropathy in primary breast cancer patients revealed three novel genes that play a role in neurite growth during development and in the regulation of actin for the formation of filopodia/lamellipodia (*FZD3*, *EPHA5* and *FGD4*).[65] *FZD3* encodes for a member of the Wnt receptor family, Frizzled-3, that controls axonal outgrowth and development of the neural crest.[71] Patients carrying variants in *FZD3* were initially linked to decreased risk of onset of paclitaxel neurotoxicity, a finding that was recently validated in an independent sequencing study.[65,72] *EPHA5* encodes the receptor tyrosine kinase ephrin receptor A5 which guides axonal growth during development.[73] Association of *EPHA5* genetic variants with increased risk of paclitaxel-induced peripheral neuropathy has been replicated in other studies and may be related to the inability to repair injured axons following paclitaxel treatment.[69,74] Although the GWAS that replicated the *EPHA5* findings was limited by sample size and included patients treated with both paclitaxel and carboplatin (Table 3), other *EPHA* genes were also independently associated to CIPN and indicate that ephrin-A signalling may be a critical function in the response to neuronal injury. rs10771973 in *FGD4*, the only variant that was validated in the initial GWAS of paclitaxel-induced peripheral neuropathy, has also been linked with increased risk of paclitaxel dose reductions in another independent candidate SNP association study.[48] Rare mutations in *FGD4*, a gene that encodes for an F-actin binding protein, cause Charcot–Marie–Tooth (CMT) disease, the most common hereditary neuropathy characterized by peripheral nerve and muscle damage.[75] Exome sequencing studies in paclitaxel-treated patients have identified other CMT genes (*ARHGEF10* and *PRX*) with known roles in the regulation of neuronal morphogenesis and function, associated with susceptibility to taxane-induced neuropathy.[76,77] In contrast to congenital CMT disease, common genetic variation in CMT genes is only associated with peripheral neuropathy when patients are challenged with neurotoxic chemotherapeutics.

The *LIMK2* (LIM domain kinase 2) genetic locus has also been associated with onset of paclitaxel-induced peripheral neuropathy.[69] LIMK2 is a protein kinase involved in the regulation of actin filament dynamics and reduced LIMK2 expression causes increased *in vitro* sensitivity to vincristine but not paclitaxel.[78] Whether the connection between LIMK2 expression and sensitivity to the cytotoxic effects of MTAs translates into sensitivity to their neurotoxic effects remains unclear. Other taxane-induced peripheral neuropathy GWAS have implicated additional nerve regeneration genes (e.g., *GPR177* and *SBF2*) as well as inflammatory genes (*RFX2* and *FCAMR*); however, with limited sample sizes, heterogeneous patient populations, and lack of replication, their importance in the pathogenesis of this dose-limiting toxicity remains unclear.[66,67,79]

TABLE 3 Summary of GWAS of MTA-induced peripheral neuropathy

Citation	Chemotherapy	Phenotype[a]	*N*	Ancestry	Gene/SNP (effect; *P*)	Replication *N*, ancestry (effect, *P*)	Biological pathway
Li et al. 2019[55]	Vincristine	Time-to-first grade 3 + PN; TNS-PV scores	1128[b]	EUR	*COCH*/rs1045644 (−1.02[d] POG, −2.36[d] ADVANCE; 8.66E-06[g]); *EYS*/rs796352/rs554669 (0.80[e] POG, 2.16[d] ADVANCE; 1.05E-05[g])	NR	Nerve innervation, photoreceptor function
Abaji et al. 2018[61c]	Vincristine	Grade 3 + PN	237[b]	EUR	*SYNE2*/rs2781377 (2.5[h]; 0.01); *MRPL47*/rs10513762 (3.3[f]; 0.01); *BAHD1*/rs3803357 (0.35[f]; 0.007)	405, EUR (NS)	Actin reorganization, mitochondrial homeostasis, transcriptional regulation
Diouf et al. 2015[62]	Vincristine	Grade 2 + PN	321[b]	MIXED	*CEP72*/rs924607 (2.43[f] St Jude Total XIIIB, 4.1[f] COG AALL0433; 6.3E-09[g])	NR	Microtubule organization
Chua et al. 2020[63]	Paclitaxel, nab-paclitaxel, ixabepilone	Time-to-first grade 2 + PN	469 (CALGB 40502), 855 (CALGB 40101)	EUR	*S1PR1*/rs74497159 (0.591[e] CALGB 40101, 0.693[e] CALGB 40502; 3.62E-07[g])	NR	Neuronal excitability and growth
Sucheston-Campbell et al. 2018[64]	Paclitaxel	Grade 3 + PN	1269 EUR; 139 AFR	EUR; AFR	*GNGT1*/rs1858826 (0.29[f,g]; 1.1E-07[g])	Meta-analysis with Baldwin et al.[65] 855, EUR (0.29[f]; 1.1E-07)	Photoreceptor function
Schneider et al. 2016[66c]	Paclitaxel	Grade 3 + PN	126	AFR	*SBF2*/rs149501654/rs117957652/rs141368249/rs146987383/rs7102464 (5.09[f]; 4.35E-06)	NR	Axonal degeneration
Schneider et al. 2015[67]	Paclitaxel	Grade 3 + PN	1357	EUR	*GPR177*/rs3125923 (1.8[f]; 4.99E-05)	789, EUR (1.8[f]; 0.0017)	Neuronal development
Schneider et al. 2015[67]	Paclitaxel	Grade 2 + PN	213	AFR	*FCAMR*/rs1856746 (5.54[f]; 1.57E-07)	NR	Inflammation

(Continues)

TABLE 3 (Continued)

Citation	Chemotherapy	Phenotype[a]	*N*	Ancestry	Gene/SNP (effect; *P*)	Replication *N*, ancestry (effect, *P*)	Biological pathway
Komatsu et al. 2015[68]	Paclitaxel	Paclitaxel-induced cytotoxicity	116 LCL	ASN	*AIPL1*/rs3892315 (0.207[d]; 8.72E-07); *AIPL1*/rs11651916 (0.207[d]; 8.75E-07); *AIPL1*/rs3892316 (0.206[d]; 9.23E-07)	NR	Photoreceptor function
Leandro-García et al. 2013[69]	Paclitaxel and/or carboplatin	Cumulative dose to first grade 2 + PN	144	EUR	*EPHA4*/rs17348202 (4.85[e]; 1.02E-06); *EPHA6*/rs301927 (2.35[e]; 3.44E-05); *EPHA5*/rs1159057 (2.01[e]; 6.84E-05); *EPHA5*/rs7349683 (1.83[e]; 3.33E-04); *EPHA5*/rs7349683 (1.68[e,g]; 1.4E-09[g]); *XKR4*/rs4737264 (1.71[e,g]; 3.1E-08[g]); *LIMK2*/rs4141404 (2.41[e]; 3.22E-06); *LIMK2*/rs5749248 (2.78[e]; 1.98E-07)	Meta-analysis with Baldwin et al.[65] 855, EUR (rs7349683, 1.63[e], 9.6E-07; rs4737264, 1.9[e], 1.0E-06)	Axonal guidance, apoptosis, actin reorganization
Baldwin et al. 2012[65]	Paclitaxel	Cumulative dose to first grade 2 + PN (onset); ordinal grades (severity)	855	EUR	*EPHA5*/rs7349683 (1.63[e]; 9.6E-07); *FGD4*/rs10771973 (1.57[e]; 2.6E-06); *FZD3*/rs7001034 (0.57[f]; 3.1E-09); *FZD3*/rs7833751 (0.58[f]; 7.5E-09)	154, EUR; rs10771973, 1.72[g], 0.013; 117, AFR; 1.93[g], 6.7E-03	Axonal guidance, neuronal development, actin reorganization
Hertz et al. 2016[70]	Docetaxel	Cumulative dose to first Grade 3 + PN	623	EUR	*VAC14*/rs875858 (3.60[g]; 2.12E-08)	NR	Neurodegeneration

Abbreviations: AFR, African America; EUR, European; GWAS, genome-wide association studies; LCL, lymphoblastoid cell lines; MIXED, multi-ethnic ancestry; MTA, microtubule-targeting agents; NR, not reported; NS, not significant; TNS-PV, Total Neuropathy Score-Pediatric Vincristine.

[a]All grading are defined by NCI-CTCAE criteria.

[b]Paediatric population.

[c]Genome-wide exome sequencing study.

[d]Coefficient.

[e]Hazard ratio.

[f]Odds ratio.

[g]Effects or *P* values of meta-analysis

A recent GWAS meta-analysis of peripheral neuropathy following treatment of breast cancer patients with paclitaxel, nab-paclitaxel or ixabepilone identified SNPs in the enhancer regions of *S1PR1* encoding the G-protein coupled receptor sphingosine-1-phosphate receptor 1 ($S1PR_1$) which were associated with an increased risk of developing CIPN.[63] *In vitro* validation studies utilizing FTY-720 (fingolimod), a functional antagonist of S1P receptors, in combination with paclitaxel in an induced pluripotent stem cell (iPSC)-derived sensory neuron model, demonstrated that S1PR1 inhibition confers neuronal protection against paclitaxel toxicity. These findings are particularly exciting given that clinical trials are ongoing to investigate the utility of fingolimod for the prevention and treatment of paclitaxel-induced peripheral neuropathy in breast cancer patients.

It is important to note that many of the genes associated with taxane-induced peripheral neuropathy identified in GWAS and sequencing studies (*FGD4*,[65] *EPHA4/5/6/8*,[65,69] *LIMK2*,[69] *ARHGEF10*,[76,77] *SBF2*[66] and *S1PR1*[63]; Figure 1) converge on RhoGTPase signalling pathways that are critical to biological processes such as axonal guidance and neuronal extension (i.e., filopodia and lamellipodia formation).[80] These findings suggest that genetic variants resulting in axonal degeneration or lack of regeneration may prevent the reinnervation of epidermal layers after chemotherapy, potentially contributing to the development and progression of CIPN.

While most of the GWAS to date are focused on paclitaxel-induced neuropathy, a few including patients treated with other MTAs have supported the hypothesis that mechanisms of CIPN are related at least in part to an inability to repair nerve damage. A GWAS investigating docetaxel-induced peripheral neuropathy identified a gene related to neurodegeneration (*VAC14*), which was functionally validated *in vitro* but has yet to be independently replicated in patients.[70] Genetic association studies of vincristine-induced neurotoxicity have also identified genes related to neuron structure, including genes involved in microtubule/actin cytoskeleton organization (*CEP72* and *SYNE2*).[61,62] Another genome-wide meta-analysis highlighted genes involved with neurogenesis (COCH), although the encoded cochlin protein has primarily been associated with function in auditory ganglion.[55] Among the genetic variants from GWAS of vincristine-induced peripheral neuropathy, rs924607 in the promoter region of *CEP72*, a gene encoding for a centrosomal protein and key regulator of microtubule organization, was associated with increased risk of neuropathy and reduced expression in HapMap samples.[62] In a blinded case–control replication study in adults receiving vincristine, carriers of rs924607 were at increased risk

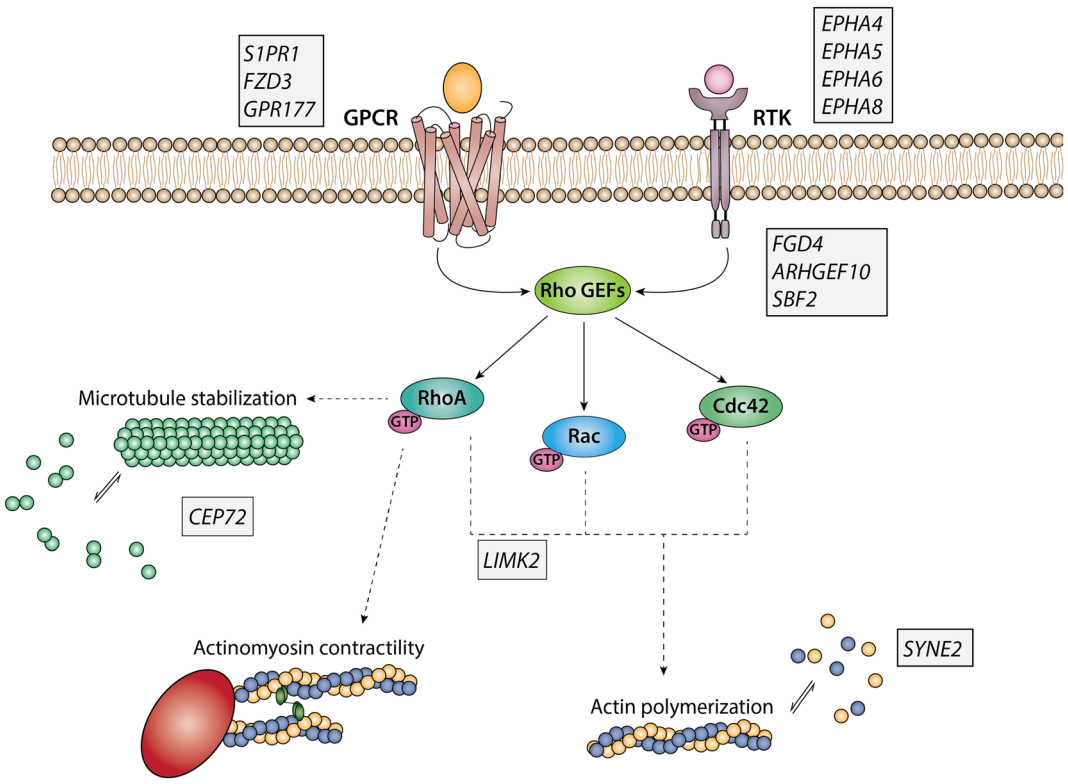

FIGURE 1 Actin cytoskeletal genes implicated in CIPN from genetic association studies. GPCR, G-protein coupled receptor; RTK, receptor tyrosine kinase

of neurotoxicity (75% of patients with the reference allele vs. 44% with the variant allele developed grade 2–4 peripheral neuropathy), validating the original GWAS findings in a paediatric population.[81]

While identification of biologically relevant genes and pathways from GWAS has generated intriguing hypotheses and supports ongoing functional studies in CIPN, finding appropriately sized and phenotyped populations for replication is challenging, and most of these GWAS findings remain unreplicated. Nonetheless, GWAS have revealed the polygenic nature of MTA-induced peripheral neuropathy and underline the potential contribution of nerve repair pathways in chemotherapy sensitivity.[82] The discovery and replication of additional genetic associations would offer innovative strategies for early detection and management of CIPN by elucidating the complex biological processes that underlie peripheral nerve damage and repair following exposure to neurotoxic chemotherapies. As genomic sequencing technologies become more affordable and neurotoxicity assessments become standardized and accessible, some reported associations will be validated and novel clinically relevant genetic associations will be uncovered.

4 | REVERSE TRANSLATIONAL STUDIES OF CHEMOTHERAPY NEUROTOXICITY

Investigations into the molecular basis of genes and pathways identified in human genetic association studies of CIPN will be critical for translating these findings into useful therapeutic approaches for prevention or treatment. Studies in both preclinical animal models and human cells can be performed, with an advantage of the latter for initial screening of GWAS findings since studies can be higher throughput and the cells are of human origin. However, the availability of appropriate cell models has been limited until recently. Below, we discuss the use of neuronal cell lines and iPSC-derived sensory neurons for reverse translational studies of CIPN GWAS findings.

4.1 | Studies in neuronal cell lines and commercial iPSC-derived neurons

Various *in vitro* cellular models have been used to investigate chemotherapy neurotoxicity such as the human neuroblastoma cell line SH-SY5Y and rat PC12 pheochromocytoma cells.[83,84] Although these models allow us to study chemotherapy-induced neurotoxicity, they present significant limitations in understanding human toxicity. The biggest limitation of these cell lines is that while they can be differentiated into 'neuron-like' cells, they are not neurons per se. To address this limitation, an increasing number of iPSC-derived neuronal models have been commercially available such as human iCell® neurons from Cellular Dynamics International, human peripheral iPSC neurons from Axiogenesis (Peri.4U®), Tempo-iSenso™ human iPSC-derived sensory neurons from Tempo Bioscience and iPSC-derived sensory neuron progenitors from Axol Bioscience. iCell® neurons represent an assortment of postmitotic GABAergic and glutamatergic cortical neurons while Peri.4U® and Tempo-iSenso™ neurons have peripheral neuron characteristics, expressing canonical markers such as β-III tubulin, peripherin, MAP2, P2X3 and vGLUT2.[85–87] Only iCell® and Peri.4U Neurons® have been used for screening of neurotoxic compounds and for functional studies on CIPN-related genes identified by GWAS.[62,68,70,86,88,89]

Important findings have been reported with the use of these models to examine the molecular basis of genetic variants associated with MTA-induced peripheral neuropathy identified in GWAS. Genetic disruption of *TUBB2A* in iCell® neurons increased paclitaxel-induced neurite retraction by ~20%.[89] Validation of *AIPL1* from a GWAS (Table 3) on paclitaxel-induced cytotoxicity using iCell® neurons demonstrated a decreased AIPL1 expression which protected against neurite morphological damage caused by paclitaxel.[68] In addition, evidence supporting the association of CEP72 with vincristine-induced neurotoxicity was reported using the same *in vitro* model.[62] In this work, genetic silencing of *CEP72*, which represents a loss of function variant in the promoter of *CEP72*, resulted in greater neuronal damage caused by vincristine (i.e., decrease in neurite length and branching).

Similar GWAS validation studies have been carried out using Peri.4U® neurons, which have more peripheral neuron-like characteristics. Peri.4U® neurons are sensitive to MTA exposure but not to platinum-based agents or thalidomide, in contrast to iCell® neurons that are sensitive to all CIPN causative drugs.[86] This finding suggests that human iPSC-derived peripheral sensory neurons are a more appropriate *in vitro* model compared to iPSC-derived central neurons to investigate MTA-induced neurodegeneration. Another study using Peri4.U® neurons for validation of *VAC14* in docetaxel-induced peripheral neuropathy (Table 3) revealed that siVAC14 Peri.4U® neurons, when exposed to docetaxel, have less neurite outgrowth and less neurite branching, compared to a non-targeting control.[70] *In vivo* studies corroborating the results found in Peri.4U® neurons, showed that after docetaxel administration, $Vac14^{-/-}$ mice have increased nociceptive sensitivity compared to wildtype mice,

emphasizing that these *in vitro* models are appropriate for validation and screening of genetic targets associated with MTA-induced neurodegeneration.

The iCell® and Peri.4U® neurons, provided from multiple vendors and with often unreported genetic background, are no longer commercially available. This negates future use as reproducible experimental models and challenges the longitudinal interpretation and comparison of data within a single laboratory and across laboratories and institutions. Human iPSC-derived sensory neurons are also available from Tempo Bioscience, but to date, there are no published studies using Tempo-iSenso™ neurons.

4.2 | Novel human iPSC-derived sensory neuron models

In the last 5 years, other cellular models to study CIPN have been established such as the use of human sensory neurons differentiated from reprogrammed fibroblasts, blood, and embryonic stem cells. These cells express canonical nociceptor and mechanoreceptor markers and functionally resemble DRG sensory neurons. One potential application for studying the effects of human genetic variation on sensory neuron response to MTAs is to establish iPSC lines from patients exposed to these chemotherapeutics and who experience variable responses with regards to neurotoxicity (i.e., no neuropathy to severe neuropathy). iPSC-derived sensory neurons from these samples could then be used to investigate underlying mechanism and to link genetic variation to causal genes and phenotypes. Analysis of gene expression in sensory neurons derived from >100 healthy donors showed more variation due to batch differentiation than to donor of origin.[90] This study highlighted the potential power of iPSC-derived sensory neurons as a tool to help understand human genetic variation but also underscores the limitation of differentiation protocols. A robust, widely available, accessible and reproducible model of human iPSC-derived sensory neurons would greatly advance mechanistic studies of chemotherapy neurotoxicity.

Sensory neurons derived from a single iPSC line could be used to investigate the role of selected genes and pathways in MTA-induced neurotoxicity. The authors have recently described the use of the iPSC line WTC11 to differentiate sensory neurons with a homogeneous genetic background.[91] This well-characterized pluripotent cell line is used worldwide for the derivation of various cell types. Human-derived sensory nociceptors are sensitive to the exposure of neuropathy-inducing antineoplastic agents. Following chemotherapy exposure, iPSC-derived sensory neurons demonstrate dose-dependent changes in neuronal morphology including reduced neurite length and neurite density. The sensory neurons also showed reduced mitochondrial transport, altered mitochondrial membrane potential and changes in glutamate-induced Ca^{2+} influx in response to paclitaxel. Overall, these iPSC-derived sensory neurons show similar nociceptive responses to MTA exposure as human sensory neurons.[91] Their utility in investigating GWAS findings was demonstrated by functional validation of a role for S1P signalling in paclitaxel-induced neurotoxicity.[63] A similar method for differentiation of human iPSCs into sensory neurons has been used to demonstrate a critical role for SARM1 in vincristine-induced axon degeneration.[92]

This iPSC-derived sensory neuron model holds great promise as the foundation of a standardized framework to understand genes and pathways underlying mechanisms of CIPN, define the contribution of genetic variation to neurotoxicity, and screen for drugs with potential clinical application. Recently, a large-scale CRISPRi-based platform from iPSC-derived central neurons was developed for genetic screens in neurodegenerative diseases.[93] The screen uncovered gene-specific effects on survival and neuronal morphology and provide a useful approach for functional and mechanistic studies. Single-cell sequencing readouts allowed the identification of genes with cell-type-specific consequences. A similar combination of CRISPRi screening and single-cell sequencing techniques in iPSC-derived sensory neurons would facilitate identification of genes and pathways involved in CIPN and would provide information at a cell-population level.

5 | LIMITATIONS AND PROMISE OF PHARMACOGENETIC STUDIES OF CIPN

Sample size and representation of non-Caucasian populations are significant limitations of most of the pharmacogenetic studies described above. Importantly, not all studies considered relevant clinical covariates such as the incidence of pre-existing neuropathy, risk factors like diabetes and excess alcohol consumption, and the use of neuropathic pain modulating agents. CIPN severity is often underestimated and underreported which also leads to the introduction of bias.[60] Clinical trials typically use the NCI-CTCAE criteria for defining peripheral neurotoxicity, which is focused on common symptoms such as burning and tingling sensations in the hands and feet and limitations to activities of daily living such as getting dressed. The use of NCI-CTCAE is also limited by the temporal collection of the data, with the

patient typically asked to report symptoms that occurred earlier in a treatment cycle. Collection of patient-reported symptoms of toxicity that are captured in real time will provide a richer phenotype that could increase the power of GWAS to detect true genetic associations. Finally, pharmacogenetic association findings can be difficult to replicate due to the lack of well phenotyped cohorts with relevant treatments and DNA samples.

To date, pharmacogenetic association studies of MTA-induced peripheral neuropathy have identified genetic variation in genes and pathways that are critical to sensory nerve function and repair. Despite the general lack of genome-wide statistical significance and replication in an independent population, these findings provide mechanistic insight into the pathogenesis of CIPN. More detailed functional studies of promising genes and pathways are warranted, with the goal to develop clinically meaningful biomarkers and to identify potential therapeutic targets for prevention and treatment of dose-limiting CIPN. It is hoped that the ongoing implementation of human iPSC-derived sensory neurons with a homogenous genetic background will allow the determination of the true contribution of patient-specific genetic variation to CIPN predisposition. Introduction of specific genetic variants into these cells will provide models to study both common and rare genetic variation identified in genetic association studies and extended to genetic variants in candidate genes that are unique to understudied populations. The iPSC-derived sensory neurons can also serve as a platform to screen potential tailored neuroprotective targets. A recent study has shown successful utilization of patient iPSC-derived sensory neurons to treat severe small fibre neuropathic pain, demonstrating that such models can more faithfully mimic target tissues and translate to patient experience.[94]

Considering that the decrease in cancer mortality is paralleled by an increasing number of cancer survivors who are prone to late effects of therapy, it is essential for the scientific community to develop standardized tools for the prediction, management, and treatment of patients genetically susceptible to CIPN. The application of pharmacogenetics to the study of CIPN will contribute to this goal.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

K.C.C., N.E.H and J.P. reviewed the literature. K.C.C., N.E.H., J.P. and D.L.K. wrote the manuscript.

ORCID

Josefina Priotti https://orcid.org/0000-0001-7493-3047
Deanna L. Kroetz https://orcid.org/0000-0001-5997-270X

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