

Gene Therapy Options as New Treatment for Inherited Peripheral Neuropathy

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Inherited peripheral neuropathy (IPN) is caused by heterogeneous genetic mutations in more than 100 genes. So far, several treatment options for IPN have been developed and clinically evaluated using small molecules. However, gene therapy-based therapeutic strategies have not been aggressively investigated, likely due to the complexities of inheritance in IPN. Indeed, because the majority of the causative mutations of IPN lead to gain-of-function rather than loss-of-function, developing a therapeutic strategy is more difficult, especially considering gene therapy for genetic diseases began with the simple idea of replacing a defective gene with a functional copy. Recent advances in gene manipulation technology have brought novel approaches to gene therapy and its clinical application for IPN treatment. For example, in addition to the classically used gene replacement for mutant genes in recessively inherited IPN, other techniques including gene addition to modify the disease phenotype, modulations of target gene expression, and techniques to edit mutant genes have been developed and evaluated as potent therapeutic strategies for dominantly inherited IPN. In this review, the current status of gene therapy for IPN and future perspectives will be discussed.

Key words: Inherited peripheral neuropathy, Gene therapy, Antisense oligonucleotide, miRNA, Gene editing

INTRODUCTION

Inherited peripheral neuropathy (IPN) is caused by genetic mutations which damage the integrity of either the axon or myelin of peripheral nerves. The prominent clinical phenotypes of IPN include progressive and symmetrical distal weakness resulting in loss of sensation, muscle wasting and gait disturbances. The symptom appears in a length dependent manner that muscle weakness and atrophy was observed from distal to proximal parts of all limbs [1]. The defects in the peripheral nerves are more severe in the

distal part than in the proximal part because smaller diameter of the nerves renders more vulnerable to the degeneration. Clinically, IPN can be subdivided into hereditary motor and sensory neuropathy or Charcot-Marie-Tooth disease, distal hereditary motor neuropathy, and hereditary sensory and autonomic neuropathy [2]. The total number of IPN patients is estimated to be more than three million globally.

Currently, the treatment options for IPN are very limited. Although several attempts have been made to lessen or ameliorate the disease phenotype after validating the efficacy of animal studies, clinical benefits remain uncertain. For example, vitamin C was proven to be successful in rodent models, however, its efficacy could not be duplicated in clinical trials [3-5]. Recently, PXT3003, a novel combination of baclofen, naltrexone hydrochloride and D-sorbitol is under clinical evaluation, yet the clinical benefits need further elucidation [6-8]. Indeed, unsatisfactory outcomes in clinical practice could be attributed to inappropriate treatments. Up to

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Table 1. IPN causative genes and the inheritance pattern

Type	Dominant	Recessive
Demyelinating type	PMP22, PMP2, MPZ*, LITAF, FBLN5, EGR2*, NEFL*	MPZ*, NEFL*, EGR2*, GDAP1*, MTMR2, SH3TC2, NDRG1, PRX, FGD4, SBF1, SBF2, FIG4, CTDPI, SURF1, ADCY6, CNTNAP1, HK1
Axonal type	MFN2, GDAP1*, LRSAM1*, MPZ*, NEFH, KIF5A, ATP1A1, VCP, TFG, DHTKD1, TUBB3, NAGLU, DCAF8, DGAT2, MORC2, HSPB1, HSPB3, HSPB8, GARS, AARS, HARS, MARS, DYNC1H1, BICD2, REEP1, BSCL2, SETX, SLC5A7, MYH14, TRPV4, RAB7	LMNA, PNKP, TRIM2, SPG11, MME, MCM3AP, SLC25A46, SCO2, MPV17, LRSAM1*, C12orf65, IGHMBP2, SIGMAR1, VRK1, ATP7A, UBA1, GLE1, LAS1L
Intermediate type (mixed type)	NEFL*, MPZ*, GJB1, YARS, INF2, DRP2, DNM2, GNB4, PDK3	GDAP1*, COX6A1, PLEKHG5, KARS, AIFM1, PRPS1
Incidence	> 95%	< 5%

*Mutations in the gene cause dominant or recessive inheritance.

now, treatment approaches have focused on modulating the disease phenotype by indirectly reducing the expression of toxic protein and/or enhancing myelination and axonal integrity. Therefore, the possibility of direct manipulation of mutant gene expression should be considered as an acceptable and effective therapeutic option.

Although IPN was first described in the 19th century, the first causative gene was isolated in 1991 [9-12]. By the advent of next-generation sequencing technology, however, more than 100 distinct genes have been identified as causative genes for IPN [13, 14]. Among the numerous causative genes, peripheral myelin protein 22 (PMP22), myelin protein zero (MPZ), gap junction protein beta 1 (GJB1) and mitofusin2 (MFN2) are prevalent in over 80% of genetically isolated IPN patients [15-18]. As such, the majority of research has been focused on these genes, revealing the pathophysiological mechanism, and developing therapeutic options.

Gene therapy is a fundamental and straightforward strategy to overcome the genetic defects in inherited disorders. Indeed, replacing a mutant gene with a functional copy through gene delivery might be the ultimate treatment strategy for IPN. Everyday gene therapy treatment options are expanding, by virtue of novel technical advances in gene manipulation. Current strategies for gene therapy can be categorized into four types: gene replacement, gene addition, gene knockdown or modulation of gene expression, and gene editing or correction [19-22]. Besides the simple delivery of functional genes, the ability to manipulate the expression of mutant genes with toxic gain-of-function, or to correct mutant genes into functional genes, is now possible. Although gene therapy has not been clinically investigated in IPN patients, a breakthrough in gene therapy with benefits for IPN is anticipated.

MAJOR TARGETS FOR GENE THERAPY IN IPN

Phenotypically, IPN can be divided into three types according to

the origin of degeneration: axonal, demyelinating, and intermediate type. Axonal type is primarily caused by degeneration of axon and demyelinating type is caused by malfunction of myelinating Schwann cell. Intermediate type shows both axonal and demyelinating features (Table 1). Thus major targets for IPN treatment has focused on either enhancing myelination of Schwann cell for demyelinating type or increasing the mitochondrial activity and axonal transport for axonal type. To enhance the myelination of Schwann cell, ascorbic acid, curcumin, a HSP90 inhibitor, and a progesterone antagonist have been evaluated in preclinical or clinical studies [3, 23-25]. Recently, a combination of preexisting drugs, PXT3003, was developed by the application of systems biology and its efficacy is currently under clinical evaluation after demonstration of the myelination enhancement in animal model [6-8]. In contrast, improvement of mitochondrial metabolism or axonal transport have been paid attention to manage the axonal type of IPN. Coenzyme Q10 and a mitofusin agonist, which might be associated the mitochondrial activity, ameliorated the axonopathic phenotype in MFN2-mutated IPN [26, 27]. In addition, inhibition of histone deacetylase 6 (HDAC6) increased acetylated alpha-tubulin and improved axonal transport in HSPB1 or GARS animal models [28-31].

As more than 100 genes have been isolated as causative of IPN, clinical interest has not been able to investigate every gene. Instead, mutations in several genes (PMP22, MPZ, and MFN2) have received major attention for therapeutic applications [4, 24, 32-34]. Interestingly, the mutations in those genes are all dominantly inherited. Indeed, more than 95% of CMT cases are dominantly inherited, with the remaining 5% of cases inherited via the autosomal recessive or X-linked recessive manner. As such, classical gene therapy, a gene replacement, cannot be applied to most IPN patients.

Recent advances in gene manipulation technology shed light on the development of novel gene therapy strategies for dominantly

inherited IPN. For example, mutant alleles can be selectively suppressed while normal alleles can regenerate the damaged peripheral nerve. Duplication of PMP22, is the major cause of demyelinating type IPN, and comprises over 40% of all IPN cases. In this subtype, the duplication of PMP22 in one haploid causes the total expression of PMP22 protein to be 1.5 times higher than normal, which in turn causes cellular stress and the demyelination of Schwann cells [35]. Thus, reducing the expression level of PMP22 protein in patients has been the major target of IPN treatment. Reduction of gene expression can be achieved by impairing the transcription and degrading the mRNA transcript, or by blocking the protein translation. Recently, gene editing technology has also proposed the conversion of a mutated gene into a normal copy might be the ultimate therapeutic option for genetic diseases (Fig. 1).

GENE REPLACEMENT THERAPY

As most genetic diseases are caused by a single gene defect, replacement of the defective gene is a straightforward approach, and the majority of gene therapy research focuses on gene replacement for recessive genetic disorders. Recently, several gene replacement methods for autosomal recessive and X-linked cases of IPN have been investigated by a European research group.

For autosomal recessive cases of IPN, Schiza et al. [36] evaluated the efficacy of gene replacement therapy for the SH3TC2 (SH3 domain and tetratricopeptide repeats 2) mutation in IPN. SH3TC2 protein is predominantly expressed in myelinating Schwann cells, and the loss-of-function mutations in the SH3TC2 gene contribute to onset of CMT type 4C, a recessively inherited demyelinating neuropathy [37]. Schiza et al. [36] generated a lentiviral vector expressing the SH3TC2 gene under the control of an MPZ promoter, a Schwann cell specific promoter. The engineered lentivirus expressing the target gene was effectively delivered to Schwann cells via intrathecal injection in a *Sh3tc2*^{-/-} mouse model and rescued the neuropathic phenotype. After 8 weeks, the mutant mice, exhibited improved myelination in the lumbar spinal roots and sciatic nerves and the motor behavior was also enhanced.

Intriguingly, the same group also tried gene replacement therapy in X-linked dominant type IPN. There, GJB1 gene mutations cause loss of Connexin 32 (Cx32) in gap junctions and lead to a severe form of inherited demyelinating CMTX1 neuropathy [38]. The mutations in GJB1 cause dysfunction in the Cx32 protein localized in the paranodal loops of non-compact myelin and the Schmidt–Lanterman incisures of Schwann cells [39]. Although the GJB1 mutation caused phenotype is considered to be dominantly inherited, the clinical phenotypes are dramatically different according to gender. An affected female with a heterozygous GJB1 mutation

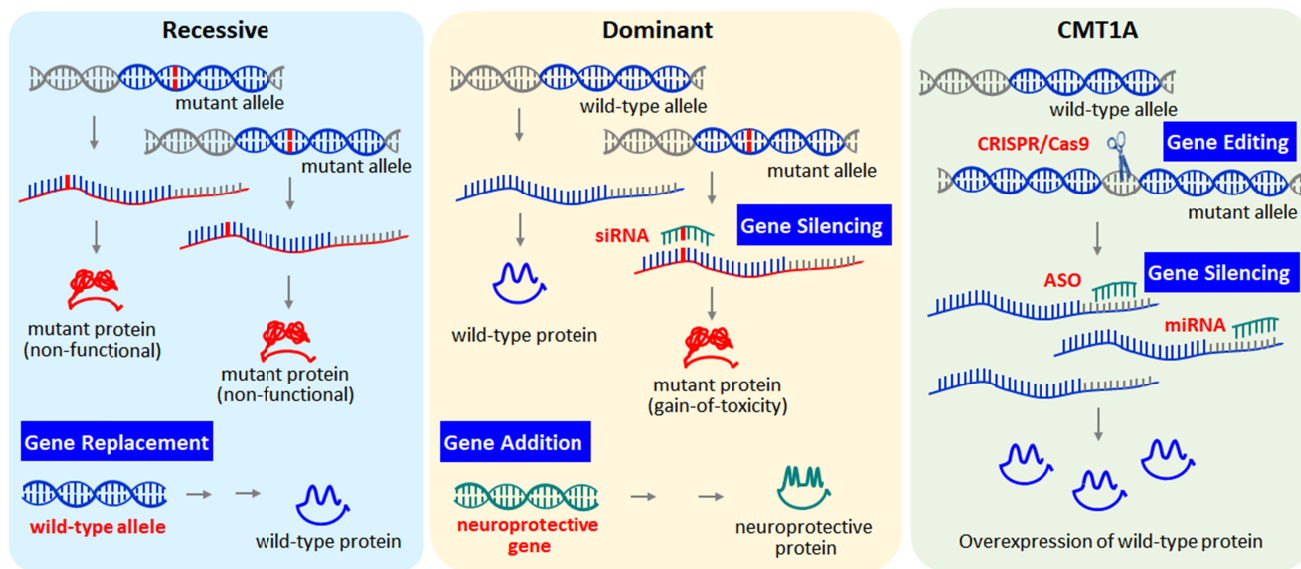


Fig. 1. Types of gene therapy and therapeutic strategies for inherited peripheral neuropathy. Based on the mode of inheritance IPN treatment can be categorized into three groups. For recessively inherited cases, gene replacement, the delivery of a functional gene, was applied to compensate for the lack of functional protein. For dominantly inherited IPN, NT-3, a neuroprotective gene, was used to ameliorate the disease phenotype. The feasibility of mutant allele-specific was also validated for suppressing the gain-of-toxicity mutant protein in dominantly inherited IPN. For CMT1A caused by the duplication of PMP22, various approaches have been developed to normalize the expression level of PMP22. Antisense oligonucleotide (ASO) or miRNA targeting the 3'-untranslated region (3'-UTR) of PMP22, and disruption of the TATA-box could effectively reduce the protein level *in vivo*.

exhibits later onset and milder phenotype than an affected male with hemizygoty due to X-inactivation [40]. To validate the therapeutic effect of gene replacement, they utilized GJB1-null/Cx32 knockout (KO) mice, which exhibit severe demyelination, as well as inflammation in the peripheral nerve [41-43]. Intraneural injection of lentivirus expressing GJB1 by MPZ promoter (LV-Mpz-GJB1) before the phenotype onset in GJB1-null mice significantly reduced the inflammation and ameliorated the peripheral neuropathic phenotype [41]. In a follow-up study, Kagiava et al. [42] also validated the efficacy of intrathecal delivery. Intrathecal administration is less invasive than intraneural delivery, and as a result the clinical feasibility of gene therapy is improved. Recently, Kagiava et al. [43] also demonstrated a therapeutic benefit even if gene therapy is performed after the onset of peripheral neuropathic symptoms. Collectively, these results increase the possibility of success in future clinical trials and positive outcomes for CMT1X patients.

GENE ADDITION THERAPY

Although demyelinating neuropathy occurs mainly due to dominant inheritance, one research group has consistently developed a phenotype modulating strategy using a neurotrophic factor. Neurotrophic factors such as nerve growth factor, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 are well-known to bind to protein tyrosine kinase receptor and activate the downstream signaling pathways in neuronal cells [44]. In addition, these neurotrophic factors also influence the survival and differentiation of Schwann cell [45]. The formation of peripheral nerve is a complex and dynamic process between neuronal axon and Schwann cell. The interaction between these cells leads to the proliferation, migration, and myelination of Schwann cell as well as axon development. During the process neurotrophic factors such as BDNF and NT-3 play an important role in the myelination and axonal growth [46, 47].

When NT-3 was subcutaneously administered into a Tr-J mouse model, a mouse model of demyelinating neuropathy with naturally occurring Leu16Pro mutation in the PMP22 gene, elevated numbers of myelinated fiber forming regeneration units were observed along with axonal regeneration [48]. In the same report, the clinical efficacy of NT-3 in CMT1A patients was evaluated. The patients treated with NT-3 exhibited enhanced nerve regeneration of the sural nerve. In a follow-up study, the administration of agonistic antibodies to NT-3 receptors TrkB and TrkC improved the neuropathic phenotype of Tr-J mice [49]. In addition, because long-term treatment with NT-3 is not clinically achievable due to its short half-life, gene delivery of NT-3 by recombinant adeno-

associated virus (rAAV) was investigated [50]. Indeed, intramuscular delivery of rAAV-NT-3 sustained the release of NT-3, as well as promoted active myelination and nerve regeneration in Tr-J mice. The clinical benefit of neurotrophic factors in modulating the disease pathogenesis of demyelinating neuropathy was also observed by other researchers. For example, the administration of neuregulin-1 enhanced myelination by stimulating myelination pathways in rodent models [51, 52].

SUPPRESSION OF MUTANT GENE EXPRESSION

As a mutated gene is translated into mutant proteins via an mRNA intermediate, inhibiting the translation of mutant mRNA into mutant protein can also be a potential therapeutic target for IPN. For this strategy, utilization of RNA interference (RNAi) has been well studied. Small interfering RNA (siRNA) is a short double-stranded RNA approximately 19~22 nucleotides long [53] which can nullify gene expression by breaking down the mRNA transcripts in a sequence-specific manner. Recently, siRNA-based technique has been a powerful research tool for gene silencing in both basic and therapeutic research [54, 55]. By introducing siRNA or short hairpin RNA (shRNA), gene expression level can be successfully modulated. In addition, because of sequence-specific mRNA breakdown, discrimination between mutant alleles and wild-type sequence is simply achieved. Since dominantly inherited genetic disorders are caused by toxic gain-of-function mutations rather than loss-of-function mutations in recessively inherited genetic disorders, mutant allele-specific targeting ought to be the primary strategy, rather than the addition of normal genes. Indeed, siRNAs have been proven to be successful at the specific targeting and silencing of the mutant allele in dominantly inherited disorders including neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Machado-Joseph disease, and amyotrophic lateral sclerosis [56-60].

For IPN treatment, Lee et al. [61] evaluated the efficacy of mutant allele-specific siRNA using Tr-J mice. Lee et al. [61] designed and isolated the mutant allele (c.47T>C, p.Leu16Pro in mouse Pmp22)-specific siRNA for Tr-J mice and evaluated the potency of allele specificity, both *in vitro* and *in vivo*. The administration of allele-specific siRNA was revealed to alleviate the neuropathic phenotype of Tr-J mice by improving myelination and restoring muscle volume. Moreover, in the sciatic nerve of treated mice, the expression level of mutant mRNA was reduced, whereas that of wild-type allele was increased. In this experiment, Lee et al. [61] validated the efficiency of a non-viral delivery method for IPN gene therapy for the first time; an important success for potential clinical applications. In addition, Lee et al. [61] also provided a

couple of potent allele-specific siRNAs for human patients with same mutation in PMP22 (CMT type 1E). These results implicate targeting mutant alleles with specific siRNA might be a potential therapeutic option for dominantly inherited IPN.

TARGETING THE GENETIC DOSAGE BY POSTTRANSCRIPTIO-NAL MODULATION

CMT1A is the most common type of IPN resulting in the demyelination of Schwann cells due to a 1.5-fold overexpression of PMP22 myelinating protein. Because over 40% of all IPN cases have CMT1A, most research has focused on exploring the novel agents capable of decreasing the expression level of PMP22. By developing a high-throughput screening method and with the aid of systems biology, some research groups were able to isolate or repurpose several drugs or drug combinations useful for downregulating PMP22 expression [6-8, 62]. However, the mode-of-action as well as the potency for these drugs in downregulating PMP22 expression remains unclear. Alternatively, two independent groups have developed novel gene therapies which directly manipulate the gene dosage of PMP22. One group isolated novel microRNAs (miRNAs) which specifically target the 3'-UTR of PMP22 mRNA and the other screened antisense oligonucleotides (ASO) which successfully downregulate PMP22 levels.

MicroRNAs (miRNA) are endogenous small noncoding RNAs, approximately 22 nucleotides in length [63], which readily bind to the 3'-UTR of target mRNAs and induce degradation. Thus miRNAs can regulate gene expression by acting as modifiers to silence overexpressed genes. The significance of regulatory function of miRNAs in the development of the peripheral nervous system has been investigated. Ablation or reduction of Dicer from Schwann cells can impair normal myelination and axonal integrity [64-67].

Regarding PMP22 gene expression, several miRNAs such as miR-9 and miR-29b are known to post-transcriptionally target the 3'-UTR of PMP22 [68]. Since miRNAs have great potential in regulating the expression level of target mRNAs, targeting PMP22 with its specific miRNA might be an excellent therapeutic option for controlling CMT1A caused by PMP22 overexpression. In this context, Lee et al. [69] reported that the administration of miRNAs downregulated the PMP22 expression levels in a CMT1A mouse model. Indeed, the expression level of several miRNAs were changed and miR-381 and miR-9 can modulate the expression level of PMP22. Using the lentiviral system, LV-miR-381 as well as LV-miR-9 were administered into the sciatic nerve of a C22 mouse, which harbors 7 copies of the human PMP22 gene and an expression level of hPMP22 1.7 fold higher than mouse Pmp22 [70-72]. Expression of both miR-381 and miR-9 enhance the loco-

motor function, electrophysiological integrity (motor nerve conduction velocity and compound action potential), and myelination through the reduction of PMP22 levels in the sciatic nerve of C22 mice. This report revealed a new way for developing potential IPN therapeutic strategies by using miRNA-mediated regulation of gene expression.

RNA transcripts can also be modulated by ASOs; which are synthetic nucleic acids in a single strand capable of binding to the target mRNA resulting in degradation, interference with pre-mRNA processing or protein binding, and alteration of RNA structure [73]. Recently, the application of ASOs has become an emerging tool to manage various degenerative neuromuscular diseases. The clinical application of ASOs has exhibited successful outcomes in spinal muscular atrophy (SMA) and Duchenne's muscular dystrophy (DMD) by modulating the splicing of the mRNA [74-78]. Developments of ASO therapy for these diseases have shifted the strategic paradigms of gene therapy. SMA is caused by mutations in survival motor neuron 1 (SMN1) gene with autosomal recessive inheritance pattern. In humans, an evolutionarily duplicated gene, SMN2, possesses almost identical nucleotide sequence to SMN1. However, a critical substitution at position 6 (C to T) of exon 7 in SMN2 causes aberrant splicing and degradation of mRNA [79]. Instead of correcting the mutated SMN1 in the patients, ASO therapy targets to recuperate SMN2 function by intervening the aberrant splicing. After identification of intron splicing silencer N1 (ISS-N1) sequence in intron 7 that is critical in the skipping of exon 7 in SMN2, ISS-N1 sequence-inhibiting ASO has been developed and successfully demonstrated the therapeutic efficacy through clinical studies [75, 76]. On the other hand, DMD gene is one of the largest human genes with 79 exons and 14kb transcripts. Generally, out-of-frame mutations in DMD gene result in DMD, and in-frame mutations lead to Becker muscular dystrophy (BMD) exhibiting a milder phenotype compared to DMD. Although DMD is an X-linked recessive disease, the huge length of the causative gene renders it difficult to treat with simple gene replacement therapy. Instead, exon skipping strategy using ASO targets and induces the deletion of exon 51 of DMD mRNA during splicing, which then results in the production of shortened but functional dystrophin protein and conversion of out-of-frame mutation into in-frame mutation. Accordingly, the DMD phenotype can be ameliorated as BMD phenotype by treatment of ASO [77, 78].

The suppression of PMP22 expression can be achieved by hybridizing the ASO to result in the specific inhibition and degradation of PMP22 through endogenous RNase H activity. Zhao et al. [80] investigated the potency of PMP22 targeting ASOs using two rodent models for CMT1A. After ASO treatment, both mouse and rat models of CMT1A showed a 35% reduction in PMP22

mRNA, which reduced disease progression and improved CMT1 phenotypes. Zhao et al. [80] also proposes that skin biopsy samples are ideal for detecting the mRNA level of PMP22 as a useful biomarker for future clinical trials on CMT1A.

MODULATION OF TRANSCRIPTIONAL ACTIVITY

Reducing the protein expression of PMP22 can also be achieved by modulating the transcriptional activity of a gene. Recently, two independent research groups demonstrated the feasibility of PMP22 reduction by disrupting either the promoter or enhancer of PMP22 with gene editing technology [81, 82]. The clustered regularly interspaced short palindromic repeats (CRISPR) and related Cas genes are now emerging as essential tools for gene editing [83, 84]. Briefly, DNA from viruses or plasmids are cut into small fragments and integrated into a CRISPR locus with a series of short repeats, around 20 bps. The loci are transcribed, and later the transcripts are processed to generate small RNAs to target foreign DNA based on the sequence complementarity principle. Using this groundbreaking new technology, numerous clinical applications have been attempted to treat various types of diseases.

Intriguingly, gene editing technology was applied to disrupt normal genes rather than to correct the mutant gene for CMT1A treatment. Pantera et al. [81] investigated the feasibility of gene editing for reducing the transcription of PMP22 *in vitro*. They deleted the potential super-enhancer or promoter located approximately 90~130 kb upstream of the Pmp22 transcription sites using CRISPR/Cas9 in S16, a rat Schwann cell line, which effectively reduced the mRNA level of PMP22.

The *in vivo* efficacy of gene editing was evaluated by another group. Lee et al. [82] targeted the TATA-box promoter of PMP22 to reduce the transcription. After intraneural delivery of CRISPR/Cas9 protein targeting the TATA-box promoter of PMP22, the

expression level of PMP22 in the sciatic nerve was effectively reduced in C22 mice. The CRISPR/Cas9 delivery also ameliorated demyelination, muscle atrophy, and defects in the locomotor function. By duplicating the experiment in accordance with the administration time-points (before onset and after onset), Lee et al. [82] also validated the efficacy of CRISPR/Cas9-mediated gene editing on reversing the neuropathic phenotype even after the onset; a crucial clinical benefit of treatment in regards to human patients. Additionally, the safety of gene editing *in vivo* was analyzed for future clinical application. Together, these two reports encourage the clinical application of gene editing technology to treat diseases with copy number variation, such as CMT1A.

OPTIMIZATION OF THERAPEUTIC STRATEGY FOR IPN TREATMENT

In accordance with the recent advances in gene manipulation technology, novel gene therapy was developed and evaluated for IPN using animal models (Table 2). To effectively translate the plausible preclinical results from gene therapy into clinical benefits for IPN patients, several aspects, such as securing the efficacy and safety, should be considered.

Securing safety is a first-line consideration for gene therapy. Although viral delivery provides the feasibility of tissue-specific targeting, long-term effects, and a large capacity for the cargo gene, they still possess the risk of virulence-mediated immunotoxicity and genotoxicity which may impede therapeutic outcomes. Innate and adaptive immune responses to the delivered vectors or the transgene are substantial challenges to the safety of the gene therapy. Although AAV is known to cause relatively weak inflammatory responses compared to other viral vectors, the possibility of activation of T cell or antigen presenting cell by AAV administration still needs to be improved for clinical trials. Genotoxicity

Table 2. Gene therapies validated in animal models of IPN

Type	Target	Therapeutic gene	Mode of action	Vector	Route	Reference
Gene addition	CMT1A	Neurotrophin-3	Stimulation of neurite outgrowth and myelination	AAV	Intramuscular	50
Gene replacement	CMT4C	SH3TC2	Replacement of the autosomal recessive gene	Lentivirus	Intrathecal	36
	CMTX1	GJB1	Delivery of a wild-type gene	Lentivirus	Intraneural (sciatic nerve)	41
Gene silencing	CMT1E	siRNA	Delivery of a wild-type gene Targeting and suppressing the mutant allele of PMP22	Lentivirus Naked (synthetic siRNA)	Intrathecal Intraperitoneal	42, 43 61
	CMT1A	miRNA (miR-381)	Downregulating PMP22 overexpression	Lentivirus	Intraneural (sciatic nerve)	69
		Antisense oligonucleotide	Downregulating PMP22 overexpression by exon skipping	Naked (synthetic oligonucleotide)	Subcutaneous	80
Gene editing	CMT1A	CRISPR/Cas9	Downregulating PMP22 overexpression by disrupting TATA-box	Naked (protein/gRNA)	Intraneural (sciatic nerve)	82

from gene delivery includes insertional mutagenesis, disruption of untargeted gene, and activation of proto-oncogenes depending on the virus type, target cells, and target sequences. Recently, several strategies have been devised to prevent the viral vector-mediated genotoxicity [85]. Activation of proto-oncogenes can be reduced by self-inactivation vector and chromatin insulator. Disruption of U3 in 3'-LTR (long terminal repeat) of the lentivirus reduced the promoter and enhancer activity on the neighboring gene [86]. Insertion of insulator after enhancer sequence in the viral vector can also block the activation of transcriptionally silent proto-oncogene [87]. The long-term effect of the delivered gene sometime causes deleterious outcomes due to uncontrolled expression. Thus regulation of the transgene expression using a molecular switch such as tetracycline-controlled transcriptional activation in animal study might be helpful to increase the safety of gene therapy in clinical application. Recently, introduction of a type III hammerhead ribozyme (HHR) at the 3'-UTR of the transgene exhibited the potency in regulating the protein expression. Since HHR possesses cis-cleaving activity, the cleavage of the 3'-UTR by HHR resulted in the degradation of transgene mRNA. Co-application of a ASO targeting HHR sequence inhibited HHR activity thereby allowing the protein expression [88]. Compared to the viral gene therapy, non-viral delivery has safety advantage. However, the cytotoxicity of their vehicle composition should be thoroughly evaluated. In addition, risk assessments for horizontal or vertical transmission should also be followed for clearing the genotoxicity concern in gene therapy.

To improve the efficacy of gene therapy, design of delivery vectors and administration route should be carefully deliberated. Especially, tropism of viral vectors is important in the effective delivery of the transgene into the target cells. Since the main target of peripheral neuropathy are Schwann cells or neuronal axon, selection of viral serotype is properly considered. Recently, the transduction efficiency of AAV and Lentivirus on the Schwann cells was compared [89]. Lentivirus showed the highest transduc-

tion efficiency on both rat and human Schwann cells, while AAV showed entirely different efficiency according to serotypes. AAV1 showed the highest transduction efficiency in rat Schwann cells, whereas AAV2 and AAV6 showed better potency on human Schwann cells. According to another previous study using mice, AAV1 transduced both Schwann cells and neurons while AAV2 and AAV8 showed selective preference on sensory neuron and Schwann cells, respectively [90]. In this regard, all the viral vector-mediated gene therapy in IPN utilized either lentivirus or AAV1. Thus further optimization of the vector system might increase the possibility of successful translation of gene therapy from preclinical studies to future clinical trials.

Increasing the target specificity is also significant issue for non-viral gene delivery. To improve transfection efficiency, increasing DNA condensation and stability, incorporating cell penetrating peptide, facilitating endosome escape, and increasing nuclear uptake and translocation by manipulating chemical composition can be considered during the design of non-viral gene delivery system [91]. Recently, application of enzymes (e.g. MMP), cell specific antibodies (e.g. anti-HER2 and anti-CD3 antibody), and aptamers have been developed to increase the target specificity [92-94]. Thus development of novel specific targets for Schwann cell or peripheral neuron is needed to increase the transfection efficiency of non-viral vectors for IPN gene therapy.

Determining the delivery route is also an important part in the efficacy and feasibility of gene therapy. Because IPN affects the peripheral nerves, the highest efficacy could be achieved via an intraneural delivery. However, the direct administration of therapeutics into a peripheral nerve can cause tissue damage and may worsen the disease phenotype. As such, intrathecal or subcutaneous delivery provide an alternative option for IPN treatment delivery.

CONCLUSION AND PERSPECTIVES

As the incidence of CMT1A is highest in IPN with therapeutic

Table 3. Strategies to improve therapeutic efficacy for IPN treatment

Type	Category	Strategy
Viral delivery	Toxicity	Reduction of genotoxicity using specific target sequence to avoid activation of proto-oncogene or using regulatory machinery for transgene expression
	Efficiency	Enhancement of viral tropism specific to peripheral nervous system
Non-viral delivery	Stability	Development of enhanced vehicle to increase the stability of oligonucleotides Chemical modification of oligonucleotide to increase the stability
	Efficiency	Development of novel chemical composition or peptide to enhance cell uptake and to facilitate endolysosomal escape or nuclear translocation
	Specificity	Isolation of novel receptors or membrane compositions in Schwann cell or axon Development of novel ligands specific to peripheral nervous system

benefits over millions of patients, therapeutic strategies for suppressing PMP22 expression urgently need to be proven safe for patients. Gene suppression mediated therapeutic strategies require relatively short nucleotides compared to the delivery of a whole gene, which enables *in vitro* synthesis of therapeutics and non-viral delivery. Indeed, most suppression strategies in IPN treatment utilized non-viral system. Although most gene suppression strategies have shown sequence-specificity *in vitro* and *in vivo*, risks of unexpected outcomes, due to off-target effects, still exist in human clinical trials. Thus, further investigation to validate safety and to enhance the specificity, stability, and efficiency of delivery system is required (Table 3).

Novel therapeutic options for IPN have been developed by virtue of the breakthroughs in RNA interference, oligonucleotide-based therapy, and genome editing technology. The development of a novel therapeutic option for CMT1A could be beneficial to the many patients affected by PMP22. Although it may be a long way until this seemingly straightforward concept comes into reality, these meaningful innovations are expected to greatly broaden the scope of gene therapy in the near future.

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CONFLICT OF INTEREST

The authors report no conflict of interest with any person or Institute.

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