ECRONICON OPEN ACCESS

EC NEUROLOGY
Review Article

Odds of Gene Therapy: Current Approaches for the Treatment of Hitherto Not Curable Neurological Diseases

Gerhard Franz Walter*

International Neuroscience Institute Hannover, Germany

***Corresponding Author:** Gerhard Franz Walter, International Neuroscience Institute Hannover, Germany.

Received: May 23, 2022; **Published:** June 28, 2022

Abstract

In this review, principles of gene expression, patterns of inheritance and different types of mutations are recalled. The progress and expanding capacity of DNA-sequencing techniques for diagnostic genetic testing is the basis for the targeted treatment of genetic diseases by means of gene therapy. Cell transplantation and stem cell therapy, antisense oligonucleotide therapy, RNA interference for gene silencing, CRISPR/Cas9-mediated gene editing and read-through therapy are options for gene modification. Particle-mediated gene delivery, plasmid transfection and viral strategies using lentiviral vectors and adeno-associated virus vectors are options for gene delivery. These approaches alone or combined have the potential for the causal treatment of hitherto not curable neurological diseases.

Keywords: *Cell Transplantation; Antisense Oligonucleotide Therapy; RNA Interference; CRISPR/Cas9-Mediated Gene Editing; Read-Through Therapy*

Introduction

Many neurological diseases are not curable to date, but gene therapy may open a new window of opportunity. Aims and objectives of the current review are to provide a comprehensive state of the art on chances but also still existing restrictions for gene therapy with a focus on neurological diseases.

Conceptually, replacing the mutant gene with a normal one would cure the disease, but this task has encountered significant challenges, especially how to deliver the normal gene to the site of malfunction. Gene therapy may permanently cure a genetic disease by deleting, silencing, or skipping of a malfunctioning gene which causes pathology (suppression of endogenous genes) and by insertion of functional genes to compensate for loss of gene function (expression of exogenous genes). Vectors (non-viral and viral) are used to deliver a functional gene region into the specific malfunctioning gene region within the nervous tissue.

For a better understanding of possible starting points for gene therapy, I would like to recall some genetic and methodological basics in an abbreviated form, even if they will be quite familiar to most readers.

Methods and genetic basics for understanding different approaches

The genome

Our genome contains the complete set of genetic instructions needed that our body grows and functions; the bearer of this instruction is the double helix of the deoxyribonucleic acid (DNA) in nearly every cell of our body. The DNA is constructed of two strands like a twisted

ladder with a backbone of alternating deoxyribose and phosphate groups. The four nucleobases of DNA, adenine (A), cytosine (C), guanine (G) and thymine (T), are bound to deoxyribose and form, due to hydrogen bonding patterns, the complementary base pairs (bp) G-C and A-T as "rungs" of the ladder, which eventually results in complementary copies of the genetic information in each of the both strands of DNA. Our genome is approximately 3 billion base pairs long. The DNA is packaged into the chromosomes, 22 pairs of autosomes and one pair of heterosomes (the sex chromosomes X and Y), in the cell nuclei.

DNA is composed of genes, coding regions which may determine disease, if a mutation of a gene causes malfunction, and non-coding satellite regions, thus, not determining health or disease. The non-coding satellite sequences flank the coding regions or genes, as if they were their satellites. Each cell in the body normally carries two copies of each chromosomal gene called "alleles" (different versions of the same gene). One allele is inherited from the father, the other from the mother.

The production of proteins

The genetic code in the DNA encodes protein molecules, for example, structural proteins (e.g. tissues) or functional proteins (e.g. enzymes). That a gene can be expressed and a protein can be produced, transcription, splicing and translation are necessary.

Transcription: The two-stranded DNA is split into its two strands. One DNA-strand serving as a template for complementary base-pairing is transcribed to a ribonucleic acid (RNA) molecule, the precursor-messenger RNA (pre-mRNA) molecule, and further processed to a messenger RNA (mRNA) molecule. In RNA, instead of thymine (T) the quite similar nucleobase uracil (U) occurs, distinguished from thymine only by one methyl group.

Splicing: RNA exon splicing is a process in which the pre-mRNA transcript is transformed into messenger mRNA. All the non-coding regions of RNA, the introns, are removed and the coding regions, the exons, are spliced together. Splicing is necessary that an mRNA molecule can be translated into a protein.

Translation: The RNA-adapted G-C/A-U message of the genetic code is brought by mRNA from the nucleus to the cytoplasm. The single stranded mRNA is translated from the "nucleotide language" into the "amino acid language". Such as words in a book, the mRNA is read in one direction, thereby, translating the genetic code of the DNA sequence to the amino acid sequence in proteins. Triplets are composed of three nucleobases, deoxyribose or ribose and a phosphate group forming a nucleotide ("the word in the nucleotide language"), thus, a codon for a codon-specific different amino acid ("the word in the amino acid language") in the cytoplasm. Therefore, ribosomes in the cytoplasm are needed. Ribosomes consist of two subunits: one subunit decodes the mRNA, reading the nucleotide that corresponds to a specific amino acid; the other subunit forms the peptide bonds. The sequence of nucleotides ("the sentence in the nucleotide language") is translated in a sequence of amino acids which form in that way a chain of amino acids, the specific protein ("the sentence of the amino acid language"), thus, the structure of DNA determines the structure of proteins. mRNA is decoded with help from transfer ribonucleic acid (tRNA). tRNA transfers one after the other corresponding amino acid to the end of the amino acid chain until the entire sequence is translated into the specific protein.

Most of the DNA in a cell is found in the cell nucleus. A small portion of DNA is found in the cytoplasm, in mitochondria which contain a circular mitochondrial DNA (mtDNA). Mitochondria, and therefore mtDNA, are inherited exclusively from the mother and are responsible for a different pattern of exclusive maternal inheritance. Each cell in the body normally carries hundreds of copies of the mtDNA.

Patterns of inheritance

Genetic diseases are associated with different patterns of inheritance, autosomal recessive or dominant, heterosomal X-linked or Y-linked, polygenic and mitochondrial.

Autosomal recessive diseases are single gene (or "monogenic") disorders that occur only when an individual carries two malfunctioning copies (alleles with the same mutation) of the relevant gene. The patient with two mutant alleles is homozygous; the parents of the patient are most frequently heterozygous, which means that they possess one normal allele and one mutant allele. Because the normal allele dominates the mutant allele, the parents seem to be healthy. The parents are carriers. Such diseases tend to affect groups of brothers and sisters, which makes it appear as if the disease is spreading horizontally. An example is ataxia telangiectasia (Louis-Bar-syndrome), a neurodegenerative disorder caused by mutations in the *ATM* gene on chromosome 11q22.3. Ataxia telangiectasia is characterized by cerebellar degeneration, telangiectasia, immunodeficiency, cancer susceptibility and radiation sensitivity [1]. A morphological hallmark is ectatic capillaries in brain and elsewhere (Figure 1).

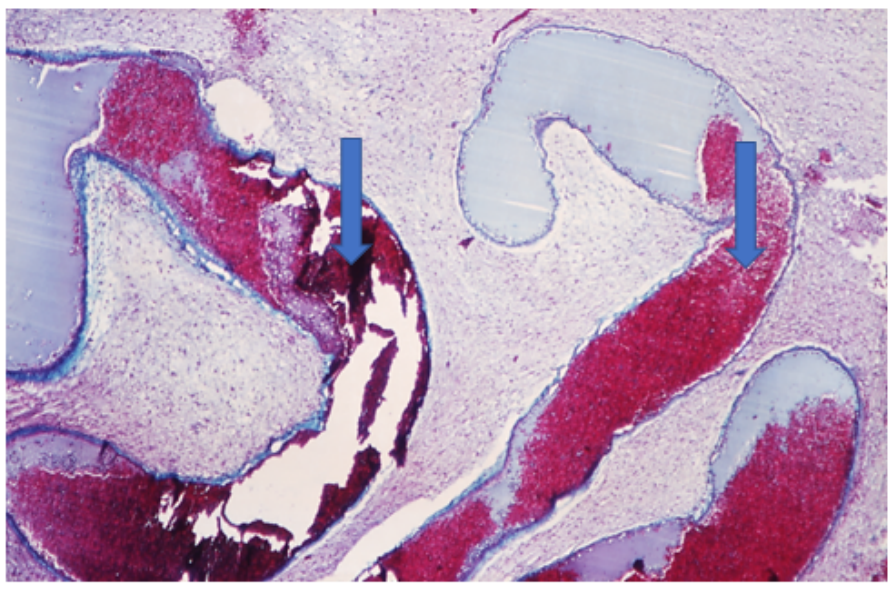

Figure 1: *Autosomal recessive ataxia telangiectasia (Louis-Bar syndrome) with intracerebral telangiectatic capillaries (arrows; Masson trichrome staining. x 18).*

Another example is Wilson disease with impaired copper metabolism. Wilson disease is caused by mutations in the *ATP7B* gene on chromosome 13q14.3, which encodes the copper-transporting ATPase 2 protein. Copper deposits can be detected especially in liver and brain (Figure 2). Wilson disease leads to neurological symptoms including tremor, dystonia, or parkinsonism [2].

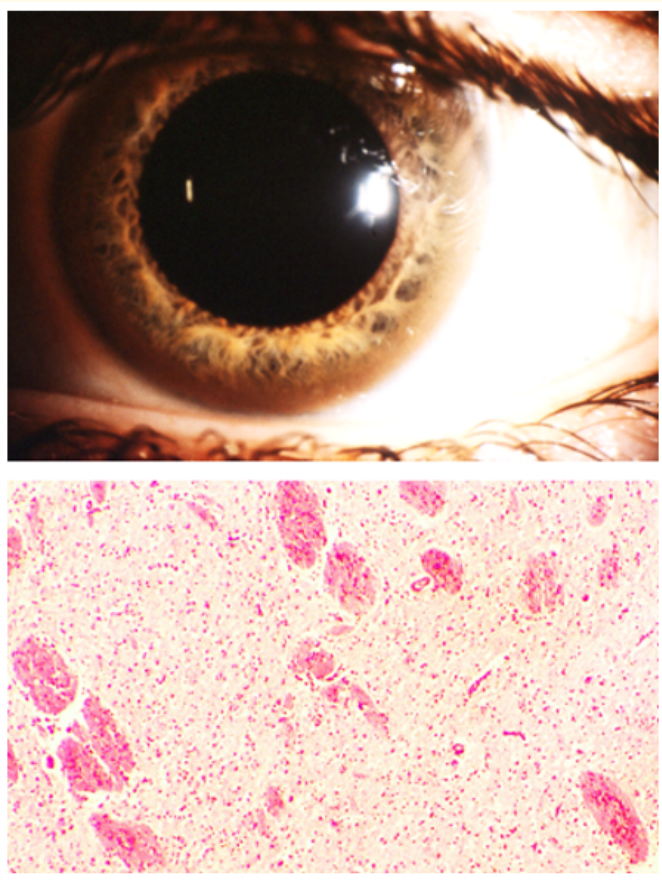

Figure 2: *Above: Autosomal recessive Wilson disease with Kayser Fleischer corneal ring caused by copper deposition in the peripheral Descemet membrane; Below: Wilson disease with abundant copper grains (all red dots) throughout the brain tissue concentrated and accumulated in the (darker) nerve cells of the putamen (rhodanine stain without counter stain. x 180).*

Autosomal dominant diseases are monogenic disorders that involve only one malfunctioning gene copy dominating the functional second copy and, thereby, causing disease. An example for an autosomal dominant single-gene disorder is spinal muscular atrophy, which is caused by mutation of the survival of motor neuron *SMN1* gene on chromosome 5q13.2. This leads to low SMN protein levels, responsible for the degeneration of motor neurons in the spinal cord and consequently muscular atrophy (Figure 3 left).

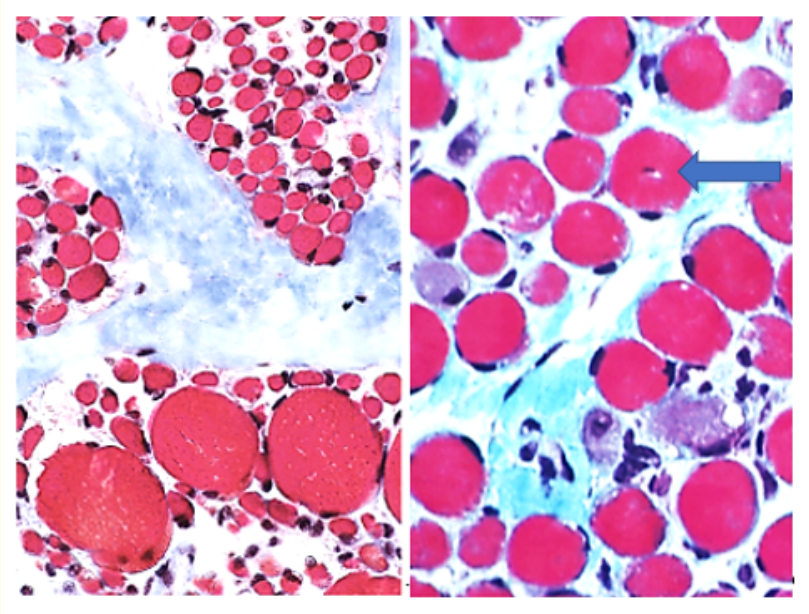

Figure 3: *Left: Autosomal dominant spinal muscular atrophy type 1 with large fields of not any longer innervated atrophic muscle fibers, the remaining innervated muscle fibers show a compensating hypertrophy; perimysial fibrosis with proliferation of fibrous tissue between the muscle fascicles (turquoise), the cell nuclei remain in subsarcolemmal position (dark blue); Right: Heterosomal X-linked Duchenne muscular dystrophy with necrotic muscle fibers (violet), endomysial fibrosis with proliferation of fibrous tissue even between single muscle fibers (turquoise), few centralized cell nuclei may occur (arrow). (Masson trichrome staining. x 180).*

Heterosomal X-linked diseases are monogenic diseases that are linked to malfunctioning genes on the X chromosome. These malfunctioning alleles are expressed especially in men who carry only one copy of the malfunctioning gene on the X chromosome (XY) which cannot be dominated by the Y chromosome. Women carry two X chromosomes (XX), so, if one allele of a gene is malfunctioning, it can be dominated by the functional allele on the second X chromosome; the woman is then a carrier. Examples for X-linked diseases are dystrophinopathies. The dystrophin gene (*DMD*) on chromosome Xp21.2 is the largest human gene and comprises more than two million base pairs with 89 exons. Mutations in the dystrophin gene lead to a lack or reduced levels of the dystrophin protein. Dystrophinopathies, dependent from site and extent of mutation, cover a spectrum of severe Duchenne muscular dystrophy (Figure 3 right) to less severe Becker muscular dystrophy [3].

Heterosomal Y-linked diseases comprise, for instance, extreme downregulation of chromosome Y as a possible male-specific pathway toward Alzheimer disease, increasing the age-related risk in men [4]. Another example is Jacob syndrome in which two Y chromosomes occur. Jacob syndrome (XYY) and Klinefelter syndrome (XXY) belong the "sex chromosome trisomies" occurring in 1 and 1.72 per 1000 male births, respectively. The patients frequently suffer from tremor and dystonia [5].

Polygenic diseases involve many genes in complex interactions, in addition to environmental influences. Since common gene variants with low predictive value and low penetrance may be associated to a specific disease, the applicability for risk prediction remains limited. One reason for this is that the prevalence of those variants in the general population is high, meaning that the majority of the populations has a "medium" risk. Also, a significant proportion of the "missing heritability" of common diseases may be attributable to low-frequency variants with intermediate penetrance effects [6]. For polygenic diseases, it might be helpful to improve the reporting standards for polygenic scores in risk prediction [7]. An example for a polygenic disease is epilepsy. More than 500 genes are associated with epilepsy [8]. Nevertheless, approaches to apply gene therapy exist [9], e.g. neuropeptide Y gene therapy is investigated for the treatment of patients with genetic generalized epilepsy [10].

Mitochondrial diseases are characterized by defects in oxidative phosphorylation and caused either by mutations in genes in the nuclear DNA (nDNA) that encode structural mitochondrial proteins, or by mutations in the circular mitochondrial DNA (mtDNA) that encode proteins regulating the mitochondrial function. Maternal inheritance means that the mtDNA in the human species is inherited exclusively from the mother. Mitochondrial dysfunction is involved in many adult-onset neurological diseases, including ALS, Alzheimer disease and Parkinson disease. Mitochondrial DNA (mtDNA) depletion syndromes (MDS) are a genetically and clinically heterogeneous group of autosomal recessive disorders that are characterized by reduction in mtDNA content leading to impaired energy production in affected tissues and organs. MDS are due to defects in mtDNA maintenance caused by mutations in nuclear genes that function either in mitochondrial nucleotide synthesis (*TK2, SUCLA2, SUCLG1, RRM2B, DGUOK, TYMP*) or in mtDNA replication (*POLG, C10orf2*). Mitochondrial depletion syndromes are phenotypically heterogeneous and usually classified as myopathic, encephalomyopathic, hepatocerebral or neurogastrointestinal [11]. An example of one of the most frequent maternally inherited mitochondrial diseases is MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes). The most common mutation associated with MELAS syndrome is in the RNA gene *MT-TL1* encoding the mitochondrial tRNA [12]. There may be an overlap with MERRF syndrome (myoclonic epilepsy with ragged red fibers), also showing the spongy encephalopathy quite typical for mitochondrial diseases (Figure 4). In MERRF, there is a mutation in the mitochondrial gene *MT-TK* in most cases; variants occur [13]. Neuropathologic alterations in the brain may show common features in disorders with different genetic background [14].

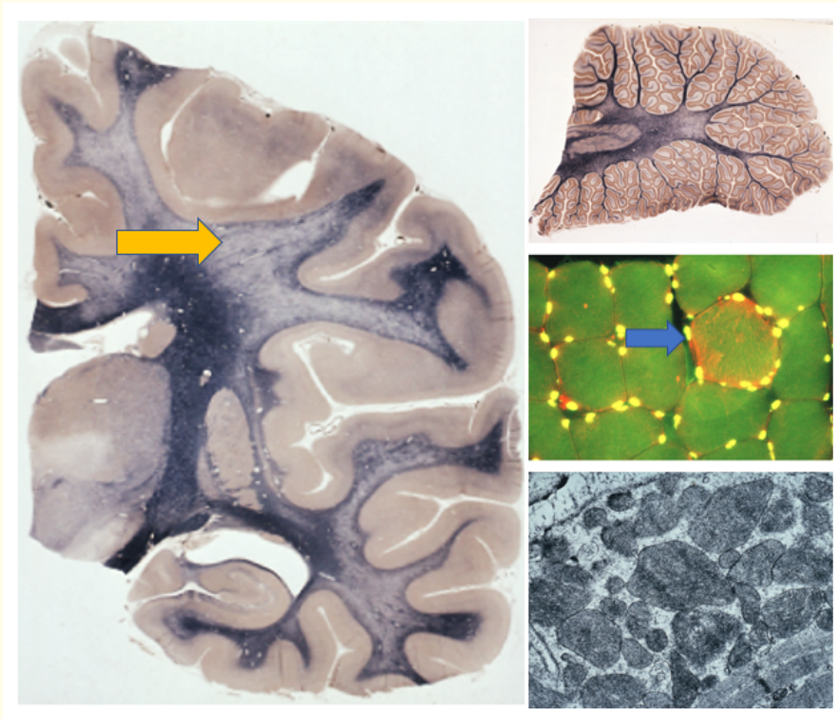

Figure 4: *Left: Mitochondrial disease MERRF syndrome with spongy encephalopathy of the white matter (arrow) of the cerebrum; Right above: MERRF with spongy encephalopathy of the white matter of the cerebellum (modified Weigert myelin staining); Middle: Skeletal muscle with one ragged red fiber (RRF; arrow) with massive multiplication of mitochondria, the nuclear DNA is yellow, the mitochondrial DNA is orange (acridine-orange staining. x 180); Below: Skeletal muscle with massive subsarcolemmal increase of mitochondria in number and size (transmission electron microscopy, x 10,000).*

Mutations

Mutations can cause disease, mutations can help in adaption to changed environmental conditions, or mutations can have no effect at all.

Point mutations occur, if one base is added and, on the complementary strand, the base pair is substituted (substitution), if one or more extra nucleotides are inserted during replication (insertion), or if one or more nucleotides are deleted during replication (deletion).

Nonsense mutations cause translation termination at in-frame premature termination codons. The substitution of a single base-pair causes a stop codon replacing a codon specifying an amino acid and terminating the production before the entire protein is formed.

Chromosomal mutations occur, if a region of a chromosome is inverted and reinserted (inversion), if a region of a chromosome is doubled (duplication), if a region of a chromosome is lost (deletion), or if a region of a chromosome is aberrantly translocated to another chromosome (translocation).

Copy number mutations occur, if the number of copies of a gene locus is increased (amplification) or if the number of trinucleotide sequences is expanded (trinucleotid repeats). An example for a trinucleotide repeats disorder is the fragile X syndrome characterized by intellectual disability and autism; its more than 200 repeats of the CGG motif in the *FMR1* gene on chromosome Xq27.3 lead to silencing of the gene and loss of the fragile X mental retardation 1 protein (FMRP). FMRP has a central role in gene expression and regulates the translation of potentially hundreds of mRNAs, many of which are involved in the development and maintenance of neuronal synaptic connections [15]. Another example for a trinucleotide repeats disorder with neurological symptoms is Huntington disease characterized by choreatic movements, psychiatric disturbances and dementia. Morphologically there is a gradual degeneration of the caudate nucleus and putamen (Figure 5). Huntington disease is caused by an elongated CAG repeat (36 repeats or more) in the *IT15* gene on chromosome 4p16.3 encoding the protein huntingtin. The longer the CAG repeat, the earlier the onset of disease. In juvenile cases the CAG repeat often exceeds 55 [16].

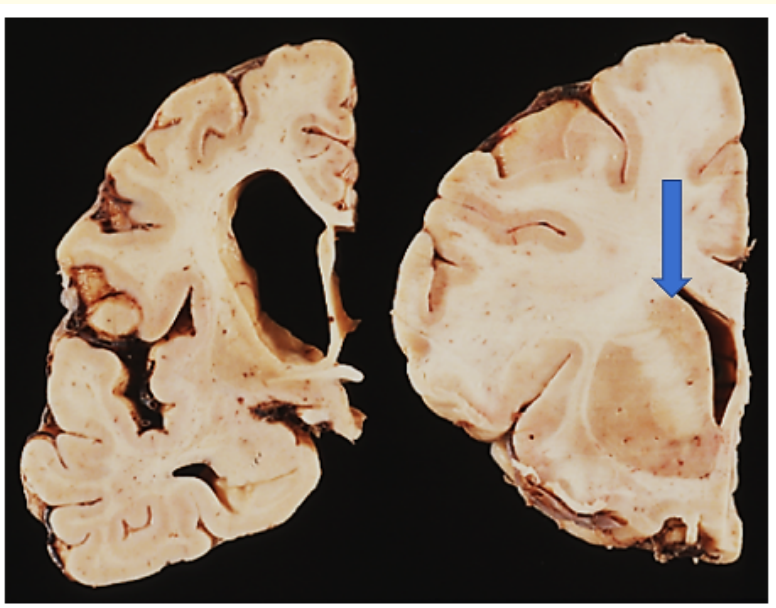

Figure 5: *Left: Trinucleotide CAG repeats Huntington disease with massive neurodegeneration of the striatum and an extended secondary hydrocephalus; Right: For comparison, normal brain showing the normal striatal structures (arrow) caudate nucleus and putamen separated by the internal capsule, and the normal slit-shaped lateral ventricle.*

Diagnostic methods

Genome-wide sequencing analysis has corroborated the estimation that over 10,000 of human diseases are single gene diseases or monogenic. The World Health Organization has estimated the global prevalence of all monogenic diseases at birth at approximately 1/100. However, striking differences in population-specific disease prevalence were described, with 101 autosomal recessive diseases (27%) being limited to specific populations, while an additional 305 diseases (68%) differed more than tenfold across major ethnogeographic groups [17].

Microarray testing uses the "normal" gene being examined as well as different variants of that gene for comparison. The DNA in a person's sample is labeled with a fluorescent dye, added to the microarray, and interpreted. Microarray-based comparative genomic hybridization (array-CGH) uses high numbers of short, synthetic, single-stranded DNA sequences.

Multiplex ligation-dependent probe amplification (MLPA) detects point mutations, single nucleotide polymorphisms (SNPs), deletions, duplications, identifies the methylation status of DNA, and quantifies DNA sequences and mRNA.

Gene expression profiling examines at which genes are turned on or off in cells by evaluating the RNA of specific genes. Gene expression profiling is ultimately aimed at developing a personalized therapeutic approach according to the individual patient's profile.

DNA sequencing determines if the sequence of nucleobases of a gene contains mutations or variants, which can be associated to a disease. Since traditional methods for detection of point mutations and other sequence variants of the estimated 22,000 genes require high cost and are time consuming, especially for larger genes, the use of next-generation sequencing (NGS) has become a useful tool available for clinical diagnosis [18]. Whole genome sequencing (both the coding and non-coding sections of the genes), and whole exome sequencing (all protein-coding sections, the exons, all of them together called the exome) have become feasible and financially viable at the individual level within hours or days. This progress has also been made possible thanks to powerful data banks. However, the extensive application of genetic testing also identifies variants of uncertain clinical significance.

Results of different therapeutic approaches

Gene transfer has long been focused on orphan genetic diseases, often monogenic. To date, more complex neurological diseases can be targeted for gene therapy.

In *ex vivo* gene therapy, to produce therapeutic factors, allogeneic cells are transplanted, or autologous cells are genetically modified outside of the body and subsequently transplanted back into patients.

In *in vivo* gene therapy, the genetic material is delivered to target malfunctioning cells that remain inside the patient's body. Gene editing allows to silence, repair, and amend endogenous genes. Safety remains a matter of discussion, which contemporaneously shows the tragical deaths of three male children with X-linked myotubular myopathy receiving high-dose gene therapy in 2020 [19]. High impact, but high risk?

Cell transplantation and stem cell therapy

Before autologous cells could be genetically modified, cell transplantation of allogeneic cells was a means to compensate malfunction. Almost 40 years ago, in the mdx mouse model of Duchenne muscular dystrophy mice, from healthy donor mice transplanted myoblasts expressing the functional donor gene were fused with host myofibers. Subsequent investigations verified the presence of dystrophin in treated animals. However, the dystrophin expression remained low, due to immune rejection and low survival of transplanted myoblasts [20,21]. In another approach, more than 30 years ago, the transplantation of dopamine neurons from human fetal brain tissue from

abortions was examined [22]. Quite apart from the difficult ethical background, the survival of the allogeneic dopaminergic graft was limited.

In a next step, to avoid immune rejection, the use of autologous cells transduced with a vector containing a healthy copy of the mutated gene should allow production of the defective protein, thereby achieving a correction of target cells in multiple tissues, including the central nervous system. For neurological diseases, autologous cells, e.g. neural stem/progenitor cells, induced pluripotent stem cells (iPSC) and mesenchymal stem cells, are tempting cell sources. Recently, human Wharton jelly mesenchymal stem cells with high expression of Aurora kinase A, a serine/threonine kinase essential for the onset and progression of mitosis, were explored and might have therapeutic efficacy [23]. The synergy between the effect of the "new" *ex vivo* cell and the additional engineered properties can often provide significant benefits to neurodegenerative changes in the brain with a focus on Parkinson disease or Alzheimer disease [24]. In Parkinson disease, the aim of cell transplantation and different gene therapy trials is to functionally recover dopaminergic signaling [25]. Glial cell line-derived neurotrophic factor (GDNF)/neurturin (NRTN) treated patients showed limited signs of restorative effects, obviously because the level of the GDNF signaling receptor Ret is overall markedly reduced relative to the non-Parkinson disease controls [26]. Promising observations made in two patients who had received AAV-NRTN gene therapy 8 - 10 years earlier, suggest the possibility that NRTN is able to restore Ret expression and upregulate its own signaling pathway, likely depending on an interaction with dopaminergic transcription factor Nurr1 [27]. Human *Nurr1* gene on chromosome 2q22-23 encode Nurr1 protein, which is predominantly expressed in central dopaminergic neurons [28]. Recently, aggregation and cellular transport of α-synuclein have become therapeutic targets. [29]. The protein alpha-synuclein (α-Syn) is a key contributor to the etiology of Parkinson disease, therefore, gene therapy by modulating gene expression could ameliorate α-Syn induced neurotoxicity [30].

Neurosurgical techniques such as interventional MRI-guided convection-enhanced delivery (iMRI-CED) are applied to distribute gene-carrying viral vectors across the blood-brain barrier. These vectors can be monitored using real-time MRI of a co-infused tracer to accurately demonstrate vector distribution and transgene expression at the perfused site [31,32]. Other innovations such as cannula design, chronically implanted infusion devices and focal opening of the blood-brain barrier with focused ultrasound have been developed or are investigated [33].

Mitochondrial diseases are an exemption again. An *ex vivo* therapeutic approach is *in vitro*-fertilization including mitochondrial donation to compensate malfunction of mtDNA. This may allow female patients with mitochondrial diseases to have healthy children [34].

The challenge of gene modification

Genome editing tools allow to repair, amend and silence endogenous genes.

Antisense oligonucleotide therapy: Antisense oligonucleotides (ASOs) are synthetic oligonucleotides, can be produced by plasmids encoding for small nuclear RNA particles and achieve wide tissue distribution with long half-lives. ASOs alter mRNA expression through different mechanisms. ASOs alter pre-mRNA splicing to promote exon exclusion, thus, alter the expression of disease-associated transcripts. ASO-mediated exon skipping therapy can be applied by targeting affected exons with predesigned ASOs, e.g. to produce a shorter but working version of the defective protein. ASOs that function through ribonuclease H or the RNA-induced silencing complex (RISC) result in enzymatic degradation of the target RNA (ribonuclease H-mediated decay of the pre-mRNA). ASOs designed to sterically block access of proteins to the RNA modulate mRNA metabolism but do not typically cause degradation; steric-blocking oligonucleotides are short, single-stranded nucleic acids designed to modulate gene expression by binding to RNA transcripts and blocking access from cellular machinery such as splicing factors [35]. A new approach is the conjugation of ASOs with lipophilic compounds to explore the influence of the lipophilic moiety on exon skipping efficiency [36].

RNA interference for gene silencing: RNA interference is applied for sequence-specific gene silencing by targeting mRNA for degradation [37]. The major barrier to realizing the full potential of RNA interference is the difficulty of delivering effector molecules, such as small interfering RNAs (siRNAs), *in vivo*. siRNAs are 19 - 23 nucleotide long and induce mRNA degradation through nuclease activity. However, siRNAs are rapidly degraded by endonucleases and require repeated invasive injection into the central nervous system. [38]. They can be continuously encoded by plasmids and can be delivered using viral vectors for stable transduction.

CRISPR/Cas9-mediated gene editing: To obtain permanent gene expression, gene editing aims to correct genomic deficits. The clustered regulatory interspaced short palindromic repeats (CRISPR)-based therapy uses the CRISPR/Cas9-based gene editing system consisting of the endonuclease Cas9 and a single-guide RNA (sgRNA). The Cas9 endonuclease produces DNA double-strand break at the editing sequence targeted by sgRNA. Obviously, the sgRNA vector dose determines the outcome of systemic adeno-associated virus-CRISPR therapy in the attempt to restore the missing protein. Subsequent non-homologous end-joining leads to exon-skipping, thus, to skip over malfunctioning exons, which eventually leads to a truncated but still functional protein. Homology-directed repair could replace gene mutations with correct sequences and generate the normal protein [39]. Recent developments comprise engineered microRNA and *in vivo* CRISPR-based therapeutics [40].

In February 2016, the Human Fertilisation and Embryology Authority in the UK approved a request by the Francis Crick Institute London, to modify human embryos by using this new gene-editing technique; the details are described by the Francis Crick Institute London as follows [41]: "The embryos used in the research project are left over from patients' fertility treatment and donated by patients. They are surplus to the patients' treatment or family-building needs, and so would otherwise be disposed of. The embryos will never be used to establish a pregnancy. The research focuses on the first seven days of development after fertilisation and none of the embryos the team do research on will ever be grown past a maximum of 14 days after fertilisation, in accordance with legislation. It is important to note that these potential improvements in infertility treatment would not be delivered through genome editing: it is illegal to implant an embryo modified in this way into a human." Thus, CRISPR/Cas9-mediated gene editing can modify disease-causing (and potentially other) genes in embryos brought to term; by some comments, as major ethical objection it is stated that this technique may be misused to genetically design the "perfect" man.

Read-through therapy: The read-through approach can overcome a stop codon. To treat nonsense mutations, an alternative amino acid is inserted at the point of the premature termination codon to allow translational read-through, so that a functional protein can be generated [42]. Nonsense suppression therapy includes read-through drugs, suppressor tRNAs, premature termination codon-pseudouridylation, and inhibition of nonsense-mediated mRNA decay [43].

The challenge of gene sequence delivery

A critical challenge is achieving the most complete transduction of the target structure while avoiding leakage into neighboring regions or perivascular spaces. For *in vivo* vector-mediated gene therapy, there are different non-viral and viral strategies how to deliver genetic material to target malfunctioning cells.

Particle-mediated gene delivery, such as by means of nanoparticles, polymers, and exosomes, can effectively transfer genes into primary neurons. The resulting gene expression can be long-term without immunological complications. Lipid- and natural polymer-based nanoparticles for genetic material transfer to the nervous system have low toxicity, but also low transfection efficiency, also due to difficult blood-brain barrier crossing. Various nanoparticles include natural inorganic particles, natural polymers, cationic lipids, polyethylenimine derivatives, dendrimers, and carbon-based nanoparticles [44]. An example is Lipofectamine™ 2000, a cationic liposome-based reagent, that provides high transfection efficiency and high levels of transgene expression in a range of mammalian cell types including neurons *in vitro* [45].

Plasmid transfection adds a DNA plasmid into a cell. Plasmids are small circular DNA molecules, resembling the circular mitochondrial DNA in humans, and naturally occur in bacteria to transfer genetic information. Plasmids can be transfected into human target cells to express a gene of interest in that specific cell line. Plasmids can be complexed with lipid-based nanoparticles for an efficient delivery into the cell's nucleus. Lipid nanoparticles containing distearoylphosphatidylcholine and ionizable amino-lipids such as dilinoleylmethyl-4-dimethylaminobutyrate are potent siRNA delivery vehicles *in vivo* [46].

Viral strategies use lentiviral (LV) and adeno-associated virus (AAV) vectors. LV vectors have a broad tropism and transduce dividing and non-dividing cells close to the injection site. They integrate into the host genome, cause a long-term transgene expression in dividing cells, but bear a mutagenic risk. In cases of brain injury following stroke and in cases of traumatic brain injury, therapeutic LV vector approaches aim to generate new neurons within the damaged brain tissue [47,48]. AAV vectors have many advantages for clinical application. They also transduce dividing and non-dividing cells, persist in the cells as extra-chromosomal episomes, are non-pathogenic and have a low risk of insertional mutagenesis. They possess a specific tropism for different cell types, including neurotropism, according to serotypes. Improved vectors could be generated from newly discovered AAV serotypes (such as AAV6, 8 and 9) which, when injected into the vasculature at high dose (in the range of 10^{14} vector genomes per kilogram), transduce the targeted tissue. The serotype AAV9 has been widely studied for its ability to cross the blood-brain barrier to transduce astrocytes, but its efficiency is limited. The variants derived from AAV9 directed evolution advances in AAV engineering, and recent developments in cargo design have been shown to have significantly higher crossing efficiency than AAV9 [49,50].

Discussion of the transition into the clinical routine

These recent developments of therapeutic approaches are recorded by the pharmaceutical industry and are increasingly mirrored in daily clinical practice. Meanwhile the first gene therapy drugs licensed in the USA and the European Union are coming onto the market.

As already mentioned above, spinal muscular atrophy is caused by low levels of the survival motor neuron protein (SMN) due to mutations in the encoding gene *SMN1.* A second duplicated gene, *SMN2*, produces little but sufficient functional protein for survival. The first *SMN2*-directed antisense oligonucleotide (ASO) therapy has recently been licensed [51]. Intravenous injection of onasemnogene abeparvovec, an AAV9 expressing *SMN1* (Zolgensma®), showed a significant increase of the lifespan and motor function of the patients younger than 4 years and has been approved for the infant form of spinal muscular atrophy by the US Food and Drug Administration (FDA) [52,53]. Another FDA-approved approach with recombinant AAV-vectors are the gene therapies for spinal muscular atrophy associated with inherited retinal dystrophy due to a mutation of the *RPE65* gene on chromosome 1p31 [54,55].

In the treatment of amyotrophic lateral sclerosis, the delivery of antisense oligonucleotides for patients with mutations in the superoxide dismutase gene *SOD1* on chromosome 21q22.1 and/or the *C9orf72* gene on chromosome 9p21.2 are involved [56].

In Duchenne muscular dystrophy, eteplirsen (Exondys 51™) is the first FDA-approved treatment and targets specific mutations of the *DMD* gene. A randomized controlled trial provided Class II evidence of the muscle cell penetration, exon skipping and induction of novel dystrophin expression by eteplirsen, as confirmed in four assays [57].

Conclusion

Especially for monogenic diseases, quite a number with neurological background, specifically targeted gene editing in combination with non-integrative vectors may expand the possibilities for a future daily clinical application of gene therapy in neurology.

For more complex neurological diseases, a multimodal approach of gene therapy combined with transplantation of *ex vivo* modified autologous cells might be hopeful. This has the potential to lead to a treatment of hitherto not curable neurological diseases with a life-changing therapy.

Conflict of Interest

The author declares that he has no conflict of interest.

Bibliography

1. Rothblum-Oviatt C., *et al*. "Ataxia telangiectasia: a review". *Orphanet Journal of Rare Diseases* 11.1 (2016): 159.
2. Samanci B., *et al*. "Neurological features and outcomes of Wilson's disease: a single-center experience". *Neurological Sciences* 42.9 (2021): 3829-3834.
3. Thangarajh M. "The dystrophinopathies". *Continuum* 25.6 (2019): 1619-1639.
4. Caceres A., *et al*. "Extreme downregulation of chromosome Y and Alzheimer's disease in men". *Neurobiology of Aging* 90 (2020): 150. e1-150.e4.
5. Davis JL., *et al*. "Tremor and dystonia in Jacob's syndrome (47,XYY)". *Movement Disorders Clinical Practice* 7.1 (2019): 107-108.
6. McCarthy MI., *et al*. "Genome-wide association studies for complex traits: consensus, uncertainty and challenges". *Nature Reviews Genetics* 9.5 (2008): 356-369.
7. Wand H., *et al*. "Improving reporting standards for polygenic scores in risk prediction studies". *Nature* 591.7849 (2021) 211-219.
8. Devinsky O., *et al*. "Epilepsy". *Nature Reviews Disease Primers* 4 (2018): 18024.
9. Turner TJ., *et al*. "Recent advances in gene therapy for neurodevelopmental disorders with epilepsy". *Journal of Neurochemistry* 157.2 (2021): 229-262.
10. Powell KL., *et al*. "Gene therapy mediated seizure suppression in genetic generalised epilepsy: neuropeptide Y overexpression in a rat model". *Neurobiology of Disease* 113 (2018): 23-32.
11. El-Hattab AW and Scaglia F. "Mitochondrial DNA depletion syndromes: review and updates of genetic basis, manifestations, and therapeutic options". *Neurotherapeutics* 10.2 (2013): 186-198.
12. El-Hattab AW., *et al*. "MELAS syndrome: clinical manifestations, pathogenesis, and treatment options". *Molecular Genetics and Metabolism* 116.1-2 (2015): 4-12.
13. Velez-Bartolomei F., *et al*. "MERRF". In: Adam MP. *et al*., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle (2003): 1993-2022.
14. Molnar MJ and Kovacs GG. "Mitochondrial diseases". *The Handbook of Clinical Neurology* 145 (2017): 147-155.
15. Hagerman RJ., *et al*. "Fragile X syndrome". *Nature Reviews Disease Primers* 3 (2017): 17065.
16. Roos RA. "Huntington's disease: a clinical review". *Orphanet Journal of Rare Diseases* 5 (2010): 40.
17. Xiao Q. and Lauschke VM. "The prevalence, genetic complexity and population-specific founder effects of human autosomal recessive disorders". *NPJ Genomic Medicine* 6.1 (2021): 41.
18. Falzarano MS., *et al*. "Duchenne muscular dystrophy: from diagnosis to therapy". *Molecules* 20.10 (2015):18168-18184.
19. Privolizzi R., *et al*. "Viral gene therapy for paediatric neurological diseases: progress to clinical reality". *Developmental Medicine and Child Neurology* 63.9 (2021): 1019-1029.

20. Watt DJ., *et al*. "Use of mononuclear precursor cells to insert allogeneic genes into growing mouse muscles". *Muscle Nerve* 7.9 (1984): 741-750.

21. Partridge TA., *et al*. "Conversion of mdx myofibres from dystrophin-negative to -positive by injection of normal myoblasts". *Nature* 337.6203 (1989): 176-179.

22. Brundin P., *et al*. "Practical aspects of the use of human fetal brain tissue for intracerebral grafting". *Progress in Brain Research* 82 (1990): 707-714.

23. Kim SJ., *et al*. "Wharton's jelly-derived mesenchymal stem cells with high Aurora kinase A expression show improved proliferation, migration, and therapeutic potential". *Stem Cells International* (2022).

24. Gowing G., *et al*. "Ex vivo gene therapy for the treatment of neurological disorders". *Progress in Brain Research* 230 (2017): 99-132.

25. Van Laar AD., *et al*. "An update on gene therapy approaches for Parkinson's disease: restoration of dopaminergic function". *The Journal of Parkinson's Disease* 11.s2 (2021): S173-S182.

26. Tenenbaum L and Humbert-Claude M. "Glial cell line-derived neurotrophic factor gene delivery in Parkinson's disease: a delicate balance between neuroprotection, trophic effects, and unwanted compensatory mechanisms". *Frontiers in Neuroanatomy* 11 (2017): 29.

27. Björklund A. "GDNF therapy: can we make it work?". *The Journal of Parkinson's Disease* 11.3 (2021): 1019-1022.

28. Jankovic J. *et al*. "The role of Nurr1 in the development of dopaminergic neurons and Parkinson's disease". *Progress in Neurobiology* 77.1-2 (2005): 128-138.

29. Poewe W., *et al*. "Parkinson disease". *Nature Reviews Disease Primers* 3 (2017): 17013.

30. Sandoval IM., *et al*. "Gene therapy to modulate alpha-synuclein in synucleinopathies". *The Journal of Parkinson's Disease* 11.2 (2021): S189-S197.

31. Lonser RR., *et al*. "Direct convective delivery of adeno-associated virus gene therapy for treatment of neurological disorders". *Journal of Neurosurgery* 134.6 (2020): 1751-1763.

32. Sudhakar V and Richardson RM. "Gene therapy for neurodegenerative diseases". *Neurotherapeutics* 16.1 (2019): 166-175.

33. Larson PS. "Improved delivery methods for gene therapy and cell transplantation in Parkinson's disease". *The Journal of Parkinson's Disease* 11.s2 (2021): S199-S206.

34. Gorman GS., *et al*. "Mitochondrial diseases". *Nature Reviews Disease Primers* 20.2. (2016): 16080.

35. Holgersen EM., *et al*. "Transcriptome-wide off-target effects of steric-blocking oligonucleotides". *Nucleic Acid Therapeutics* 31.6 (2021): 392-403.

36. Marchesi E., *et al*. "Antisense oligonucleotides conjugated with lipophilic compounds: synthesis and in vitro evaluation of exon skipping in Duchenne muscular dystrophy". *International Journal of Molecular Sciences* 23.8 (2022): 4270.

37. Gavrilov K. and Saltzman WM. "Therapeutic siRNA: principles, challenges, and strategies". *Yale Journal of Biology and Medicine* 85.2 (2012): 187-200.

38. Han H. "RNA interference to knock down gene expression". *Methods in Molecular Biology* 1706 (2018): 293-302.

39. Babačić H., *et al*. "CRISPR-cas gene-editing as plausible treatment of neuromuscular and nucleotide-repeat-expansion diseases: a systematic review". *PLoS One* 14.2 (2019): e0212198.

40. Pena SA., *et al*. "Gene therapy for neurological disorders: challenges and recent advancements". *Journal of Drug Targeting* 28.2 (2020): 111-128.

41. Niakan K. "Human embryo genome editing licence". *The Francis Crick Institute* London, (2016).

42. Malik V., *et al*. "Gentamicin-induced readthrough of stop codons in Duchenne muscular dystrophy". *Annals of Neurology* 67.6 (2010): 771-780.

43. Keeling KM., *et al*. "Therapeutics based on stop codon readthrough". *Annual Review of Genomics and Human Genetics* 15 (2014): 371-394.

44. Pérez-Martínez FC., *et al*. "The use of nanoparticles for gene therapy in the nervous system". *Journal of Alzheimer's Disease* 31.4 (2012): 697-710.

45. Dalby B., *et al*. "Advanced transfection with Lipofectamine 2000 reagent: primary neurons, siRNA, and high-throughput applications". *Methods* 33.2 (2004): 95-103.

46. Kulkarni JA., *et al*. "Design of lipid nanoparticles for in vitro and in vivo delivery of plasmid DNA". *Nanomedicine* 13.4 (2017): 1377-1387.

47. Vandeputte C., *et al*. "Bioluminescence imaging of stroke-induced endogenous neural stem cell response". *Neurobiology of Disease* 69 (2014): 144-155.

48. Geribaldi-Doldán N., *et al*. "Specific inhibition of ADAM17/TACE promotes neurogenesis in the injured motor cortex". *Cell Death and Disease* 9.9 (2018): 862.

49. Liu D., *et al*. "Crossing the blood-brain barrier with AAV vectors". *Metabolic Brain Disease* 36.1 (2021): 45-52.

50. Zhu D., *et al*. "Adeno-associated virus vector for central nervous system gene therapy". *Trends in Molecular Medicine* 27.6 (2021): 524-537.

51. Bowerman M., *et al*. "SMA Research Consortium. Therapeutic strategies for spinal muscular atrophy: SMN and beyond". *Disease Models and Mechanisms* 10.8 (2017): 943-954.

52. Barkats M. "Amyotrophie spinale infantile - De la découverte du gène à la thérapie génique". *Medical Sciences* 36.2 (2020): 137-140.

53. Kariyawasam D., *et al*. "Great expectations: virus-mediated gene therapy in neurological disorders". *Journal of Neurology, Neurosurgery, and Psychiatry* 91.8 (2020): 849-860.

54. Flotte TR., *et al*. "Gene therapy for rare neurological disorders". *Clinical Pharmacology and Therapeutics* 111.4 (2022): 743-757.

55. Tang PH., *et al*. "RPE65 is present in human green/red cones and promotes photopigment regeneration in an in vitro cone cell model". *The Journal of Neuroscience* 31.50 (2011): 18618-18626.

56. Cappella M., *et al*. "Gene therapy for ALS - a perspective". *International Journal of Molecular Sciences* 20.18 (2019): 4388.

57. Charleston JS., *et al*. "Eteplirsen treatment for Duchenne muscular dystrophy: exon skipping and dystrophin production". *Neurology* 90.24 (2018): e2146-e2154.

Volume 14 Issue 7 July 2022