Review

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A New Era Has Begun in Neurology Thanks to Gene and Biotechnologies

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Abstract

Human genetics has evolved considerably since the discovery of DNA. New gene- and biotechnologies invented for the last three decades have inspired novel treatments for diseases that were once accepted as untreatable. Congenital neuromuscular disorders and neurodegenerative diseases are typical examples of these diseases. Novel emerging trials demonstrate promising results to alter the poor prognosis of these unfortunate patients.

Keywords: Genetics, neurogenetics, gene technologies, biotechnology, gene therapy

INTRODUCTION

Human genetics has covered remarkable advances over the last three decades to be merited as a genetic revolution. Actually, this advancement has been achieved in conjunction with tremendous progress in the field of molecular biology. The first identification of human disease-associated genes in the 1980s and the sequencing of whole human genome in 2001 were two major steps in this development. These advances stimulate hope-inspiring approaches for the practice of medicine in general and the practice of neurology in particular. The leap in the field is great, so the main theme of the 4th Congress of the European Academy of Neurology in 2018 was neurogenetics (1).

Advances in Gene- and Biotechnologies

In 1953, the genetics era started with the discovery of the double helix DNA. In 1983, polymerase chain reaction was discovered and entirely modified DNA studies. Molecular analysis of mammalian genes has changed greatly ever since. In 1977, Sanger published an article about his DNA sequencing method. Sanger's approach widely spread across the research community and finally integrated into clinical diagnostics. The sequencing of the first human genome-Human Genome Project-was accomplished by using the Sanger method. The sequencing lasted 13 years and was completed in 2001, with an estimated cost of $2.7 billion. In 2008, the time needed to sequence human genome declined to 5 months, and the cost decreased to $1.5 million. Today, the sequencing can be performed in a couple of days with a cost of $10,000. In 2005, the launch of the first massively parallel pyrosequencing platform for commercial use made the subsequent progress possible. This led to high-throughput genomic analysis now referred to as next-generation sequencing (NGS). NGS platforms share a common technological feature, namely, massively parallel sequencing of clonally amplified or single DNA molecules. This design is much faster than Sanger sequencing and surveys high numbers of specimen. It is expected that the time and cost will decrease more in the very near future, making these technologies affordable for more researchers (2).

Table 1 summarizes the major milestones in gene- and biotechnologies.

Technologies for Gene Therapy

Gene therapy is designed to introduce the genetic material into the cells to repair abnormal genes. If a mutated gene damages an essential protein, the functionality of that protein may recover through gene therapy. Gene therapy can be successful by refinement of gene delivery

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Table 1. Milestones in gene technologies

1953	Discovery of the DNA double helix
1983	Discovery of PCR- changing the DNA study method
1977	Sanger's DNA sequencing method (technology)
1994 1998	Emerging of Massive parallel sequencing (MPS) - high throughput approach to DNA sequencing.
2001	Sequencing of the first human genome
2002	First successful GWAS (about myocardial infarction) was published
2005	Commercialising of MPS - Next generation sequencing
2007	Array- based hybrid capture method: target enrichment strategy to be applied to whole exome sequencing
2011	Third generation sequencing Cheaper and faster sequencing of human genome

Table 2. Technologies for gene therapy

Virus mediated gene therapy	Viruses capable of invading selected tissues deliver desired gene to target cell populations and induce long-term expression
Short synthetic nucleotids	Antisense oligonucleotids (ASO) and RNA interference (RNAi) modify disease proteins by targeting RNA/DNA precursors
Genomic DNA editing / engineering	Uses the CRISPS/CAS9 system to remove sections of malfunctioning (mutated) genomic DNA and replace these with normal sequences
Polymer encapsulated cell technology	A nonviral approach -uses a semipermeable membrane that allows free exchange of nutrients, oxygen and therapeutic gene products while shielding the implanted cells from host immune system and preventing uncontrolled cellular proliferation and mass formation
Convection-enhanced delivery technique	circumvents the BBB in delivering agents directly into the brain

systems. Researchers have been concentrating on nonviral and viral gene transfer vectors. Various physical and chemical nonviral methods exist to introduce DNA and mRNA to mammalian cells. Much of these methods have been developed for gene therapy procedures.

Viruses are the most eligible vectors for the delivery of therapeutic agents, and significant numbers of clinical trials employ this technology. Adenoviruses (Ads) are the most commonly researched viral vectors. Recombinant adeno-associated virus (AAV) vector-mediated gene therapy has demonstrated to be effective in certain conditions (3).

In recent years, genome editing technologies have been developed based on engineered or bacterial nucleases. Genome editing methods provide opportunities for gene addition, gene ablation, and gene correction in contrast to viral vectors that can mediate only gene addition. Multiple clinical genome editing trials aiming to integrate these new technologies to patient care are ongoing (4).

Table 2 outlines the current technologies for gene therapy.

Gene- and Biotechnologies in Neurology

In 1986, the identification of the Duchenne muscular dystrophy (DMD) gene was the first for neurological disorders. Others followed in rapid succession. Disease-associated genes have been discovered perpetually. As a consequence, genetic testing should be integrated into clinical practice. In 1995, barely 10 commercially available tests relevant to neurology were available. Now, there are several hundred tests to all areas of clinical neurology, including neuromuscular disorders, dementias, movement disorders, strokes, and white matter diseases. Comprehensive open sources for these tests are being updated regularly. For instance, Gene Reviews currently comprises 721 chapters focused on a single gene or phenotype. There are also overviews summarizing the genetic causes of common conditions, such as Alzheimer's disease (5).

New Perspectives in Neurology in the Context of Gene- and Biotechnologies

More genes are being discovered constantly relevant to neurological disorders. As a consequence, new options are revealed day by day in diagnosing and treating these disorders. This is becoming truer as the use of clinical exome and genome sequencing becomes increasingly widespread. Technological advancements pave the way for the genomic medicine era. Clinicians are now faced with the problem of associating this new genetic information with daily clinical practice (6).

New genetic and molecular biology information has promoted new approaches in diagnosis, genetic counseling, prognosis, and treatment.

Evaluation and Diagnosis

The vast number of genetic testing available for single gene disorders and for genomic variation makes the evaluation and diagnosis increasingly easier. Many commercial laboratories provide tests for Mendelian disease genes, and in some instances, genetic testing has been routinely used similar to other common blood tests (7). For instance, in a Germany-based laboratory with a large panel approach, 351 genes that are associated with hereditary neurodegenerative diseases are sequenced by NGS. Twenty-nine sets of genes are engaged due to main disease types. For example, they can scan 40 genes for the diagnosis of dystonia. Using a large panel approach, this laboratory revealed that its sensitivity and specificity are 99.7% and 99.9%, respectively, in diagnosis (8).

Clinical examination is a prerequisite to define the patient's phenotype, which will in turn propose the most proper conditions for genetic testing. As bio- and gene technologies proceed, the secrets of human genetic variation are increasingly revealed as well. Therefore, it is easier to associate these findings to clinical phenotype nowadays. Traditionally, in single gene (Mendelian) disorders, patients were assumed to be either healthy or diseased depending on their genetic condition. Friedreich ataxia (FA) or Huntington disease is an example of Mendelian disorder. However, common neurodegenerative dis-

Table 3. Gene therapy trials for neurological disorders

Alzheimer's disease	Immunotherapy Vaccine Neurotrophic factors Anti-inflammation	Aß antibody Aß cDNA IGF2 IL-4	Decreased Aß deposition in AD mouse models Decreased Aß deposition, improved memory and cognition ability Promoted dendritic spine formation, restored normal hippocampal excitatory synaptic transmission in AD mouse model Reduced astro-/microgliosis, enhanced neurogenesis, improved spatial learning in AD mouse model
Parkinson's disease	Dopamine biosynthesis enzyme STN activity modulation Neurotrophic factors	AADC GAD NTN	AAV2-AADC delivery into putamen alleviated motor symptoms in moderate and advanced PD patients Stable and persistent transgene expression Bilateral delivery of AAV2-GAD65/67 into STN of advanced PD patients provide modest improvement İntraputaminal injection of CERE-120 (AAV2-NTN) resulted in improvement in motor function
Spinal muscular atrophy	Stimulate SMN2 exon 7 to increase SMN protein concentrations	SMN1	Nusinersen has recently been licenced for treatment for SMN
Duchenne muscular distrophy	Skipping of exons to correct reading frame disruptions using antisense oligonucleotides (ASO)	Dystrophin	Antisense oligonucleotide Eteplirsen reduce the severity of DMD and produce a milder phenotype
Familial amyloid polyneuropathy	Degradation of thransthyretin mRNA	Transthyretin (TTR)	Antisense oligonucleotide inotersen and RNAi patisiran both completed phase III clinical trials. Aim is to deplete total TTR levels to restrict amyloid deposition
Friedreich ataksia	Restoring wild-type gene expression levels and reversing cellular transcription changes	Frataxine	Correction of changes induced by frataxin downregulation, sustained elevation of frataxin mRNA and protein a phase I study to increase frataxin levels in peripheral blood mononuclear cells
Fabry disease	Recombinant enzyme replacement Increasing GLA activity	Alpha-galactosidase A (GLA)	Agalsidase alfa (Fabrazyme) decreases globotriaosylceramide (GL-3) accumulation, was approved by FDA in 2003. Oral migalastat (Galafold) was approved by FDA in August 2108
Pompe disease	Recombinant enzyme replacement	Acid α-glucosidase	Myozyme enabled all patients to live to the age of 18 months, a 99% reduction in death, was approved by FDA in 2006
Huntington's disease	Mutant HTT Knockdown Neurotrophic factors	shRNA siRNA GDNF CNTF	Reduced brain atrophy, rescue of motor deficits and increase in survival in HD mouse model Complete elimination of mutant HTT-positive inclusions with improved behavioural deficits in HD mouse model Improved motor function and increased striatal neuron survival Transplantation of polymer-encapsulated BHK cells secreting CNTF into the lateral ventricles of HD patients improved electrophysiologically
Amyotrophic lateral sclerosis	Mutant SOD1 Knockdown Nonsense mediated mRNA decay Neurotrophic factors	shRNA UPF1 GDNF VEGF	Delayed disease onset, enhanced survival of spinal motor neurons, expanded lifespan in ALS SOD1 rat model Preservation of forelimb function and improved motor scores in ALS TDP43 rat model İntramuscular injection of AAV-GDNF delayed disease onset and prolonged survival in ALS mouse model Ex vivo delivery of GDNF andVEGF showed synergistic effect in ALS SOD mouse model
Stroke	Anti-ischemia induced apoptosis Anti-inflammation Neurotrophic factor Blocking BBB disruption Prevention of vasospasm	Bcl-2 and Bcl-w IL-1 sTNFR1 BDNF MMP-9 shRNA CGRP eNOS	Delayed ischemia neuronal death, reduction in infarct size and improvement in neurological function Reduced cerebral infarct volume Smaller infarct size and decreased inflammation Intrastriatal delivery of rAAV-NGF and BDNF can lessen neuronal death and save function after middle cerebral artery occlusion in rat model İmproved ischemic brain injury Ameliorated ischemic brain injury Reduced vasospasm, partly restored vasodilator response in canine model

orders, such as Alzheimer disease (AD) and Parkinson's disease (PD) in particular, and more common neurological diseases, such as epilepsy and stroke, might stem from the interaction of multiple genes. Each of these genes might play distinctive roles in disease susceptibility and conceivably interact with environmental factors (7). Genetic susceptibility factors-a variety of genes and biomarkers that present a risk of illness-have been identified in a fast pace. For instance, many biomarkers, such as amyloid beta (Aβ) (1-42), total tau, and phosphorylated tau 181 in the cerebrospinal fluid, were described that are currently being used as surrogate markers for presymptomatic AD. Many studies are currently in the pipeline to discover dependable blood biomarkers for AD (9).

Genome and/or exome sequencing tests are being integrated into clinical practice gradually. The cost would decrease to that of a magnetic resonance imaging study within 5 years (10).

Prognosis and Treatment

A diagnosis can be helpful to predict the prognosis. It may also warn the clinician about potential life-threatening comorbidities, such as cardiomyopathy in FA.

Currently, the majority of genetic diseases are incurable. However, while symptomatic relief and even prevention of disease progression could be achieved for many of them, genetic etiology in these cases should be identified as soon as possible. Phenylketonuria is an excellent example of this, since dietary restriction of phenylalanine initiated soon after birth will prevent cognitive impairment and enable virtually normal development (11).

Treatment in the Biotechnology Era

Translational medicine aims to integrate new information about disease pathology at the molecular level to clinical practice-treatment in particular. Recent new treatments, which take advantage of the molecular aspects of these disorders, show promise in the clinic. Even some have been licensed and showed results unimaginable previously. Some outstanding and dramatic examples will be discussed below.

Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is a disabling, progressive X-linked neuromuscular disorder for which there is no cure. It is caused by a lack of functional dystrophin protein resulting from mutations in the 2.2 Mb DMD gene. Several promising gene therapies are currently under investigation (12). One of these drugs, eteplirsen, an antisense oligonucleotide, received accelerated approval by the Food and Drug Administration (FDA) on September 19, 2016. Eteplirsen is specifically indicated for patients who have a confirmed mutation of the dystrophin gene due to exon 51 skipping, which affects approximately 13% of the population with DMD. The accelerated approval of eteplirsen is based on the consequence of dystrophin increase in the skeletal muscle observed in some treated patients although the clinical benefit of eteplirsen has not been established (13).

Nusinersen for Spinal Muscular Atrophy

Nusinersen is one of the great success stories of the biotechnology era. Previously, spinal muscular atrophy (SMA) was a disabling and progressively fatal disease of the spinal motor neurons. Nusinersen-an antisense oligonucleotide drug that modifies pre-messenger RNA splicing of the SMN2 gene-promotes the increased production of full-length SMN protein that is deficient in SMA (14). It exhibits dramatic consequences on SMA. These patients who were previously immobilized and succumbed to death acquired normal development by nusinersen therapy. They can walk and even run independently. Their life expectancy is considerably prolonged. It was licensed and approved by the FDA in 2016 (15).

Vaccine for Alzheimer's Disease

Alzheimer's disease (AD) is a devastating dementing disorder characterized by age-related Aβ deposition, neurofibrillary tangles, and synapse and neuronal loss. Late onset AD is the most common form and becomes symptomatic in later life. However, pathogenic protein Aβ is known to commence accumulating as earlier as 20 years at least. A treatment that could be used at this phase has been expected to prevent or slow down the inevitable fate of the patients. A delay of 5 years if available by 2025 would decrease the total number of patients with AD by 50% in 2050. Therefore, there is an urgent need to develop a disease-modifying therapy that could be given 20 years prior to symptom onset. A growing body of evidence shows that immunotherapy targeting Aβ holds great promise for reducing Aβ in the brain. Several phase 1 trials applying AAV vectors encoding anti-Aβ monoclonal antibodies caused a significant decrease in Aβ levels in the brain of mice models that showed great promise for their use for the prevention and treatment of AD (16).

Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder that can be treated symptomatically but cannot be cured. Loss of the substantia nigra cells that produce dopamine is the primary cause. There are several trials targeting the gene coding aromatic l-amino acid decarboxylase-a key dopamine biosynthesis enzyme. Virus vector delivery of this enzyme into the bilateral putamen reported regression of symptoms in patients with moderate and advanced PD and provides stable and persistent transgene expression for >4 years (17).

Table 3 outlines the gene therapy trials for these and additional neurological disorders (3, 8, 18).

CONCLUSION

Neurological diseases had been conventionally admitted as incurable. Accelerating new inventions in gene- and biotechnologies have been changing this pessimistic paradigm for three decades. New disease biomarkers are being detected continuously that allow to diagnose neurodegenerative diseases in the prodromal phase that could be >20 years. A great deal of trials is in progress to restore this phase to prevent these hopeless diseases. Recently licensed therapies for congenital neuromuscular diseases promote these ambitious expectations to come true in the very near future.

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