GENE THERAPY FOR NEURODEGENERATIVE DISEASES

A PROJECT WORK (BP812PW) SUBMITTED TO

NIRMA UNIVERSITY

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy

BY

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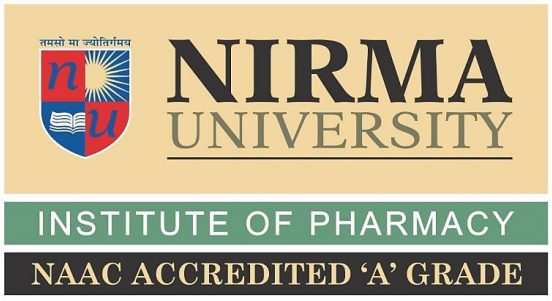
Semester VIII

UNDER THE GUIDANCE OF

DR. JIGNA S. SHAH



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CERTIFICATE

This is to certify that Project Work (BP812PW) entitled ***"GENE THERAPY FOR NEURODEGENERATIVE DISEASES"*** *is the bonafide work carried out by* ***GOSWAMI MAYANK*** *(17BPH055),* ***PATEL HITANSHI*** *(17BPH033),* ***PATEL DHRUVI*** *(17BPH017),* ***RAMANANDI MANALI*** *(17BPH050),* ***SAVALIYA NEEL*** *(16BPH060), B.Pharm semester VIII under my guidance and supervision in the Institute of Pharmacy, Nirma University, Ahmedabad during the academic year 2020-2021. This work is up to my satisfaction.*

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CERTIFICATE OF SIMILARITY OF WORK

This is to undertake that the B.Pharm. Project work (BP812PW) entitled ***"GENE THERAPY FOR NEURODEGENERATIVE DISEASES"*** *Submitted by* ***GOSWAMI MAYANK (17BPH055), PATEL HITANSHI (17BPH033), PATEL DHRUVI (17BPH017), RAMANANDI MANALI (17BPH050), SAVALIYA NEEL (16BPH060)****, B.Pharm. Semester VIII is a bonafide review/research work carried out by us at the Institute of Pharmacy, Nirma University under the guidance of "Dr. Jigna S. Shah". We are aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, the review/research work carried out by us is not reported anywhere as per best of our Knowledge.*

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Yours sincerely;

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ABSTRACT

In today's world, genes are very useful components for the treatment of many diseases. Gene therapy can be considered as the insertion of a gene to the cell in order to correct a cellular function or to treat a disease. Vectors like viral and non-viral vectors are used in gene therapy as carriers. It has the potential to deliver therapeutic advantage to lot of people with neurodegenerative diseases by several means, counting direct improvement of pathogenic mechanisms, neuroprotection, neuro-restoration, and symptoms control. Therapeutic efficacy is therefore reliant on knowledge of the disease pathogenesis and the required temporal and spatial specificity of gene expression. An additional critical challenge is achieving the most complete transduction of the target structure while avoiding leakage into neighboring regions or perivascular spaces. The gene therapy field has recently entered a new technological era, in which interventional MRI-guided convection-enhanced delivery (iMRI-CED) is the gold standard for verifying accurate vector delivery in real time. Gene therapy can be promising treatment goal for many neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, multiple sclerosis and epilepsy. With the help of vectors like viral and non-viral vectors, Gene therapy can bring treatment of these diseases to reality. The availability of this advanced neurosurgical technique may accelerate the translation of the promising preclinical therapeutics under development for neurodegenerative disorders, including Parkinson's, Huntington's, and Alzheimer's diseases.

Keywords: Gene therapy; Alzheimer's disease; Multiple Sclerosis; Epilepsy; Parkinson's disease; viral vector.

1. Gene therapy for neurodegenerative diseases

1.1 Introduction:

In this era, the health care industry has experienced a lot of advancement because of the research and innovation in genetics and molecular science. As we all know, genes and chromosomes are the foundation of heredity. Biotechnology has opened a broad spectrum in the field of medical research. Alteration in genes can lead to change in the human body to a significant level. Thus, mutations or change in the genetic material can trigger improvement in cellular system of the body which may lead to cure of many diseases and as a result it can emerge as an important treatment method for many diseases. In this review article, the importance of gene therapy for treatment of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, epilepsy and multiple sclerosis is reviewed and described.

Gene therapy:

Typically, gene therapy can be understood as the introduction of a gene into cells in order to achieve a proper functioning of a mutated cell or to obtain a new ability of a cell. In other words, gene therapy is a technique for the betterment of a gene in a way to improve the diseased gene to have proper function of a body system.

Gene therapy focuses on the appropriate use of genetic material for the betterment of a disease. In this method, either a normal gene or chromosome is added or altered or affected gene is replaced for a desired function.

1.2 Why do we need gene therapy?

Genes are one of the foundational factors of human body. By modifying genes, we can achieve many desired functions and we can have cure, prevention and mitigation of many diseases. The actions, for which gene therapy is useful are:

Replacing dysfunction genes: by replacing defected genes with genes working correctly can prevent a dysfunction of a cell. This can lead to cure of many diseases. This can be useful in the inhibition of tumor growth also.

Gene silencing: by gene therapy we can prevent the expression of certain genes so that we can stop the cell growth of unwanted cells, which can lead us to the cure and prevention of cancer diseases.

To improve immunity: some times our immune system can't recognize the diseased cells, pathogens and other harmful agents. By using gene therapy, we can train the immune system to recognize these agents or we can make these harmful cells exposed to the immune system so that we can have better immunity.

***1.3 Process of gene therapy*:**

The first step of the method is the release of the gene. In order to replace genetic material by an intended gene, the gene is first of all released with the help of carrier cell known as 'vector'. Then the vector containing the therapeutic genetic material is introduced to the host cell by ex-vivo or in-vivo methods so that the intended gene can be expressed to the host cell.

1.4 Types of gene therapy:

1) **Somatic gene therapy:-** in this method, the genetic material is introduced to any cell in the body except the egg and the sperm cells. This kind of therapy gives its effect to the patient only. The next generation of that patient will not be benefited from the therapy. This type of gene therapy encounters wide range of research.
2) **Germline gene therapy:-** this type of therapy involves the insertion of the gene into the sperm and fertilized eggs. This kind of changes can be passed to the next generation. Thus, hereditary diseases can be prevented by this method. Unfortunately, this area encounters very less research.
3) **Ex-vivo gene therapy:-** in this method the target cells for the vector are isolated from the host and the vector containing therapeutic gene is reacted to the isolated cells and the gene expressed cells are now introduced to the host cells. Which leads to therapeutic activity.
4) **In-vivo gene therapy:-** in this method, vector containing the intended genetic material is inserted to the host and the gene expression occurs inside the host which leads to therapeutic activity.

***1.5 Gene therapy methods based on vectors*:**

Mainly, two methods based on vectors are there in gene therapy.

1) Viral vectors
2) Non-viral methods

Viral methods:

This method in widely used currently. In this method, some viruses or modified viruses having no pathogenic activity are used as a vector to carry the therapeutic genetic material. Some viruses do not enter the cell but transfers the intended genes to the body, while some viral vectors cross the semi permeable membrane and enters the cell and expresses the therapeutic gene. Different types of viruses are used in this type of gene therapy:

Retrovirus: This type of vectors contains single stranded RNA as a genetic material which can be injected into the host cell and from this single strand the double stranded DNA is formed and the desired function is obtained or the diseased function of the cell can be corrected. For example, retrovirus-based gene therapy in treatment of gene therapy. The drawback of this type of vectors can be the chances of uncontrolled cell growth leading to cancer.

Adenovirus: in this method, viruses having genetic material containing double strands are used. These vectors are intended to cause infection and by this, they transcript their genetic material in the nucleus of the host cell in free form. Then the cell adopts the intended function through this free genetic material. The side effect of this kind of therapy is unwanted allergic reactions.

Adeno-associated viruses (AAV): in this method, vector viruses are used, which are having DNA which is single stranded. They infect both type of cells which can multiply themselves and also the cells which cannot undergo the multiplication. The major use of this ability of the therapy is that we can use this therapy for treatment of diseases related to brain also as they can deliver genes to neurons.

Herpes simplex virus (HSV) is also used as a vector in the gene therapy these days. Mainly they are used for the nervous system.

Non-viral methods:

In these type of methods different methods is used and being explored in the clinical trials. The methods are: direct injection, by electroporation, gene guns and receptor mediated gene transfer.

Non-viral methods include vectors like liposomes, microspheres and plasmid DNA are being used nowadays.

Oligonucleotide approaches:

Besides introducing a complete whole gene, we can insert short part of the genes in the form of oligonucleotides in order to achieve the desired function and minimize undesired actions. Another approach is the use of antisense oligonucleotides, which are complementary part of the gene fragment which is important for the desired function. These fragments of genetic material help to synthesize required genes and eventually lead to the desired functions of the cell.

1.6 Areas of gene therapy:

Gene therapy for cancer:

Gene therapy can be proven as effective approach for the cancer prevention and cure. The approaches for cancer gene therapy are:

1) By promoting the tumor suppressor gene. This can lead to inhibition of tumor growth.
2) By inhibition of tumor expression genes.
3) By suicide gene approach, in this method the cancer cells are more efficiently exposed to anti-cancer drugs which can cure the cancer.

Gene therapy for cardiovascular diseases:

Gene therapy is a promising approach to the cardiovascular treatments. The research is being conducted in vascular diseases in which the gene is delivered to the diseased coronary or other arteries in order to achieve their proper functioning. The angiogenic gene therapy can be a useful approach for these diseases.

Gene therapy for infectious diseases:

We can use gene therapy for the prevention of infectious disease. We can improve the immunity by gene therapy by improving immune cell functions. DNA vaccines are useful approach for the prevention of some infections. The potential interest of gene therapy is the prevention of HIV. We can improve functions of stem cells and make them HIV resistant before they get mature.

Gene therapy for CNS diseases:

Nowadays, gene therapy is a potential approach for the neurological diseases. Because of gene therapy we can achieve treatment goals like neuroprotection, neuro-restoration, and direct disease control and symptom management.

Due to technological advancement, we have an advance gene delivery system like interventional MRI-guided convection- enhanced delivery which can introduce the therapeutic gene to the neurological targets which can be helpful to treat many CNS diseases. There is a potential approach for Disease related to motor function can also be treated by converting glial cells into motor neurons in order to restore motor function.

***1.7 Gene therapy for neurodegenerative diseases*:**

Gene therapy can be a promising treatment for neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, multiple sclerosis, epilepsy, Huntington's disease etc.

Gene delivery of nerve growth factor using AAV2 can be useful to prevent memory loss in Alzheimer's disease. Reprogrammed glial cells can also prevent brain damage and memory loss in this disease.

Neurturin is a molecule that is useful in the dopaminergic neuron protection which can improve behavioral function in Parkinson's disease. The AAV2-AADC gene therapy can be used as aid in the conversion of levodopa to dopamine which can be useful for the Parkinson's disease patients. The AAV2-GAD gene therapy can also be a promising treatment for Parkinson's disease by providing GAD to the subthalamic nucleus in order to reduce the symptoms.

The AAV9-SMN gene therapy can deliver the survival motor neuron1 (SMN) gene for the treatment of spinal muscular atrophy. RNAi based gene therapy can be the promising treatment of epilepsy. In other words, gene therapy can be a potential treatment for some currently incurable diseases.

2. ALZHEIMER'S DISEASE*:*

It is a neurodegenerative disease that causes decline in the behavioural pattern, memory loss, thinking ability and overall an individual's capability to function independently. The brain cells are likely to shrink and die eventually.

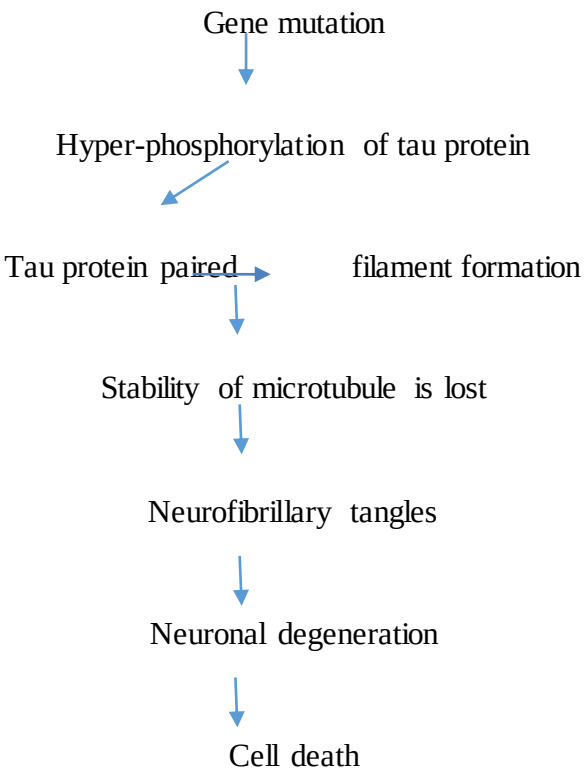
There is no particular treatment available on Alzheimer's but several medications that can enhance the results and slow the process of degeneration or dementia.

2.1 PATHOPHYSIOLOGY:

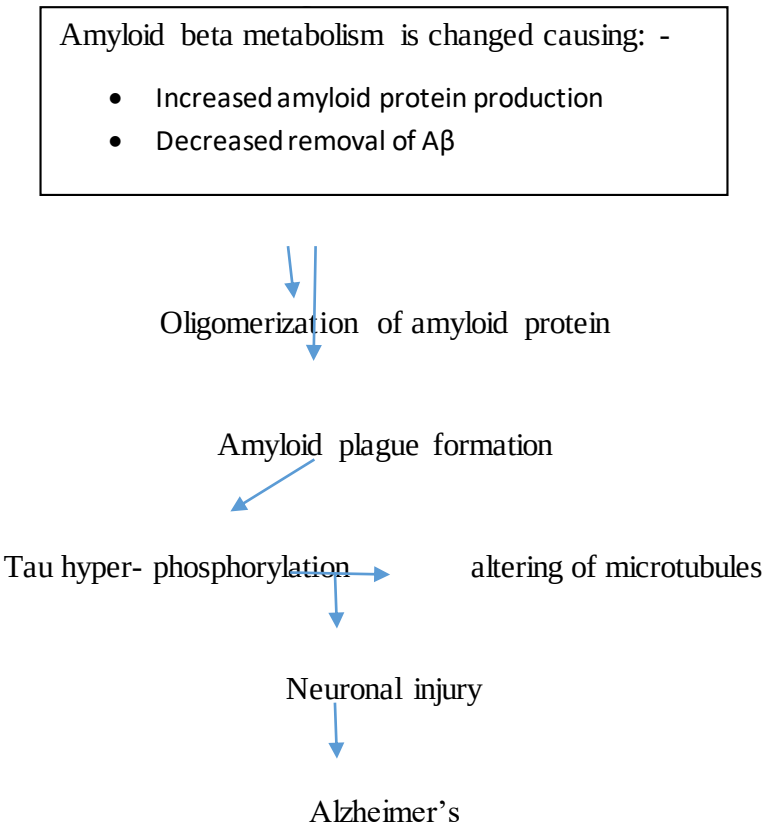
There are three main pathological features observed in the AD: -

1. Hyper phosphorylated Tau protein
2. Amyloid beta hypothesis
3. Oxidative stress

HYPERPHOSPHORYLATED TAU PROTEIN:



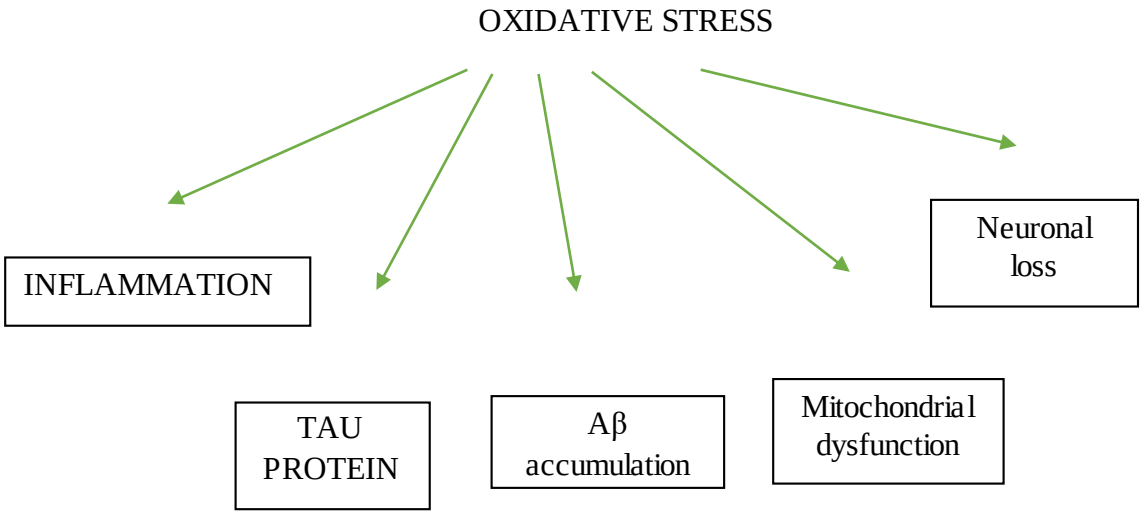
AMYLOID BETA HYPOTHESIS:



OXIDATIVE STRESS:

Reactive oxygen species are produced normally by the body but have dual roles to play which is beneficial and also harmful. It is useful in the cell signal pathways while having a detrimental effect by damaging the cell structure.

These damage in the cells causes increased consumption of CO_2 by the brain cells causing Oxidative stress.



There is a large amount of poly-unsaturated fatty acid that reacts with oxygen and lead to lipid peroxide causing programmed cell death followed by neuronal injury and Alzheimer's disease.

2.2 SYMPTOMS BASED ON STAGES OF ALZHEIMER'S DISEASE:

- **MILD ALZHEIMER'S DISEASE:**

  Feeling more exhausted, mood swings, decreased interest in doing any task

  Difficulty doing day to day task, problems with language, driving issues

  Coordination issues

- **MODERATE ALZHEIMERS:**

  Severe memory loss

  A person cannot remember his/her own family members

  Confusion about time weather places

  Problem solving issues

  Trouble sleeping and even in speech

- **SEVERE ALZHEIMERS:**

  Cannot swallow food

  Loss of bladder control

  Hallucinations

  Seizers

2.3 DIAGNOSIS AND TREATMENT OF ALZHEIMER'S DISEASE:

The first and foremost diagnosis is signs and symptoms like difficulty remembering things, behaviour issues, how all these symptoms affect day to day life. Also, finding reasons for all these symptoms.

Neurologist might order some other lab tests, memory tests, brain scanning to figure out other conditions that might also cause similar symptoms.

These includes:

- Physical examination
- Evaluation of memory: - here the doctor asks you to perform various tasks related to your thinking ability, problem solving, and language and also may ask several questions.
- Neuropsychological test

Brain scanning images helps identifying other disease for instance;

MRI, CT-SCANS, PET-SCANS provides a detailed scanning of brain. PET scans which are developed recently detects cluster of amyloid plaques that are allied with Alzheimer's.

2.4 GENE THERAPY, A POTENTIAL TREATMENT FOR ALZHEIMER'S DISEASE:

2.4.1 INTRODUCTION: -

Gene therapy is a technique used for the treatment of various disease and is still under study. In these, the disease is treated by inserting the gene directly into the cells. There are various

approaches by which this can be done. It can either be done by replacing the mutated gene with a healthy one or removing the defected gene or by introducing the new gene that can fight the disease. Reduction of bio-active compounds and increased activity of enzymes can directly be achieved by gene therapy. This is accomplished by transferring the trans-gene facilitated by a vector, the gene is articulated in the infected host cell.

2.4.2 VECTORS USED IN GENE THERAPY:

Table 2.1;

VECTORS	LOCATION	TYPE OF VIRUS
AAV	DNA	ssDNA
ADENO-VIRUS	CHROMOSOMES	dsDNA
HSV	DNA	dsDNA
LENTI-VIRUS	CHROMOSOMES	ssRNA
MLV	DNA	ssRNA

For neurodegenerative disorders like Alzheimer's a substitute vector is developed called recombinant adeno associated viral vector and HIV derived lenti-viral vector. These vectors offer elongated period expression, safe but weak immune-reactive.

2.4.3 VIRAL VECTORS USED IN NEURODEGENERATIVE DISEASE AND THEIR ROLES:

AAV serovar are majorly used in the AAV type gene therapy and have been successful. Which serotype is intended for the specific delivery is the most important step to find an expectable gene therapy approach?

Table 2.2

VIRAL VECTORS	ROLE
AAV	Neutralize antibody generate in-vivo
AAV2	Safe, show constant expression in neurons
AAV9 & AAVrh.10	Can cross BBB
AAV-PHP.B	Transduce half of the astrocytes and neurons and also transport high no of AAV genomes into CNS by IV injection.
Lentivirus (LVS)	Incorporate DNA into host genome by reverse transcription Stable Provides long trans-gene expression
Adenovirus	High safety profile

There are several drawbacks of using the viral vectors like problems in production of vectors, loading capacity is limited also, host inflammatory reaction and tropism.

2.4.4 NON-VIRAL VECTORS FOR NEURODEGENERATIVE DISEASE:

Lipid base vectors are the most widely used non-viral genomes for gene therapy. Lipids like:

Cholesterol

Dopamine

Distearoylphosphatidylethanolamine

These enhances the liposomal stability and transfection capability. There are three main fields of the cationic lipids naming: -

Water repelling tail, linking group and cation cap group. These groups have certain negative aspects like rapid clearance rate, cytotoxicity and non- satisfactory bio-distribution. But to solve this issue there has been a development of lipids with proper pka value.

***2.4.5 SELECTION OF TARGET FOR ALZHEIMER'S DISEASE*:**

1. **Mechanistic target of rapamycin (MTOR) :-**
   It regulates growth, catabolic and anabolic processes. Also regulates metabolism, autophagy and translation.
   Any defect in this signaling may cause distinctive effect in several neural cells like caudate nucleus, retina, and sub-stantial nigra.
   For Alzheimer's gene therapy used is AAVI-AKT.
   Route of delivery is Intrastriatal injection.

2. **Epigenetic regulation:-**
   Epigenetic controlling mechanism, such as chromatin remodeling, DNA methylation, histone variant is said to control plentiful characteristic of axonal growth and neuron survival. An evidence in a study showed that alterations in h3k27ac and h3k4me3 happened in connection with gene alternatives in Alzheimer's, signifies a vital purpose for immune-associated accompaniments and supporter proteins in defining Alzheimer's liability.
   Gene therapy used for Alzheimer's disease: AAV2-P1NK1
   AAV2-PSD95-6ZF-VP64
   Route of administration: Intrahippocampal injection

3. **Microglial and astrocyte function:**

   Microglia is the chief neuron immune cell that perform abundant perilous task like an organization that sustain neuron welfare and neuronal links, sentry purpose allied with continuous insight of ecological variations, and self-protective purpose which is important for neuron-protection.

   Neuronal damage in Alzheimer's, Parkinson, Huntington's disease and the deterioration connected with prolonged and acute shock stems from disturbances of these

   Microglial roles and neuron inflammation.
   Gene therapy used in Alzheimer's: AAV2/8-STREM2
   Lentivirus-pgrn
   Route of administration: Intra-cerebral injection
   Unilateral brain injection

2.4.6 GENOME EDITING TOOLS USED IN ALZHEIMER'S DISEASE:

There are several pathways that are used in genome editing to correct the mutations in the pathogenesis of AD and are also a potential target.

Pathways that are used as targets for genome editing studies are:

Main use of CRISPR/CAS9 to edit the mutated gene in several pathways

Table 2.3:

MUTANT	PATHWAY
APP, PSEN1, PSEN2	Amyloid beta toxicity

GSK-3BETA, P25/CDK5	Tau phosphorylation
nACHR, ChAT	Cholinergic transmission
Usp7, usp47	Protein ubiquitation
GMF, cd33, trem2	Neuroinflammation

These showed the demolition of mutant cells and there were no mutations on the normal alleles.

There was a diminished production of Abeta40 and 42. Modifications in gamma-secretases can also reduce the making of these toxic Aβ40and42.

BACE1 is another potential target for Alzheimer and is also required to develop Aβ.

All these factors are studied to correct the known mutation in the early onset of Alzheimer.

The foremost factor that leads to late onset of AD is APOE4 which is to be converted to APOE3 for the cure.

So, to conclude, genome editing can be used to correct various mutations. Followed by using CRISPR/CAS9 is the tool that is used for the mutations in the genes. However, the disease progression is still no completely understood. Also, the treatment is not fully developed and research upon in accordance to gene therapy. The research and clinical trials are still under study.

3. MULTIPLE SCLEROSIS:

Multiple sclerosis (MS) is an illness it has the potential to harm the brain and spinal cord (central nervous system - CNS). In MS; the system outbreaks the protective sheath (myelin) which shelters nerve fibers and origins communication difficulties among the brain and other essentials of the body. Lastly, the illness will cause permanent nerve damage.

Multiple sclerosis means "scarring in multiple areas.

When the myelin sheath damages different areas, it leaves scars or sclerosis. Experts refer to this area as plaque or wound.

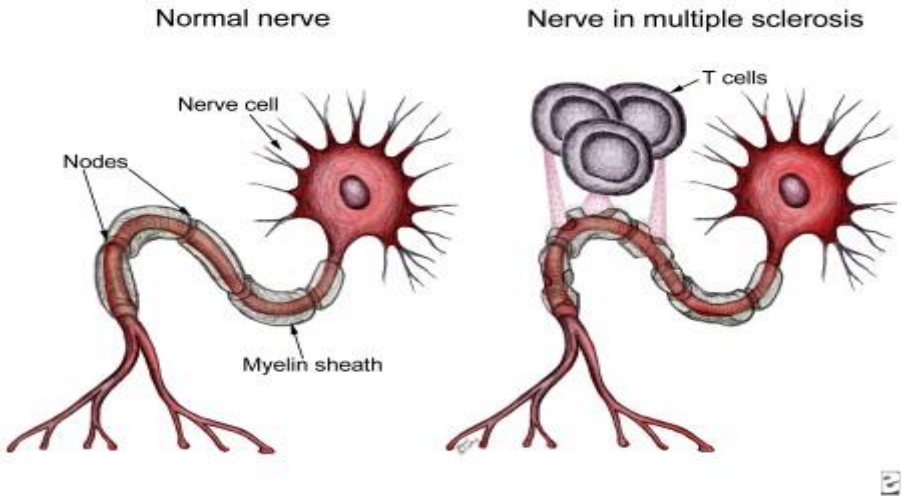


Figure 3.1

Indicates the difference between normal nerve cell and nerve cell in MS

3.1 SYMPTOMS:

- Fatigue
- Vision problems
- Mobility problems
- Sexual difficulties
- Bladder difficulties
- Language and swallowing problems
- Difficulties with thinking, learning and weakness

3.2 PATHOPHYSIOLOGY:

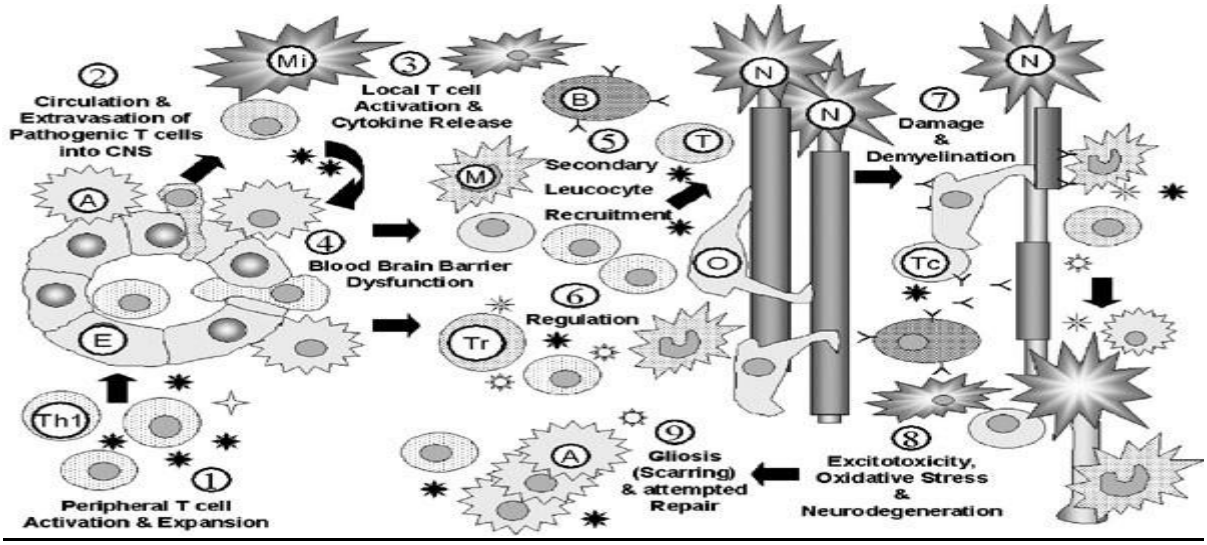


Figure 3.2

Overview of Pathophysiology of MS

***3.3 DIAGNOSIS*:**

It is difficult to identify the different types of sclerosis. There is no test that can analyze this. Under the same conditions, conclusions usually require different tests to rule out different conditions with comparative indications. After your PCP has made the actual assessment, they'll likely run several separate tests if they suspect you have MS.

MS can be time consuming and a series of diagnostic tests. It starts when you or your doctor see certain signs or symptoms that mentioned above.

Diagnosis test for Multiple Sclerosis:

MRI

Lumber puncture

Eye exam

Blood test

***3.4 GENE THERAPY; A POTENTIAL TREATMENT FOR MULTIPLE SCLEROSIS*:**

The key problems for any a success gene remedy method is the character of the vector and characterization of targets. This remedy been tried in MS, however there had been some of research in EAE which have always proven a few degrees of efficacy at inhibiting the sickness, even though in lots of instances this has simplest been an amelioration in preference to removal of sickness. Greater number of the disorders related to CNS are postmitotic, which places restrictions on the vector's character that might be applied, and thus far insertion of the plasmid DNA, DNA, viral infection, in addition retrovirally transduced cell (RVC)-transporters had been inspected in EAE.

Exogenous gene transport gives a beneficial device to investigate the biological reason behind sickness in 'physiologically ordinary' grownup creatures. Significantly in particular such as gene transference of cytokines may be proven to be greater successful rather than transportation of bolus protein, it additionally gives a path for remedy.

- ***DIFFERENT VECTORS USED IN GENE THERAPY OF CNS AUTOIMMUNE DISORDERS:***

  Table 3.1

TRANSGENE	GENE VECTOR	INDUCING ANTIGEN	THERAPEUTIC EFFECT	CLINICAL EFFICACY
IL-4-Ig	Naked DNA	MBP	Prophylactic	+
IL-2	Vaccina virus	SCH	Prophylactic	+
IL-4	Naked DNA	MBP peptide	Prophylactic	=
	DNA-liposome	SCH	Therapeutic	+
	Naked DNA	PLP peptide	prophylactic	=
IL-1β	Vaccina virus	SCH	Prophylactic	+
IL-6	Vaccinia virus	SCH	Prophylactic	+
IL-10	DNA-liposome	SCH	Prophylactic	=
	Naked DNA	MBP peptide	Prophylactic	=

	Adenovirus	SCH	Prophylactic	=
	HSV-1	SCH	Prophylactic	=
IFN-β	DNA-liposomes	SCH	Prophylactic	+
IFN-γ	Vaccinia virus	SCH	Prophylactic	=
	HSV-1	MOG peptide	Prophylactic	+
TNF-α	Naked DNA	MBP peptide	Prophylactic	=
	DNA-liposomes	SCH	Preventive	=
	Vaccinia virus	SCH	Prophylactic	+
TGF-β	NAKED DNA	MBP	Prophylactic	+
	DNA-liposomes	SCH	Prophylactic	=
	DNA-liposomes	SCH	Therapeutic	+

Cytokine transgene contained vectors used in EAE studies: IL (interleukin), TGF (transforming growth factor), IFN (interferon), TNF (tumor necrosis factor);

Chemokine transgenes: MCP (Macrophage Chemotactic Protein), elements indicating the CAT (complex oxygen species scavenger catalase), (Ig) the immunosuppressive CTLA4 immunoglobulin myelin antigens and fusion protein.

Antigens used: MBP (myelin basic protein), PLP (myelin proteolipid protein), MOG (Myelin oligodendrocyte glycoprotein)

The route of administration was intravenous (iv) routes and were associated with agents inserted straight into the CNS, either as liposome (with or without cationic lipids) or naked plasmid DNA or HSV (Herpes Simplex Virus) or adenoviral vectors of transgene either delivered by using retrovirally transduced cell vectors (RCV) of the B cell, fibroblast of myelin precise originated by T-cells.

Therapeutic situation through relapsing disease (Therapeutic R) and whichever bettered (+), get worse (–) or had no result of the clinical sickness (=)

3.4.1 LOCAL IMMUNOGENE THERAPY:

In the course of the CNS injury of MS arise and the protein might should be brought through numerous, aggressive injections as there may be restricted parenchymal diffusion, that's unworkable. Such therapeutic molecules bathe the CNS via the cerebrospinal fluid (CSF), this can be resolved. Presently, in CNS distribution of medicine is carried out via the osmotic pumps which can be steeply-priced and inconvenient. Gene therapy can be boon in such problem. CNS administration shows superior efficiency than the systemic administration has been done by delivery of cytokine exploitive gene vectors and inhibitory cytokine. Significantly, the BBB restrictions not solitary influx, but also way out of particles from the brain, which are able to generate the local concentration slope in the CNS, reliant on the amount inserted, that can make local therapy, nonetheless does not trigger circulating stages which origin peripheral immune destruction. As a result, the CNS might be a special tissue for delivering effectiveCTLA4-Ig (immunosuppressive agent) because of their undesirable effects of comprehensive immunosuppression, for example infection growth they may not be endured if distributed to other tissues.

Though viral vectors are admirable for transferring gene into the CNS, there are chances of negative implication like direct infection. The CNS has certain immune privilege characteristics, allowing potentially immunogenic allogeneic viral vectors or cell donors to be delivered and overlooked, allowing for longstanding CNS expression.

3.4.2 SYSTEMIC IMMUNOGENE THERAPY:

The migrant possible of informed T lymphocytes has located attached and so permits for the systemic transfer as a substitute method to accomplishing local CNS delivery. As a consequence of their bond molecule in addition chemokine receptor phenotype, the bulk of T cells inside inflammatory scratches are doubtless not CNS-precise, but are secondarily enlisted to the inflammatory location. Uncertainty these cells have affinity for myelin or supplementary CNS antigens, they will be guided to lesioned areas and stimulated/preserved locally. They will then prevent neighboring infective cells through a bystander effect by releasing neuroprotective growth factors immunosuppressive and by generating antagonizing molecules in situ.

However, in vitro pre-activation, which is needed for T cell entry into the CNS, might result in cytokine release in the lymphoid tissue or bloodstream, which can have negative consequences. If the inborn TGFβ is used, as this is classically covert until slashed at the sites of inflammation; this could be avoided. To reject disease-worsening so it is advisable to engineer such type of cells by suicide genes.

3.4.3 SYSTEMIC VACCINATION GENE THERAPY:

Current developments in genetics and genetics have staggeringly multiplied the tilt of possible marks in MS and could be adapted outline autoantigenic goals in specific humans presumably for polymer vaccination converse genomics. A variation of methods intended to avoiding the generation of encephalitogenic T cells are evaluated. In few EAE models equivalent to the Lewis rat and PL/J mouse, the sickness is beginning by action of cells by terribly restricted T-cell receptor (Tcr) subtype heterogeneousness. The most of the encephalitogenic cells definite TcrVb8 too preventive, general DNA vaccination in contrast to Tcr subtype has encouraged EAE enhancement. Even in inherited animals whereas inadequate TcrVb heterogeneity might not continuously occur, in selected patients clinical research with recurrent TcrVb amide insertions are by now current and seem harmless.

Additional research is needed to prove that they're effective in a very really beneficial situation in semipermanent recognized disease. Though, one research has exposed some achievement during this deference and applied co-administration of myeline polymer besides IL-4 to initiative the initiation of response by Th2.

3.4.4 INDUCTION OF TOLERANCE:

Immunological resistance: it is the state in which the resistant scaffold cannot retort to certain antigens due to previous findings. These interactions can stop or reduce the immune retort to the antigen itself. Focal resistance happens in the thymus and marginal resistance arises in the blood. In cooperation cycles involve utilitarian removal and lethargy from the automatically responding lymphocytes. Administrative T-cell elongation was observed in a similar manner during the increase in resistance. By killing these damaging cells via resistance, autoimmunity can be barred. However, nearly T cells

might emerge from the ends and keep on in the collection. If these T cells are re-energized by causal influences, they might subsidize to the pathogenesis of immune system diseases, counting MS. Of course, myelin-responsive T cell positions made out of solid subjects, showing frustration with the focal ability to carry T cells to myelin antigen receptors. Acceptance and enhancement of resistance strips to eliminate or alter receptive T cells alone are accepted as possible techniques for treating diseases of the immune system. To stimulate resistance, tolerogenic antigens could be managed via various courses (oral, intranasal, intravenous route).

3.4.5 STEM-CELL BASED THERAPIES FOR MS:

Bone-marrow depending mesenchymal foundational micro-organisms relocation can affect MS in both way: neurodegenerative and immunomodulatory. These cells just like hematopoietic foundational microorganisms are handily got from grown-ups; healing application is believed to be protected and have the ability of assuming the critical part for MS treatment. By way of compliments immunomodulation in addition neuroprotection applied by undeveloped cells, here technique could have probable for remedial methodology for MS.

3.5 FUTURE PROSPECTS:

Yet, these treatments have had optimistic results in preventing lesion development and therefore the degeneration rate. It is getting down to highlight the new certainty that the foremost reason behind everlasting advanced incapacity is by reason of neurodegeneration. Whereas this happens initial in MS and EAE, it is perhaps going that as a consequence of inflammation-tempted demyelination, a structure microenvironment is formed wherever the nerve cells are remarkably sensitive to toxin invectives, reminiscent of salt excitotoxicity, aerophilous free-radical harm and toxic particle changes, and a sluggish chronic method which will last casual of the inflammatory response is activated. Even though some achievement has been publicized by cistron-transported development factors that give biological process provision (e. g. growth factor and PDGF) neuroprotection via any direction in system autoimmunity, notably in long-established sickness, is actually unknown. Though, there's resistant that gene delivery of growth factors will endorse remyelination from alternative neurodegenerative systems.

To focus on changed parts of the disease progression it is necessary that actual medical care would need a mix of mediators. Currently, monotherapies are for the most part being inspected and these have pointing the immune response. Although this immunomodulatory treatment is useful for preventing forthcoming CNS tissue damage, neurodegenerative methods to treat damaged CNS tissue effectively such as demyelination, axonal and neurological disasters will be very important in accelerating neurological recovery.

4. Epilepsy: -

Seizure: Electrical impulses between neurons/nerve cells in the brain fire rapidly and uncontrollably. With or without loss of consciousness, this causes changes in a person's muscle tone, gestures, feelings, and behavior.

Epilepsy: It is a brain disease characterized by unprovoked seizures that are caused by an unknown medical condition. Seizures happen to someone who has epilepsy, but not everyone who has seizures has epilepsy.

As per the International League against Epilepsy (ILAE), an individual is said to have epilepsy if they are having:

A. More than two non-provoked seizures that occurs a day apart

B. After 2 unprovoked seizures in the next ten years, an unprovoked seizure with a probability of more seizures equivalent to normal reappearance (minimum 60%).

C. Epilepsy disease is diagnosed.

4.1 Prevalence and Incidence of Epilepsy:

- Epilepsy affects more than half a million people all over the world.
- Epilepsy has an estimated prevalence of 3.0-11.9 per 1,000 people in India, with an annual incidence of 0.2-0.6 per 1,000 people.
- People with epilepsy have a three-fold increased chance of dying prematurely as compared to the general population.
- About 80% of epileptics reside in low- and middle-income nations. Furthermore, 34% of epileptic patients in low-income countries do not receive adequate care.

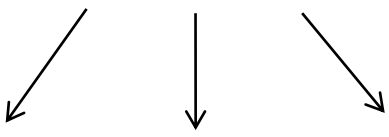
4.2 Classification based on Etiology:

Epilepsy is divided into three categories based on the cause:

1. Idiopathic: means "without a cause." These epilepsies are caused by a genetic mutation.
2. Cryptogenic: An exact etiology is unknown in these cases. Though there is a probability of genetic interference in this community of epilepsies.
3. Symptomatic epilepsy: As per International Classification of Epileptic Syndromes, about 25% of epilepsies are related to the CNS or grey matter, such as trauma, attack, palsy, CNS infection, metabolic insults, bleeding, and so on.

4.3 Classification of Seizures:

Epileptic Seizures



Generalized Partial (Focal) Epileptic Spasms

Seizures	Seizures
↓	↓
- Absence	- Simple Partial
- Typical	- Complex Partial
- Atypical	- Partial seizures secondary generalizing
- Tonic-Clonic	
- Myoclonic	
- Atonic	

A. Generalized Seizures: -

The brain's bilateral neuronal networks are the source of generalized seizures. These begin with Cerebral Cortex involvement and are characterized by an unconsciousness. Convulsive or non-convulsive seizures are possible.

- ❑ Absence seizure (Petit-Mal seizure):

  It depicts less intense seizure disorder in which the patient ends up losing consciousness and becomes slow to respond to the environment for 5-30 seconds.

- ❑ Tonic-Clonic seizure (Grand-Mal seizure):

  This is thought to happen to people who have irregular electrogenic circuitry in their brain. Excessive discharges in various parts of the brain trigger it.

- ❑ Atonic seizures: This is characterized by a lack of body tone that causes the head to drop or fall.

- ❑ Myoclonic seizures: This may be broad or generalized entails quick, brief movements that aren't associated with any visible disruption of conscious awareness.

B. Partial (Focal) Seizures: -

The symptoms of a focal seizure may vary on the basis of the area of cortex involved. Seizures that arise from temporal lobe are mostly dyscognitive.

C. Epileptic Spasms: -The onset of epileptic spasms is unknown.

4.4 Pathophysiology of Epilepsy:

- The onset of seizures:
  - Little clusters of abnormal neurons go through:
  a) Longer depolarization phase
  b) Quick firing of continuous action potentials
  - This action potential afterwards spreads to nearby neurons or to the neurons with which they are connected in the process. When a large group of cells' electrical discharges are linked together abnormally, developing a blast of electrical impulses in the brain then a clinical seizure takes place. Then the seizures can spread to nearby areas of the brain or to other distant areas via developed anatomic pathways.
- At molecular level:
  - Due to cell membrane instability at the cortical level, abnormalities in polarization occur to sensitive cell membranes. Lower threshold (Polarization abnormalities) like Ionic Imbalance in the instant chemical environment of neurons will lead to:

    1- Excessive excitation (Ach, Glutamate)

    2- Decreased inhibition (GABA)

***4.5 Diagnosis*:**

History and evaluation:

The clinical context of the seizure, as well as premonitory signs, seizure specifics, order of phenomena, receptivity, focal features, and postictal state, are the most important historical features. The aim of a neurological examination is to look for specific signs that may indicate or localize cerebral disease.

EEG: It is the most commonly used procedure for epilepsy diagnosis. Concurrent video-EEG monitoring can improve diagnostic yield or help distinguish between epileptic and non-epileptic seizures.

Neuroimaging: CT and MRI scans are important supplementary measures for the diagnosis of an individual with seizures. CNS structural abrasions are particularly vulnerable to neuroimaging techniques.

- ➔ **Computerized tomography (CT) scan: -** It can expose the abnormalities of the brain that may be the reason for seizures, tumors', cysts, and hemorrhaging, to name a few.
- ➔ **Magnetic Resonance Imaging (MRI): -**In patients with abnormal neurologic findings, focal seizures, or focal discharges on EEG, an MRI can reveal an irregularity.
- ➔ **Functional MRI (fMRI): -** Prior to surgery, doctors can use fMRI to identify the specific area of core activities such as speech and action in order to avoid harming those areas while operating.
- ➔ **Positron emission tomography (PET): -**PET creates a picture of the brain's local glucose use, as well as asymmetries that indicate sections of interictal/ictal abnormality.
- ➔ **Single-Photon Emission Computerized Tomography (SPECT): -** At the time MRI and EEG were unable to pinpoint brain regions from which the seizures originate, this test was used. When registered during a seizure, SPECT compares variations in local blood flow; this information is extremely useful.

Metabolic Evaluation:

The metabolic workup needed depends on the seizure and disorder type. Many conditions such as incomprehensible emesis, coma, and perhaps developmental delays often accompany seizures in metabolic disorders.

Genetic testing:

Genetic testing is currently available for some single genes as well as complex genetic disorders. A simple karyotype (image of an individual's chromosome) may be used to identify chromosomal abnormalities, which is especially useful in patients with dysmorphic features.

4.6 Treatment:

The following are some of the treatments:

- anti-epileptic drugs (AEDs)
- removal of a small part of the brain that is causing the seizures through surgery
- A seizure control procedure that includes inserting a small electrical device inside the body.
- Ketogenic diet is a form of diet which can help with seizure control.
- Gene therapy can also be a potential for the treatment of epilepsy

4.7 Gene Therapy for epilepsy

- Since there is an unmet need for experimental therapies in focal drug-resistant epilepsy, developing innovative methods to combat the disorder has been a priority. A few other focal targeted strategies are being developed due to the complexity of focal epilepsies, especially TLE.
- The pharmacotherapy agent could be delivered via gene therapy directly to epileptic target or inside a seizure generating cascade to overcome the BBB penetration barrier.
- Increased expression of endogenous neuropeptides as well as neurotrophic factors is said to lower seizure frequency and raise seizure threshold.

- Seizure suppression has been demonstrated using optogenetics, chemo genetics, and modified potassium channels.
- Gene therapy for focal epilepsy has been identified as a promising solution, with increased expression of neuropeptide Y being examined for seizure-suppressing impact.

4.7.1 Optogenetics:

Gene therapy for epilepsy treatment focuses on the impacted brain area.

In a recent research, Wykes et al. employed two different gene therapy approaches to control focal epilepsy caused by tetanus toxin injection inside the motor cortex within the rat brain.

A) Infusing a viral vector carrying the gene both for halorhodopsin (chloride pump which is sensitive to light) as well as tetanus toxin into the rat brain was done using an optogenetics process. They used laser light to activate halorhodopsin, which is expressed through excitatory chief nerve cells in the epileptogenic region. The presence of stimulated halorhodopsin allowed chloride ions to reach neurons, resulting in hyperpolarization of membrane and a reduction in excitability of neutrons, which reduced epileptic activity.

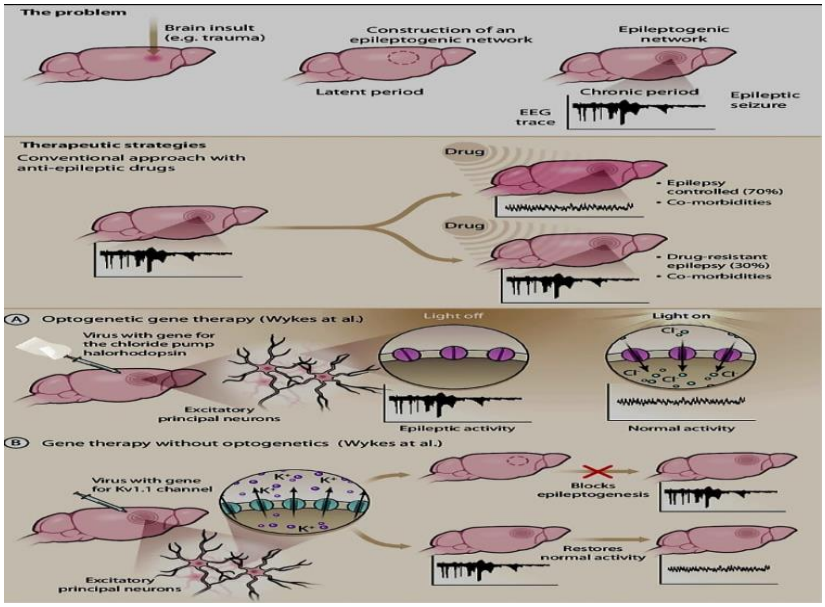


Figure 4.1

Gene therapy for treating epilepsy

B) The second gene therapy technique involved introducing a viral vector containing a gene which encodes for the local potassium ion channel - Kv1.1 (encoded by KCNA1) into the rat brain. Kv1.1 Increased expression in rat neurons resulted in increased potassium ion outflow, enhanced hyperpolarization of membrane, and reduced excitability of neurons.

   If a virus containing Kv1.1 was administered into the brain of a rat simultaneously as tetanus toxin, epileptogenic was halted. Epileptic activity steadily decreased over a few weeks after a virus containing Kv1.1 was administered into the brain of rats after epilepsy had been identified, eventually resulting in a normal EEG.

4.7.2 An Engineered Potassium Channel for Epilepsy Gene Therapy:

In a procedure of FNE stimulated by tetanus neurotoxin (TeNT) injection into the motor cortex of rat's brain, it was previously reported that increased expression level of human voltage-gated potassium channel Kv1.1 mediated by lentivector, reduces pathologically enhanced electrocorticography (ECoG) activity.

Gene therapy centered on Kv1.1 overexpression necessitates successful transgene expression targeting of excitatory neurons. The potent viral promoter CMV successfully drives KCNA1 increased expression in rat pyramidal nerve cells.

A gene of engineered potassium channel (EKC) was used to enhance Kv1.1 expression and minimize inactivation, while use of cell type-specific promoter (CAMK2A) to improve protection from harm and other non-desirable outcomes was done. The structure was evaluated in cases of

FNE and temporal lobe epilepsy (TLE). It was structured into a nonintegrating lentiviral vector or even an AAV2/9 vector.

Within a motor cortex TeNT model of EPC, it was previously demonstrated that Kv1.1 increased expression can decrease the occurrence of epileptiform discharges which are short (i.e., less than 1 s) and of high-frequency. However, the study did not look into whether overexpression of Kv1.1 could prevent discrete seizures that could last 1–2 minutes, which are much more common in focal epilepsy.

Three independent studies indicate that overexpression of Kv1.1 is effective in reducing the occurrence of isolated, long-lasting seizures. According to in vitro studies, increased expression of Kv1.1 reduces both underlying excitability of neurons and release of glutamate from transduced pyramidal neurons, which could be a mechanism for preventing initiation of seizures. Both of these effects evaluated properties of neurons, and both neuronal excitability and neurotransmitter release are not fully removed.

In the TLE model, AAV-CaMKII-EKC therapy decreased both the duration and frequency of seizures, whereas therapy involving Lenti-CaMKII-EKC only decreased the frequency of seizures inside the FNE model. The AAV and lentiviral vector distribution throughout the parenchyma of the brain in connection to networks which generate seizures may explain this difference.

The nonintegrating EKC lentivirus used in this study directed effective, localized expression of transgene after injecting it directly into the rat neocortex and also inhibited focal seizure activity quickly and persistently. This gives credence to integration-deficient vectors' utilization as secure and reliable delivering vehicles for neurological gene therapy.

In the case of epilepsy, an additional safety concern is the possibility of increased expression of potassium channels in interneurons, which could intensify seizure activity by increasing instead of decreasing localized excitability. To reduce this risk, a human CAMK2A promoter is utilized - which resulted in less activity in GABAergic cells in rats.

Neuropeptide Y and its receptors-based in-vivo gene therapy:

Memory formation, dietary patterns, stress, heart rate, angiogenesis, neurogenesis, and sleep patterns are just a handful of good physiological functions that NPY has been linked to. Furthermore, NPY is involved in a variety of pathological conditions, including several neurodegenerative diseases as well as alcoholism, stress, PTSD and depression. NPY receptors are G-protein coupled receptors with five forms (Y1, Y2, Y4, Y5, and Y6).

Modifications in the Neuropeptide Y framework for epilepsy:

After prolonged seizure activity, amounts of NPY (as well as other neuropeptides) have been seen to rise, like SE in rats which are induced by electrical stimulation or by KA. Similarly, NPY upregulation is caused by frequent brief seizures which are stimulated by chemical or electrical kindling.

In humans, increased expression of NPY is only seen between neurons with axonal sprouting, leading to higher GABAergic and NPY innervation of mossy fibers in tissue resected from subjects with TLE. The Y1 and Y2 receptors are shown to have a variety of opposite hyperexcitability effects. As a result, these adaptive improvements in Y1-2 receptors and NPY expressions is said to have an endogenous anti-seizure strategy.

NPY system used in Gene Therapy:

In 2004, this was discovered that injecting bilaterally NPY enhanced expressing AAV vectors-decreased acute seizures which were induced by KA as well as dramatically prolonged kindling.

Since these initial experiments were conducted in induced seizure models, it was unclear if SRSs would be inhibited by focal AAV-derived NPY overexpression. In 2008, it was found that bilateral overexpression of NPY could suppress spontaneous seizures after electrically induced SE.

Since the activation of Y2 receptor mediates the seizure-suppressing effect of NPY, it had been speculated that increased expression of such a receptor would have a seizure-suppressing effect. Overexpression of Y2 reduced the severity of KA-induced acute seizures and slowed the growth of kindling's.

Interestingly, when Y2 receptor were conjugated with NPY, its expression levels increased. The threshold which was observed after-discharge was considerably higher than when Y2 was overexpressed individually or when the control was empty.

This suggested that enhanced expression of NPY and Y2 receptor in combination may exhibit a beneficial result on seizures.

Absence seizures could be prevented with AAV-mediated gene therapy that increases NPY expression.

Overexpression derived from viral vectors was directed to the somatosensory cortex or else the thalamus, leading to decrease in seizure recurrence and length.

4.7.3 Combinatorial gene therapy of NPY and Y2 in suppressing seizures:

Combinal gene therapy of NPY and Y2 was conducted in the KA rat model, in which two separate AAV vectors were administered independently in the presumed seizure focus. The treated rats had a disease-modifying effect, as their SRS frequency was maintained.

The control group, on the other hand, experienced a rise in SRS incidence, demonstrating the disease's progression in the model. Furthermore, as opposed to the control animals, the treated

animals demonstrated a significant difference in the amount of time spent in SRSs due to propensity for shorter seizures combined with a decrease in SRS duration.

There is a 20 percent responder value (more than 50% reduction in SRS frequency) and a 20 percent seizure-free average, according to a medical assessment of the tests. These requirements were not met by any of the control group animals.

Furthermore, a single vector for transgene overexpression was used in an efficacy trial.

The findings of individual AAV1-NPY-IRES-Y2 gene therapy conducted with the aid of MRI revealed a responder rate of 31.3% in 5 out of 16 results and no seizure rate of 12.5% in 2 out of 16 results, that is approximately equivalent also to outcomes of vagal nerve stimulation. Once more, none of the animals in the test group were able to meet these criteria. The likelihood that Y2 receptor not only disrupts glutamate production, but can also modulate NPY discharge through self-regulation is a result of simultaneous increased expression of Y2 and NPY receptor.

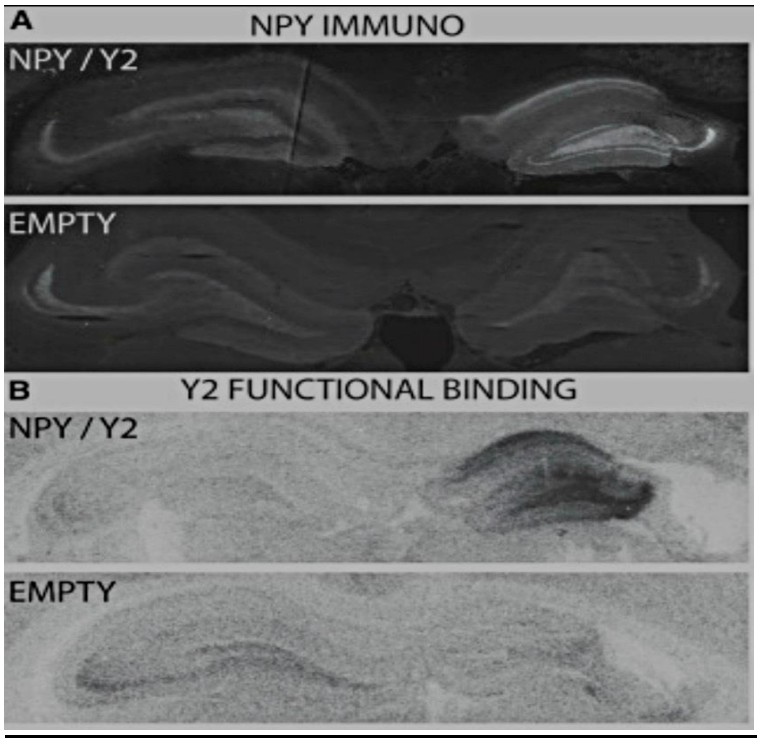


Figure 4.2

NPY and Y2 were significantly increased in NPY/Y2- Treated Animals

Among NPY/Y2-treated animals, Y2 and NPY levels were considerably higher.

The transgender NPY activity in the hippocampus was investigated using immunohistochemical staining. In most of the NPY/Y2-treated animals, a raised level of NPY activity was found by their immunofluorescent rates, ipsilateral to injection site of the viral vector. Endogenous NPY immunoreactivity has been found within the hippocampus in the brain of control group animals and among the NPY/Y2 community contralateral to the vector injection.

4.7.4 GDNF-based ex-vivo gene therapy:

Neurotrophic factors are biomolecules that are essential for neuron growth and survival. There are several sub-groups, with neurotrophins, such as BDNF and NGF, as one of the most studied. The GDNF Family Ligands (GFLs), consisting of GDNF, which was derived from a glial cell line from a pig, are another subgroup.

With a little low affinity interaction, each GFL binds with a different family receptor of GDNF i.e., GFRa1, GFRa2, GFRa3 or GFRa4.

GDNF role in Epilepsy:

The fact that KA or pilocarpine-induced seizures raise both protein and mRNA levels of GDNF in the brain region of the hippocampus suggests that GDNF is related to epilepsy.

Seizures affect the GDNF receptors as well. The increased level of hippocampal GFRa1 can be induced by either systemic KA administration or by electrical stimulation.

GDNF administered intracerebroventricularly (i.c.v.) prevents KA-induced seizures and slows kindling's behavioral progression.

GDNF system used in Gene Therapy:

GDNF transmission by viral vector and ECB (Encapsulated cell bio delivery) into the hippocampal region of the brain, have demonstrated reduction in seizures in different ways.

In the hippocampal region, GDNF activity mediated by adenoviral inhibited acute epilepsy which were induced by KA along with restoring expression of GAD (Glutamic Acid Decarboxylase), which is involved in GABAergic signaling pathway.

In pre-kindled rats, increased GDNF expression (which was driven by AAV vectors) reduced the frequency of generalized seizures in SE, generated by electrical stimulation and raised the induction threshold of seizures.

Encapsulated Cell Bio delivery of GDNF demonstrated a correlation which is dependent on the dose between the GDNF amounts released from the implants and the re-kindling responsiveness.

And, on fifth day of rekindling, animals inserted with devices that released low levels of GDNF had reduced after-discharge time and milder seizures than animals inserted with devices that released higher quantities.

4.7.5 Encapsulated cell bio delivery of GDNF in seizure suppression:

In the pilocarpine model, the GDNF's seizure depressing ability discharged by Encapsulated Cell Bio delivery is seen to prevent SE which are electrically induced as well as prevent SRSs.

Since encapsulation of cells allows explanation of the system, Encapsulated Cell Bio delivery has some substantial benefits over direct cell therapy as well as in-vivo gene therapy. This allows them

to cancel the procedure if there are questions about compliance or if the treatment is unsuccessful. Furthermore, it provides a mechanism for delivering the therapeutic agent to the parenchyma in a targeted manner.

The mechanism underlying GDNF's inhibition effect over SRSs is still unknown. In the pilocarpine model, Encapsulated Cell bio delivery of GDNF has demonstrated to partially resist changes in hippocampal volume as well as pathology related neurodegeneration. It also helped GABAergic parvalbumin positive interneurons survive longer. Since GABAergic dysfunction has been linked to seizure generation it is likely that restoring GABAergic signaling could help explain therapeutic effects.

A research was conducted to see whether GDNF ECB has a seizure-suppressing effect when administered independently into the suspected seizure focus. With a response rate of 50% (reduction of more than 50 percent in SRS frequency), the findings show a strong inhibitory action on SRSs.

Zero animals throughout the control group showed this extent of decrease, similar to efficacy analysis with Y2 and NPY receptors. Rather, disease progression was seen in all animals except one in the control group of 7 animals, whereas SRS frequency was reduced in 5 among 6 animals in the treated group. These findings indicate that GDNF ECB alone is sufficient to suppress SRSs.

5. Parkinson's Disease: -

Parkinson is an ordinarily neurodegenerative disorder that have an effect on the movement, regularly inclusive of tumour.

PD is pathologically characterized via way of means of the lack of dopaminergic neurons in the substantia nigra and the making sure inadequate dopamine in striatum.

5.1 Pathophysiology: -

While PDs are sporadic in maximum cases, and chronic kinds of the disorder additionally exist. Point Mutation of mutations and gene encoding d-synuclein, The protein required in synaptic transmission, generates autosomal recession.

Even in sporadic PD, diagnostic function of the disorder -the lewy body containing the Alpha Synuclein.

The linkage among Alpha synuclein and pathogenesis of PD is unclear, however genetics structure of the PD offers clues. Clues come from affiliation of PD with the mutation with protein kinase referred to as LRRK2.

Some shapes of familial PD are related to mutation PARK7 or PINK I genes, each of which seem to be vital for ordinary mitochondrial function.

Figure 5.1.1

Normal substantia nigra

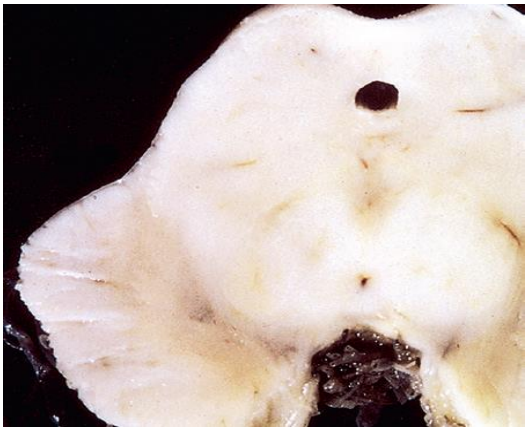


Figure 5.1.2

Decolourized substantia nigra

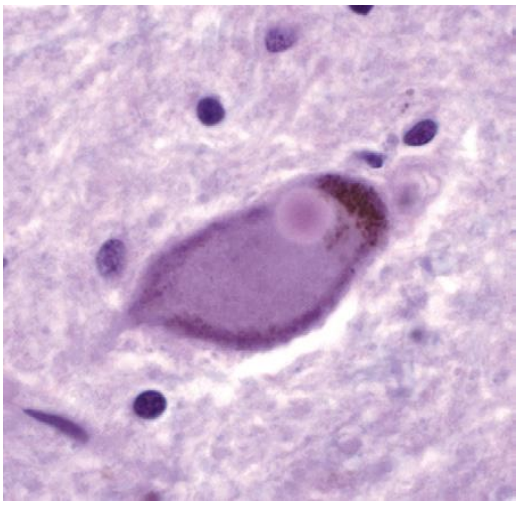
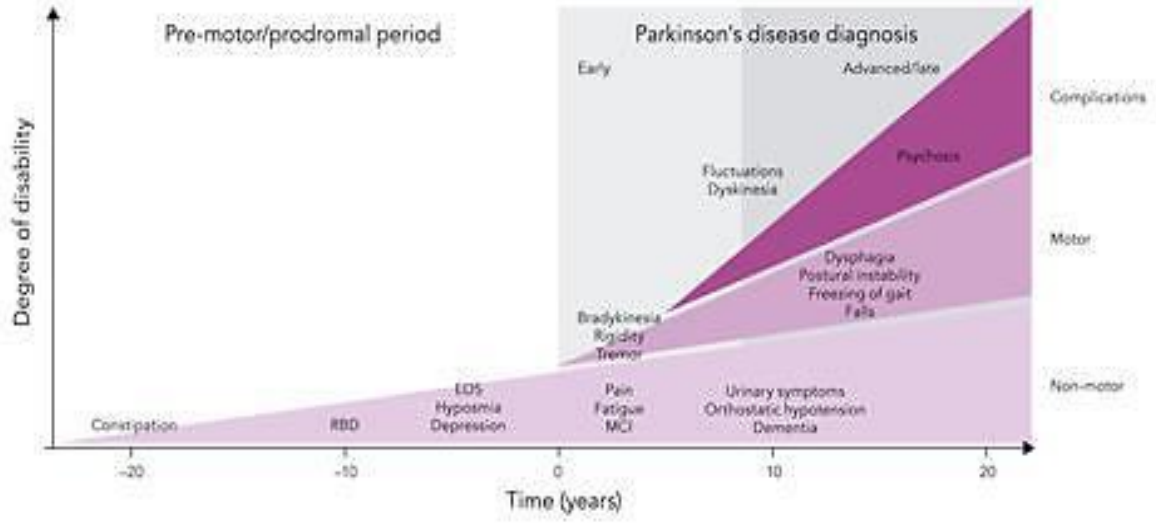


Figure 5.1.3

Lewy body in neuron

5.2 OCCURANCE OF THE DISEASE (WITH RESPECT TO TIME):-



***5.3 STAGES OF THE DISEASE*:-**

Stages	Severity	Symptoms
1	Mild	Tremor of one hand Rigidity Clumsy leg
2	Mild	Loss of facial expression both side Decrease blinking Speech abnormalities
3	Moderate	Balanced is compromised Inability to make Rapid, autonomic and involuntary adjustment

4	Severe	Patient is enable to live an independent life and need assistances
5	Severe	Patient falls when stand or turning Hallucination and delusions

Table 5.3

***5.4 Treatment*:-**

Mediation:-

Use of levodopa as continuous therapy for early and late disease. Addition agent like catechol O-methyltransferase inhibitor or/and monoamine oxidase are used for the treatment of PD. This therapy are for the symptomatic relief therapy there is no effect on the pathogenesis of the disease.

Exercise:-

For improvement in the movement of the upper and lower limb exercise is beneficial medication for PD patient

***5.5 Gene therapy for Parkinson's*:-**

For gene therapy isolated target is mention below

1. Glutamate decarboxylase
2. Glial cell line-derived neurotrophic factor
3. Neurturin
4. Aromatic-L-amino decarboxylase (AADC)
5. Prosavin

***5.5.1 Glutamate decarboxylase*:**

In the preclinical animal models of Parkinson's disease, glutamic acid decarboxylase (GAD) gene transfer and other approaches which modify GABA synthesis in the subthalamic nucleus enhance basal ganglia function.

Changes in basal ganglia network connections, such as disinhibition of the subthalamic nucleus (STN) and increased stimuli of the primary output nuclei, induce motor impairments in Parkinson's disease (PD).

In the subthalamic nucleus of rats, GAD was expressed using AAV- vector; GAD plays a role in catalyzing the synthesis of GABA. When electrical stimulation was applied to the transduced neurons, GABA realease associated mixed inhibitory responses were seen. This phenotypic shift led to substantial neuroprotection of nigral dopamine neurons as well as in rescue of the behavioural phenotype in parkinsonian patients. Thus this approach suggested that in the mammalian brain there is plasticity amonst excitatory and inhibitory neurotransmission which can be targeted for therapeutic effect.

5.5.2 Glial cell line derived neurotropic factor:-

By increasing the levels of growth factors which occur naturally, GDNF gene therapy aims to improve the longevity and functionality of susceptible brain cells which gets deteriorated in Parkinson's disease.

This gene therapy uses:

1) A delivery vector : made up of AAV2

It gives the attachment characteristics, carrying capacity, and genetic payload transmission to specific brain cell types

2) Specific genetic payload: It has a unique DNA sequence (gene) that codes for the GDNF protein.

5.5.3 Neuturin:-

In preclinical animal models of Parkinson's disease, neurotrophic factors such as neurturin (NTN) have been demonstrated to successfully boost the efficiency and reduce the dopaminergic nigrostriatal neurons death.

As a result, NTN treatment could potentially improve Symptoms & decrease adverse Effects as well as may change the course of treatment in Parkinson's disease.

In the Phase I clinical test in people with significant Parkinson's disease, CERE-120, a gene transfer vector for delivering NTN to the striatum, was shown to be safe and effective. A Phase II clinical research is now being planned to see if CERE-120 can help those with severe Parkinson's disease.

5.5.5 Prosavin:-

ProSavin, a gene treatment targeted at reviving the production of dopamine in patients with severe Parkinson's disease, was examined for safety by the scientists from France and UK. ProSavin is a viral vector which provides major three enzymes which are involved in dopamine synthesis to the brain. Although the ProSavin method was safe, it did not appear to be very effective.

6) CONCLUSION: -

Personalized treatment is the most progressive creation in the news today. In addition, in the forthcoming time, gene therapy will be a regular word utilized in our family. Personalized treatment can turn into a successful remedy for different deadly illness particularly neurodegenerative infection like Alzheimer's disease, PD, epilepsy and some more. With the continuous examination on quality and their capacity, the capability of gene therapy seems to be boundless. Soon gene therapy can totally change the essence of the medical services framework.

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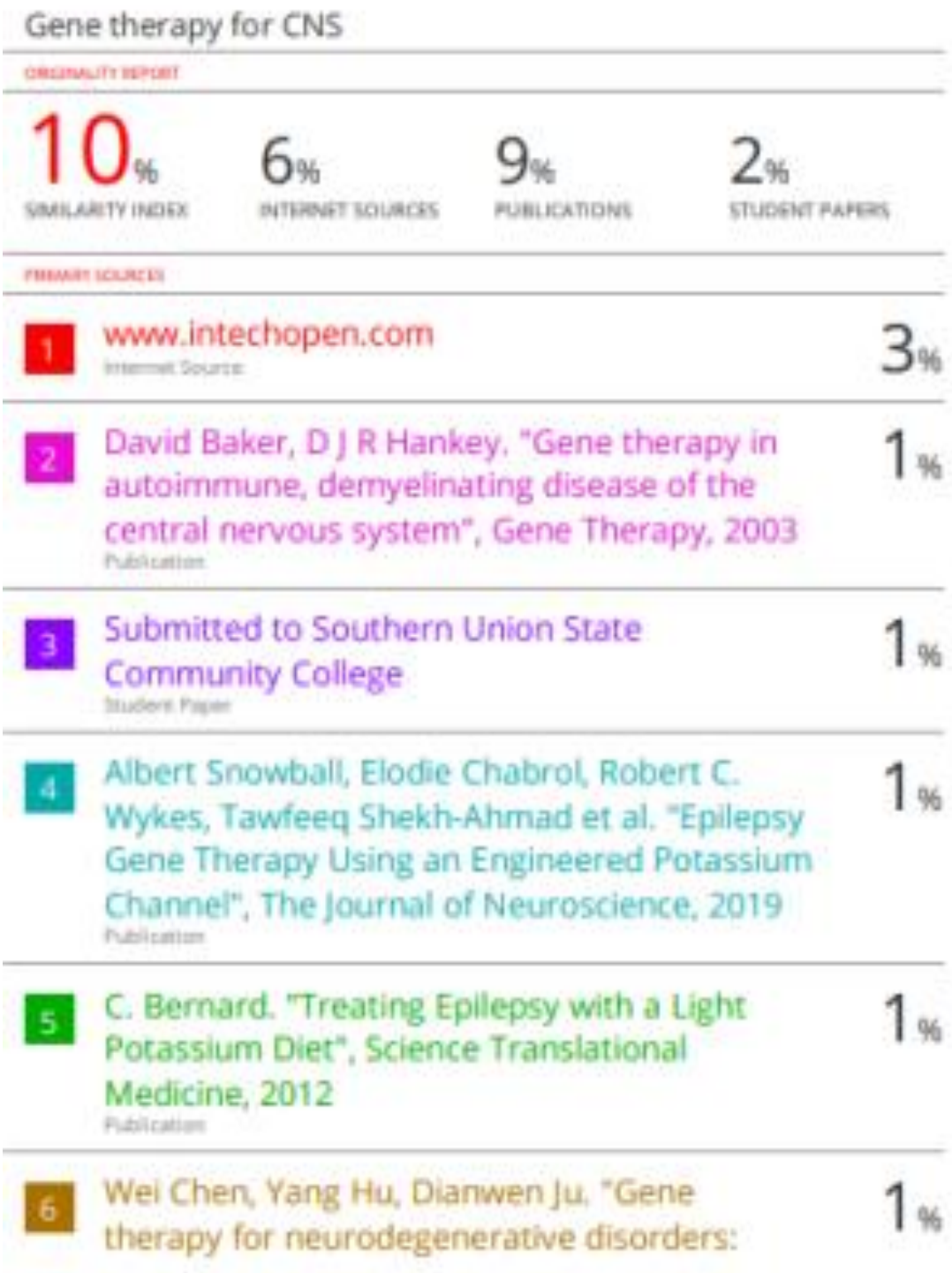

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