Chapter 2
Literature Review

2 Literature Review

2.1 Schizophrenia

2.1.1 Background

Schizophrenia is chronic debilitating mental disorder characterized by positive and negative symptoms including delusions, hallucinations, disorganized speech or behavior and impaired cognitive ability [1]. It also results in disintegration of thinking process and emotional responsiveness and leads to comorbid disease. Schizophrenia causes disability in the patients due to early onset, negative symptoms and cognitive symptoms resulting in loss of attention, memory and working capability. Additionally, there are huge chances of relapse when patients halt the treatment due to positive symptoms like delusions and hallucinations [2].

2.1.2 Pathophysiology

2.1.2.1 Neurotransmitter abnormalities

Various theories are described to explain pathophysiology of schizophrenia which mainly concerns with abnormalities in neurotransmission. These theories focus mainly on excess or deficiency of neurotransmitters like dopamine, serotonin, and glutamate. Other theories associate aspartate, glycine, and gamma-aminobutyric acid (GABA) as part of the neurochemical imbalance of schizophrenia [3, 4].

Abnormal dopamine levels especially at D_2 receptor sites is implicated with symptoms of schizophrenia [5]. Dopamine neurotransmission in brain is regulated by 4 pathways, all of which have been linked with onset of various schizophrenic symptoms. Low dopamine levels in nigrostriatal pathway affect extrapyramidal systems and cause motor symptoms. Excess dopamine in mesolimbic pathway causes positive symptoms. Low levels of dopamine in mesocortical pathway leads to negative and cognitive defects. Decreased or blocked tuberoinfundibular dopamine results in galactorrhea, ammenorrhea, and reduced libido [6, 7].

Serotonin affects several important brain functions like perception, mood, sleep, appetite, cognition and pain. Additionally, lysergic acid diethylamide (LSD) which enhances effects of serotonin in brain developed psychosis like syndrome and thus serotonin was implicated in the pathophysiology of schizophrenia. Furthermore, clozapine and risperidone, newer antipsychotic agents and serotonin antagonists, proved better in treatment of negative symptoms and treatment resistant schizophrenia [2, 8].

Currently, researchers have diverted their focus on glutamate neurotransmitter and some even believe schizophrenia as a hypoglutamatergic disorder. This was supported by the fact that phencyclidine and ketamine, NMDA receptor antagonist, can lead to psychotic illness even in healthy humans [9]. NMDA receptors were also linked to negative, affective and cognitive symptoms of schizophrenia [2].

Additionally, neurochemical imbalance in the levels of aspartate, glycine and gamma-aminobutyric acid (GABA) have been implicated in the pathophysiology of schizophrenia [4].

2.1.2.2 Anatomic abnormalities

Schizophrenia is also associated with anatomical changes in the brain which may be considered as structural features of the disease. There has been reduction in brain volume in medial temporal areas with larger ventricles along with some structural changes in hippocampus [10-12]. Magnetic resonance imaging (MRI) studies found some structural anomalies in neocortical and limbic regions and interconnecting white-matter tracts [13]. Additionally, loss of whole-brain volume in both gray and white matter and increase in lateral ventricular volume was observed in patients [14].

2.1.2.3 Immune system and inflammation

It is hypothesized that changes in brain structure and function may be due to excessive production of inflammatory cytokines which may be outcome of hyperactive immune system [15].

2.1.3 Etiology

Even though exhaustive research over a century has been carried out to understand the primary cause of schizophrenia, the researchers have not found marvelous success in it and thus this disease is considered to be caused by interaction among multiple factors including genetic susceptibility and environmental influence [4, 16].

It is believed that schizophrenia begins to develop in fetal stage i.e. it develops in utero [17]. Various obstetric complications including gestational diabetes, low birth weight, asphyxia, bleeding during pregnancy and emergency cesarean section have been implicated to cause schizophrenia [16]. Infection and excessive stress during second trimester of pregnancy viz. considered important for fetal neurodevelopment doubles the risk of schizophrenia in developing fetus [18].

The findings of various studies come to common point of conclusion that genetic factor have central and primary role in causation of schizophrenia [16]. The statistics also states about 10% risk for a first-degree relative while 3% risk for a second-degree relative supporting hereditary cause for schizophrenia. Additionally, for monozygotic twins, the risk of schizophrenia is 48% while it reduces to 12% to 14% in dizygotic twins. Furthermore, if both the parents have schizophrenia then the risk of schizophrenia increases by 40% in the child [19, 20]. Secondly, studies conducted to distinguish whether schizophrenia is caused by genetic factors or environment led to unbiased results that environment do not prevent emergence of the disease in the children born to biological parents with illness [17, 18]. Moreover, siblings with schizophrenia experience the onset of symptoms at the same age which further supports the genetic basis of schizophrenia [16].

Environmental and social factors have also been implicated to cause schizophrenia especially in subjects vulnerable to this disease. Environmental factors involved in schizophrenia includes residence in an urban area, childhood trauma, social isolation and minority ethnicity. Additionally, social factors like discrimination or economic adversity make individuals more prone to develop delusion and paranoia [3].

2.1.4 Epidemiology

Currently, schizophrenia is included in the top 10 causes of disability worldwide. It has prevalence of 0.6% to 1.9% [21] worldwide and diagnosed in about 5.1 per 1000 lives, annually [22]. The lifetime morbidity of schizophrenic patients is about 7.2 per 1000 [23]. It is equally prevalent in males and females but differs in onset of symptoms which is early 20s in males while early 30s or late 20s in females [24]. There is bias whether its occurrence varies according to the geographic location. A study carried out in 10 countries by World Health Organization concluded that occurrence of schizophrenia is comparable across populations of different geographical locations [25]. But later when similar study was carried out in 33 countries the results indicated effect of geography on incidence of schizophrenia [26].

2.1.5 Clinical presentation

Schizophrenia is chronic mental disorder that results in disintegration of thinking process and emotional responsiveness making it difficult for individual to participate in social events and meaningful relationships [1, 16]. Social withdrawal is the first symptom presented in the psychotic attack but sometimes patients present no

symptoms at all. Additionally, psychotic episodes are characterized by patient specific signs and symptoms creating false reality in the patient's mind. The symptoms of schizophrenia can be classified as positive, negative and cognitive symptoms.

Positive symptoms include delusions, hallucinations and abnormal motor behavior. Negative symptoms present with high morbidity as they disturb patient's emotions and behavior. It generally includes flat expressions or little emotion, alogia (poverty of speech), anhedonia (inability to experience pleasure), lack of desire to form relationships, lack of motivation, inability to exhibit goal directed behavior. Cognitive symptoms are characterized by disordered attention, thought and speech which mainly affect individual's communication skill and thus affect social relationships of the individual.

Additionally, the individuals are prone to substance abuse disorder towards substances like alcohol, tobacco and other medications. The patients are more prone to other neurological disease like depression, anxiety, obsessive compulsive disorder etc [27]. Generally, the patients are not aware of their illness which results in therapy non-compliance, poor hygiene, worsening of the disease conditions and possibly relapse [16]. Furthermore, most of the patients respond poorly to the administered antipsychotics and only 20% patients respond well to the given therapy which has restricted efficacy of antipsychotic medications.

2.1.6 Diagnosis

Diagnosis of schizophrenia is done following the guidelines of Diagnostic and Statistical Manual of Mental Disorders, Fifth edition (DSM-5). According to DSM-5, persistence of two or more of the symptoms like delusions, hallucinations, disrupted speech, disorganized behavior and negative symptoms for more than one month indicate emergence of schizophrenia. Amongst all these one of the qualifying symptom is delusion, hallucination or disorganized speech. The individual also loses interest in work, relationship and self-care with reduction in working potential. The symptoms should persist for minimum of 6 months with more than 1 month of active phase symptoms. The delusions and hallucinations period and severity can be used to differentiate it from other psychotic illness including depression, obsessive compulsive disorder, post-traumatic stress disorder etc. Additionally, it is important to determine whether the symptoms are due to schizophrenic episode or other conditions like substance abuse or other medical conditions [28].

2.1.7 Treatment

The primary aim of treatment of schizophrenia includes symptomatic relief, avoidance of relapse and improvement of the functioning capability of the individual. All these may lead to improvement of patient's social and communication skills which would help them to integrate smoothly back into the society. Despite of rigorous pharmacotherapy patients rarely return to their normal functioning and thus both pharmacological and non-pharmacological treatments are required for better and sustainable outcomes over long term [16].

2.1.7.1 Nonpharmacological therapy

Psychotherapy is classified mainly into 3 categories: Cognitive behavioral, individual and group therapy. Morken et al. conducted a case study and found out that 37 to 74% of schizophrenic individuals fail to co-operate with the treatment regimen [29]. This non-adherence of therapy may be result of individual being non-aware of their medical condition, severe adverse effects of antipsychotic medications or paranoia. This may lead to worsening of disease condition or relapse. Thus, cognitive behavioral therapy concerns mainly with patient compliance and thus assures that patient adheres to treatment regimen willingly [30]. Group therapy focuses on interaction of the affected individual with others and thus helpful to improve social relationships of the individual. Individual therapies are very important and they focus mainly for betterment of affected individual along with their rehabilitation. This includes therapies like counselling, personal therapy, social skill therapy, vocational sheltered employment rehabilitation therapy etc. Emerging psychotherapies include meta-cognitive training, narrative therapies, and mindfulness therapy [31]. All these psychotherapies have been effective in reducing rehospitalization and improving social functioning of affected individuals.

2.1.7.2 Pharmacological therapy

It is hypothesized that structural changes in brain is observed in first 5 years after onset of first acute episode of schizophrenia and thus initiation of treatment during this period is very important [27]. Rigorous observations are required in individuals consuming amphetamines and other CNS stimulants as these substance results in poor prognosis of schizophrenia. Additionally, substance abuse of alcohol, caffeine and nicotine may cause adverse drug interactions [32].

The treatment of schizophrenia should be done in two stages i.e. acute therapy and maintenance therapy. Prompt pharmacotherapy within seven days of first psychotic episode would ensure and help the individual to return to their normal functioning especially for sleeping and eating. Dose of the antipsychotic medications during acute treatment should be adjusted according to the patient's response to administered medications. Acute phase treatment is followed by maintenance therapy for minimum 12 months viz. aimed to improve social behavior of the patients and to prevent relapse. Maintenance therapy reduces chances of remission or relapse of schizophrenia by almost 50% [27, 33].

Currently, medications used for the treatment of schizophrenia are classified into 2 groups - the typical antipsychotics or first generation antipsychotics (FGA) and the atypical antipsychotic medications or second generation antipsychotics (SGA). Currently available drugs act either by D_2 antagonism or $5HT_2A$ antagonism or combination of both mechanisms [34, 35].

Table 2. 1: Clinically used antipsychotic drugs with their trade names

Typical Antipsychotics		**Atypical Antipsychotics**	
Drug	**Marketed products**	**Drug**	**Marketed products**
Chlorpromazine	Thorazine®	Aripiprazole	Abilify®
Fluphenazine	Prolixin®	Clozapine	Clozaril®
Haloperidol	Haldol®	Iloperidone	Fanapt®
Loxapine	Loxitane®	Olanzapine	Zyprexa®
Perphenazine	Trilofan®	Paliperidone	Invega®
Thioridazine	Mellaril®	Quetiapine	Seroquel®
Thiothixene	Navane®	Risperidone	Risperdal®
Trifluoperazine	Stelazine®	Ziprasidone	Geodon®

The Texas Medication Algorithm Project (TMAP) has proposed 6 stage therapeutic algorithm for management of schizophrenia [36] which is depicted as follows:

- Stage 1 consist of monotherapy with atypical antipsychotics.
- Stage 2 involves treatment with another SGA or typical antipsychotic.
- Stage 3 comprises clozapine monotherapy along with WBC count monitoring.
- Stage 4 therapy includes combination of clozapine with FGA, SGA or electroconvulsive therapy (ECT).

- Stage 5 treatment deals with monotherapy by either FGA or SGA that has not been tried in previous stages.
- Stage 6 consist of combination therapy of SGA, FGA, ECT and/or a mood stabilizer.

Progression to succeeding stage of therapy depends only on failure or inadequate response to therapy in the previous stages. Clozapine is avoided in initial phase of treatment due to its risk of causing agranulocytosis but is preferred drug of choice for treatment resistant schizophrenia. According to American Psychiatric Association SGAs are preferred over FGA as they have less potential to cause extrapyramidal side effects. But these agents have been associated with metabolic side effects like diabetes mellitus, hyperlipidemia, weight gain etc. which may increase the risk of cardiovascular diseases [37]. Additionally, combination therapy is generally preferred in the later stages of therapy as it may result in drug interactions and patient non-compliance. Moreover, to avoid compliance issues, long acting injectables are administered in the schizophrenic individuals.

2.1.8 Adverse effects

The main adverse effects that proves to be significant in patient non-compliance towards antipsychotic medications are weight gain and extrapyramidal symptoms. SGAs are associated with weight gain and other metabolic adverse effects while FGA are concerned mainly with extrapyramidal side effects. Other side effects affecting several systems are as follows:

2.1.8.1 Endocrine system

Risperidone and paliperidone can lead to hyperprolactinemia in 87% cases which further leads to decreased libido, sexual dysfunction, menstrual abnormalities or gynecomastia. Olanzapine, and to some extent risperidone and quetiapine has potential to cause diabetes.

2.1.8.2 Cardiovascular system

Orthostatic hypotension is found in 75% of the patients treated with antipsychotics. Additionally, metabolic side effects like weight gain, diabetes and hyperlipidemia may increase the risk of cardiovascular disease. Antipsychotics like thioridazine, clozapine, iloperidone, and ziprasidone may lead to Electrocardiographic changes like QTc prolongation [38].

2.1.8.3 Lipid changes

SGAs or phenothiazine treatment have potential to increase concentration of serum triglycerides and cholesterol.

2.1.8.4 Central nervous system

FGAs have been associated with variety of side effects including dystonia, akathisia, pseudo-parkinsonism, tardive dyskinesia etc. All antipsychotic medications have sedating effect and the patients are more prone to seizures, delirium and psychosis. Poikilothermia and neuroleptic malignant syndrome are rare but fatal side effects of antipsychotic treatment.

2.1.8.5 Miscellaneous

This includes cataract, urinary retention, leucopenia, hematological complications, dermatological allergic reactions, photosensitivity, sialorrhea etc.

2.2 Gene therapy for schizophrenia

Current drug treatments for schizophrenia are not curative, since people who stop taking them have a very high chance of having a full psychotic relapse (i.e., 70% or greater) within a few years. Also, antipsychotics generally do not treat the cognitive deficits (attention, memory) or functional disabilities (social, occupational) that are often the most chronic and intractable features of schizophrenia. Furthermore, antipsychotic medications are accompanied with several severe side effects resulting sometimes in discontinuation of therapy. Typical antipsychotics results in neurological side effects and extrapyramidal side effects including tardive dyskinesia, muscle stiffness, temporary paralysis etc. while atypical antipsychotics presents side effects including drowsiness, sexual dysfunction, weight gain etc.

Although, medical science has evolved greatly, mortality rate in schizophrenic patients has increased which may be attributed to lifestyle (i.e., lack of exercise, unhealthy diet, excessive smoking and alcohol intake), adverse effects, suboptimal treatments of the related adverse effects and suicide [39]. Additionally, pathophysiology of this disease still remains unclear which is considered as the main cause for failure of currently available therapies [40]. Even though, improvements on existing drugs and drugs acting at novel neurotransmitters will yield improved therapy, this approach to therapy is not as intellectually satisfying as efforts to find causes. Thus, researchers have diverted their attention for alternative form of treatment for schizophrenia which can target the root cause of the disease and have focused on

developing gene therapy. With these efforts various susceptibility genes for schizophrenia were discovered some of which are depicted in Table 2. 2

Table 2. 2: Susceptible genes in schizophrenia [41]

Gene	Chromosome	Role
RGS4	1q23.3	Modulates G-protein signaling downstream of receptors for dopamine and other neurotransmitters
DISC-1	1q42.2	Interacts with citron, which possibly links to PSD95/glutamate (NMDA) receptor
NRG1	8p12	Decreases NMDA-R expression and modulates glutamatergic neurotransmission
DAOA	13q34	Modulates DAAO, influencing D-serine metabolism
CHRNA7	15p14	Acetylcholine receptor
COMT	22q11.21	Influences metabolism of catecholamines such as dopamine
PRODH	22q11.21	Functions as a neuromodulator for glutamatergic neurotransmission and serves as precursor for glutamate
AKT1	14q32.33	Participates in neurodevelopment, synaptic plasticity, protein synthesis and neurotransmission in the central nervous system.
DRD3	3q13.31	Encodes the D_3 receptor viz. localized to limbic areas and associated with cognitive, emotional, and endocrine functions
DTNBP1	6p22.3	Plays a role in synaptic vesicle trafficking and in neurotransmitter release. Also modulates prefrontal cortical activity via the dopamine/D_2 pathway.
G30/G72	13q33.2	Interaction of G72 with d-amino acid oxidase acts as a modulator of N-methyl-d-aspartate receptors through regulation of d-serine levels
HTR2A	13q14.2	Encodes one of the receptors for serotonin
SLC6A4	17q11.2	Transports the neurotransmitter serotonin from synaptic spaces into presynaptic neurons. The

		encoded protein terminates the action of serotonin and recycles it in a sodium-dependent manner.
ZDHHC8	22q11.21	Encodes a putative palmitoyltransferase at 22q11

2.3 Neuregulin1 (NRG1)

2.3.1 NRG1 and schizophrenia

Human NRG1 is located at chromosome 8p12. It consists of about 1.4 megabases, has more than 20 exons, several large introns and gives rise to at least 15 isoforms. Genetic studies carried out recently have enlightened the possible etiological mechanism of schizophrenia. With this approach several putative genes as mentioned in Table 2. 2 have been implicated in schizophrenia [42].

Meta-analyses identified 8p chromosome as susceptibility locus for development of schizophrenia [43, 44]. Mapping of this chromosome and association studies narrowed down this region to 8p12-8p21 concluding the role of NRG1 in pathogenesis of schizophrenia as it is located in that susceptible region [45]. This was further confirmed by association studies in multiple populations in Ireland, Scotland, Korea, UK, China and Netherland [46-49]. Furthermore, the fact that about 80 schizophrenia associated short nucleotide polymorphisms (SNPs) are located at 5' and 3' region of NRG1 [50, 51].

In accordance with these evidences, polymorphisms in gene that encode NRG1 and its receptor ErbB4 has been considered to play an important role in pathogenesis of schizophrenia. NRG1 has important role in neurodevelopment, neurotransmission and synaptic plasticity and thus is considered potential candidate gene for schizophrenia [52-54].

Overexpression of NRG1 gene was considered to cause schizophrenia [48, 55-59]. It was hypothesized to regulate NMDA receptor expression and thus was considered as a vital therapeutic target for management of schizophrenia [60].

2.3.2 NRG1 signaling

Neuregulin 1 acts as a ligand for ErbB receptor tyrosine kinases, which binds to either ErbB3 or ErbB4 receptors via its EGF domain. Neuregulin 1 does not interact directly with ErbB2, but this receptor is preferred partner in heterodimerization that follows NRG1-ErbB3/4 binding. ErbB dimerization is followed by autophosphorylation of tyrosine residues in cytoplasmic domain of the receptor which creates docking sites for various adaptor proteins such as Shc, Grb2 and the regulatory

subunit of phosphoinositide-3-kinase (PI3-kinase). These in turn activate the mitogen-activated protein (MAP) kinase and PI3kinase pathways and modulate transcriptional activity in the cell [61-63].

The release of NRG1, synthesized as proproteins, is controlled by cell-surface metalloproteases and neuronal activity [64, 65]. NRG1 is released either by paracrine or juxtacrine mechanism depending on the region beyond EGF domain and linking of NRG1 to ErbB receptor [66, 67]. When Ig-NRG1 is cleaved between EGF and transmembrane domain, soluble N-terminal fragment (NTF), containing EGF and Ig domains, is released which diffuses through extracellular space and thereby released by paracrine mechanism. But, type III NRG1, on cleavage, generates membrane bound NTFs which acts on ErbB receptor by juxtacrine signaling mechanism [68, 69].

2.3.3 NRG1 functions

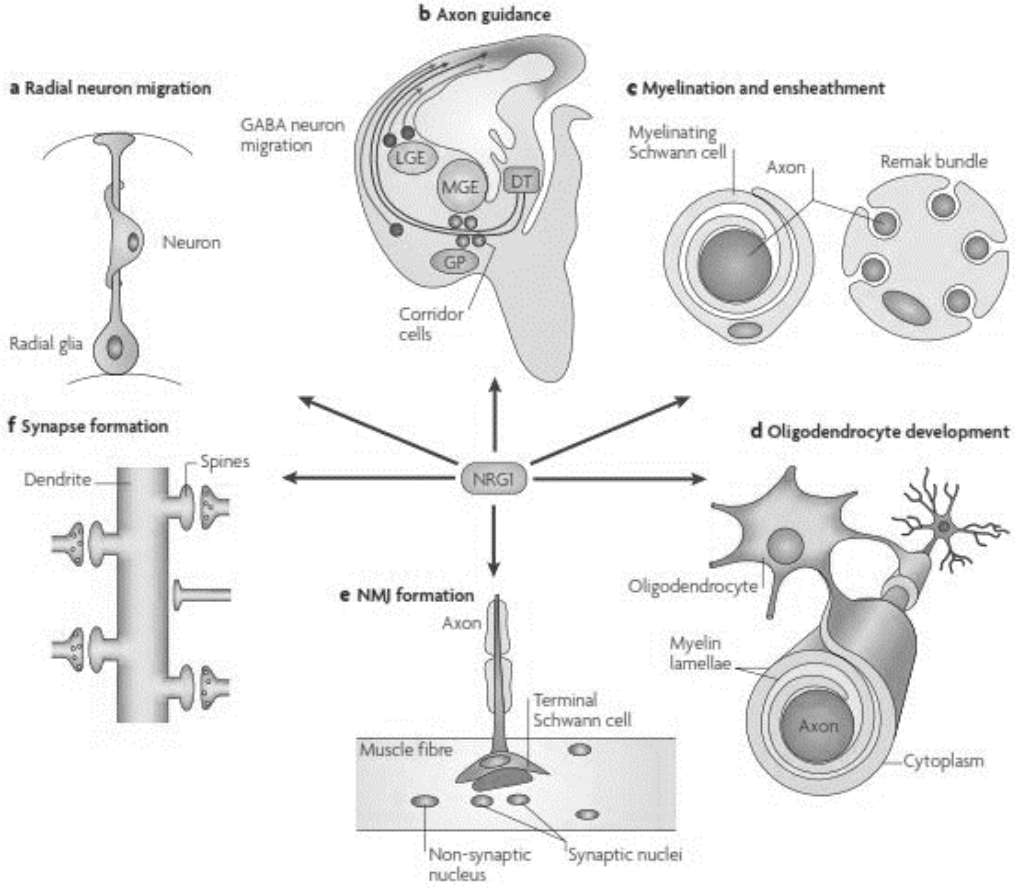

Figure 2. 1: Functions of NRG1 in central nervous system

NRG1 plays important role in neuronal development in CNS which can be depicted as follows:

- Neuregulin 1 promotes formation and maintenance of radial glial cells, which are necessary for the radial migration of neurons from ventricular zones to the pial surface.
- Presence of NRG1 in cortical region results in tangential migration of GABAergic neurons.
- Expression of NRG1 in corridor cells causes thalamocortical axon navigation through the diencephalon.
- NRG1 produced in axons regulates myelination and ensheathment of peripheral nerves
- Even oligodendrocyte formation and myelination of CNS neurons is controlled by NRG1 released in axons.
- NRG1 modulates Schwann cell differentiation and survival and thus proposed to play a role in neuromascualr junction formation.
- NRG1 also has potential role in CNS synapse formation.

2.4 RNA interference (RNAi)

2.4.1 Background

Presently, researchers are focusing to develop alternative treatment approaches as currently available therapeutics fails, in some cases, to provide desired therapeutic efficacy. Additionally, mutation of infectious agents has outdated therapy for infectious disease. This has aroused need to develop RNAi therapeutics which can prove as pioneering technology, revolutionizing the field of therapeutics [70]. RNA interference (RNAi), employing antisense therapeutics to knock down overexpressed genes post-transcriptionally, can be employed to develop novel class of therapeutics [71-73]. It is unique regulatory mechanism controlling gene expression by preventing translation of genes to proteins [74].

2.4.2 Pathway

RNA interference involves degradation or translational arrest of target RNA by binding of short RNA sequence with endogenous mRNA target [75, 76]. The enzyme dicer present in living system initiate RNAi mechanism by causing breakdown of longer RNA transcripts into short double stranded RNAs (dsRNAs). These dsRNAs are incorporated into RNA-induced silencing complex (RISC) viz. comprised of several proteins, amongst which Argonaute (Ago) proteins have active catalytic domain for cleavage [77, 78] and thus plays central role in RNAi. Passenger strand from dsRNAs

is cleaved by Ago protein and the released guide strand is directed to pair with complementary mRNA sequence by RISC complex [79]. The outcome of pairing depends on degree of complementarity of dsRNA and mRNA i.e. if there is perfect or near-perfect complementarity then mRNA target is cleaved by Ago proteins while partial complementarity results in translational repression [80].

2.4.3 RNAi strategies

RNAi can be achieved by delivery of small RNA duplexes including short hairpin RNAs (shRNAs) short interfering RNAs (siRNAs) and microRNA (miRNA).

Exogenous siRNA, as described earlier, are initially cleaved by Ago proteins and the sense strand is incorporated into RISC complex. This activated RISC complex mediates complementary base pairing of siRNA guide strand to mRNA target [79]. Later, under influence of Ago protein, degradation of mRNA occurs which ultimately causes gene silencing [81].

In case of miRNA, RNA polymerase II (pol II) generates primary miRNA (pri-miRNAs) form genome [82]. This pri-miRNA is recognized by and acted upon by microprocessor complex in nucleus, containing Drosha and DiGeorge critical region 8 protein, generating stem-loop hairpin structure called precursor miRNA (pre-miRNA) [83-86]. Later, transport of pre-miRNA from nucleus to cytoplasm is assisted by exportin-5 [87-89]. In cytoplasm, dicer causes cleavage of pre-miRNA generating double stranded miRNA [90, 91]. The guide strand of miRNA, formed as a result of cleavage by Ago proteins, gets associated with RISC complex and causes translational repression [92]. miRNA mimics, delivered exogenously, gets directly incorporated into RISC and causes gene silencing by translational repression [70].

shRNA is transcribed through RNA polymerase II or III which generates hairpin like structure called pri-shRNA. This is acted upon by complex in nucleus, containing Drosha and DiGeorge critical region 8 protein converting pri-shRNA to pre-shRNA [93]. This pre-shRNA under influence of exportin-5 is transported to cytoplasm [94, 95] where it is processed by complex containing RNase III enzyme dicer and TRBP/PACT which results in formation of ds-siRNAs [88, 89]. The subsequent steps of the pathway are identical to that of exogenous siRNA. The pathway followed by all the RNAi therapeutics and their expression mechanism is summarized in Figure 2. 2

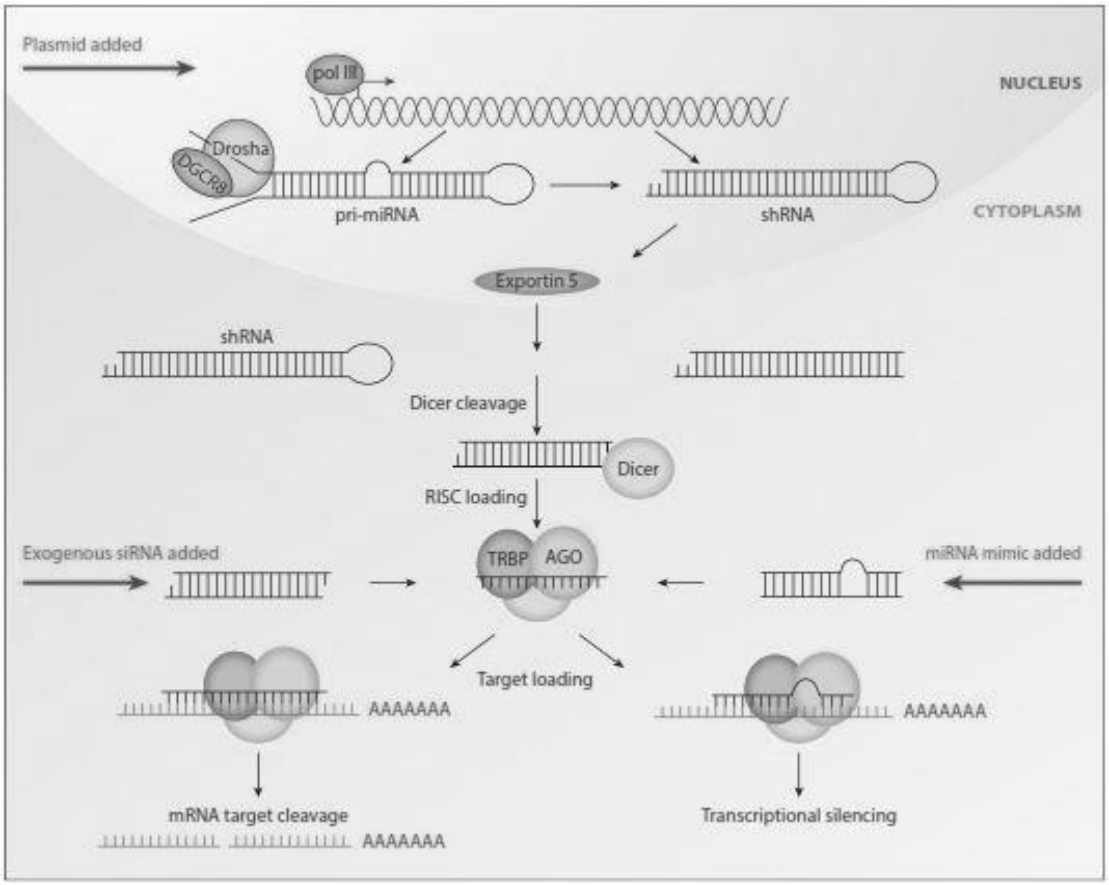

Figure 2. 2: Pathways for RNAi therapeutics

2.4.4 Clinical trials of RNAi therapeutics

All the strategies mentioned above for RNAi therapeutics have evolved to such an extent that all of them have model drugs in clinical trials which are depicted in Table 2. 3.

Table 2. 3: RNAi based drugs in clinical trials [70, 96]

Drug	Delivery system	Disease	Target	Phase
ALN–VSP02	Lipid nanoparticles	Solid tumours	KSP and VEGF	I
siRNA–EphA2–DOPC	Intravenous infusion of DOPC lipid nanoparticles	Advanced cancers	EPHA2	I

Atu027	Lipid nanoparticles	Solid tumours	PKN3	I
TKM–080301	Intravenous injection of lipid nanoparticles	HCC, neural endocrine cancer	PLK1	I/II
TKM–Ebola	Intravenous infusion of lipid nanoparticles	Ebola-virus infection	VP24, VP35, Zaire Ebola L-polymerase	I
ALN–RSV01	Naked siRNA	Respiratory syncytial virus infections	RSV nucleocapsid	II
ALN–PCSSC	SC injection of lipid nanoparticles	Hypercholesterolaemia	PCSK9	I
ALN–TTR02/ patisiran	Intravenous infusion of lipid nanoparticles	Familial amyloidotic polyneuropathy	TTR	I, II, III
CALAA-01	Cyclodextrin NP	Solid tumours	RRM2	I
TD101	Intradermal injection of naked siRNA	Pachyonychia congenita	K6a (N171K mutation)	I
QPI-1007	Intravitreal injection of naked siRNA	Optic atrophy, non-arteritic anterior ischaemic optic neuropathy	CASP2	II/III
I5NP	Naked siRNA	Kidney injury, acute renal failure	p53	I
PF-655 (PF-04523655)	Intravitreal injection of naked siRNA	Choroidal neovascularization, diabetic retinopathy,	RTP801 (Proprietary target)	II

		diabetic macular oedema, glaucoma		
siG12D LODER	Surgical implantation of LODER polymer	Pancreatic cancer	KRAS-G12D	I, II/III
Bevasiranib	Naked siRNA	Diabetic macular oedema, macular degeneration	VEGF	II
SYL1001	Ocular-topical of naked siRNA	Ocular pain, dry-eye syndrome	TRPV1	I, II
SYL040012/ bamosiran	Eye drops od naked siRNA	Ocular hypertension, open-angle glaucoma	ADRB2	II
CEQ508	*Escherichia coli*-carrying shRNA	Familial adenomatous polyposis	CTNNB1	I, II
RXi-109	Intradermal injection of self delivering RNAi compound	Cicatrix, scar prevention	CTGF	II
ALN–TTRsc/ revusiran	SC injection of ESC-GalNAc	Familial amyloidotic cardiomyopathy	TTR	I
ARC-520	Dynamic polyconjugate	HBV	Conserved regions of HBV	I, II, III
ALN-AT3	SC injection of ESC-GalNAc	Hemophilia	AT	I
ALN-AS1	SC injection of ESC-GalNAc	Hepatic porphyrias	ALAS1	I

ALN-CC5	SC injection of ESC-GalNAc	Paroxysmal nocturnal hemoglobinuria	C5	I/II
DCR-PH1	Intravenous injection of lipid nanoparticles	Primary hyperoxaluria type 1	HAO1	Preclinical
ALN-AAT	SC injection of ESC-GalNAc	Alpha-1 antitrypsin deficiency	AAT	I/II
ARC-AAT	Intravenous injection of dynamic polyconjugate	Alpha-1 antitrypsin deficiency	AAT	I
ND-L02-S0201	Intravenous injection of vitamin A targeting lipid nanoparticles	Hepatic fibrosis	HSP47	I/II
TKM-ALDH	Intravenous injection of lipid nanoparticles	Alcohol use disorder	ALDH	Preclinical
APN401	Intravenous infusion of ex vivo electroporation	Metastatic tumors not removable by surgery	Cbl-b/DC cancer vaccine	I
FANG	Intravenous infusion of ex vivo electroporation	Multiple types of cancer	Furin/DC cancer vaccine	I, II, III
MRX34	Intravenous injection of lipid nanoparticles	Liver cancer	miR-32 mimic	I

pbi-shRNA STMN	Intratumoral injection of lipid nanoparticles	Advanced/ metastatic cancer	STMN	I
DCR-MYC	Intravenous injection of lipid nanoparticles	HCC, solid tumor, myeloma, lymphoma	MYC	I, II/III
TT-034	Intravenous infusion of AAV	Hepatitis C virus	Three conserved regions of HCV	I/II
TKM-HBV	Intravenous infusion of lipid nanoparticles	Hepatitis B virus	Three conserved regions of HBV	I

2.4.5 Challenges for RNAi therapeutics

Presently, there are several limiting factors hindering application of RNAi therapeutics clinically which along with strategies to circumvent them are discussed below.

2.4.5.1 Intravascular degradation

Naked siRNA is degraded in serum due to presence of RNase A type nucleases and rapid renal clearance resulting in shorter half-life [97]. Additionally, with each cell division, concentration of siRNA reduces in the cell and thus have transient effect in gene silencing [98]. All these can be overcome by employing nanoparticulate carriers for safe and efficient delivery of dsRNAs. Still, these carriers may pose problems of thrombogenicity, hemolysis and complement activation leading to toxicity issues and altered biodistribution due to their physicochemical properties like size and surface charge [99].

2.4.5.2 Tissue penetrance and intracellular delivery

As evident from several studies, nanocarriers having size >200 nm are trapped by reticuloendothelial system resulting in their degradation by activated monocytes and

macrophages [100]. Additionally, solid tumor pose problems of higher interstitial fluid pressure which restricts diffusion of nanocarriers [101]. This can be bypassed by optimizing size of nanocarriers to avoid RES uptake and employing the concept of enhanced permeability and retention (EPR) [102, 103].

2.4.5.3 Intracellular trafficking

RNAi therapeutics, when delivered by nanocarriers, enter the cells by endocytosis forming endosomes [104]. Later, the contents are transferred to lysosomes which are acidified and nucleases present inside degrade RNA. Thus, if delivered RNAi therapeutics doesn't undergo endosomal escape, the delivered therapeutics become ineffective. Fusogenic lipids/peptides, photosensitive molecules and pH-sensitive lipoplex/polyplex have capacity to enhance endosomal escape of delivered RNAi therapeutics. The carriers either interact with endosomal membrane or alter pH of endosomes by proton sponge effect resulting in endosomal rupture and release of its contents [105].

2.4.5.4 Extracellular factors

After extravasation, RNAi therapeutics carriers must move through extracellular matrix, which may present problems in achieving desired concentration of therapeutics at intended site of action [106]. Several physico-chemical and biological properties of ECM may cause premature release of encapsulated RNAi therapeutics [107]. Various three dimensional models can be employed to estimate the effect of ECM components on RNAi therapeutics carriers [108]. Immune system also act as extracellular barrier by acting as extracelllar traps through phagocytosis of delivered RNAi carriers by monocytes and neutrophils and thus limiting successful delivery of RNAi therapeutics to target site [109, 110].

2.4.5.5 Immune mediated toxicities

Systemic delivery of RNAi therapeutics may cause innate immune response which may be either Toll like receptor (TLR) mediated or non TLR mediated immune response. Cell surface interaction resulting in endocytosis of nucleic acids followed by endosomal acidification and maturation causes TLR mediated immune response. This subsequently leads to activation of IFNα, IFNγ, IL-12 and other inflammatory cytokines [111, 112]. Such immune response may be avoided by delivering RNAi therapeutics in suitable carriers which hinders cell surface interaction of RNAi therapeutics.

The non-TLR mediated immune response is activated by retinoic acid-inducible gene 1 (RIG1) and dsRNA binding protein kinase. When dsRNA binds with RIG1, inflammatory cascade is activated which finally results in production of IFNβ and other inflammatory mediators [113]. This immune response is seen in various cells while TLR mediated immune response is observed only in hematopoietic cells. Thus, employing targeted carriers may help to predict immune response due to delivered RNAi therapeutics. Additionally, some chemical modification to dsRNA backbone improves intravascular stabilization which evades initiation of immune response while retaining activity of RNAi therapeutics [98, 112].

2.4.5.6 Carrier mediated toxicities

Although, cationic lipids which presents lucrative approach for RNAi therapeutics delivery through lipoplexes, these have been implicated in type I and II interferon response [114]. They can also change gene expression levels by themselves independent of RNAi therapeutics which may raise safety issues [115]. Additionally, generation of reactive oxygen species due to cationic lipids causes toxicity and pulmonary inflammation [116]. Negatively charged lipids are also responsible for immune response and reduced efficacy due to repulsion between negatively charged carrier and cell membrane [117].

Application of polymers for RNAi therapeutics delivery has also presented distinct approach through formation of polyplexes but this also faces some limitations. PEI, one of the most widely used polymer has shown cytotoxicity and biocompatibility issues. PAMAM dendrimers, evolving formulation for RNAi delivery, also results in cell membrane disruption through membrane thinning and erosion [118]. These issues can be overcome by using modified polymers having desired properties and acceptable toxicity profile [118, 119]. Additionally, use of biodegradable polymers like chitosan, cyclodextrins and collagen may solve toxicity related issues. Formation of lipidoids by amino-acrylates and amino-acrylamide compounds has presented novel approach for RNAi therapeutics delivery which has proven safety and also require less payload, improving therapeutic efficacy [120, 121].

2.4.5.7 Non-immune off target effects

miRNAs have partial complementarity to target mRNA and thus it is non-selective which can potentially regulate the expression of many different genes. Similarly, poorly designed siRNA can also undergo cross hybridization with

unintended targets and causes gene silencing [122]. Such phenomenon may lead to unintended phenotypic changes which may have severe implications on therapeutic efficacy of RNAi therapeutics [123]. These off-target effects can be avoided by 2'-O-methylation of siRNA which also reduces immune activation [112] and intravascular degradation [124], retaining its efficacy on target mRNA [125].

2.4.5.8 Oversaturation of RISC

Even though RNAi therapeutics are successfully delivered intracellularly, there are some barriers which hinder gene silencing. In some cancer condition, there is alteration in expression level of dicer and drosha which affects processing of miRNA and shRNA. Low expression of dicer attenuates treatment response of delivered shRNA and miRNA [126]. Conversely, overexpression of shRNA in liver may result in hepatic failure and death [127]. Furthermore, several reports suggest that exogenously delivered siRNA or miRNA can compete with endogenous miRNA for RISC which finally causes re-expression of miRNA regulated genes [128, 129].

2.5 siRNA delivery

The foremost cause of concern in delivering siRNA to their targeted site is their degradation by nucleases which may render gene therapy ineffective. Additionally, various challenges described above are also applicable to siRNA and thus conjugation approaches of siRNA to suitable vector is sought for effective delivery of siRNA to intended site [130]. siRNA can be efficiently delivered employing either viral or non-viral vector [130]. Although, viral vectors have shown good transfection efficiency at targeted site, currently their use has become obsolete as they have potential toxicity issues like immunogenicity, carcinogenicity and mutagenicity [131]. This has increased popularity of non-viral vectors to be employed as gene delivery carriers. Non-viral siRNA carriers include (i) cationic vectors like cell penetrating peptides, cationic polymers or lipids and dendrimers (ii) conjugation with small molecules like cholesterol, antibodies and bile salts (iii) nanoparticulate formulations etc. Additionally, siRNA backbone can be modified to improve its stability without affecting RNAi efficiency [131]. Suitable choice of vector is primary requirement for efficient delivery of siRNA which depends on siRNA properties, delivery route and target site.

2.5.1 Cationic cell penetrating peptides (CPP)

Such carriers have wide applications in delivering biomolecules like antibodies, proteins, peptides, plasmid DNA, siRNA, antisense oligonucleotides etc. They are responsible for intracellular delivery of above mentioned biomolecules either via endocytosis or direct crossing of cell membrane. They form positively charged complexes with nucleic acids through electrostatic attraction by forming non-covalent bonds [132]. CPP can also form complex with siRNA by forming covalent disulfide linkages which degrades in-vivo and releases siRNA intracellularly. Additionally, CPP plays important role in altering cellular localization of siRNA complexes [133]. Several reports indicate that such CPP-siRNA complexes formed are efficient than cationic liposomal carriers [134].

2.5.2 Polymeric and dendrimeric carriers

Linear or branched cationic polymers can form polyplexes by electrostatic attraction between positively charged groups of polymer and negatively charged phosphate groups of siRNA [135]. Apart from polyplexes, polymers can also be employed in non-viral siRNA carriers like nanoplexes, micelles, nanogels and nanocapsules [136]. Cationic polymers prevents oligonucleotide degradation due to lysosomal enzymes by promoting endosomal escape through proton sponge effect leading to osmotic swelling and endosomal rupture, releasing nucleic acids in cytoplasm [137-140].

Various cationic polymers used in formulation of polyplexes include poly-ethylenimine, poly-L-lysine, polyD,L-lactide-co-glycolide (PLGA), gelatin, poly-alkylcyanoacrylate, protamine, chitosan, pluronic block copolymers, poly-N-isoporpylacrylamide copolymers etc [141]. Amongst all the polymers listed, PEI is broadly explored for nucleotide-based therapies [142, 143]. The transfection efficiency and toxicity of polyplexes prepared using PEI depends on 2 characteristics – (i) whether the selected PEI is linear or branched (ii) molecular weight of PEI (ranges from 1 to 1000 kDa). Generally, more branched structure produces complexes with higher transfection efficiency while higher molecular weight PEI leads to augmented toxicity. Several studies reported increased stabilization of siRNA when complexed with PEI along with improved intracellular delivery [144].

Additionally, PLGA, being biodegradable and biocompatible, is studied widely as carrier for siRNA delivery viz implicated to improve stability of encapsulated siRNA

and also provide sustained release [145]. Surface modification of PLGA carriers with cationic polymers have been reported to increase cellular uptake [146, 147]. When PEI and PLGA are used in combination, the resulting carriers gave better loading efficiency with higher gene silencing and better protection from nuclease degradation activity [148, 149].

Dendrimers are synthetic star shaped macromolecules having radially symmetric structure which are synthesized from branched chains. It has core-shell structure which favors gene loading by encapsulation, adsorption or chemical conjugation. Surface charge, size and structure of dendrimer affect biocompatibility, cytotoxicity and immunogenicity [150]. Polyamidoamine (PAMAM), polypropyleneimine (PPI), cyclodextrin etc. can be employed in synthesis of dendrimers which can provide efficient delivery of siRNA intracellulary.

2.5.3 siRNA bioconjugates

Various molecules including small molecules like cholesterol, lipids and bile acids; peptides; proteins (antibody); aptamers and polymers can be conjugated with siRNA and the resulting conjugates provides better formulation characteristics, depending on conjugating moiety, compared to naked siRNA [151]. Conjugation of siRNA to cholesterol has been shown to improve stability and the conjugate targets encapsulated siRNA to liver, lungs, kidney, heart and fat tissue [152]. siRNA when conjugated to endo-osmolytic agent results in endosomal escape and augmented transfection efficiency [153]. Similarly, conjugation of siRNA with antibodies or aptamers causes site specific delivery and thus minimizing off-target effects [154, 155].

2.5.4 Lipid carriers

Various formulations like micelles, liposomes, solid lipid nanoparticles and microemulsion are categorized as lipid carriers for siRNA delivery [156]. Being biocompatible, biodegradable and simple formulation, use of liposomes as siRNA carriers is highly prevalent. These lipidic carriers have important role in protecting siRNA from nucleases, improves endosomal escape and thus augments siRNA accumulation in cytoplasm, causes endocytosis and thus promotes cellular uptake.

2.6 Barriers in the brain

Delivery of drugs and other therapeutics to brain is complicated as there are several barriers which limits entry of such therapeutics to brain. These barriers include

blood brain barrier, blood cerebrospinal fluid barrier and the ependyma which are represented schematically in Figure 2. 3.

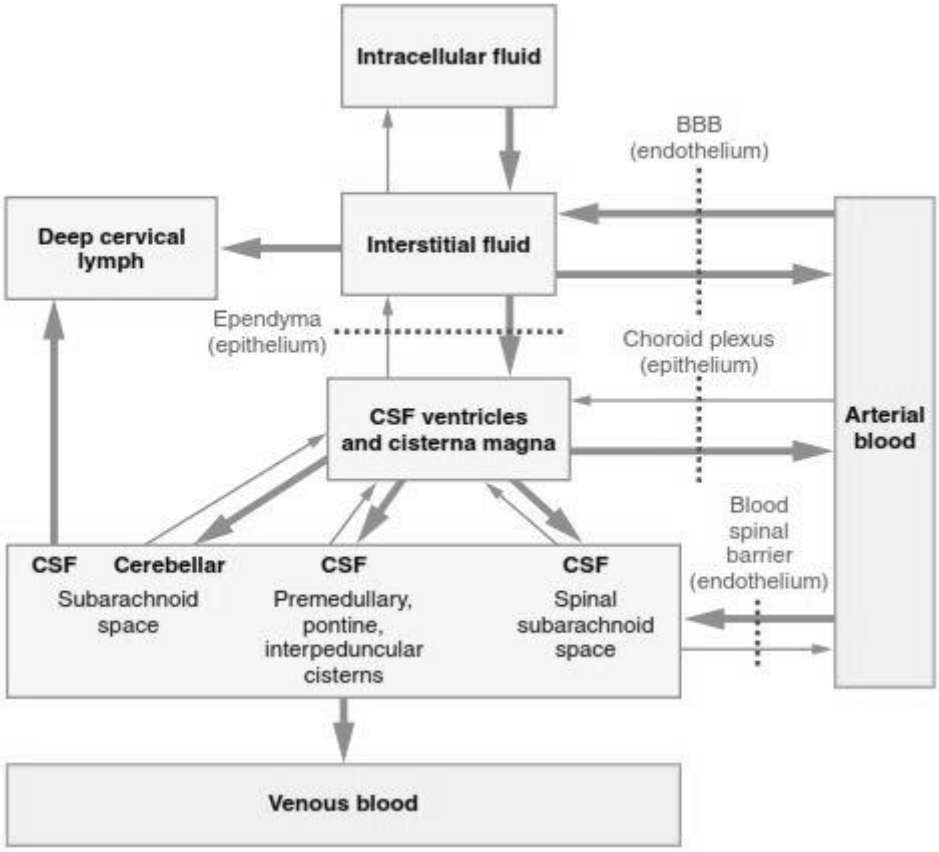

Figure 2. 3: Schematic representation of barriers present in brain

2.6.1 Blood brain barrier

The concept of existence of a barrier between blood and brain was postulated and evidenced by Ehrlich [157]. Blood brain barrier (BBB) is situated in brain capillaries localized at interface of brain and blood. It is considered as primary barrier which prevents entry of solutes in brain and thus protects brain along with maintaining its homeostasis [158, 159]. Various cells including brain capillary endothelial cells, pericytes, astrocytes and neuronal cells have important function in regulating BBB [160-163]. Endothelial cells form tight junction and thus prevent to and fro transport of solutes while astrocytes (surrounding endothelial cells) and neuronal cells (innervating endothelial cells) have important role in regulating BBB. Phagocytosis by pericytes, present on the basement membrane along with endothelial cells, augments BBB property [158].

2.6.2 Blood cerebrospinal fluid barrier

This barrier comprises of arachnoidal and choroidal epithelium, amongst which choroidal epithelium constitute important component of blood cerebrospinal fluid barrier (BCSFB). The activity of BCSFB is regulated by phase I-III enzymes. Phase I enzymes functionalize the drugs; phase II enzymes conjugates drug molecules while phase III enzymes transport drug molecules. Choroidal plexus, where choroidal epithelium is formed, is involved in neurogenesis and thus involved in repair of neurons. It also plays important role in signaling as various receptors are expressed at choroidal plexus. Choroidal plexus epithelium is important for drug transport as it possess several desirable characteristics over BBB which includes presence of gap junction imparting more permeability, higher blood flow in choroidal plexus capillaries and higher surface area. Still, drug transport employing this path is hindered as BCSFB faces CSF and not blood [164].

2.6.3 Ependyma

Ependyma consist of single layer of epithelial cells connected by gap junctions which presents additional barrier to drug molecules already entered in CSF. Compared to BBB, ependyma is more permeable as it consists of gap junction which allows passage of small lipophilic molecules. Various reports also suggest passage of macromolecular drugs through ependyma [165].

2.7 Intranasal route for brain delivery

2.7.1 Nasal structure and physiology

The nose is divided into two symmetrical nasal cavities by nasal septum and the resulting nasal cavities have surface area of 150 cm^2. Each nasal cavity is further divided into three regions namely vestibule, respiratory region and olfactory region [166, 167]. Vestibule is the anterior most region of nasal cavity which contains nasal hairs responsible for filtration of inhaled air. Additionally, it contains nasal valve which consist of squamous cells, involved in filtration of air [168]. The respiratory region consists of three turbinates – superior, middle and inferior turbinate, which projects from lateral wall of nasal cavity. These turbinates are covered by respiratory epithelium which consist of ciliated and non-ciliated columnar cells increasing surface area of nasal cavity and thus improve systemic drug absorption, goblet cells forming mucus layer by secreting mucin, and basal cells [169]. The olfactory region is composed of three types of cells – olfactory neuronal cells, sustentacular cells and basal cells.

Amongst all these, olfactory neuronal cells act as potential site for nose to brain delivery of therapeutics [170]. The pH of nasal cavity ranges from 5.5 to 6.5 [171] and several enzymes including cytochrome P450 isoforms, glutathione transferase etc. are present in nasal cavity [172, 173]. The nasal cavity plays important role in olfaction process i.e. sensation of smell and filtration of solutes and particulates from the inhaled air.

2.7.2 Pathways and mechanism

Nose to brain delivery of therapeutics primarily employs pathways that involves nerves connecting nasal cavity and brain. Additionally, pathways including vasculature, CSF and lymphatics have also been explored for brain delivery of therapeutics. All the pathways and mechanism employed for nose to brain delivery are represented schematically in Figure 2. 4.

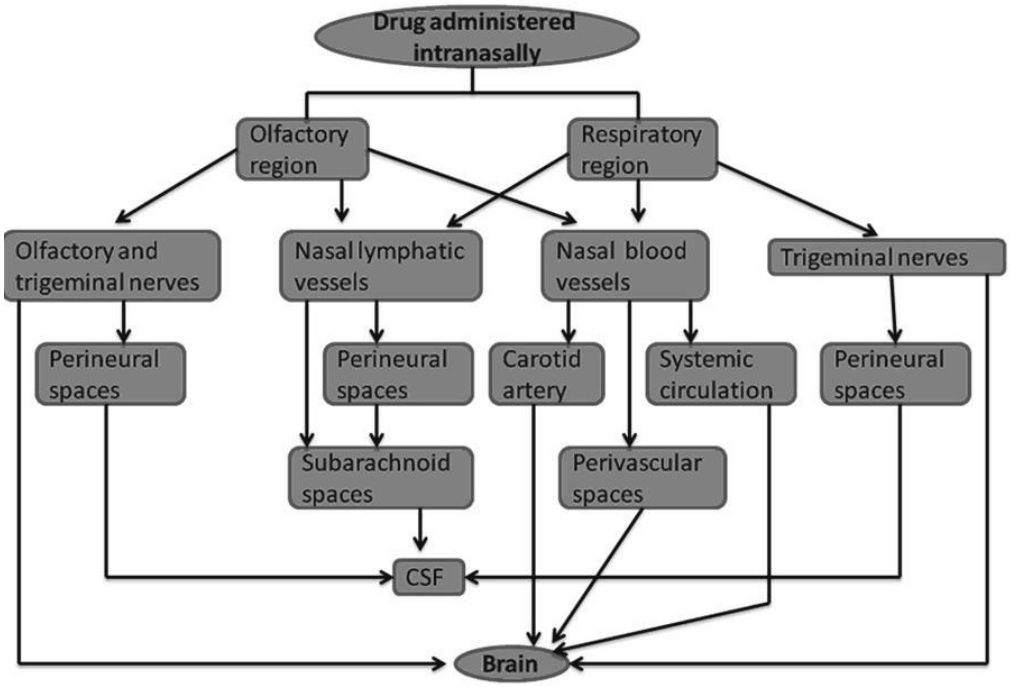

Figure 2. 4: Pathways for brain targeting by intranasal route

2.7.2.1 Olfactory nerve pathway

The drug or similar therapeutic agent administered intranasally has to pass olfactory epithelium to reach CSF or brain parenchyma. This passage across olfactory epithelium can occur by any of the three mechanisms – (i) transcellular pathway across supporting cells by passive diffusion which mainly transports lipophilic drugs (ii) paracellular pathway through tight junctions between supporting cells which mainly

employs diffusion through aqueous channels for transport of hydrophilic moieties (iii) olfactory nerve pathway which employs uptake of therapeutic moiety by endocytosis or pinocytosis and transports that moiety to olfactory bulb via. axonal transport [174, 175]. Thus, main mechanisms involved in CNS delivery through nasal route are – transcellular passive diffusion, paracellular passive diffusion, transcytosis, carrier mediated transport and efflux transport [176].

2.7.2.2 Trigeminal nerve pathway

Trigeminal nerve, largest cranial nerve, innervates respiratory and olfactory epithelium of nasal cavity which on the other end connects to pons and olfactory bulb in CNS. The ophthalmic and maxillary division of trigeminal nerves are important for brain delivery of therapeutics by intranasal route as these neurons traverse nasal mucosa. Trigeminal nerve has entry points in both rostral and caudal areas of brain and thus it overlaps with olfactory neuronal pathway for nose to brain delivery of therapeutics [177].

2.7.2.3 Vascular pathways

The respiratory mucosal region of the nasal cavity is highly vascularized by sphenopalantine artery [178]. Additionally, respiratory epithelium consists of both continuous and fenestrated endothelium which augments permeability of small as well as large molecules in systemic circulation which further crosses BBB and enters CNS [179]. Another mechanism namely counter current transfer involves entry of therapeutics in nasal cavity venous supply which further transfer them to carotid artery delivering blood to brain and spinal cord. This may cause nose to brain delivery of therapeutics bypassing systemic circulation [180]. Perivascular transport of therapeutics delivered intranasally, driven by arterial pulsations [181, 182], act as important transport pathway for nose to brain delivery [183].

2.7.2.4 CSF pathway

Circulation of cerebrospinal fluid and its drainage occurs through pathway connecting subarachnoid space and nasal lymphatics viz. also responsible for nose to brain delivery of therapeutics. The evidence for such transport was given by several studies which depicts direct transport of therapeutics, delivered intranasally, to CSF and their distribution in CNS. This pathway is dependent on several characteristics of therapeutic molecules including molecular weight, lipophilicity and pKa [184-186].

2.7.2.5 Lymphatic pathway

Last two decades have transformed the understanding of CSF production and absorption with evidence of involvement of extracranial lymphatics. Several reports indicate connection of nasal submucosal lymphatics to subarachnoid space through perineural space and cribriform plate. This provides direct shortcut pathway for nose to brain delivery [187, 188].

2.7.3 Factors affecting nasal absorption and absorption enhancement

Several factors have important effects on absorption of therapeutics via nasal route which are summarized and represented in Figure 2. 5.

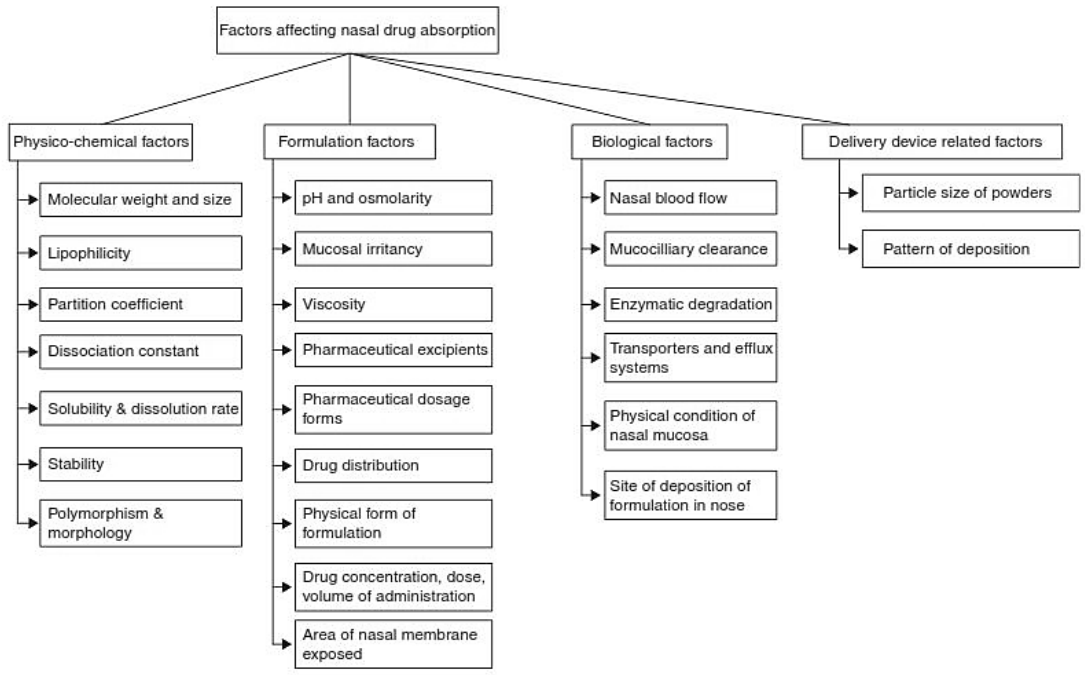

Figure 2. 5: Factors affecting absorption and permeability of therapeutics across nasal mucosa

Several strategies can be employed to improve nasal absorption [168, 189, 190] which can be summarized as follows:

- Enzyme inhibitors: Proteases and peptidases can be employed to restrict enzymatic activity in nasal cavity and thereby prevent metabolism of peptide and protein therapeutics administered intranasally.
- Structural modification: This can enhance nasal absorption of therapeutics but care should be taken to preserve the pharmacological activity of active moiety.

- Permeation enhancer: Various substances like fatty acids, bile salts, cyclodextrin, lipids, surfactants etc. have been employed to increase permeability of nasal epithelium either by disrupting nasal epithelium or increasing its permeability by making the epithelium porous and leaky.
- Particulate drug delivery: Several carriers like liposomes, nanoparticles etc. prevent exposure of encapsulated therapeutics to nasal environment and also retain formulation in nasal cavity for longer time and thus augment absorption of therapeutics.
- Bioadhesive polymer: Formulations prepared using mucoadhesive substances increase nasal residence time of the delivered formulations and thus reduce their mucociliary clearance. This ultimately results in enhancement of nasal absorption of delivered therapeutics.

2.7.4 Formulations for nasal delivery

Various formulations [191] which can be employed for intranasal delivery of therapeutics for brain targeting can be divided into 2 categories – conventional and novel as represented in Figure 2. 6

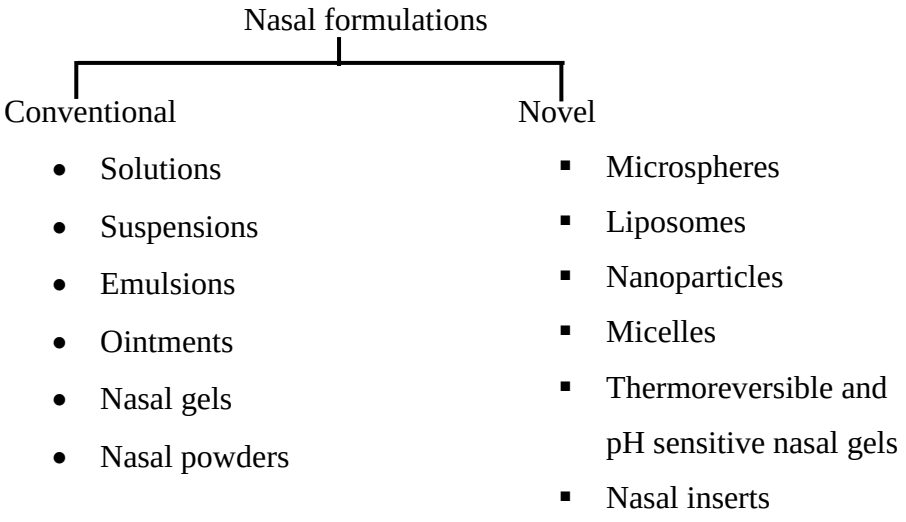

Figure 2. 6: Formulations for nasal drug delivery

2.7.5 Advantages of nose to brain delivery

- Along with being rapid and safe technique, it is non-invasive which enhances patient compliance.
- Bypasses first pass metabolism due to hepatic and gut-wall enzymes.
- Systemic entry of delivered therapeutics is negligible which diminishes chances of systemic side effects.

- Brain targeted therapeutic agents can be administered via nasal route without any structural modifications avoiding complicated approval procedure.
- Can solve bioavailability issues for low molecular weight drugs and act as better alternative for parenteral route.
- Nasal mucosa is highly vascularized and more permeable as compared to BBB and thus it provides rapid action.

2.7.6 Disadvantages of nose to brain delivery

- The administered therapeutic agents may be eliminated rapidly due to mucociliary clearance.
- Some absorption enhancers act by disrupting nasal mucosa and thus associated with toxicity issues.
- Various factors are involved for nose to brain transport and thus there is huge variation in concentration of therapeutics reaching CNS.
- Permeability across nasal mucosa is inversely proportional to molecular weight and thus difficulty arises in delivering high molecular weight agents.
- Some pathological conditions including cold or allergy may interfere with nose to brain delivery of therapeutics.
- Enzymatic degradation or metabolism in nasal mucosa may render therapy ineffective. Also, some agents may cause either irritation to nasal mucosa, infection or anosmia.
- Patient may employ improper administration technique which may cause loss of therapeutic agents and inaccurate dosage administration.
- Pathways and mechanism for nose to brain delivery are still unclear.

2.8 Patents on nose to brain delivery systems

Till date, various researchers have focused on targeting CNS via. Intranasal administration of therapeutics and the work done by them is depicted in Table 2. 4.

Table 2. 4: List of patents for nose to brain delvery

Disease	Drug	Formulation	Patent
Brain disorders	Neurological agents	-	[192]
Brain disorders	Insulin	Solution	[193]
Neurodegenerative disorders	Therapeutic cells	-	[194]
Brain disorders	Neurotropic agents	-	[195-198]
Parkinson and Alzheimer disease	NMDA receptor Antagonist	Extended-release dosage form	[199-201]
Neurodegenerative disorders	Proteasomes Glatiramer	Nanoemulsion	[202]
Epilepsy	Diazepam	Microemulsion	[203]
Epilepsy	Lorazepam	Nasal spray	[204]
Insomnia	Zolpidem	Cyclodextrin/chitosan sols	[205]

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