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3.1. DELUSIONAL DISORDER: Characteristic features

3.1.1. Historical overview of delusional disorder :

Historically, the concept of delusional disorder is derived from the classic Greek concept of *paranoia.* The term paranoia, from which the modern adjective paranoid is derived, has a long and chequered history. It has probably given rise to more controversy and confusion of thought than any other term used in psychiatry. The word paranoia was derived from the Greek *Para* (beside) *nous* (mind). It was used in ancient Greek literature to mean "out of mind", i.e., of unsound mind or insane. Paranoia was historically used to describe a variety of mental states, including dementia and delirium.

The disease entity, delusional disorder, was first delineated by Karl Ludwig Kahlbaum (1863). He used the name paranoia and first applied the term to a chronic delusional disorder. Karl Kahlbaum classified paranoia as a separate mental illness and referred to the condition as a partial insanity, which throughout the course of disease, principally affected the sphere of the intellect but not other areas of mental functioning. His work also led to recognition that paranoid features are nonspecific characteristics of many medical diseases.

Emil Kraepelin (1856-1926) also recognized a condition that he called *paranoia,* characterized by a persistent delusional system in the absence of hallucinations and personality deterioration. He recognized subtypes with delusional contents of grandiosity, persecution, erotomania and jealousy, and also allowed for the possibility of a hypochondriacal content. He clearly differentiated paranoia from *dementia precox* (later named schizophrenia by Bleuler).

Subsequently, Kraepelin introduced the concept of paraphrenia, an illness similar to paranoid schizophrenia but with significantly better preservation of affect and of personality. He regarded paranoia, paraphrenia, and paranoid schizophrenia as a relatively discrete group of illness, later referred to as the paranoid spectrum.

Kraepelin, like Kahlbaum, was concerned with the longitudinal course and gradually altered his formulation of paranoia. By the eighth revision of his *Lehrbuch der Psychiatrie,* he had restricted the term to describe persons with systematized delusions, an absence of hallucinations and a prolonged course without recovery but not leading to mental disorientation (Kendler, 1988).

Eugen Bleuler (1906) broadened the definition of paranoia to include cases with hallucinations –a paranoid form of dementia praecox for which he coined the term schizophrenia and an intermediate group. Bleuler's contributions reinforced a trend toward the diagnosis of paranoid illness as a form of schizophrenia.

Despite this, various workers continued to contribute to speculation on the nature of delusions and paranoia. Karl Jaspers (1883-1969) wrote outstandingly on the phenomenology and psychopathology of delusions (Jaspers, 1963). Kretschmer (1988-1964) proposed that paranoid symptoms tended to occur in abnormally sensitive individuals who suffered from lifelong conflict between feelings of inadequacy and of unrequited self-importance and who, after undergoing some 'key experience', were participated in to a delusional psychosis. Kretschmer's observations tended to emphasize the importance of pre-existing personality disorder in paranoid illness. Sigmund Freud (1856-1939) wrote extensively on paranoia (Freud, 1958), proposing 'latent homosexuality as the underlying psychopathology', a view no longer widely accepted.

Although this and many other speculations have contributed much to the descriptive phenomenology of delusions, little or nothing about the mechanisms underlying delusions and their associated illnesses have been explored (Maher, 1992).

From the 1970s onwards, interest in paranoia began to reappear (Winokur, 1977) and a more optimistic view of treatment emerged (Munro, 1982). In 1987, DSM-IIIR returned to a description of the illness which was essentially that of Kraepelin, except that non-prominent hallucinations were allowable, and renamed it delusional (paranoid) disorder, now simplified to delusional disorder in DSM-IV, and ICD-10. The definition

of delusion by Mullen (1979) is widely quoted and its implications are largely accepted by DSM-IV and ICD-10. He characterizes delusion as follows: i) they are held with absolute conviction, ii) the individual experiences the delusional belief as self-evident and regards it as of great personal significance, iii) the delusion cannot be changed by an appeal to reason or by contrary experience, iv) the content of delusions is unlikely and often fantastic, and v) the false belief is not shared by others from a similar socio-economic group.

3.1.2. Comparative account of the diagnostic features of delusional disorder :

Delusion, as "the basic characteristic of madness" (Jasper, 1963), has appropriately attracted an enormous amount of theoretical interest but remarkably little is known with any certainty. Arthur (1964) in his classic review concluded: "delusion can still claim to be the most outstanding and baffling behavior symptom of mental illness"(Jasper,1963), has appropriately attracted an enormous amount of theoretical interest but remarkably little is known with any certainty. Arthur (1964) in his classic review concluded: "delusion can still claim to be the most outstanding and baffling behavioural symptom of mental illness". The problem of delusion is one of the basic problems of psychopathology and has impaired an impressive diversity of theoretical speculation. The last few decades have seen a renewed interest in studying the psychological phenomena of psychoses. Considerable effort has been devoted to establish reliable diagnoses through operational definitions and multiaxial classifications. However, these have failed to shed any further light on the symptom used to construct them because their origins are unclear and the literature consists largely of unsupported speculations, a small number of experimental studies and some associations with demonstrable pathology. The current concept of delusion is increasingly being challenged. It seems unlikely that delusion is a single entity, and attempts to dissect out its component parts have demonstrated that they vary with considerable independence from each other over time and during the progression of a delusional disorder (Brett-Jones *et al*,1987).

Presently, there are two classificatory systems for all the mental illnesses managed by two different organizations. The International Classification of Diseases (ICD) is a disease classification system developed by World Health Organization (WHO) to promote international comparability of healthy care statistics. Diagnostic and statistical manual of Mental Disorders (DSM) is another such system which is managed by American Psychiatric Association. The long developmental history of psychiatric classification along with the subsequent improvement of different diagnostic schemes have been observed in different editions of ICD and DSM. However, a comparative account of the diagnostic features emphasized by the 10^{th} edition of DSM (DSM-IV) are represented below.

Diagnostic and Statistical Manual of Mental Disorders Text Revisions (DSM-IV-TR) was published in 1994 and it defines the core psychopathological features of delusional disorder as persistent, nonbizarre delusions not explained by other psychotic disorders.

The delusions are unusual yet they refer to different aspects of life that might occur, such as being conspired against, cheated on, physically ill, in love, jealous and so forth. Delusions are categorized according to their content. The delusions re fixed (persistent) and unarguable.

The person's emotional contact and behaviours are generally intact. The emotional response is usually consistent with the delusional concern, and the mood is often appropriately depressed, frustrated, or even intensely angry or elated. Social and marital functioning is more likely to be compromised than intellectual and occupational functioning.

Associated features in delusional disorder include those of the paranoid syndrome. The degree of hostility and suspiciousness may be such that violent or aggressive behaviour results. Litigious behaviour is common among such patients. However, some patients, notably those with somatic delusions, may not display hostility,

anger, or even suspiciousness to any considerable degree. DSM-IV diagnostic features are represented in Table-1.

Table -1: DSM-IV diagnostic criteria and subtypes of delusional disorder (297.1)

Principal features

a) Non-bizzare delusions of at least 1 month's duration.

b) Criterion A for Schizophrenia has never been met, although tactile and olfactory hallucinations may be acceptable if they are related to the delusional theme.

c) Apart from the impact of the delusion(s) or its consequences, functioning is not markedly impaired and behaviour is not obviously odd or bizarre.

d) Concurrent mood episodes, if present, are belief relative to the duration of the delusional disorder.

e) The disturbances are not the direct outcome of a drug or medication or of a medical disorder.

Subtypes:

Erotomanic
Grandiose
Jealous
Persecutory
Somatic
Mixed
Unspecified or other

International Classification of Diseases (Tenth Edition) was published in 1992. delusional disorders are characterized by the development either of a single or of set of related delusions, which are usually persistent and sometimes lifelong. Onset is commonly in middle age but sometimes, particularly in the case of beliefs about having a misshapen body, in early adult life. Apart from actions and altitudes directly related to the delusion or delusional system, affect, speech and behaviour are normal. Table 2 depicts the ICD-10diagnostic features as well as subtypes of delusional disorder.

Table-2: ICD-10 diagnostic criteria for persistent delusional disorders

Subtypes
Persecutory
Litigious
Self-referential
Grandiose
Hypochondriacal
Jealous
Erotomanic
Other persistent delusional disorders (F22.8)
This is a category for persistent disorders with delusions or schizophrenia. Illness with prominent delusions accompanied by persistent hallucinatory voices or by psychotic symptoms insufficient to satisfy the criteria for schizophrenia are included here. A delusional disorder of less than 3 months' duration is coded under Acute and Transient Psychotic Disorders (F23) until proven otherwise.

Summary of the diagnostic features of DSM-IV and ICD-10

DSM-IV and ICD-10 descriptions are very similar in overall outline but with a number of rather striking minor differences. These are noted below:

1. DSM-IV uses the term 'non-bizzare' delusion; this criterion has been shown to have little or no validity (Flaum *et al*, 1991).

2. DSM-IV allows the presence of tactile and olfactory hallucinations, while ICD-10 mentions only auditory hallucinations; in practice most modalities may be represented but the important point is that they are relatively non-prominent and usually parallel the content of the delusion(s).

3. DSM-IV says that delusions should have been present for I month and ICD-10 insists on 3 months.

4. Both classifications exclude delusional illnesses due to organic brain disorder, medical illnesses, medication effects or psychoactive substance abuse. In essence this is correct, especially in an illness of acute onset.

5. DSM-IV and ICD-10 agree emphatically that delusional disorder is not schizophrenia and DSM-IV notes that general functioning is not impaired. Both say that mood disturbance may accompany the delusional illness but is not a cause of it.

6. The list of subtypes according to delusional content is similar in both classifications, although ICD-10 adds self referential and litigious theme.
7. Neither classification specifies that the essence of delusional disorder is a highly organized delusional system, largely encapsulated from normal aspects of the personality, although DSM-IV hints at this when it comments that functioning is not markedly impaired and behaviour is not obviously odd or bizarre.

8. The ICD-10 category of other persistent delusional disorder is vaguely described and is largely a catch-all heading.

9. Overall, DSM-IV and ICD-10 give rather laconic descriptions of delusional disorder and it will be necessary to flesh them out with relevant clinical details.

The essence of the modern concept of delusional disorder is that of a permanent and unshakable delusional systems developing insidiously in a person in middle or late life. Recent study has found that the diagnosis of delusional disorder was temporarily consistent in only about 50% of the patients (Fenning *et al.*,1996). This suggests that an initial diagnosis of delusional disorder should be considered as provisional, and that patients need to be reassessed longitudinally.

3.1.3. Classifications of delusion :

Delusions are sufficiently idiosyncratic that sharp distinctions and inclusive classifications have remained elusive. Nash (1983) listed 44 varieties of delusions and admitted that the list is not yet complete. Vast differences can be seen in the content of any single variety of delusions. The current classificatory systems bypass the etiological discussion by implementing pure symptomatological criteria closely to the Kraepelinian category of paranoia (Gabriel, 1999). A general scheme of descriptions of delusions has been suggested by many as given in **Table-3** (Gelder *et al*, 1996).

Table-3 : Descriptions of delusions

1. **According to Fixity**
   - Complete
   - Partial
2. **According to onset**
   - Primary
   - Secondary
3. **Other delusional experiences**
   - Delusional
   - Delusional perception
   - Delusional memory
4. **According to theme**
   - Persecutory (Paranoid)
   - Delusions of reference
   - Grandiose (expansive)
   - Delusions of guilt and worthlessness
   - Nihilistic
   - Hypochondriacal
   - Jealous
   - Sexual or amorous
   - Delusions of control
   - Delusions concerning possession of thought
     - Thought insertion
     - Thought withdrawal
     - Thought broadcasting
5. **According to other features**
   - Shared delusions

While no single classification of the varieties of delusion is satisfactory, most emphasize on alterations of thought content. Nine of the most common and dramatically distinct types of delusion are presented in Table-4.

Table-4: Showing nine of the most common and dramatically distinct types of delusion

1. Delusion of Persecution	
	Delusions of Reference
	Delusions of Loss of Property
	Delusions of Poison or infection
	Delusions of Influence
	Delusions of Innosence
2. Nihilistic Delusions	
3. Delusions of ill health	
	Hypochondriasis
	Monosymptomatic hypochondriasis
	Somatic Delusions
4. Delusions of Grandeur	
5. Delusions of Poverty	
6. Delusions of Love	
7. Delusions of Jealousy	
8. Delusions of Possession	
9. Delusions of Reduplication	

3.1.4. Epidemiology of delusional disorder :

Overall, delusional disorders are rare; the prevalence in the population is estimated at 0.03%. The epidemiological information is meager, however, it is now increasingly being realized that although the prevalence is low, delusional disorder is not rare (Manschreck, 1996). Most studies suggest that the disorder account for 1-4% of psychiatric admissions and from 2%-7% of admissions for functional psychosis. The prevalence of this disorder is probably much higher than commonly recognized, since the delusions often remain concealed for years and may be manifested only in non medical situations, where they can go unrecognized as a medical condition (Dyke, 2000). The

demographic evidence covering a period from 1912 to the 1970s has been represented in Table-5, which provides an estimate of incidence, prevalence and related statistics (Kendler, 1982).

Table-5: Epidemeological features of delusional disorder

Incidence	0.7-3
Prevalence	24-30
Age of onset	18-80 (mean 34-45 years)
Type of onset	Acute or gradual
Sex ratio	Somewhat more frequently among female
Prognosis	Best with early, acute onset
Associated features	Widowhood, celibacy often present, history of substance abuse, head injury not infrequent.

The age of onset of delusional disorder is generally middle or late adult life, with a peak frequency of first admissions between 35 and 55 years of age but can be at younger age with variable patterns of course. Delusions are exceptional until puberty (Werry,1992). Recent studies have highlighted the relationship between age and delusional proneness, younger subjects scoring higher on most dimensions of delusional ideations of delusional ideation, such as persecution, thought disturbances, grandiosity and paranormal beliefs. However, 'religiosity' was the only dimension that was positively associated with age (Verdoux *et al*, 1998).

The age of onset may differ significantly according to the type of delusional disorder, the oldest age at onset was associated with the persecutory type where as the youngest with somatic type (Yamada *et al*, 1998).

More women than men develop the disorder as documented, while 60-70% of patients are married, up to one-third are widowed, divorced or separated. Few studies have reported that the female / male ratio is 2:1 (Jorgensen & Munk- Jorgensen,1986). Recent study reveals that delusional persecution is predominant in female patients while males present significantly with delusions of jealousy and grandiosity (Gutierrez-bobos *et al*, 2001).

Persons with delusional disorder are economically and educationally disadvantaged, and immigrants seem especially prone to develop the disorder. Once established, delusional disorder is generally chronic and life long. However, it appears to have a better long term prognosis (Opjordsmoen,1989). Remission is reported in one-third to one-half of cases (Jorgensen, 1994).

3.1.5. Familial pattern of delusional disorder :

Family studies have been consistent in showing that most major disorders such as schizophrenia, bipolar disorder and depression are common in relatives of affected individuals than in the population at large (Tandon & McGuffin, 2002). Family studies that have begun to appear in the literature indicate the possible specific family transmission of delusional disorder. It has been reported that delusional disorder is more likely to be associated with a family history of such traits as suspiciousness, jealousy, secretiveness and the presence of paranoid behaviour or delusions (Winokur,1986). Some studies have found that delusional disorder is more common among relatives of individuals with schizophrenia than would be expected by chance, whereas other studies have found that the families have no increase in schizophrenia or mood disorders (Watt,1985;Winokur,1985). There is limited evidence that avoidant and paranoid personality disorders may be especially common among first degree of biological relatives of individuals with delusional disorder.

3.1.6. Clinical heterogeneity, Paranoid spectrum and Differential diagnosis of delusional disorder :

Delusional disorder is probably a heterogeneous group of illness and delusion occurs in a variety of psychiatric and medical conditions. Delusional symptomatology has been poorly investigated with factor analytic studies. Delusional symptomatology consisted of four independent factors like core depressive symptoms, hallucinations,delusions and irritability symptoms which indicate a substantial heterogeneity of this diagnostic category (Serretti *et al*, 1999). The clinical heterogeneity of delusional disorder has also been supported by other studies (Campana *et al*, 1998).

Paranoid symptoms are commonly seen in various psychiatric disorders, known as "Paranoid spectrum" (Fujinawa,1981). Delusional disorder, a psychosis previously called "paraphrenia" (Roth, 1987), is characterized by monosymptomatic paranoid symptoms. Delusional disorder possess features characteristic of the full range of paranoid illnesses. There are many conditions to consider (**Table-6**), especially the more common disorders associated with paranoid (delusional) features are alcohol abuse, drug abuse (especially CNS stimulants), anticholinergic toxicity, sedative-hypnotic withdrawal, delirium, dementia, HIV infection, brain tumor, epileptic disorder, mood disorders and schizophrenia/schizoaffective disorders. Among these paranoid associated disorders, dementia of Alzheimer's type, mood disorders and schizophrenia are reviewed to understand the psychopathological basis of formation of delusion in these disorders. Delusions in depression, if present, are frequently related to mood, called mood congruent delusions and usually indicate severe depression (Coryell, 1996). Grandiose delusions are the most common symptoms (Dunayevich & Keck, 2000). Delusional thoughts are common in patients with Alzheimer's disease and contribute prominently to morbidity (Rao &Lyketsos,1998). It has been reported that the prevalence of delusion ranged from 10% to 73% (median 33.5%) in the patients with Alzheimer's disease (Cooper et al, 1990; Gormley & Rizwan, 1998). The pathophysiologic underpinnings for delusions in Alzheimer's disease are not well understood. Delusional disorder are usually thought to overlap with schizophrenic disorders, and there may be a continuum with paranoid schizophrenia (Munro,1988). Although paranoid schizophrenia is invariably grouped with other schizophrenia subtypes, there is still justification for Kraepelin's original concept of its belonging with the delusional disorders (Munro, 2003).

Table-6: Conditions and agents associated with delusions and other Paranoid features

Neurological Disorders

Adrenoleukodystrophy
Arteriosclerotic psychoses
Blunt head trauma
Brain tumors
Cerebrovascular disease
Cerebral anoxia
Complex partial seizure disorder
Delerium
Dementia
Fat embolism
Hearing loss
Hungtington's disease
Hydrocephalus
Hypertensive encephalopathy
Idopathic basal ganglia Calcification
Idiopathic Perkinson's Disease
Intracranial hemorrhage
Marchifava-Bignani disease
Menzel type ataxia
Metachromatic leukodystrophy
Migraine
Motor-neurone disease
Multiple sclerosis
Muscular dystrophy
Narcolepsy
Postencephalitic parkinsonism
Presenile dementia
Roussy-Levy syndrome
Senile psychoses
Spinocerebellar degeneration
Subarachnoid hemorrhage
Subdural hematoma
Sydenham's chorea
Temporal arteritis

Infections

AIDS
Encephalitis lethargia
Creutzfeldt-Jakob disease
Malaria
Syphilis
Toxic shock syndrome
Trypanosomiasis
Typhus
Viral encephalitides

Alcohol and other Substances

Alcohol withdrawal
Amphetamine
Anesthetic nitrous oxide
Atropine toxicity
Barbiturate
Chronic alcohol Hallucinosis
Chronic bromide intoxication
Cocaine
Ephedrine
Marijuana
Mescaline & other Hallucinogens
Perbitine
Withdrawal from minor tranquilizers & hypotonic
Medications

Pharmacological Agents

Adrenocorticotropic hormone
Amphetamine and related compounds
Atiperkinson agents
Anabolic steroids
Antiarrhythmic drugs
Hyponatremia
Antibiotics
(cephalosporin, penicillin)
Anticholinergic drugs
Antihypertensive agents Antimalarials
Antitubercular drugs
Bromocriptine
Bupropion
Chemotherapeutic agents
(asparaginase)
Cimetidine
Corticosteroids
Diphenylhydantoin
Disulfiram
Imipramine & other tricyclic drug
Levodopa
Mephentermine
Methydopa & imipramine
Pentazocine
Phenylpropanolamine and sympathomimetic agents

Metabolic and endocrine disorders

Acute intermittent pophyria
Addison's disease
Complication of surgical Portcaval anastomosis
For cirrhosis
Cusing's syndrome
Folate deficiency
Hemodialysis
Hypercalcemia
Hypoglycemia
Hyponatremia
Hypopituitarism
Liver failure
Malnutrition
Niacin deficiency
Pancreatic encephalopathy
Parathyroid disorders
Pellagra
Pernicious anaemia
Phenylketonuria
Systemic leupus erythrematosus
Thiamine deficiency
Thyroid disorders
Uremia
Vitamin B_{12} deficiency
Wilson's disease

Psychiatric disorders

Brief psychotic disorder
Delusional disorder
Shared psychotic disorder
Mood disorders
Psychotic disorders not otherwise specified
Schizoaffective disorders
Schizophrenia(all types)
Schizophreniform disorder

Sex Chromosome Disorder

47XXY
Klinefelter's syndrome
Turner's syndrome

Propylhexedrine

A careful assessment is necessary in order to rule out other functional or medical causes for the delusions. This work up should include a physical examination to rule out alcohol-, cocaine-, and other drug induced conditions, dementia and infectious, metabolic and endocrine disorders (Manschreck,1996). The major diagnostic task remains in separating delusional disorder from mood disorders, schizophrenia and paranoid personality. Associated features of delusional disorder include anger, social isolation and seclusiveness, eccentric behaviour, suspiciousness, hostility and sometime violence prompted by the delusion (Kennedy *et al,* 1992). Winokur (1997) reported that patients with delusional disorders frequently develop sexual problems and depression and described many as over talkative and circumstantial. Clinical wisdom suggests that many patients become litigious and end up as lawyer's clients rather than as psychiatrist's patients.

3.1.7. Etiological Models of Delusional Disorder :

The knowledge of etiology of delusional disorder is scanty and highly speculative, as little modern research has been conducted. A general outline of the etiological factors is described as below.

Organic Brain Factors -

Recent evidence from the study of delusional misidentification syndrome indicates that delusions of very specific type may arise in association with certain well-defined brain insults. There are strong hints, but much less supportive evidence, to suggest that organic brain factors may also be important in cases of

Delusional disorder, for example, head injury may lead to the development of marked paranoid symptoms, and there is a long-established association between chronic alcoholism and pathological jealousy (Michael *et al,* 1995). Old age itself may be linked to the onset of symptoms typical of delusional disorder and early evidence of brain changes, especially in subcortical areas, is starting to appear in studies of various kinds of senile 'paranoid' illness (Feinstein & Ron, 1990). Amphetamine and cocaine abuse (Satel & Edell,1991) can induce delusional illness. Delusional illness induced by the brain

effects of AIDS infection has been documented in recent years (Reilly & Batchelor, 1991).

Gorman and Cummings (1990) have proposed that delusional illnesses of organic origin have underlying features in common, particularly temporal lobe or limbic involvement and an excess of dopamine activity in certain areas of the brain. It is very possible that organic brain factors are much more common than we suspect in delusional disorder, especially in younger males who have previously abused alcohol or drugs or have suffered a head injury in the past, and in older patients (more commonly female) who suffer from effects of an aging brain (Munro, 1988).

Risk factors of delusional disorder -

The cause of delusional disorder is unknown. Epidemiological and clinical literature suggests that certain risk factors may be relevant to etiology and deserve further research elaboration. Whether they are risk predictors or simply characteristics or markers of the disorder are unknown. Familial psychiatric disorder, including delusional disorder, is the best-documented risk factor at present. Advanced age, sensory impairment/ isolation, family history social isolation personality features (e.g. unusual interpersonal sensitivity), and recent immigration may act as risk factors associated with delusional disorder (Manschreck, 2000).

Genetic basis of delusional disorder -

Human genetics research has generated enormous amount of data about the genetic differences among individuals and groups. Investigation of these differences has transformed our understanding of the origins and nature of human diseases (Cavalli-Sforza, 1971;Bamshad *et al.*,2004;Collins *et al*, 2003). Immunogenetic studies considering HLA system has been carried out for a long time for a number of psychiatric disorders like Schizophrenia, bipolar disorders, delusional disorder etc. The discovery of HLA associations with delusional disorder implies that at least part of their genetic basis lies in the MHC region (Debnath *et.al.*, 2005). The first HLA association study was reported in delusional disorder by Chaudhri *et.al.*, 1997.Since then several studies from

our laboratory have implicated that involvement of gene polymorphisms of HLA system in the etiopathology of delusional disorder. Debnath et al, (2000) demonstrated a significant association between the HLA-A*03 allele and delusional disorder in Indian patients. In other studies, Debnath et al,(2003) have shown that apart from HLA-A*03 allele, several other alleles such as HLA-A*11 and HLA-B*5001, HLA-B*37,HLA-B*53 are also associated positively with delusional disorder. Debnath et al, have also tried to investigate the haplotypic association of HLA alleles in delusional disorder in Indian patients. They have shown that there is a significantly higher positive association of haplotypes like A*24-B*3701, A*26-B*08 and A*26-B*44 with delusional disorder.

Few isolated studies have also been implicated the possible involvement of dopamine receptor genes with delusional symptomatology in major psychosis. Serretti et.al.,(2000) demonstrated a significant association between the Ser311Cys variant and delusional features in major psychoses in Italian patients. But surprisingly enough no data are available on the association between other dopamine receptor genes and their variants with delusional disorder. The isolated studies are not consistently uniform and need to be replicated on a large sample size.

In case of psychiatric disorders, the largest hurdle in the process of assigning the disease traits. The situation was further muddled by the lack of objective, quantifiable tests for delusional disorders. Moreover, because familial clustering in certain behavioural traits can be due to genetics (nature) or upbringing (nurture), the construction of accurate pedigrees, strictly according to genetic criteria may be impossible.

Now the broad agreement that there should have a substantial genetic predisposition to psychotic disorders, constituting a biological vulnerability and that the most common and successful 'antidelusional' treatment remains various medications. However, the successful neuroleptic treatment of delusions may be mediated through a cognitive reappraisal (Hole *et al*,1979), and there appears to be complex and uncertain interrelationship between organic factors and delusional beliefs. The tension between genetic, psychodynamic and cognitive perspectives continue and seem to be best resolved

by a model incorporating the insights of each and acknowledging the breadth and limitation of the data.

3.1.8. Pathogenesis of Delusional Disorder :

There is no generally accepted model of delusional development and there remains a need to develop a complex integrated model, able to incorporate factors, at present subsumed under different disciplines, which may exert their effect at different stages. A review of literature essentially reveals that there interwinning trends of theories- cognitive, psychodynamic and psychophysiological were proposed in varied forms regarding the development, maintenance and the content of delusional beliefs (Malancharuvil,2004). Three approaches are suggested for the formation and development of delusion, these are psychodynamic mechanisms, disordered of reasoning and psychobiological mechanisms.

Psychodynamic Mechanism -

Freud (1911) in his classic psychoanalytic view stated that all delusions are a protection against homosexual urges. Homosexual feeling unacceptable to the individual are transformed by projection into suspiciousness and rejection –in this theory, an understandable warding-off of supposed homosexual advances. This scenario involving repressed homosexuality is assumed with no convincing proof and there seems to be no established connection between homosexuality and delusional disorder, although cases of delusional disorder in homosexuals are recorded (Ovesey, 1954; Aronson, 1989).

Klein (1957) postulated a fixation at the paranoid-schizoid position, said to occur between the sixth and ninth months of life, including profound hatred of the mother of the infant, symbolically represented by the maternal breast, and envy of other women, ultimately leading to paranoia. Paranoid delusions have been described as an escape, via projective mechanisms, from shame, guilt and inadequacy, with persecutory and grandiose beliefs attempting to overcome a prevailing sense of inferiority. A recurring suggestion of weakness counteracted by paranoid aggressiveness which is projected on to

the external object who can then be perceived and blamed as an aggressor (Hesselbach,1962).

Disordered Reasoning -

The definition of delusion emphasizes the operation of reasoning processes that have been made to establish that disorder of reasoning processes that have gone haywire, but it is not surprising that a number of attempts have been made to establish that disorder of reasoning is related to delusion formation and that such disorders can be observed among deluded patients. Related to psychodynamic formulation is the proposal that delusions arise on the basis of defects in formal logical reasoning. However, Kemp *et al.*,(1997) reported that differences in reasoning between deluded patients and controls were small.

Two other proposals involving disturbances in reasoning have been studied recently. The first portrays the difficulty underlying delusion formation as a failure in the application of Bayesian reasoning. According to this model of developing beliefs, making choices, and drawing conclusions, delusional patients accept conclusions at level of probability too low for acceptance by non delusional persons. The second proposal suggests that the reasoning processes of delusional patients are influenced by the subject's tendency to assign meaning in a biased manner. Application of this model reflecting motivational and reasoning difficulties (based on social attribution theory) has been tested, but the results do not provide sound support for the formulation. However, a recent study on the two components of social cognition (attentional and attributional biases) that contribute to the formation and maintenance of delusion has been supported by fMRI imaging approach (Blackwood *et al*, 2000).

Psychobiological Mechanism -

Delusional thinking may have proved difficult to explain by consistent psychological mechanisms but the nature of the disorder has led many to suggest psychological explanations (Strauss,1988). Anomalous perceptual experiences that lead to thought disorder are often suggested (Maher & Ross,1984) and the relationship between psychotic delusions and less deviant but still aberrant been explored (Chapman

et al,1982). Disturbing social experiences establishing delusional defense mechanisms has been a suggested explanation (Higgins,1987) and reasons for an individual's vulnerability to delusional belief have been explored (Neale *et al*,1985). Personality disorder, situational stresses (particularly recurrent) and cultural background have been proposed as significant in the formation of delusional beliefs (Westermeyer,1985). Despite the many psychological approaches proposed over the years, a consistent psychological explanation for delusional beliefs remains elusive. Psychological factors are, nonetheless, obviously significant in the content and quite possibly govern the formation and longitudinal consistency of a delusion. Psychological factors clearly affect both the genesis and course of delusional thinking.

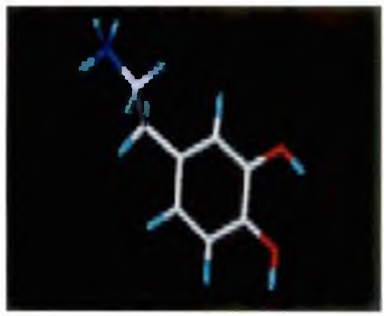
There is broad acceptance among many psychological theorists that delusions are derived from some unknown constitutional predisposition and initiated by some unaccountable change, which is probably organic (Freeman,1988). There is also agreement among organic theorists that where delusions are associated with known pathology, the association is neither characteristics of any particular lesion nor any specific delusions are characteristically produced. Hence, the evolution, expression, and elaboration of delusions depend chiefly on psychological factors.

A general model of delusion formation has been proposed by different schools, which has been used as a means of ordering the wide range of disparate theories and data and are represented in **Fig.1.** It is intended as a general framework based on the assumption that the formation of normal belief follows a temporal sequence and according to the complexity and persistence of the belief, it may progress through a number of stages (Bleuler,1951; Cameron, 1959;Arieti,1964 and Cutting,1989). It has been divided into pre-psychotic, acute and chronic phases representing the vulnerability, inception and increasing complexity of delusional development. It allows the multiplicity of proposed causal factors to be located where they are considered to exert their effect.

3.2. DOPAMINE RECEPTORS

3.2.1. Historical perspective :

Dopamine (DA) is the predominant catecholamine neurotransmitter in the mammalian brain, where it controls a variety of functions including locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation. This catecholamine also plays multiple roles in the periphery as a modulator of cardiovascular function, catecholamine release, hormone secretion, vascular tone, renal function, and gastrointestinal motility.



The dopaminergic systems have been the focus of much research over the past 30 years, mainly because several pathological conditions such as Parkinson's disease, schizophrenia, Tourette's syndrome, hyperprolactinemia and many more have been linked to a dysregulation of dopaminergic transmission.

A new impetus to the search in the DA field came from the application of gene-cloning procedures to receptor biology one-half a decade ago, which revealed a higher degree of complexity within DA receptors than previously thought. The complementary DNAs of five distinct DA receptor subtypes (D_1-D_5) have been, in fact, isolated and characterized.

The first evidence for the for the existence of dopamine receptors (DR)in the central nervous system came in 1972 from biochemical studies showing that dopamine was able to stimulate adenylyl cyclase (AC) (Keefe and Gerfen, 1995). In 1978, dopamine receptors were first proposed on the basis of pharmacological and biochemical evidence, to exist as two discrete populations, one positively coupled to AC and the other one independent of the adenosine 3′,5′-cyclic monophosphate (cAMP) generating system (Spano et al.,1978). It was shown, in fact, that in the pituitary DA inhibited prolactin secretion but did not stimulate cAMP formation (Caron et al., 1978; Kebabian et al., 1979) and that although the antipsychotic drug sulpiride was a DA antagonist when tested

in the anterior pituitary, it was not able to block the striatal DA-sensitive AC (Kebabian et al., 1979; Spano et al.,1978). In 1979, Kebabian and Calne summarized these observations and suggested to call D_1 the receptor that stimulated AC and D_2 the one that was not coupled to this effector.

Subsequent studies confirmed this classification scheme, and D_1 and D_2 receptors were clearly differentiated pharmacologically, biochemically, physiologically, and by their anatomic distribution.

Concurrently in the late 1970s, by means of functional tests such as renal blood flow and cardiac acceleration measurements in the dog, the existence of specific peripheral receptors for DA was demonstrated. These receptors were named DA_1 and DA_2 on the basis of some pharmacological properties distinguishing them from their central counterparts (Goldberg et al., 1978). This led to a long-standing controversy concerning the identity or nonidentity of peripheral versus central receptors. However, subsequent biochemical and molecular biology studies in peripheral tissues pointed to extensive similarities between central and peripheral DA receptors so that the DA_1/DA_2 classification has been dropped (Andersen et al., 1990; Missale et al., 1988; Nash et al., 1993; O'Connell et al., 1995). For a decade, the dual receptor concept served as the foundation for the study of DA receptors. However, after the introduction of gene cloning procedures, three novel DA receptors subtypes have been characterized over the past five years. These have been called D_3 (Sokoloff et al., 1990) D_4 (Van tol et al., 1991) and D_5/D_{1b} (Tiberi et al., 1991 ; Sunahara et al., 1991). Detailed structural, pharmacological, and biochemical studies pointed out that all DA receptor subtypes fall into one of the two initially recognized receptor categories. The D_1 and D_5/D_{1b} receptors share, in fact, a very high homology in their transmembrane domains. Similarly, the transmembrane sequences are highly conserved among D_2 , D_3 , and D_4 receptors (Civelli et al., 1993; Giros et al., 1996; Jackson et al., 1994; Seeman and Van Toll, 1994; Sokoloff and Schwartz, 1995) Pharmacologically, although the profiles of D_1 and D_2 receptors are substantially different, the D_5/D_{1b} receptor exhibits the classical ligand-binding characteristics of D_1 receptors, and the D_3 and D_4 receptors bind the hallmark D_2-selective ligands with relatively high affinity (Civelli et al., 1993; Gingrich and Caron, 1993; Jackson and

Danielsson, 1994; Seeman and Van Toll, 1994; Sokoloff and Schwartz, 1995). In addition, the initial distinction between D_1 and D_2 receptors in terms of signaling events, that is, positive and negative coupling to AC, appears to apply, in broad terms, also to the novel members of the DA receptor family, the D_5/D_{1b} receptor being coupled to stimulation of AC (Dearry et al., 1990; Grandy et al., 1989; Sunahara et al.,1991; Tiberi et al.,1991) and the D_3 (Chio et al.,1994; Mc Allister et al., 1995; Potenza et al., 1994; Robinson and Caron, 1996) and D_4 receptors (Chio et al., 1994a; Cohen et al., 1992; Mc Allister et al., 1995;Mc Hale et al., 1994;Tang et al., 1994) to inhibition of cAMP formation.

The D_1/D_2 classification concept developed in the late 1970s thus is still valid, and D_1 and D_5/D_{1b} receptors are classified as D_1-like and D_2, D_3, and D_4 receptor subtypes as D_2-like. The mammalian D_{1b} receptor, originally named on the basis of its high homology with the D_1 receptor, is now commonly referred to as the D_5 receptor.

3.2.2. Evolutionary perspective of dopamine receptor genes :

The D_2 receptor cDNA was first isolated in 1988 (Bunzow et al, 1988) and subsequently, in 1989, the existence of splice variants of this receptor was demonstrated (Toso et al, 1989; Giros et al, 1989; Monsma et al., 1989) The D_3 receptor was identified by screening a rat cDNA library with the D_2 receptor sequence followed by PCR extension and genomic library screening (Sokoloff et al, 1990). The D_4 receptor was cloned by screening a library from the human neuroblastoma cell line SK-N-MC (Van Tol et al., 1991) The D_1 receptor was cloned by using either low-stringency screening of libraries or PCR based on the sequence of the D_2 receptor (Dearry et al., 1990; Monsma et al.,1990; Zhou et al., 1990). The second member of the D1-like receptor family was isolated using the sequence of the D1 receptor and was referred to as D5 (Sunahara et al., 1991), D1b (Tiberi et al., 1991) and D1 (Weinshank et al., 1991) It is now well established that the D_5 and D_{1b} are the human and rat equivalents of the same receptor.

The genomic organization of the DA receptors supports the concept that they derive from the divergence of two gene families that mainly differ in the absence or the presence

of introns in their coding sequences. As summarized in Table 7, the D_1 and D_5 receptor genes do not contain introns in their coding regions (Civelli et al., 1993; Gingrich and Caron , 1993; O'Wood B F, 1993), a characteristic shared with most G protein-coupled receptors (Dohlman et al., 1987). In contrast, and by analogy with the gene for rhodopsin (Nathans and Hogness, 1983) , the genes encoding the D_2-like receptors are interrupted by introns (Civelli et al., 1993; Gingrich and Caron, 1993; O'Wood, 1993) It appears likely that many of the genes in the G protein-coupled receptor family have arisen from a single primordial gene, suspected to be one of the opsin genes, that lost its introns by a gene-processing event (O'Wood, 1993). Two main evolutionary mechanisms may have created and amplified the molecular diversification within the two gene families: *1*) gene duplication mechanisms that gave rise to different, but nevertheless similar, sister genes encoding receptor subtypes or pseudogenes and 2) speciation that originated species homologs and the development of genetic polymorphism that provided receptor variants found in individuals within the same species (Vernier et al., 1995; Civelli, 2000)

3.2.3. Molecular Biology of the Dopamine Receptors :

The genes for the D1 (DRD1) and D5 (DRD5) receptors are intronless but pseudo genes of the D5 exist. The D2 and D3 receptors vary in certain tissues and species as a result of alternative splicing and the human D4 receptor gene (DRD4) exhibits extensive polymorphic variation. Analysis of the gene structure of D2 like receptors revealed that the DRD2 coding region contains six introns (Toso et al., 1989; Giros et al., 1989; Grandy et al., 1989; Monsma et al., 1989) and the DRD3 coding region five (Sokoloff et al., 1990) and DRD4 3 (Van Tol et al., 1991). Interestingly, the localization of introns is similar in the three receptor genes and in the opsin gene. The DRD3 lacks the fourth introns of the DRD2 and the DRD4 lacks the third and fourth introns of the DRD2. the third intron of the DRD4 has an unusual intron/exon junction in which the conventional splice junction donor and acceptor sites are missing (Van Tol et al., 1991, 1992).

The presence of introns within the coding region of D2like receptors allows the generation of receptor variants. Indeed, the D2 receptor has two main variants, called D2S and D2L, which are generated by alternative splicing of a 87-bp exon between

introns 4 and 5 (Toso et al., 1989; Gingrich and Caron, 1993; Giros et al., 1989; Monsma et al., 1989). Splice variants of the D3 receptor encoding nonfunctional proteins have been also identified (Fishburn et al., 1993; Giros et al., 1991; Snyder et al., 1991).

Analysis of the gene for the human D4 receptor revealed the existence of polymorphic variations within the coding sequence. A 48bp sequence in the third cytoplasmic loop exists either as a direct repeat sequence, as a fourfold repeat, or a sevenfold repeat. DRD4 containing up to 11 repeats have been found (Van Tol et al., 1992).The DRD5 has two related pseudogenes on human chromosome 1 and 2. they are 98% identical to each other and 95% identical to the human DRD5 and code for truncated, nonfunctional forms of the DRD5 (Grandy et al., 1989; Weinshank et al., 1991.

TABLE 7: DOPAMINE RECEPTOR GENES

	D1-Like		D2-Like			
	D1	D5	D2		D3	D4
			D2S	D2L		
Amino acids	446	477	414	443	400	387-515*
Amino acids in 3rd Cytoplasmic loop	57	50	134	443	120	101-261*
Introns	0	0	6		5	3
Chromosomal Localization	5q 35.1	4p 15.1-16.1	11q 22-23		3q 13.3	11p 15.5

3.2.4. Structure of Dopamine receptors:

Analysis of the primary structure of the cloned DA receptors revealed that they are members of the seven transmembrane (TM) domain G-protein coupled receptor family and share most of their structural characteristics (Fig. 1).

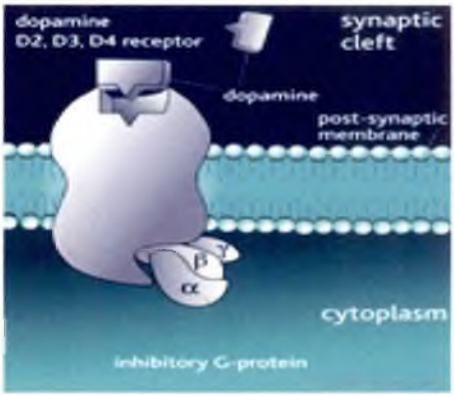


Fig1:Transmembrane domain of the dopamine D2 like receptors

Members of this family display considerable amino acid sequence conservation within TM domains (Probst et al., 1992).

Analysis of DA receptor structure pointed to similarities and dissimilarities between D_1-like and D_2-like receptors (Civelli et al., 1993; Gingrich and Caron, 1993; Jackson and Danielsson, 1994; O'Wood, 1993). Members of the same family share considerable homology. The D_1 and D_5 receptors share a 80% identity in their TM domains.

The D_2 and D_3 receptors have a 75% identity in their TM domains, and the D_2 and D_4 receptors share a 53% identity in the TM domains.

The NH_2-terminal stretch has a similar number of amino acids in all the receptor subtypesand carries a variable number of consensus *N*-glycosylation sites. The D_1 and D_5 receptors possess two such sites, one in the NH_2 terminal and the other one in the second extracellular loop. The D_2 receptor has four

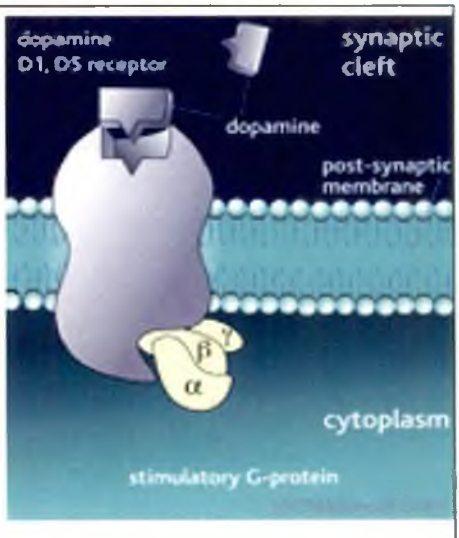


Fig 2: Structure of dopamine D1 and D1 like receptors

potential glycosylation sites, the D_3 has three, and the D_4 possesses only one (Civelli and Grandy, 1993; Gingrich and Caron, 1993; Jackson and Danielsson, 1994; O'Wood, 1993).

The COOH terminal is about seven times longer for the D_1-like receptors than for the D_2-like receptors, is rich in serine and threonine residues, and contains a cysteine residue that is conserved in all G protein-coupled receptors and that has been shown to be palmitoylated in the β_2-adrenergic receptors and in rhodopsin probably to anchor the cytoplasmic tail to the membrane (O'Wood et al., 1989; Ovchinnikov et al., 1988).

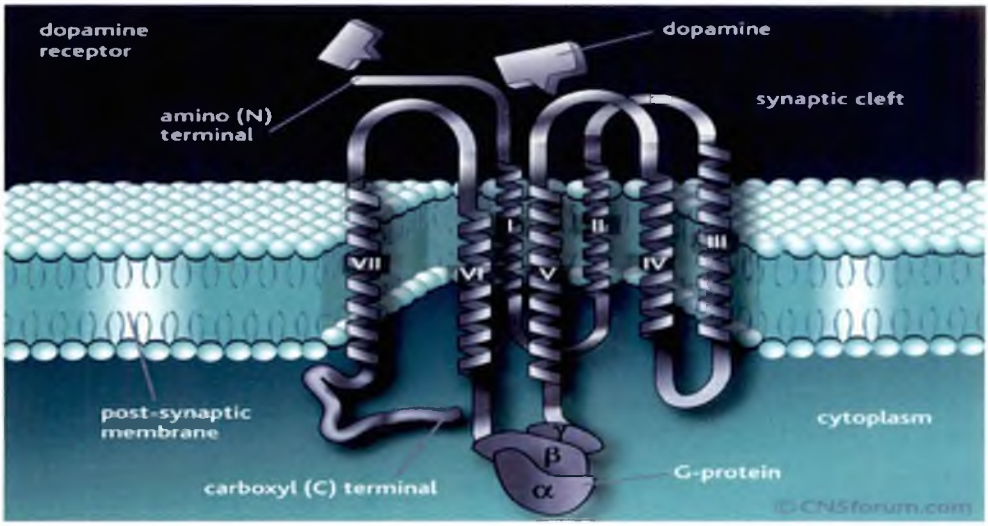


Fig. Dopamine receptors – 7-transmembrane spanning, G-protein coupled receptors

In the D_1-like receptors, this cysteine residue is located near the beginning of the COOH terminus, whereas in the D_2-like receptors, the COOH terminus ends with this cysteine residue (Fig. 1). Likewise, as in all G protein-coupled receptors, DA receptors possess two cysteine residues in extracellular loops 2 and 3 (Civelli et al., 1993; Gingrich and Caron, 1993; Jackson and Danielsson, 1994; O'Wood, 1993), which have been suggested to form an intramolecular disulfide bridge to stabilize the receptor structure (Dohlman et al., 1990; Fraser, 1989). The D_2-like receptors have a long third intracellular loop, a feature which is common to receptors interacting with G_i proteins to inhibit AC,

whereas the D_1-like receptors are characterized by a short third loop as in many receptors coupled to G_s protein (Civelli et al., 1993; Gingrich and Caron, 1993; O'Wood, 1993).

The D_1 and D_5 receptor third intracellular loop and the COOH terminus are similar in size but divergent in their sequence. In contrast, the small cytoplasmic loops 1 and 2 are highly conserved so that any difference in the biology of these receptors can be probably related to the third cytoplasmic loop and the COOH-terminal tail (Civelli et al., 1993; Gingrich and Caron, 1993; O'Wood, 1993).

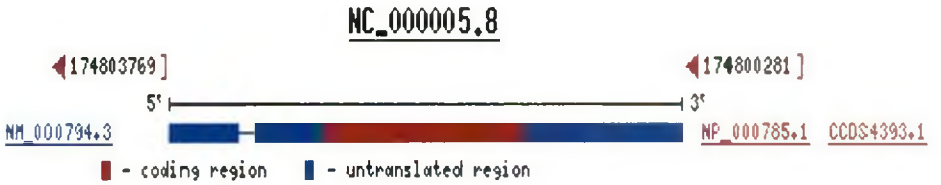
The external loop between TM4 and TM5 is considerably different in the two receptor subtypes, being shorter (27 amino acids) in the D_1 receptor than in the D_5 receptor (41 amino acids). The amino acid sequence of this loop, in addition, is divergent in the D_5 and in its rat counterpart D_{1b} (Sunahara et al., 1991; Tiberi et al., 1991).

Site-directed mutagenesis for catecholamine receptors (Kjelsberg et al., 1992; Strader et al.,1988, 1989) and protein modeling with the β_2-, α_2-, and D_2 receptors (Hibert et al., 1992, 1993; Trumpp et al., 1992) suggested that the agonist binding likely occurs within the hydrophobic TM domains (Fig. 1). Highly conserved residues are present in the core of the protein and define a narrow binding pocket that most probably corresponds to the agonist binding site (Hibert et al., 1993). In particular, an aspartate residue in TM3 is most probably involved in binding the amine group of the catecholamine side chain (Hibert et al., 1993; Strader et al., 1988) Two serine residues in TM5 have been shown to be hydrogen bond donors to bind the hydroxyl groups of the catechol moiety for the β_2- (Strader et al., 1989), α_2- (Wang et al., 1991), D_2 (Cox et al.,1992; Mansour et al., 1992), and D_1 (Tomic et al., 1993) receptors. A phenylalanine in TM6 is highly conserved in all receptors interacting with catecholamine neurotransmitters and can make a stabilizing orthogonal interaction with the aromatic moiety of the ligand. A highly conserved aspartate residue in TM2 has been shown to play a crucial role in β_2-adrenergic (Chung et al., 1988; Hibert et al., 1993; Strader et al., 1988α_2-adrenergic (Wang et al., 1991), and D_1 (Tomic et al., 1993) and D_2 dopaminergic (Neve et al., 1991) receptor activation and to affect agonist binding (Hibert et al., 1993;

Sdhu et al., 1992; Tomic et al., 1993). It has been suggested that the interaction between this aspartate and the agonist is allosteric and can be modulated by Na^+ or H^+ (Hibert et al., 1992; Horstman et al., 1990; Neve et al., 1991). A number of cytoplasmic residues, such as the DRY sequence in the second intracellular loop or the alanine residue in the third intracellular loop of the β-adrenoceptor, also play a role in receptor activation (Kjelsberg et al., 1992; Strader et al., 1988).

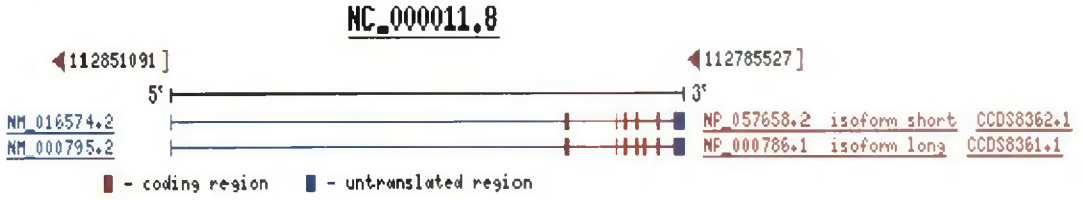
3.2.5. Polymorphism and Linkage disequilibrium

A. D1 receptor



Dopamine D1 receptor (DRD1) is part of the superfamily of G-protein coupled receptors. In 1990, Grandy et al. reported that the DRD1gene was localized at 5q35.1.The DRD1 gene has one small intron of 116 bp in the 5′ untranslated region (Minowa et al,1992; Mouradian et al., 1993). Several polymorphisms in the DRD1 gene have been detected however Grandy et al, identified a two allele *Eco* RI restriction fragment length polymorphism. Cichon et al, detected four SNPs (-94G/A *Mst*NI,1263G/A *Pvu*I, 1403T/C *Bsp*12861 and -48A/G *Dde*I) in DRD1 gene.

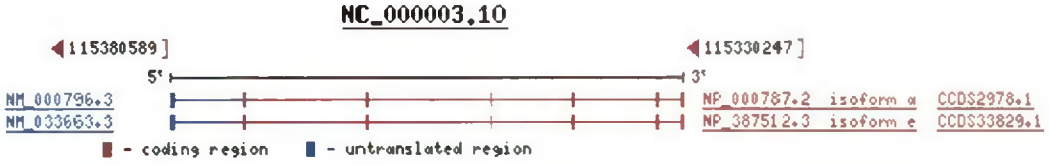
B. D_2 Receptor



The D_2 receptor exists as two alternatively spliced isoforms differing in the insertion of a stretch of 29 amino acids in the third intracellular loop (D_{2S} and D_{2L}) (Toso et al., 1989; Giros et al., 1989;Monsma et al., 1989). Because this loop seems to play a central role in receptor coupling, the existence of a splicing mechanism at this level could imply functional diversity. However, in spite of the efforts of several groups, no obvious

differences have emerged so far between the two D_2 receptor isoforms. The two D2 receptor forms are neither species- nor tissue-specific; they coexist in all tissues analyzed but at a highly variable ratio. Both variants share the same distribution pattern, with the shorter form less abundantly transcribed (Toso et al., 1989; Giros et al., 1989;Monsma et al., 1989; Neve et al.,1991). Both isoforms revealed the same pharmacological profile, even if a marginal difference in the affinity of some substituted benzamides has been reported(Castro et al.,1993; Malmberg et al., 1993). When expressed in host cell lines, both isoforms inhibited AC (Toso et al., 1989; Giros et al., 1989;Monsma et al., 1989). However, the D_{2S} receptor isoform displayed higher affinity than the D_{2L} in this effect (Toso et al., 1989; Montmayeur et al., 1991). Both isoforms mediate a phosphatidylinositol-linked mobilization of intracellular calcium in mouse Ltk fibroblasts. Protein kinase C (PKC), however, differentially modulates D_{2S}- and D_{2L}-activated transmembrane signaling in this system with a selective inhibitory effect on the D_{2S}-mediated response (Liu et al., 1992). Attempts to identify the preferred G protein β-subunit for D_{2S} and D_{2L} have led to conflicting results. One group suggested, in fact, that the 29-amino acid insertion in the D_{2L} receptor directs its interaction with $G_{i\text{-}2}\beta$ (Guiramand et al., 1995; Montmayeur et al., 1993), whereas another report showed that in transfected cell lines the D_{2S} isoform signals preferentially through $G_{i\text{-}2}\beta$ and the D_{2L} through $G_{i\text{-}3}\beta$ (Senogles,1994). The two receptor variants, in addition, appear to differ in their mode of regulation (Kukstas et al., 1991; Martres et al., 1992; Zhang et al., 1994).

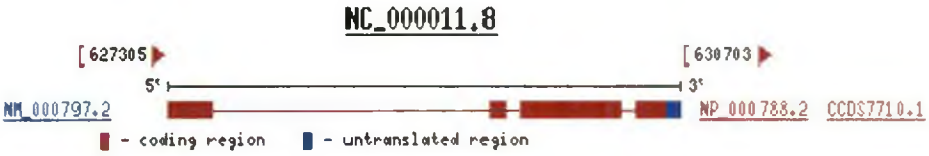
C. D_3 Receptor



Splice variants of the D_3 receptor have also been identified. One transcript carries a 113-bp deletion in TM3 and a frame shift in the coding sequences generating a stop codon shortly after the deletion and encodes a 100-amino acid-long truncated form of the receptor (Synder et al., 1991). A second variant derives from a deletion of 54 bp between

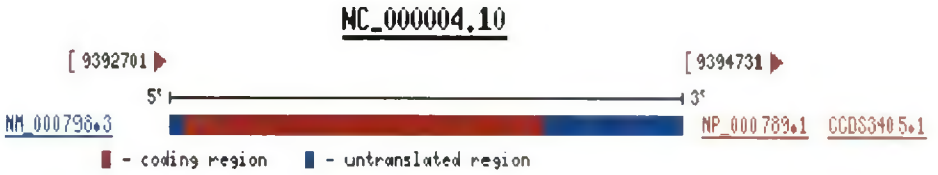
TM5 and TM6 of the D_3 receptor. Although this structure may be compatible with the occurrence of seven transmembrane domains, cell lines transfected with this cDNA failed to show any binding (Giros et al., 1991). Two alternatively spliced forms of the D_3 receptors have been identified in the mouse (Fishburn et al, 1993) but not in other species (Giros et al., 1991). These differ by a stretch of 21 amino acids in the third intracellular loop and are generated by a splicing mechanism that uses an internal acceptor site inside an exon, rather than a separate exon like the D_2 receptor. Both isoforms bind dopaminergic ligands with a D_3 pharmacological profile and have the same distribution pattern with the longer form predominant (Fishburn et al, 1993).

D. D_4 Receptor



Analysis of the deduced amino acid sequence of the D_4 receptor reveals that it is the most distantly related of the D_2-like receptors. In human polymorphic variants, the D_4 receptor exists with different insertions in the third intracellular loop. This loop contains repeat sequences of 16 amino acids with the number of repeats differing in the different forms of the receptor. The four-repeat form ($D_{4.4}$) is the predominant in the human population (60%). The $D_{4.7}$ variant is present in 14% of the population and the $D_{4.2}$ in 10% (Seeman and Van Tol, 1994; Van Tol et al., 1992). Receptor forms with up to 10 repeats have also been identified but are much less frequent (Seeman and Van Tol, 1994). The functional significance of these variants has not been elucidated. They display a slightly different affinity for the neuroleptic clozapine, but none of them has been related to an increased incidence of schizophrenia (Seeman and Van Tol, 1994; Van Tol et al., 1992).

E. D5 receptor:



Finally, the D5 receptor gene is peculiar among the G-protein-coupled receptors because it is associated with two pseudogenes in the human genome (26). The three D5-related genes are found on different chromosomes (24). Only one gene (DRD5, chromosome 4 q15.1-q15.3) codes for the active receptor; the two others contain an 8-base-pair insertion which leads to a frame shift and are genuine pseudogenes. Interestingly, these pseudogenes appear to be specific to humans, suggesting that the evolution of the D5 pseudogenes is a very recent event which may be restricted to primates.

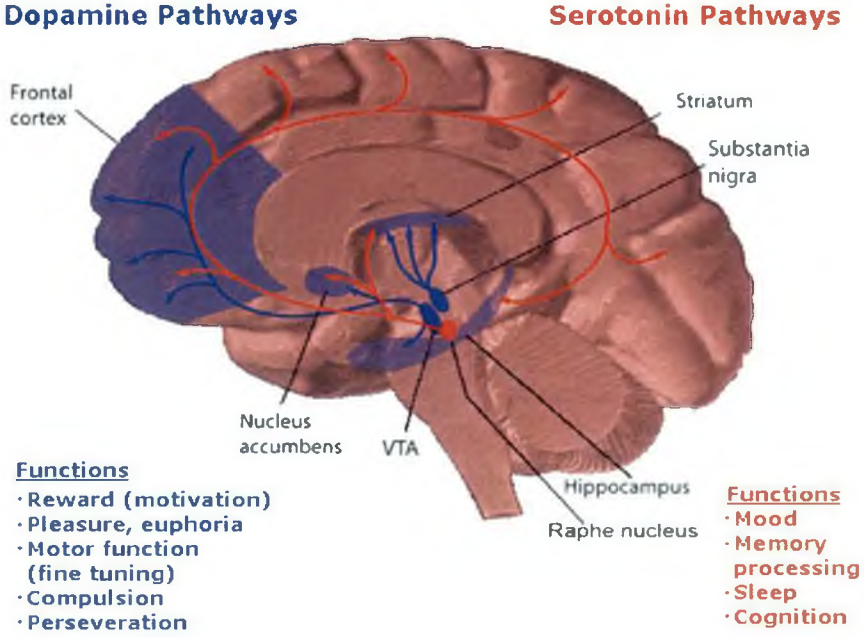
Although the human genome contains five dopamine receptor genes, the number of dopamine receptor mRNA species that it encodes is higher. This results from the fact that polymorphism and alternative splicing events play a role in dopamine receptor gene expression and leads to the existence of more than five different receptor binding sites.

First was the discovery that there exist two forms of D2 dopamine receptors (10, 14, 23, 25, 40, 42, 48, 52). These two forms differ in 29 amino acid residues located in the putative third cytoplasmic loop of the receptor. They are generated by an alternative splicing event which occurs during the maturation of the D2 receptor pre-mRNA (14, 25, 48). The two D2 receptor forms are neither species- nor tissue-specific; they coexist in all tissues analyzed but at a highly variable ratio. Because of its location in the third cytoplasmic loop, the 29-residue addition was expected to affect G protein coupling and consequently second messenger systems. It has been shown that both forms can inhibit cAMP accumulation (14) and that their efficiencies are somewhat variable (28,43). Alternative splicing events have also been shown to occur during the maturation of the D3 dopamine receptor pre-mRNA (22 , 53).

The existence of different variants of the human D4 receptor has also been demonstrated, although their generation is not by alternative splicing. These variants differ in the number of 48 base-pair repeats contained in their putative third cytoplasmic loop (59) and they have been detected in the genomes of different individuals, showing that a genetic polymorphism is responsible for the generation of the D4 receptor variants. These repeats are not present in the rat gene, making the polymorphism specific to humans. When expressed by DNA transfection, the variants containing 2, 4, and 7 repeats bind clozapine with equal affinities in the presence of sodium chloride. In the absence of sodium ions, however, the variants containing 2 and 4 repeats had a six- to eightfold lower dissociation constant for clozapine, while the affinity of the variant containing seven repeats was practically unaffected (59). Although it is not understood what effects the sodium ions have on receptors, these data indicate that the variants can behave differently with respect to the mechanism of ligand recognition.

3.2.6. Biological functions of dopamine receptors :

Dopaminergic neurons form a neurotransmitter system which originates in substantia nigra pars compacta, ventral tegmental area, and hypothalamus. Thease neurons basically acts through four major pathways: 1) Mesocortical pathway,2) Mesolimbic pathway,3) Nigrostriatal pathway and 4)Tuberoinfundibular pathway.



Via the dopamine receptors D1, D2, D3, D4 and D5 dopamine reduces the influence of the indirect pathway and increases the actions of the direct pathway within the basal ganglia. It has been found that D1 receptors are responsible for the cognitive-enhancing effects of dopamine (Heijtz et al, 2007). In general, the analgesic capacity of dopamine occurs as a result of dopamine D2 receptor activation, however, exceptions to this exist in the periaqueductal gray, in which dopamine D1 receptor activation attenuates pain presumably via activation of neurons involved in descending inhibition (Wood P B,2008). In addition, D1 receptor activation in the insular cortex appears to attenuate subsequent pain-related behaviour. The degree of forward locomotion is primarily controlled by the ventral striatum through activation of D1,D2 and D3 receptors.

Activation of presynaptic D2 receptors which results in decreased dopamine release, has been shown to decrease locomotor activity (Jackson and Westlind, 1994). Whereas activation of postsynaptic D2 receptors slightly increases locomotion.

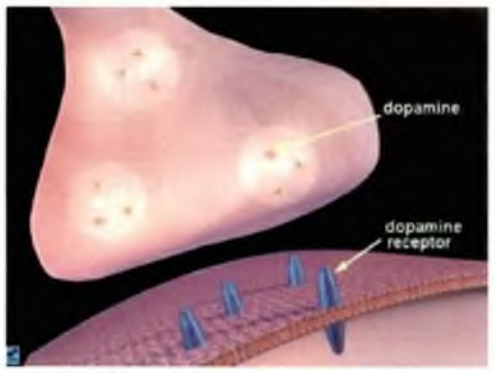


Fig. Structure of pre- and post-synaptic dopamine receptors

Activation of D1 receptors has little or no effect on locomotor activity (Gershanik et al, 1983; Jackson and Westlind, 1994).

The D3 receptor, which has been shown tobe mainly postsynaptically located in the nucleus accumbens (Diaz et al, 1995), seems to play an inhibitory role on locomotion.

The D1 like receptors appear to modulate intracellular calcium levels by a variety of mechanisms. One mechanism is via phophatidyl- inositol triphosphate (PI). It is fount to stimulate PI hydrolysis in Ltk cells (Liu et al, 1992). D1 receptor appears to affect the activity of calcium channels.In both rat striatal neurons and D1 receptor transfected GH_4C_1 cells, D1 agonists increase calcium currents by L-type calcium channels. In both

cases, the effect is mimicked by cAMP analogs (Liu et al., 1992; Surmeier et al.,1995). And blocked by
PKA inhibitors (Surmeier et al.,1995), suggesting that it may be the result of phosphorylation of calcium channels by PKA.

D2 like receptors also mediate changes in intracellular calcium levels. D2 receptors in the pituitary have been shown to inhibit PI metabolism (Canonico et al, 1983 ;Enjalbert et al, 1990;Simmonds et al., 1985). Neither D3 nor D4 receptors increase PI hydrolysis in any cell line tested. D2 receptors have also been shown to cause release of intracellular calcium stores in NG108-15 cells (Castellano et al., 1993).

D2 like receptors can also cause a decrease in intracellular calcium levels by inhibition of inward calcium currents (Seabrook et al., 1994; Vallar et al, 1990). D3 receptors also inhibit calcium currents in differentiated NG108-15 cells (Meister et al., 1991), whereas D4 receptors have this effect in GH_4C_1 cells (Liu et al., 1992).

The Na^+-K^+- ATPase, which pumps sodium out of cells and potassium in, is essential for maintaining the electrochemical gradient that is responsible for the excitability of nerve and muscle cells and drives the transport of fluid and solutes across

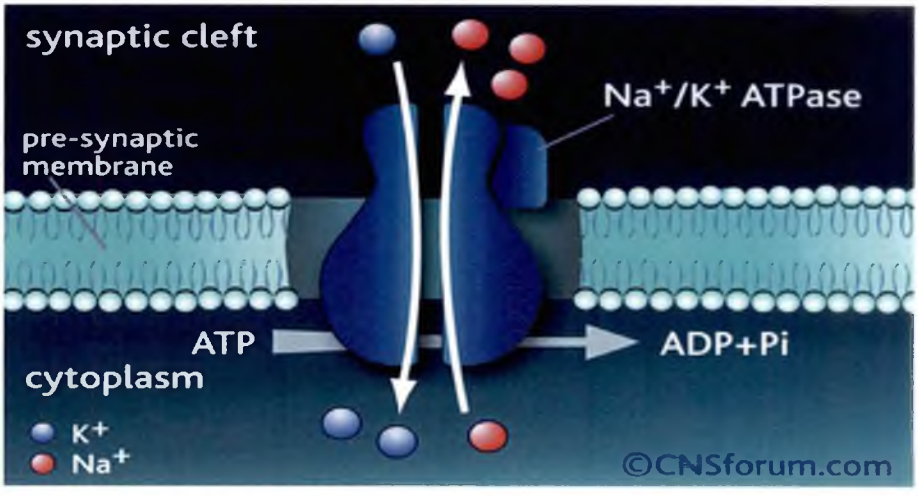


Fig. Membrane transport of small molecules and the electrical properties of membranes

epithelial membranes.It has been known that dopamine receptors influence the activity of this ion pump.In this manner dopamine regulates fluid absorption in the kidney and neuronal excitability in the brain. Most work has suggested that dopamine effects on the Na^+-K^+-

ATPase are mediated through the D1 receptor (Laitinen J T,1993). However, some reports also Suggested that activation of both D1 and D2 receptors may be required (Bertorello et al.,1990).

Table 8: Tissue distribution of dopamine receptors

Tissue	Receptor type	Function
Synaptic ganglia	D2	Inhibition of NE release
Adventitia	D2	Inhibition of NE release
Media	D1	Vasodialation
Glomerulosa	D1	Unknown
Medulla	D1	Stimulation of E/NE release
Juxtaglomerular	D1	Stimulation of rennin secretion Apparatus
Proximal tubule	D1	Inhibition of Na+ reabsorbtion
Ascending loop of Henle	D1	Inhibition of Na+ reabsorbtion
Heart	D4	Unknown

3.2.7. Dopamine receptors and disease associations

Dopamine is a chemical substance that plays an important role in the transmission of certain nerve signals. It is thought that abnormalities related to dopamine and its actions could be responsible or partially responsible for some neurological and psychiatric disorders. Different associations have been reported with dopamine receptors

and different neuropsychiatric diseases in different populations (Table 8). However, none of the studies so far have reported the association of a particular dopamine receptor gene with monosymptomatic psychiatric disorder in particular.

Table 9: Neuropsychiatric and neurological disorders and their associated dopamine receptor genes/ alleles:

Diseases	Dopamine receptor gene/ allele	Reference
Acoholism	DRD2	Klein et al.,1991, Ernest P. Noble,2002
Tourette's syndrome	DRD2	Klein et al.,1991
Attention Deficit Hyperactivity Disorder (ADHD)	DRD2, DRD4 7-repeat allele	Klein et al.,1991; Shaw et al., 2007
Posttraumatic Stress Syndrome	DRD2	Ernest P. Noble, 2002 Fujiwara et al., 1997
Perkinson's Disease	DRD3, DRD4	Nanko et al., 1993
Psychosis	DRD1 B2, DRD3 1	Holmes et al.,2001
Alzheimer's disease	DRD1 B2, DRD3 1	Holmes et al.,2001
Schizophrenia	DRD2, DRD3	Arinami et al, 1996; Fujiwara et al., 1997; Morimoto et al, 2004; Dargham et al, 2000; Ernest P. Noble,2002;
Bipolar disorder	DRD1	Weglarz et al., 2006
Reward Deficiency Syndrome	DRD2	Blum et al., 1996
Manic depression	DRD1	Jensen et al., 1992

3.3. TYROSINE HYDROXYLASE

3.3.1. Historical perspective :

Tyrosine hydroxylase (EC 1.14.16.2; tyrosine 3-monooxygenase; TH) catalyzes the first and rate-limiting step in the biosynthesis of catecholamine neurotransmitters (dopamine, norepinephrine, and epinephrine) (Nagatsu *et al.*, 1964).

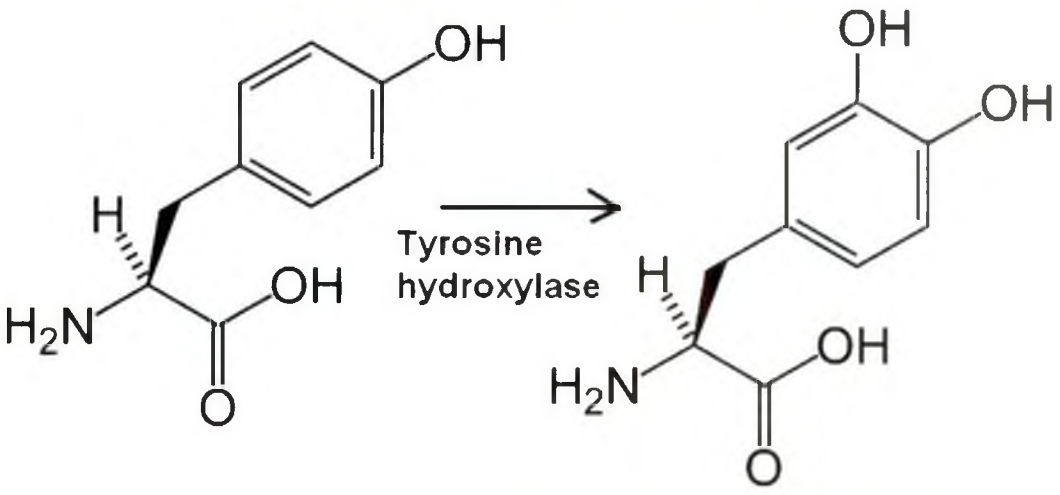
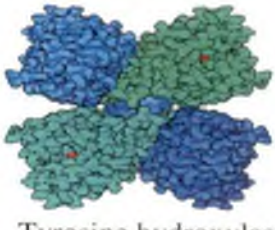


Fig 1: Tyrosine hydroxylase catalyzes tyrosine to dihydroxyphenylalanine

TH is expressed in catecholaminergic neurons in discrete regions of the brain, noradrenergic neurons of sympathetic ganglia and sympathetic nerves, and norepinephrine and epinephrine cells of the adrenal medulla. TH was purified from various tissues of various species, including bovine adrenal medulla (Nagatsu and Oka, 1987), rat adrenals (Fujisawa and Okuno, 1987), and rat pheochromocytoma (Tank and Weiner, 1987)

Human TH was purified from adrenals (Mogi *et al.*, 1984) and brain (striatum) (Mogi *et al.*, 1986).The results indicate the presence of the active form and inactive or less active form of human TH in both adrenals and brain. TH is a homotetramer requiring a tetrahydropterin cofactor and Fe^{2+} .

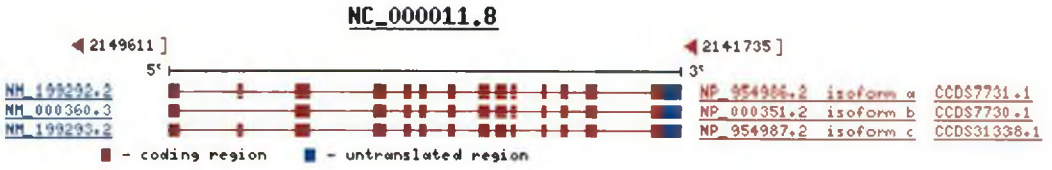
The molecular weight of the subunit of TH from various sources is about 60 kDa, and each subunit may contain one catalytic site, where the substrates, L-tyrosine


Tyrosine hydroxylase

and oxygen, and a tetrahydropterin cofactor bind to form the products, L-3,4-dihydroxyphenylalanine (DOPA), H20, and a quinonoid dihydropterin. The natural cofactor for tyrosine hydroxylase is *(6R)-L-erythro-tetrahydrobiopterin* (Brenneman and Kaufman, 1964; Matsuura *et al.*, 1980).

3.3.2. Molecular Genetics, DNA Sequence and gene content

Genomic regions, transcripts, and products



Genomic context

chromosome: 11; **Location:** 11p15.5

Tyrosine hydroxylase (TH) is a rate-limiting enzyme for catecholamine biosynthesis, responsible for catalyzing the conversion of the amino acid L-tyrosine to dihydroxyphenylalanine (DOPA) (Kaufman, 1995) and it is a pterin-requiring monooxygenase. Tyrosine hydroxylase (TH), has been mapped to human chromosome 11 p15.5 in the vicinity of the loci for insulin (INS) and for the oncogene Harvey Ras 1 (HRAS).

Both cDNAs and genomic DNA of human TH have been cloned and the nucleotide sequence has been determined (O'Malley and Rotwein, 1988). Four similar but distinct mRNAs encode human TH. The results of Southern blot analysis and the nucleotide sequence of the human TH genomic DAN indicate that the four types of human TH mRNA are produced through alternative splicing from a single gene (O' Malley et al., 1987). The human TH gene was split into 4 exons and 13 introns. The 12-

bp insertion sequence is encoded by the 3'-terminal portion of the first exon. The 81-bp insertion sequence corresponds to the second exon. Two kinds of alternative splicing are involved: the alternative use of two donor sites in the first exon and the inclusion/exclusion of the second exon. The four types (type 1-4) were expressed in COS cells, and all had enzymatic activities. The type 1 enzyme had the highest homospecific activity (activity per enzyme protein), the values for the other enzymes ranging from 30 to 40%. The *Km* values of the four types for L-tyrosine and 6-methyl-5,6,7,8-tetrahydropterin were similar (Nagatsu, 1989).

3.3.3. Biologic function of tyrosine hydroxylase

Tyrosine hydroxylase plays a key role in the physiology of adrenergic neurons. Tyrosine hydroxylase is regularly used as a marker for dopaminergic neurons, as tyrosine hydroxylase, in a manner parallel to the values reported for dopamine turnover (Blum et al., 1987).

3.3.4. Tyrosine hydroxylase and disease associations

TH may play an important role in the etiology of some diseases attributed to the impairment of central catecholaminergic neurons such as Parkinson's disease. In mental diseases, linkage analysis of an autosomal dominant type of manic-depressive illness indicated that a mutant gene is closely linked to the TH gene locus on chromosome 11, which suggests that some defect in the TH gene may cause the manic-depressive illness (Egeland *et al.,* 1987). It may be involved in the pathophysiology of psychiatric disorders. Mogi *et al.*1987; Nagatsu and Oka, 1987) reported that the homospecific activity of TH in the parkinsonian human brain was significantly increased. The increase in the homospecific activity of residual TH in parkinsonian brain suggests such molecular changes in TH molecules as result in a compensatory increase in TH activity and could be related to changes in types of TH isoenzymes.

Mutations in the TH gene outside exon 3 resulting in an amino acid exchange have been almost traced to the neurological disorders L-DOPA-responsive dystonia

(Segawa's syndrome) (Gln381Lys) (Lüdecke et al., 1995) and Arg233His (Van Den et al., 1998) L-DOPA-responsive parkinsonism (Leu205Pro) (Lüdecke et al., 1996) and severe form of TH deficiency (Cys359Phe) (Bräutigam et al., 1999) due to the lowered catalytic activities of the mutated enzymes.

Different associations have been reported with TH genes and different neuropsychiatric diseases in different populations (Table 9). However, none of the studies so far have reported the association of a particular TH allele with monosymptomatic psychotic disorder.

Table 10: Neuropsychiatric and neurological disorders and their associated tyrosine hydroxylase gene/ allele

Disease	TH gene/ allele	Reference
Mood disorder	TH2	Serretti et al, 1998
Schizophrenia	TH1	Jacewicz et al, 2008
Progressive Supranuclear Palsy (PSP)	TH isoform lacking exon3	Dumas et al, 1996
Personality traits	TH1	Giegling et al.,2009

3.4. DOPAMINE TRANSPORTER GENE (DAT)

3.4.1. Historical perspective :

The dopamine transporter (also dopamine active transporter, DAT, SLC6A3) is a membrane-spanning protein that binds the neurotransmitter dopamine; DAT provides the primary mechanism through which dopamine is cleared from synapses, transporting dopamine from the synapse into a neuron (Torres et al., 2003). DAT is present in the peri-synaptic area of dopaminergic neurons in areas of the brain where dopamine signaling is common. The initial determination of the membrane topology of DAT was based upon hydrophobic sequence analysis and sequence similarities with the GABA transporter. These methods predicted twelve transmembrane domains (TMD) with a large extracellular loop between the third and fourth TMDs (Kilty et al., 1991). Further characterization of this protein used proteases, which digest proteins into smaller

fragments, and glycosylation, which occurs only on extracellular loops, and largely verified the initial predictions of membrane topology (Vaughan and Kuhar, 1996). Regional distribution of DAT has been found in areas of the brain with established dopaminergic circuitry including: mesostriatal, mesolimbic, and mesocortical pathways (Ciliax et al., 1999).

The nuclei that make up these pathways have distinct patterns of expression.DAT in the mesocortical pathway, labeled with radioactive antibodies, was found to be enriched in dendrites and cell bodies of neurons in the substantia nigra pars compacta and ventral tegmental area. This pattern makes sense for a protein that regulates dopamine levels in the synapse.

Staining in the striatum and nucleus accumbens of the mesolimbic pathway was dense and heterogeneous. In the striatum, DAT is localized in the plasma membrane of axon terminals. Double immunocytochemistry demonstrated DAT colocalization with two other markers of nigrostriatal terminals, tyrosine hydroxylase and D2 dopamine receptors. The latter was thus demonstrated to be an autoreceptor on cells that release dopamine.

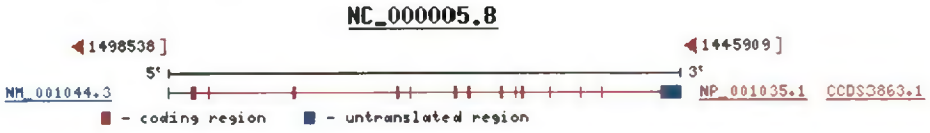
Surprisingly, DAT was not identified within any synaptic active zones. These results suggest that striatal dopamine reuptake may occur outside of synaptic specializations once dopamine diffuses from the synaptic cleft.

In the substantia nigra, DAT appears to be specifically transported into dendrites, where it can be found in smooth endoplasmic reticulum, plasma membrane, and pre- and postsynaptic active zones. These localizations suggest that DAT modulates the intracellular and extracellular dopamine levels of nigral dendrites.

Within the perikarya of pars compacta neurons, DAT was localized primarily to rough and smooth endoplasmic reticulum, Golgi complex, and multivesicular bodies, identifying probable sites of synthesis, modification, transport, and degradation (Hersch et al., 1997).

3.4.2. Molecular Genetics, DNA Sequence and gene content

Genomic regions, transcripts, and products



Genomic context

chromosome: 5; **Location:** 5p15.3See SLC6A3 in MapViewer

The gene that encodes the DAT protein is located on human chromosome 5, consists of 15 coding exons, and is roughly 64 kbp long. Evidence for the associations between DAT and dopamine related disorders has come from a genetic polymorphism in the DAT gene, which influences the amount of protein expressed. The gene for DAT is located on chromosome 5p15 (Venderbergh et al., 1992).The protein encoding region of the gene is over 64 kb long and is comprised of 15 coding segments or exons (Kawarai et al., 1997).

The human dopamine transporter (hDAT) gene (SLC6A3) contains 15 exons and encodes a 620-aminoacid protein with 12 transmembrane domains and cytosolic NH2 and COOH termini (Giros et al.,1992).

This gene has a variable number tandem repeat (VNTR) at the 3' end (rs28363170) (Sano et al, 1993). Differences in the VNTR have been shown to affect the basal level of expression of the transporter; consequentially, researchers have looked for associations with dopamine related disorders (Miller et al., 2002).

Etiological factors and pathogenetic processes involved in schizophrenia has focused on the "dopamine hypothesis," despite its many limitations (1). The doparnine transporter is primarily responsible for terminating doparninergic activity in the synapse

by taking released neumotransmitter back up into the presynaptic terminal (Antonio, 1995) dopamine transporter gene location on chromosorne 5p15.3.(Vandenbergh, 1992). The pivotal role played by the dopamine transporter in dopaminergic neunotransmission thus makes it a candidate gene for these disorders.

The neurotransmitter dopamine (DA) plays an important role in many behaviors including mood, reward, cognition, and motor function (Carlsson, 1987). In humans,dysfunction of the DA system is believed to contribute to conditions such as schizophrenia, drug abuse,attention deficit hyperactivity disorder (ADHD) and Parkinson's disease (Bannon et al., 2000)

Dopaminergic cells in the ventral tegmental area and substantia nigra project to the cortex, nucleus accumbens, and striatum where DA is released and acts at multiple dopamine receptors, including postsynaptic D1eD4 receptors, as well as presynaptic D2 receptors (Cooper et al., 1996).

An important mechanism for inactivation of DA following release is reuptake via the dopamine transporter (DAT). DAT is a member of the Na^+/Cl^- coupled cotransporter gene family that also includes the transporters for norepinephrine (NE) and serotonin (5-HT) (Nelson,1998).

The human dopamine transporter (hDAT) gene (SLC6A3) contains 15 exons and encodes a 620-aminoacid protein with 12 transmembrane domains and cytosolic NH2 and COOH termini (Giros et al., 1992)

3.4.3. Biological function of dopamine transporter :

DAT is an integral membrane protein that removes dopamine from the synaptic cleft and deposits it into surrounding cells, thus terminating the signal of the neurotransmitter. Dopamine underlies several aspects of cognition, including reward, and DAT facilitates regulation of that signal (Schultz, 1998).

DAT is a symporter that moves dopamine across the cell membrane by coupling the movement to the energetically-favorable movement of sodium ions moving from high to low concentration into the cell. DAT function requires the sequential binding and co-transport of two

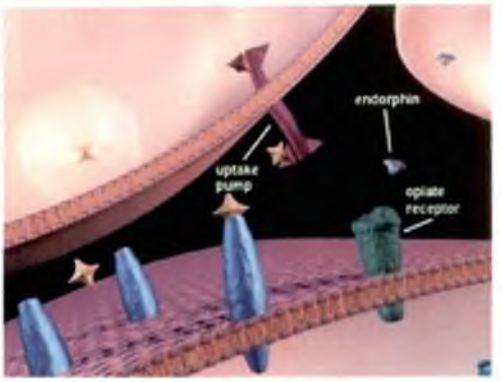


Fig. Reuptake of dopamine by DAT

Na+ ions and one Cl- ion with the dopamine substrate. The driving force for DAT-mediated dopamine reuptake is the ion concentration gradient generated by the plasma membrane Na+/K+ ATPase (Torres et al., 2003).

In the most widely-accepted model for monoamine transporter function, sodium ions must bind to the extracellular domain of the transporter before dopamine can bind. Once dopamine binds, the protein undergoes a conformational change, which allows both sodium and dopamine to unbind on the intracellular side of the membrane (Sonders et al., 1997).

Studies using electrophysiology and radioactive-labeled dopamine have confirmed that the dopamine transporter is similar to other monoamine transporters in that one molecule of neurotransmitter can be transported across the membrane with one or two sodium ions. Chloride ions are also needed to prevent a buildup of positive charge. These studies have also shown that transport rate and direction is totally dependent on the sodium gradient (Wheeler et al.,1993). Dopaminergic cells in the ventral tegmental area and substantia nigra project to the cortex, nucleus accumbens, and striatum where DA is released and acts at multiple dopamine receptors, including postsynaptic D1,D4 receptors, as well as presynaptic D2 receptors (Cooper et al., 1996)

Because of the tight coupling of the membrane potential and the sodium gradient, activity-induced changes in membrane polarity can dramatically influence transport rates.

In addition, the transporter may contribute to dopamine release when the neuron depolarizes (Wheeler, 1993).

3.4.4. Dopamine transporter and disease associations:

Because DAT terminates the dopamine signal, it is implicated in a number of dopamine-related disorders, including attention deficit hyperactivity disorder, bipolar disorder, clinical depression, and alcoholism (Vandenbergh et al., 1992).

The rate at which DAT removes dopamine from the synapse can have a profound effect on the amount of dopamine in the cell. This is best evidenced by the severe cognitive deficits, motor abnormalities, and hyperactivity of mice with no dopamine transporters (Gainetdinov et al., 1999). These characteristics have striking similarities to the symptoms of ADHD.

Differences in the functional VNTR have been identified as risk factors for bipolar disorder (Greenwood, 2001) and ADHD (Yang et al., 2007) Data has emerged that suggests there is also an association with stronger withdrawal symptoms from alcoholism, although this is a point of controversy (Sander et al., 1997;Ueno, 1999) Interestingly, an allele of the DAT gene with normal protein levels is associated with non-smoking behavior and ease of quitting (Ueno, 2003). Additionally, male adolescents particularly those in high-risk families (ones marked by a disengaged mother and absence of maternal affection) who carry the 10-allele VNTR repeat show a statistically significant affinity for antisocial peers (Beaver et al., 2001).

Increased activity of DAT is associated with several different disorders, including clinical depression (Lassonen et al., 1999). Decreasing levels of DAT expression are associated with aging, and likely underlie a compensatory mechanism for the decreases in dopamine release as a person ages (Bannon et al., 1992).

Etiological factors and pathogenetic processes involved in schizophrenia has focused on the "dopamine hypothesis," despite its many limitations (1). The doparnine transporter is primarily responsible for terminating doparninergic activity in the synapse

by taking released neumotransmitter back up into the presynaptic terminal.(Antonio *et al.*, 1995), dopamine transporter gene location on chromosorne 5p15.3.(Vandenbergh *et al.*, 1992). The pivotal role played by the dopamine transporter in dopaminergic neurotransmission thus makes it a candidate gene for these disorders.

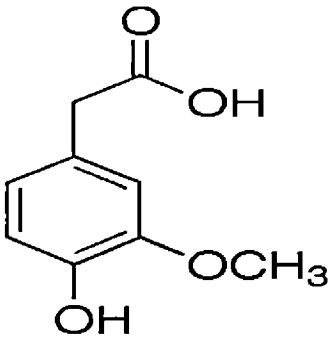
The neurotransmitter dopamine (DA) plays an important role in many behaviors including mood, reward, cognition, and motor function (Carlsson, 1987). In humans, dysfunction of the **DA** system is believed to contribute to conditions such as schizophrenia, drug abuse, attention deficit hyperactivity disorder (ADHD) and Parkinson's disease (Bannon *et al.*, 1998).

Table 11: **Neuropsychiatric and neurological disorders and their associated DAT gene /allele**

Disease	DAT gene/ allele	Reference
ADHD	DAT	Curran et al, 2001
Schizophrenia	DAT	Jacewicz et al, 2008
Bipolar Disorder	DAT	Greenwood et al,2006
Alcoholics	DAT	Wernicke et al.,2002
Alcohol Withdrawal Seizures	DAT1	Gorwood P.2008
dyskinesia	DAT1	Kaiser et al, 2003

3.5. PLASMA HOMOVANILLIC ACID AND DISEASE ASSOCIATION

Homovanillic acid is a major catecholamine metabolite. It is used as a reagent to detect oxidative enzymes, and is associated with dopamine levels in thebrain. Plasma levels of homovanillic acid (pHVA) are used as a peripheral measure of central dopaminergic activity (Sumiyoshi et al., 2000). It is also a potential index of central dopamine turn over and can therefore be used as the most suitable



Structure of Homovanillic acid

instrument currently available to assess dopamine activity under relatively neutral behavioural conditions, i,e., without any pharmacological manipulations or inducing stress from study conditions (Amin et al., 1998).

In psychiatry and neuroscience, brain and cerebrospinal fluid levels of HVA are measured as a marker of metabolic stress caused by 2-deoxy-D-glucose (Marcelis et al., 2006). HVA presence supports a diagnosis of neuroblastoma and malignant pheochromocytoma.

Biological pathway of homovanillic acid synthesis:

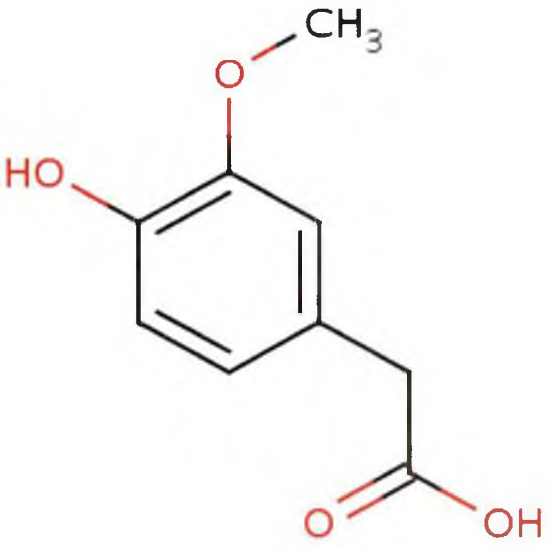
dopamine

↓

Dopac · 3-Methoxytyramine

↓

Homovanillic acid



Homovanillic acid (Biological Magnetic Resonance Data Bank, A Repository for Data from NMR Spectroscopy on Proteins, Peptides, Nucleic Acids, and other Biomolecules).

Table 12: Plasma homovanillic acid and disease associations

Disease	Phva concentration high/ low	Reference
Schizophrenia	High	Sumiyoshi et al., 2000; Morimoto et al., 2002
ADHD	Low	Coccaro et al., 2007
Alcoholism	Low	Kohnke et al., 2003
Delusional disorder	High	Bandopadhyay et al., 2009

3.6. BRIEF PSYCHIATRIC RATING SCALE (BPRS) :

A psychiatric assessment is a process of gathering information about a person within a mental health service, with the purpose of making a diagnosis. The assessment is usually the first stage of a treatment process. The assessment includes social and biographical information, direct observations, and data from specific psychological tests. It is typically carried out by a psychiatrist (Trzepacz et al., 1993).

A psychiatric assessment is most commonly carried out for clinical and therapeutic purposes, to establish a diagnosis and formulation of the individual's problems and to plan the individual's care and treatment. This may be done in a hospital (or in-patient) setting, in an ambulatory (or out-patient) setting, or in a community setting (as a home-based assessment).

The Brief Psychiatric Rating Scale (BPRS;Overall & Gorham, 1962) is one of the most frequently used instruments for evaluating psychopathology in patients with psychiatric disorders (Leucht et al.,2005). The score for a particular symptom varies between 0-7.

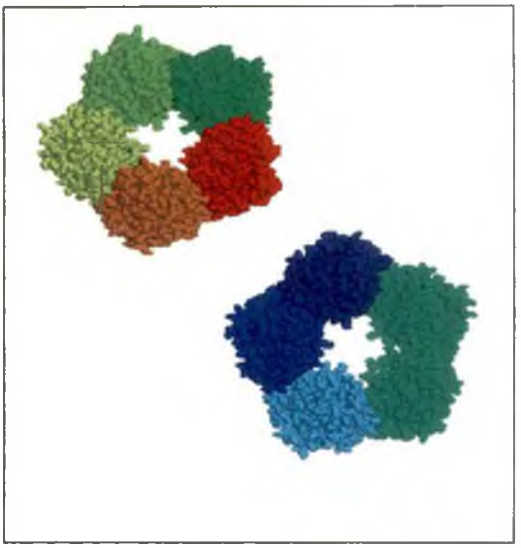
The interpretation of the scoring is as follows--

0 = not assessed, 1 =not present, 2 = very mild, 3 = mild, 4 = moderate, 5 = moderately severe, 6 = severe, 7 =extremely severe

The detailed scoring sheet is shown in Annexure IV.

3.7. C- REACTIVE PROTEIN (CRP)

3.7.1. Historical perspective :



C-reactive protein (CRP) is a protein produced as part of the inflammatory process. It is a routine test for heart failure; high levels of CRP may predict a bad outcome.CRP is a plasma protein, an acute phase protein produced by the liver (Pepys &Hirschfield,2003) and by adipocytes (Lau et al., 2005). It is a member of the pentraxin family of proteins (Pepys & Hirschfield, 2003). It is not related to C-peptide or protein C.

C-reactive protein was originally discovered by Tillett & Francis in 1930 as a substance in the serum of patients with acute inflammation that reacted with the *C* polysaccharide of *pneumococcus*. Initially it was thought that CRP might be a pathogenic secretion, as it was elevated in people with a variety of illnesses, including carcinomas. Discovery of hepatic synthesis and secretion of CRP closed that debate. It is thought to bind to phosphocholine, thus initiating recognition and phagocytosis of damaged cells (Pepys &Hirschfield,2003).

3.7.2 Genetics and biochemistry:

The *CRP* gene is located on the first chromosome (1q21-q23). CRP is a 224 residue protein (NCBI Entrez Protein #CAA39671) with a monomer molar mass of 25106 Da. The protein is an annular pentameric disc in shape. Proteins with this type of configuration are known as pentraxins. Native CRP is a bit different as it has 10-subunits making two pentameric discs, with an overall molecular mass of 251060 Da.

3.7.3. Biologic function of C-reactive protein :

CRP is a member of the class of acute phase reactants as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages (Pepys &Hirschfield,2003) as well as adipocytes (Lau et al., 2005). CRP binds to phosphorylcholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages, which express a receptor for CRP. It is also believed to play an important role in innate immunity, as an early defense system against infections.

CRP rises up to 50,000 fold in acute inflammation, such as infection. It rises above normal limits within 6 hours, and peaks at 48 hours. Its half-life is constant, and therefore its level is mainly determined by the rate of production (and hence the severity of the precipitating cause). Serum amyloid A is a related acute phase marker that responds rapidly in similar circumstances (Pepys &Hirschfield, 2003).

Measurement of acute phase proteins, especially C-reactive protein, is a useful marker of inflammation in both medical and veterinary clinical pathology. It correlates with the erythrocyte sedimentation rate (ESR) (Lloyd *et al.*,2006).

3.7.4. CRP and disease association :

CRP is used mainly as a marker of inflammation. Apart from liver failure, there are few known factors that interfere with CRP production (Pepys &Hirschfield, 2003).

Measuring and charting C-reactive protein values can prove useful in determining disease progress or the effectiveness of treatments. Recent research suggests that patients with elevated basal levels of CRP are at an increased risk for diabetes (Das, 2003;Lloyd *et al.*,2006), hypertension and cardiovascular disease.

Blood samples of persons with colon cancer have an average CRP concentration of 2.69 milligrams per liter. Persons without colon cancer average 1.97 milligrams per

liter. The difference was statistically significant (Zacho *et al.*, 2008). These findings concur with previous studies that indicate that anti-inflammatory drugs could lower colon cancer risk (Pepys *et al.*, 2006).

Table 13: C-reactive protein and disease associations

Disease	CRP concentration (high/ low)	Reference
Schizophrenia	High	Hanson and Gottesman, 2005; Fan *et al.*, 2006; Singh *et al.*, 2009.
Cognitive disorder	High	Hsu *et al.*, 2005; Zacho *et al.*, 2008
Depression	High	Hsu *et al.*, 2005
Diabetes	High	Pfutzner and Forst, 2006
Hypertension	High	Esther L G, 2005
Cardiovascular diseases	High	Bassuk *et al.*, 2004;
Colon Cancer	High	Baron *et al.*, 2003; Pepys *et al.*, 2006