Section - 3

Review of The Literature

3. REVIEW OF THE LITERATURE

3.1 HISTORICAL OVERVIEW OF SCHIZOPHRENIA

The symptoms relating to schizophrenia have been noted since the age of antiquity. The history of schizophrenia can be traced back to documents written by the Pharaonic Egyptians as far back as 2000 B.C. In these texts thought disturbances are mentioned that are commonly seen in schizophrenia. At that time it was thought that these mental disturbances were caused by demons and evil spirits and could be cured by exorcising. Many signs and symptoms of schizophrenia have been described in ancient Greek, Roman, and Chinese scripts. Hindu description of schizophrenia dates back to approximately 1400 BC and can be found in the Atharva Veda. It has been posited that health resulted from a balance between 5 elements (Buthas) and 3 humours (Dosas) and that an imbalance between these various elements might result in madness (Kyziridis, 2005).

In the modern times schizophrenia history is better known and recorded since 1700s. It was during this time that more detailed and accurate descriptions of abnormal mental behavior were recorded. These included changes in a person's speech, gestures and emotions. In 1851 Falvet first described a 'Folie Circulaire' or cyclical madness, and some twenty years later Hecker referred to a 'Hebephrenia', or a silly, undisciplined mind after Hebe, goddess of youth and frivolity. In 1874, Kahlbaum referred to both catatonic and paranoid disorders of the mind. The term catatonia is used to describe a movement disorder characterized by a mannequin-like muscle stiffness associated with unusual postures and a pervading fear. Then in 1878 Emil Kraepelin, perhaps auspiciously, combined these various 'disorders' into a single disease entity which he termed dementia praecox, or 'dementia of early onset' reflecting a decline of cognitive processes. This has been divided by him into four subtypes: (1) simple, marked by slow social decline concomitant with apathy and social withdrawal, (2) paranoid, with its attendant fear and 'persecutory' delusions, (3) hebephrenic and (4) catatonic, characterized by a poverty of movement and expression.

The inevitable inexactitudes of this emerging science continued with the dawn of the 20th Century when in 1908 Eugen Bleuler criticized the use of the term dementia praecox, arguing for an absence of evidence supporting a global dementing process. It was Bleuler who first coined the divisive term 'schizophrenia' in 1911. Bleuler defined schizophrenia with his four "A's", referring to the blunted Affect (diminished emotional response to stimuli), loosening of Associations (by which he meant a disordered pattern of thought, inferring a cognitive deficit), Ambivalence (an apparent inability to make decisions, again suggesting a deficit of the integration and processing of incident and retrieved information) and Autism (a loss of awareness of external events, and a preoccupation with the self and one's own thoughts).

After the publication of his classic textbook "Dementia Praecox, or the Group of Schizophrenias" in 1911, Bleuler's ideas enjoyed increasing acceptance and became an influential description of schizophrenia in most of Europe, England and the United States for decades. Consequently, the influence of Bleuler led to an increasingly broad definition and conceptualization of schizophrenia as psychiatry gathered strength and momentum during the post war years and through the 1950s and 1960s. This phenomenon was particularly apparent in the United States, where concepts such as "latent schizophrenia" and "pseudoneurotic schizophrenia" became popular (Black and Bofelli, 1989). These concepts were reflected in the initial editions of the "Diagnostic and Statistical Manual of Mental Disorders", published by the American Psychiatric Association. The first edition (American Psychiatric Association, 1952) emphasized intrapsychic mechanisms rather than classes of disease, whereas the second edition (American Psychiatric Association, 1968) shifted the emphasis to classification, but without listing specific criteria.

By the late 1960s, a number of factors intervened to introduce a climate of change. Studies of comparative diagnostic practices in the United States, England and other nations alerted American psychiatrists to the fact that their diagnostic habits were out of step. For example, the United States/United Kingdom diagnostic project (Cooper *et al.*, 1972) set out to determine why the prevalence of schizophrenia was greater in New York than London, while the reverse was true for manic depressive illness. The investigators discovered that the same patients received different diagnoses in different countries due to conceptual and theoretical differences between their

respective diagnostic systems. At about the same time, findings were reported from the International Pilot Study of Schizophrenia (WHO, 1975), in which schizophrenia was studied in nine countries. The major finding to emerge was that similar criteria were used in seven of the nine countries, but broader criteria were used in the United States and the Soviet Union.

In the context of these studies, an interest in reliable diagnosis emerged, leading to the development of structured interviews such as the Present State Examination (Wing *et al.*, 1967) and operational diagnostic criteria such as the St. Louis Criteria (Feighner *et al.*, 1972).The Present State Examination helped to introduce the concepts of the German psychiatrist Kurt Schneider (1887-1967) and his emphasis on "first-rank symptoms" to the English-speaking world. These forces helped reshape the concept of schizophrenia into one of a relatively severe psychotic disorder, bringing it closer to the original ideas of Kraepelin, but lacking Kraepelin's emphasis on a longitudinal definition that used course and outcome as a diagnostic guide.

Finally, and perhaps more importantly, the development of effective treatments such as neuroleptics, anti depressants and eventually lithium carbonate made diagnosis an important clinical issue. If a patient with bipolar disorder or major depression were misdiagnosed with schizophrenia because of an excessively broad diagnostic concept, that patient might be deprived of the most appropriate treatment available, potentially condemned to an unnecessarily chronic course of illness and perhaps condemned to needlessly suffer permanent and irreversible medication side effects.

All of these developments led to a reassessment of how schizophrenia and other mental disorders were diagnosed, culminating in the third edition of the "Diagnostic and Statistical Manual of Mental Disorders" (DSM-III)(American Psychiatric Association, 1980), which enumerated specific criteria for all recognized psychiatric disorders. DSM-III and its revision (DSM-III-R) (American Psychiatric Association, 1987) represented a convergence of these various points of view. The criteria included the Kraepelinian emphasis on course through the requirement that the illness be present for at least 6 months, the emphasis on specific delusions and hallucinations thought important by Schneider, and the emphasis on the importance of fundamental

Bleulerian symptoms (thought disorder in the form of associative loosening or incoherence and affective blunting).

The DSM-III and DSM-III-R compromise stirred debate among investigators interested in understanding the pathophysiology and etiology of schizophrenia. New technologies, such as molecular genetics or brain imaging, reemphasized the importance of careful and precise definition of the disease, as has occurred with the introduction of neuroleptics. Geneticists interested in familial patterns of transmission wondered whether the DSM-III and DSM-III-R definitions, with their requirement of florid psychotic symptoms, were too narrow to pick up subclinical cases in family pedigrees. They considered whether the concept of schizophrenia should be expanded to include nonpsychotic forms (e.g., simple and latent schizophrenia and "schizotaxia"), much as Bleuler originally suggested (Tsuang *et al.*, 2000). Studies of the neurobiology of schizophrenia, made possible by brain imaging and postmortem-brain banks, blurred the distinction between schizophrenia and "organic" disorders. Nearly four decades of psychopharmacological treatment of schizophrenia demonstrated that florid psychotic symptoms are probably not the core defining features after all, since crippling negative or deficit symptoms persist and seem fundamental, much as Bleuler observed. These observations have now been given more weight in DSM-IV (American Psychiatric Association, 1994) and DSM-IV-TR (American Psychiatric Association, 2000). Research conducted in the 1980s, as well as field trials conducted specifically for the Task Force on DSM-IV, showed that deficit symptoms can be reliably defined and should be considered as core features of the disorder.

3.2 DIAGNOSTIC CRITERIA FOR SCHIZOPHRENIA

Several sets of operational criteria were developed in the United States during the 1970s to increase the reliability of diagnosis. The St. Louis Criteria developed in 1972 include both longitudinal and cross-sectional criteria designed to identify schizophrenic patients with poor prognosis. The criteria require the exclusion of affective illness, drug abuse, or alcoholism and exclusion of cases of less than 6 months duration. The Research Diagnostic Criteria (RDC)(Spitzer *et al.*, 1978) were introduced later and differ from the St. Louis criteria mainly in emphasis on course of

illness. The St. Louis criteria require only a 2-week history. These two sets of criteria were instrumental in the development of DSM-III in 1980 and DSM-III-R in 1987 and include definitions of schizophrenia requiring both longitudinal and cross-sectional features.

The concept of including both cross-sectional and longitudinal features remains in DSM-IV-TR (Table 1), but more prominence is given to Bleulerian fundamental symptoms, reconceptualized as disorganized or negative symptoms. According to DSM-IV-TR, schizophrenia consists of the presence of characteristic positive or negative symptoms for of at least 1 month (unless successfully treated). The negative symptoms are characterized by the loss of motivation, impaired concentration, and the inability to express emotions, while positive symptoms include delusions, hallucinations, anxiousness and distorted perceptions of reality (Greer *et al.,* 2005; Schwartz *et al.,* 2004).This symptoms are continuous for at least 6 months.

If an illness otherwise meets the criteria but has a duration of at least 1 month but less than 6 months, it is termed a schizophreniform disorder. If it has lasted less than 4 weeks, it may be classified as either a brief psychotic disorder or a psychotic disorder not otherwise specified which is a residual category for psychotic disturbances that cannot be better classified.

Changes made in the diagnostic criteria of schizophrenia from DSM-III to DSM-IV are shown in Table 2. The major changes involve the description of and time requirement for active-phase symptoms, the introduction of the concept of negative symptoms, the deletion of the age at onset criterion, various exclusions, and the expansion of choices for classification of course.

Table 1: DSM-IV-TR diagnostic criteria for schizophrenia.

A.	Characteristic symptoms: Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated):
	(1) delusions
	(2) hallucinations

(3) disorganized speech (e.g., frequent derailment or incoherence)

(4) grossly disorganized or catatonic behaviour

(5) negative symptoms, i.e., affective flattening, alogia or avolition

Note: Only one Criterion A symptom is required if delusions are bizarre or hallucinations consist of a voice keeping up a running commentary on the person's behavior or thoughts, or two or more voices conversing with each other.

B. Social/ occupational dysfunction: For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).

C. Duration: Continuous signs of the disturbance persist for at least 6 months. This 6- month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

D. Schizoaffective and Mood Disorder exclusions: Schizoaffective disorder and mood disorder with psychotic features have been ruled out because either (1) no major depressive, manic, or mixed episodes have occurred concurrently with the active- phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.

E. Substance/general medical condition exclusion: The disturbance is not due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition.

F. Relationship to a Pervasive Developmental Disorder: If there is a history of autistic disorder or another pervasive developmental disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).

Table 1: DSM-IV-TR diagnostic criteria for schizophrenia

Classification of longitudinal course (can be applied only after at least 1year has elapsed since the initial onset of active-phase symptoms):
Episodic With Interepisode Residual Symptoms (episodes are defined by the reemergence of prominent psychotic symptoms); also specify if: **With Prominent Negative Symptoms**
Episodic With No Interepisode Residual Symptoms
Continuous (prominent psychotic symptoms are present throughout the period of observation); also specify if:
With Prominent Negative Symptoms
Single Episode In Partial Remission; also specify if:
With Prominent Negative Symptoms
Single Episode In Full Remission
Other or Unspecified Pattern

Table 2: Differences among DSM-III, DSM-III-R and DSM-IV/DSM-IV-TR criteria for schizophrenia.

DSM-III	DSM-III-R	DSM-IV/DSM-IV-TR
Characteristic active-phase symptoms	Characteristic active-phase symptoms for 1 week or more	Characteristic active-phase symptoms 1month or more (less if treated)
Deterioration in functioning	Impairment in functioning	Social/occupational dysfunction
Duration at least 6 months or more (including active phase)	Duration at least 6 months or more (including active phase)	Duration at least 6 months or more (including active phase)
Depression and mania ruled out	Schizoaffective disorder and psychotic mood disorder ruled out	Schizoaffective disorder and psychotic mood disorder ruled out
Organic mental disorder and mental retardation ruled out	Organic mental disorder ruled out	Effects of substance or general medical condition ruled out

Onset before age 45	Autistic disorder ruled out	If there is a history of pervasive developmental disorder, prominent delusions, hallucinations must also be present for at least 1 month (or less if successfully treated)
Classification of course Subchronic (>6months but<2years) Chronic (>2 years)	Classification of course Subchronic (>6months but<2years Chronic (>2 years)	Classification of course Episodic with interepisode residual symptoms Episodic with no interepisode residual symptoms
Subchronic with acute exacerbation	Subchronic with acute exacerbation	Continuous
Chronic with acute exacerbation	Chronic with acute exacerbation	Single episode in partial remission
In remission	In remission	Single episode in full remission
	Unspecified	Other or unspecified pattern

3.2.1 DIFFERENTIAL DIAGNOSIS

The major task in differential diagnosis involves separating schizophrenia from scizoaffective disorder, mood disorder with psychotic features, delusional disorder, or a personality disorder. To rule out schizoaffective disorder and psychotic mood disorders, major depressive or manic episodes should have been absent during the active phase, or the mood episode should have been brief relative to the total duration of the psychotic episode. Unlike delusional disorder, schizophrenia is characterized by bizarre delusions and hallucinations are common. Patients with personality disorders, particularly those in the "eccentric" cluster (e.g., schizoid, schizotypal, and paranoid personality) may be indifferent to social relationships and display restricted affect. They also have bizarre ideation and odd speech, or may be suspicious and hypervigilant, but they do not have delusions, hallucinations, or grossly disorganized behavior. Furthermore, patients with schizophrenia may develop other symptoms, such as a profound thought disorder, behavioral disturbances and enduring personality

deterioration. These symptoms are uncharacteristic of the mood disorders, delusional disorder, or the personality disorders.

Other psychiatric disorders also must be ruled out, including schizophreniform disorder, brief psychotic disorder, factitious disorder with psychological symptoms, and malingering. If symptoms persist for more than 6 months, schizophreniform disorder can be ruled out. The history of how the illness presents will help to rule out brief psychotic disorder, since schizophrenia generally has an insidious onset and there are usually no precipitating stressors. Factitious disorder may be difficult to delineate from schizophrenia, especially when the patient is knowledgeable about mental illness or is medically trained, but careful observation should enable the clinician to make the distinction between real or feigned psychosis. Likewise, a malingerer could attempt to simulate schizophrenia, but careful observation will help to distinguish the disorders. With the malingerer, there will be evidence of obvious secondary gain, such as avoiding incarceration, and the history may suggest antisocial personality disorder. The differential diagnosis for schizophrenia is summarized in Table 3.

Table 3: Differential diagnosis of schizophrenia.

Psychiatric illness	General medical illness	Drugs of abuse
Psychotic mood disorders	Temporal lobe epilepsy	Stimulants (e.g., amphetamine, cocaine)
Schizoaffective disorder	Tumor, stroke, brain trauma	Hallucinogens (e.g., phencyclidine
Brief reactive psychosis	Endocrine/metabolic disorders (e.g., porphyria)	Anticholinergics (e.g., belladonna alkaloids)
Schizophreniform disorder	Vitamin deficiency (e.g.,B_{12})	Alcohol withdrawal delirium
Delusional disorder	Infectious (e.g., neurosyphilis)	Barbiturate withdrawal delirium
Induced psychotic disorder	Autoimmune (e.g., systemic lupus	
Panic disorder	erythematosus)	
	Toxic (e.g., heavy metal	
Depersonalization disorder	poisoning)	
Obsessive-compulsive disorder		

Personality disorders (e.g., "eccentric" cluster) Factitious disorder with psychological symptoms Malingering		

3.2.2 CLINICAL FINDINGS

Clinical manifestations of schizophrenia and schizophreniform disorders are diverse and can change over time. Because of their variety, it has been said that to know schizophrenia is to know psychiatry. Whereas many symptoms are obvious, such as hallucinations, other symptoms like affective blunting or incongruity are relatively subtle and can be easily missed by a casual observer.

Various methods have been developed to describe and classify the multiplicity of symptoms in schizophrenia. Traditionally, schizophrenia is considered to be a type of "psychosis," yet the definition of psychosis has been elusive. Older definitions stressed the subjective and internal psychological experience and defined psychosis as "impairment in reality testing." More recently, psychosis has been defined objectively and operationally as the occurrence of hallucinations and delusions. Because schizophrenia is characterized by so many different types of symptoms, clinicians and scientist have tried to simplify the description of the clinical presentation by dividing the symptoms into subgroups. The most widely used subdivision classifies the symptoms as positive and negative.

3.2.2.1 Positive and negative symptoms

The concept of positive and negative symptoms was originally formulated by the British neurologist John Hughlings Jakson (1931). Jackson believed that positive symptoms reflected release phenomenon occurring in more phylogenetically advanced brain regions, due to injury to the brain at a more primitive level. Negative symptoms on the other hand, simply represented a "dissolution," or a loss of brain function. Current definitions of positive and negative symptoms are an amplification of these earlier ideas.

The positive symptoms, including hallucinations, delusions, marked positive formal thought disorder (manifested by marked incoherence, derailment, tangentiality, or illogicality) and bizarre or disorganized behavior reflect a distortion or exaggeration of functions that are normally present. For example, hallucinations are a distortion or exaggeration of the function of the perceptual systems of the brain. The person experiences a perception in the absence of an external stimulus.

Negative symptoms reflect a deficiency of a mental function that is normally present. For example, some patients display alogia (i.e., marked poverty of speech, or poverty of content of speech). Others show affective flattening, anhedonia/associality (i.e., inability of experience pleasure, few social contacts), avolition/apathy (i.e., anergia, impersistence at work or school) and attentional impairment. These negative or deficit symptoms not only are difficult to treat and respond poorly to neuroleptics than positive symptoms, but they are also the most destructive because they render the patient inert and unmotivated. The schizophrenic patient with prominent negative symptoms may be able to raise his or her performance under supervision but cannot maintain it when supervision is withdrawn.

Recent research suggests that the positive and negative symptoms reflect dimensions rather than discrete categories of psychopathology and that there are probably three dimensions rather than two (Andreasen *et al.*, 1995; Arndt *et al.*, 1995; Bilder *et al.*, 1985; Liddle *et al.*, 1989). Positive symptoms can be further divided into dimensions of psychoticism (i.e., delusions and hallucinations) and disorganization (i.e., disorganized speech and behavior and inappropriate affect). Negative (or deficit) symptoms represent a third dimension. The relationship between these three dimensions and their underlying pathophysiology continues to be studied and discussed (Andreasen *et al.*, 1995). The positive and negative symptoms of schizophrenia is summarized in Table 4.

Table 4: Positive and negative symptoms of schizophrenia.

Positive Symptoms of Schizophrenia
Delusions are firmly held erroneous beliefs due to distortions or exaggerations of reasoning and/or misinterpretations of perceptions or experiences. Delusions of

being followed or watched are common, as are beliefs that comments, radio or TV programs, etc., are directing special messages directly to him/her.

Hallucinations are distortions or exaggerations of perception in any of the senses, although auditory hallucinations (?hearing voices? within, distinct from one?s own thoughts) are the most common, followed by visual hallucinations.

Disorganized speech/thinking, also described as ?thought disorder? or ?loosening of associations,? is a key aspect of schizophrenia. Disorganized thinking is usually assessed primarily based on the person?s speech. Therefore, tangential, loosely associated, or incoherent speech severe enough to substantially impair effective communication is used as an indicator of thought disorder by the DSM-IV.

Grossly disorganized behavior includes difficulty in goal-directed behavior (leading to difficulties in activities in daily living), unpredictable agitation or silliness, social disinhibition, or behaviors that are bizarre to onlookers. Their purposelessness distinguishes them from unusual behavior prompted by delusional beliefs.

Catatonic behaviors are characterized by a marked decrease in reaction to the immediate surrounding environment, sometimes taking the form of motionless and apparent unawareness, rigid or bizarre postures, or aimless excess motor activity.

Other symptoms sometimes present in schizophrenia but not often enough to be definitional alone include affect inappropriate to the situation or stimuli, unusual motor behavior (pacing, rocking), depersonalization, derealization, and somatic preoccupations.

Negative Symptoms of Schizophrenia

Affective flattening is the reduction in the range and intensity of emotional expression, including facial expression, voice tone, eye contact, and body language.

Alogia, or poverty of speech, is the lessening of speech fluency and productivity, thought to reflect slowing or blocked thoughts, and often manifested as short, empty replies to questions.

Avolition is the reduction, difficulty, or inability to initiate and persist in goal-directed behavior; it is often mistaken for apparent disinterest. (examples of avolition include: no longer interested in going out and meeting with friends, no longer interested in activities that the person used to show enthusiasm for, no longer interested in much of anything, sitting in the house for many hours a day doing

nothing.)

Anhedonia is the few recreational interests/activities. Little sexual interest/activity. Impaired intimacy/closeness. Few relationship with friends/peers.

Attention is the social inattentiveness. Inattentiveness during testing.

3.3 SUBTYPES OF SCHIZOPHRENIA

According to the presence of the symptoms (Table 5) DSM-IV-TR recognizes five classic subtypes of schizophrenia i.e., paranoid, disorganized, catatonic, undifferentiated and residual. The main purpose of subtyping schizophrenia are to improve predictive validity, to help the clinician select treatments and predict outcome and to help the researcher delineate homogeneous subtypes. However, these goals remain largely unfilled. The reliability and validity of the classic subtypes are poor. Data from the International Pilot Study of Schizophrenia also failed to substantiate their usefulness (Strauss and Carpenter, 1981). Furthermore, many patients seem to fit several of these subtypes during the course of their illness. Hence, other subtyping strategies have been investigated, including paranoid versus nonparanoid forms, deficit versus nondeficit, Kraepelinian versus non-Kraepelinian, and early versus late onset.

Table 5: DSM-IV-TR subtypes of schizophrenia.

Subtype	Criteria	Associated features
Paranoid	Preoccupation with one or more delusions or frequent auditory hallucinations.	Often associated with unfocused, anger, anxiety, argumentativeness, or violence.
	Relative preservation of cognitive functioning and affect.	Stilted, formal quality or extreme intensity of interpersonal interactions may be seen.
	None of the following is prominent: disorganized speech, disorganized or catatonic behavior, flat or inappropriate affect.	
Disorganised	All of the following are prominent: disorganized speech; disorganized	Silly and childlike behavior is common;

	behavior; flat or inappropriate affect. The criteria are not met for catatonic type.	associated with extreme social impairment, poor premorbid functioning and poor long-term functioning.
Catatonic	The clinical picture is dominated by at least two of the following: Motoric immobility as evidenced by catalepsy (including waxy flexibility)or stupor.	Marked psychomotor disturbance present (stupor or agitation), and unusual motor disturbances may be present.
	Excessive motor activity (that is apparently purposeless and not influenced by extreme stimuli)	May need medical supervision due to malnutrition, exhaustion, hyperpyrexia or self-injury.
	Extreme negativism (an apparently motiveless resistance to all instructions or maintenance of a rigid posture against attempts to be moved) or mutism.	Sodium amobarbital interview may be helpful diagnostically.
	Peculiarities of voluntary movement as evidenced by posturing (voluntary assumption of inappropriate or bizarre postures), stereotyped movements, prominent mannerisms or prominent grimacing Echolalia or prominent grimacing	
Undifferentiated	Symptoms meeting criterion A are present, but criteria are not met for paranoid, disorganized or catatonic types.	Probably the most common presentation in clinical practice.
Residual	Absence of prominent delusions, hallucination, disorganized speech and grossly disorganized or catatonic behavior.	Active-phase symptoms (i.e., psychotic symptoms) are not present, but patient still exhibits emotional blunting, eccentric behavior, illogical thinking and mild loosening of associations.
	Continuing evidence of the disturbance, as indicated by the presence of negative symptoms or two or more symptoms listed in criterion A for schizophrenia, present in an attenuated form (e.g., odd beliefs, unusual perceptual experience).	

In validating the paranoid/nonparanoid subtypes, Kendler *et al.*, (1984) found that across all four diagnostic systems (i.e., DSM-III, RDC, ICD-9 and the Tsuang-Winokur criteria [Tsuang and Winokur, 1974]), patients with the paranoid subtype had better short-and long-term outcomes than nonparanoid patients (hebephrenic or undifferentiated subtypes). There was also moderate stability and reliability of subtype diagnosis at follow-up, with the paranoid subtype being the most stable and reliable (Kendler *et al.*, 1985). However, these subtypes did not appear to "breed true" within families (Kendler *et al.*,1988). Carpenter *et al.*, (1988) proposed dividing schizophrenia into deficit and nondeficit forms based on the presence or absence of "primary enduring negative symptoms." A body of literature has accumulated in support of the reliability and validity of this construct (Roy *et al.*, 2001). The deficit syndrome represents a promising strategy for reducing the heterogeneity of schizophrenia, identifying more homogeneous subgroup(s) and enhancing the power of research design at each level of inquiry (Carpenter *et al.*, 1999).

3.4 EPIDEMIOLOGY OF SCHIZOPHRENIA

Schizophrenia presents a challenge to the epidemiologist due to disagreement about the definition of its core features and the breadth of its spectrum. The development of operational criteria such as those in DSM-IV-TR has provided greater specificity for the diagnosis of schizophrenia and has resulted in a more cautious use of the concept. This has led to a reassessment of earlier epidemiological studies that generated rates based on older conceptualizations of schizophrenia. Despite these advances, case identification remains an ongoing problem among epidemiologists. Efforts to standardize the diagnosis have met with some success, such as with the present state examination used in the international pilot study of schizophrenia and the composite interview diagnostic instrument used in the National Comorbidity Study (Kessler *et al.*,1994).

Schizophrenia occurs equally in male and females but it appears earlier in males. The peak ages of onset are 20-28 years for males and 26-32 years for females (Castle *et al.*, 1991). Negative symptoms tend to predominate in men, whereas depressive episodes, paranoid delusions and hallucinations tend to predominate in women. Onset in childhood is much rarer (Kumra *et al.*, 2001), as is onset in middle or old age

(Hassett *et al.*, 2005). The lifetime prevalence of schizophrenia, the proportion of individuals expected to experience the disease at any time in their lives is commonly given at 1%. However, a systematic review of many studies found a lifetime prevalence of 0.55% (Goldner *et al.*, 2002). Table 6 shows the prevalence of schizophrenia in across the various countries. Despite the received wisdom that schizophrenia occurs at similar rates worldwide, its prevalence varies across the world (Jablensky *et al.*, 1992), within countries (Kirkbride *et al.*, 2006) and at the local and neighborhood level (Kirkbride *et al.*, 2007). One particularly stable and replicable finding has been the association between living in an urban environment and schizophrenia diagnosis, even after factors such as drug use, ethnic group and size of social group have been controlled for (Van Os, 2004).

Among the epidemiological studies on psychoses conducted in India, one of the largest has been the longitudinal Study of Functional Psychoses in an Urban Community (SOFPUC) in Chennai carried out by SCARF and the Department of Psychiatry, Madras Medical College. A population of over 100,000 was screened. The age corrected prevalence rate of schizophrenia was 3.87/1000. Other studies in India have reported prevalence of 0.7/1000 to 14.2/1000. However comparability among studies has been limited by variations in population size, geographical area and diagnostic criteria. The SOFPUC study also reported a higher prevalence of the illness in urban slums, in those living alone, with no schooling and unemployed (Rajkumar *et al.*, 1993). This study did not report any difference in male-female incident rates in contrast to other studies, which had reported a higher preponderance among males. The paucity of incidence studies in India could be due to the absence of demarcated catchment areas for health service delivery and lack of case registers and costs involved in conducting community surveys.

Table 6: Prevalence of schizophrenia across the continents and countries (Extrapolated Statistics).

Schizophrenia in America	Extrapolated Prevalence	Population Estimated Used
USA	2,375,154	293,655,405[2]
Canada	262,931	32,507,874[2]
Belize	2,207	272,945[2]

Brazil	1,489,053	184,101,109[2]
Chile	127,987	15,823,957[2]
Colombia	342,219	42,310,775[2]
Guatemala	115,504	14,280,596[2]
Mexico	848,937	104,959,594[2]
Nicaragua	43,350	5,359,759[2]
Paraguay	50,077	6,191,368[2]
Peru	222,784	27,544,305[2]
Puerto Rico	31,527	3,897,960[2]
Venezuela	202,346	25,017,387[2]
Schizophrenia in Africa	**Extrapolated Prevalence**	**Population Estimated Used**
Angola	88,797	10,978,552[2]
Botswana	13,258	1,639,231[2]
Central African Republic	30,270	3,742,482[2]
Chad	77,149	9,538,544[2]
Congo Brazzaville	24,248	2,998,040[2]
Congo kinshasa	471,681	58,317,030[2]
Ethiopia	576,986	71,336,571[2]
Ghana	167,887	20,757,032[2]
Kenya	266,767	32,982,109[2]
Liberia	27,424	3,390,635[2]
Niger	91,886	11,360,538[2]
Nigeria	143,569	12,5750,356[2]
Rwanda	66,636	8,238,673[2]
Senegal	87,774	10,852,147[2]
Sierra leone	47,590	5,883,889[2]
Somalia	67,169	8,304,601[2]
Sudan	316,639	39,148,162[2]
South Africa	359,509	44,448,470[2]
Swaziland	9,457	1,169,241[2]
Tanzania	291,749	36,070,799[2]
Uganda	213,450	26,390,258[2]
Zambia	89,178	11,025,690[2]
Zimbabwe	29,698	1,2671,860[2]

Schizophrenia in Australasia and Southern Pacific	Extrapolated Prevalence	Population Estimated Used
Australia	161,062	19,913,144[2]
New Zealand	32,302	3,993,817[2]
Schizophrenia in Europe	**Extrapolated Prevalence**	**Population Estimated Used**
Austria	66,119	8,174,762[2]
Belgium	83,699	10,348,276[2]
Britain (United Kingdom)	487,483	60,270,708[2]
Czech Republic	10,079	1,0246,178[2]
Denmark	43,784	5,413,392[2]
Finland	42,176	5,214,512[2]
France	488,725	60,424,213[2]
Greece	86,119	10,647,529[2]
Germany	666,669	82,424,609[2]
Iceland	2,377	293,966[2]
Hungary	81,144	10,032,375[2]
Liechtenstein	270	33,436[2]
Ireland	32,106	3,969,558[2]
Italy	469,582	58,057,477[2]
Luxembourg	3,742	462,690[2]
Monaco	261	32,270[2]
Netherlands (Holland)	131,985	16,318,199[2]
Poland	312,418	38,626,349[2]
Portugal	85,121	10,524,145[2]
Spain	325,800	40,280,780[2]
Sweden	72,684	8,986,400[2]
Switzerland	60,264	7,450,867[2]
United Kingdom	487,483	60,270,708[2]
Wales	23,601	2,918,000[2]
Azerbaijan	63,641	7,868,385[2]
Belarus	83,393	10,310,520[2]
Bulgaria	60,807	7,517,973[2]
Estonia	10,851	1,341,664[2]

Georgia	37,965	4,693,892[2]
Kazakhstan	122,485	15,143,704[2]
Latvia	18,653	2,306,306[2]
Lithuania	29,181	3,607,899[2]
Romania	180,816	22,355,551[2]
Russia	1,164,496	143,974,059[2]
Slovakia	43,867	5,423,567[2]
Slovenia	16,269	2,011,473[2]
Tajikistan	56,711	7,011,556[2]
Ukraine	386,068	47,732,079[2]
Uzbekistan	213,613	26,410,416[2]
Schizophrenia in Asia	**Extrapolated Prevalence**	**Population Estimated Used**
Bangladesh	1,143,195	141,340,476[2]
Bhutan	17,677	2,185,569[2]
China	10,505,385	1,298,847,624[2]
East Timor	8,243	1,019,252[2]
Hong Kong	55,445	6,855,125[2]
India	8,614,541	1,065,070,607[2]
Indonesia	1,928,663	238,452,952[2]
Japan	1,029,899	127,333,002[2]
Laos	49,080	6,068,117[2]
Macau	3,601	445,286[2]
Malaysia	190,255	23,522,482[2]
Mongolia	22,253	2,751,314[2]
Philippines	697,543	86,241,697[2]
Papua New Guinea	43,840	5,420,280[2]
Vietnam	668,596	82,662,800[2]
Singapore	35,215	4,353,893[2]
Pakistan	1,287,617	159,196,336[2]
North Korea	183,583	22,697,553[2]
South Korea	390,125	48,233,760[2]
Sri Lanka	160,997	19,905,165[2]
Taiwan	184,006	22,749,838[2]
Thailand	524,647	64,865,523[2]

Afghanistan	230,625	28,513,677[2]
Egypt	615,655	76,117,421[2]
Gaza strip	10,716	1,324,991[2]
Iran	545,981	67,503,205[2]
Iraq	205,236	25,374,691[2]
Israel	50,139	6,199,008[2]
Jordan	45,384	5,611,202[2]
Kuwait	18,259	2,257,549[2]
Lebanon	30,551	3,777,218[2]
Libya	45,549	5,631,585[2]
Saudi Arabia	208,643	25,795,938[2]
Syria	145,724	18,016,874[2]
Turkey	557,230	68,893,918[2]
United Arab Emirates	20,414	2,523,915[2]
West Bank	18,693	2,311,204[2]
Yemen	161,965	20,024,867[2]

3.5 ETIOLOGY OF SCHIZOPHRENIA

In his classic monograph on schizophrenia published early in the 1900s, Eugen Bleuler said, "We do not know what the schizophrenic process actually is." Today a century later, Bleuler's comment is equally true. The problem of etiology in the schizophrenia is complicated by the fact that this group of disorders includes a variety of conditions. It is unlikely that there is a single cause common to all the schizophrenias. One would hardly expect, on the basis of the symptom picture, to find the same causative factors responsible for such widely divergent clinical types of schizophrenia.

While the reliability of the diagnosis introduces difficulties in measuring the relative effect of genes and environment, evidence suggests that genetic and environmental factors can act in combination for the causation of schizophrenia (Harrison and Owen, 2003). Evidence suggests schizophrenia has a significant heritable component but that onset is significantly influenced by environmental factors or stressors (Day *et al.*, 1987). The various theories of causations of schizophrenia are mentioned below.

3.5.1 GENETIC

Twin, family and adoption studies have suggested that genetics play a major role in the transmission of schizophrenia. Irwing Gottesman compiled over 40 studies in order to work out the risks of developing schizophrenia among the people with different familial relationships to the schizophrenic person. Two classes of relatives have especially high risks of developing schizophrenia. These are the offspring of two schizophrenic parents and a monozygotic (MZ) co-twin (Fig.1). Apparently, people who share the greatest number of genes with the people who have schizophrenia, have an increased risk of developing schizophrenia themselves.

Gottesman and Shields reviewed the results of 5 twin studies looking for concordance rates for schizophrenia (Gottesman and Shields, 1976). It was found that in MZ twins there was a concordance rate of 35-58% compared with dizygotic (DZ) twin rates that ranged from 9-26%. They also found a concordance rate in MZ twins of 75-91% when the sample was restricted to the most severe form of schizophrenia (Gottesman and Shields, 1982). The milder forms of schizophrenia had concordance rates of 17-33% suggesting that there may be greater genetic loading with severe forms of schizophrenia. The twin studies have all assumed that the shared environmental effects for MZ and DZ twins are equal which may be incorrect.

Environmental factors is thought to influence the development of schizophrenia. On the other hand adoption studies support the genetic theory of transmission. In 1994, a study looked at schizophrenia in the biological and adoptive relatives of schizophrenic adoptees and compared this to a demographically matched group of control adoptees (Kety *et al.*, 1994). In the sample of adoptees with chronic schizophrenia, the disorder was found exclusively in their biological relatives and not their adoptive relatives. The prevalence of the disorder was 10 times higher in the biological relatives of the schizophrenic adoptees than in the biological relatives of the control group. These studies make a clear case for the involvement of genetics in schizophrenia.

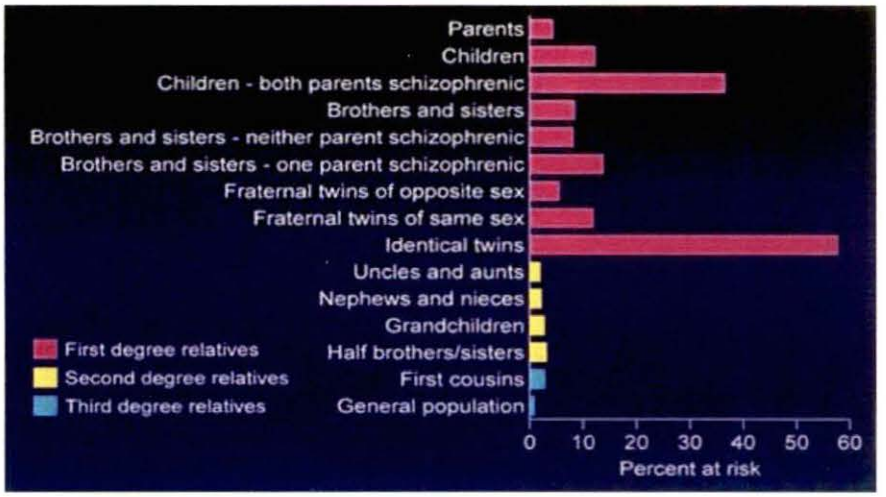

Figure 1: Risk of developing schizophrenia in percentage among relatives of schizophrenic patients.

3.5.1.1 Modes of Transmission

Since the concordance rate among MZ twins is not 100% and people can apparently carry the genotype for schizophrenia without ever developing the disease, there probably is not a single dominant gene for schizophrenia.

One model of transmission that has been suggested is the single gene model with incomplete penetrance (O'Rourke *et al.*,1982). Another model of transmission is the polygenic model which suggests that the 'liability to develop the disorder' is continuously distributed within the population (Bebbington and McGuffin, 1988). Only those individuals whose liability exceeds a certain threshold show symptoms of the disorder. This model is appealing because it could explain why concordance in twins increases with severity of illness and why the risk of schizophrenia increases with the number of relatives affected. Resolving the mode of inheritance of schizophrenia is complicated. Studies using genetic models have not been able to exclude either the single gene or polygenic models. This is most probably due to the problems in defining the schizophrenic phenotype.

3.5.1.2 Schizophrenia Loci

Genetic marker studies can greatly improve the prospect of detecting a major gene effect. A study published in 1998, conducted a genome wide search for evidence of

loci linked to schizophrenia (Shaw *et al.*, 1998). Genetic maps were constructed for each chromosome using genotype data. The genome wide search did not find evidence for a major genetic loci for schizophrenia but, it did find 12 chromosomes that had one statistically significant region at a 5% level (Shaw *et al.*, 1998; Nolen-Hoeksema *et al.*, 1998; Gottesman and Shields, 1976; Gottesman and Shields, 1982; Bebbington and McGuffin, 1988; Bogerts, 1993; Bogerts *et al.*,1985; Harrison, 1999; Crow, 1997; Powchik, 1998; Verdoux, 1997) and 2 of these chromosomes had statistical significance to a 1% level (Harrison, 1999; Powchik, 1998).

From these results, it seems unlikely that one major locus exists for schizophrenia. It could be that schizophrenia belongs to a class of complex disorders that have a genetic predisposition. This could be due to more than one gene that could produce illnesses independently in different families or, genes that act together to cause illnesses in susceptible people.

3.5.2 NEUROPATHOLOGY

A number of brain-imaging and post-mortem studies have shown that abnormal brain morphology and physiology appear to be involved in the development of schizophrenia. Figure 2 shows the specific areas of the brain involved with schizophrenia.

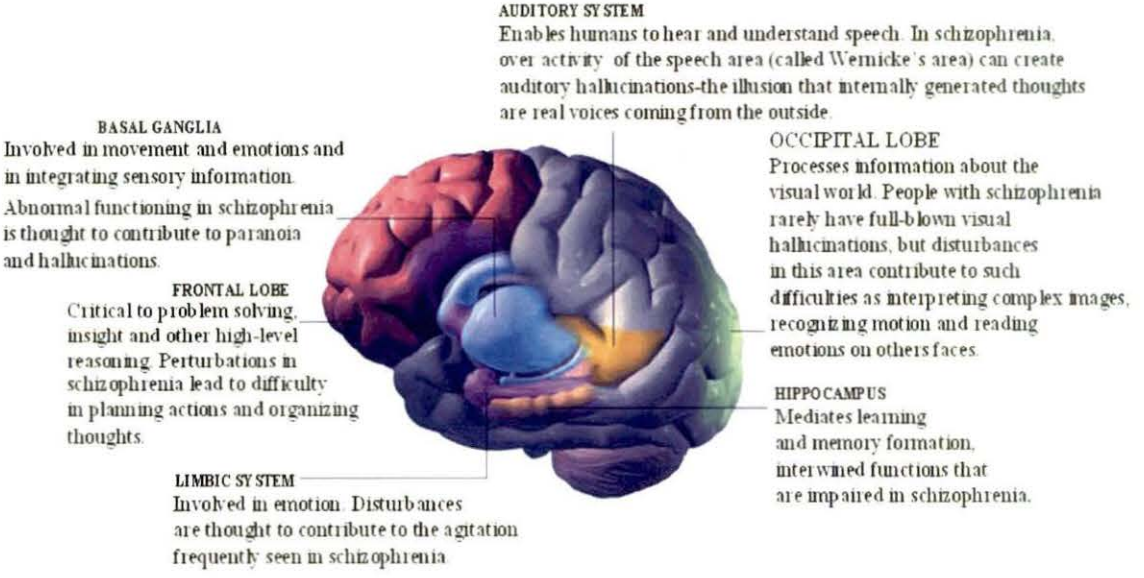

Figure 2: Brain structures involved in schizophrenia.

3.5.2.1 Limbic system

Limbic and paralimbic regions are highly organised regions of the brain and are involved in association and integration functions. If there is a structural and functional deficit in this region it will cause associative and integrative problem, as are experienced by schizophrenic patients. These problem leads to distorted interpretations of reality. The hippocampus and amygdala are the key regions in sensory interpretation, processing and comparing past and present experiences. They also control the basic drives and emotions that are generated in the neuronal networks of the septum hypothalamus complex (Harrison,1999). In addition to disturbed sensory information processing, limbic pathology could explain the dyscontrol syndrome of basic drives and emotions that are seen in schizophrenic patients. There is a particularly strong association between the left temporolimbic pathology and the positive symptoms of schizophrenia (Crow, 1997).Therefore it appears that the limbic system pathology is involved in the pathology of schizophrenia.

3.5.2.2 Neurodevelopment

The neurodevelopmental hypothesis states that the origins of schizophrenia are in abnormal brain development and that the pathology originates in the middle stage of intrauterine life (Murray, 1987). An earlier timing for the pathology of schizophrenia can be ruled out since abnormalities in the structure of the cerebral cortex would be expected if neurogenesis were affected. The absence of gliosis can be taken to mean that the changes must have occurred prior to the third trimester (Harrison, 1999). However, this hypothesis is weak because (a) gliosis is difficult to recognise and (b) the cytoarchitectural changes that have been found have not been proven to be a feature of schizophrenia (Arnold, 1996). Overall however, the cytoachitectural abnormalities and the lack of gliosis appear to be indicative of neurodevelopmental problems rather than a neurodegenerative process.

The neurodevelopmental hypothesis can only account for a minority of cases of schizophrenia. Most of the evidence in support of this theory is circumstantial, but the neuropathological findings in the brains of schizophrenics fit in well with this theory.

Another way of studying the etiology of schizophrenia is to use neuropathology to investigate cortical architecture. Abnormal architectural arrangements of single nerve cells, cell clusters or cortical layers are strong indicators of disturbed early brain development. Abnormal cell clusters have been found, more frequently in the left hemisphere of schizophrenic patients, but these abnormalities are not as extensive as in other disorders of cortical development like developmental dyslexia (Bogerts, 1993).

3.5.3 BRAIN ASYMMETRY

Studies of cerebral asymmetry are also used to study the etiology of schizophrenia. In the normal brain, there is a structural asymmetry which includes larger right frontal and temporal lobes. Several CT and MRI studies suggest that in schizophrenia, the normal structural asymmetry is absent (Fig.3), and left temporal horn and left ventricular enlargement have been reported (Fig.4 and 5) (Bogerts,1993; Crow, 1989).

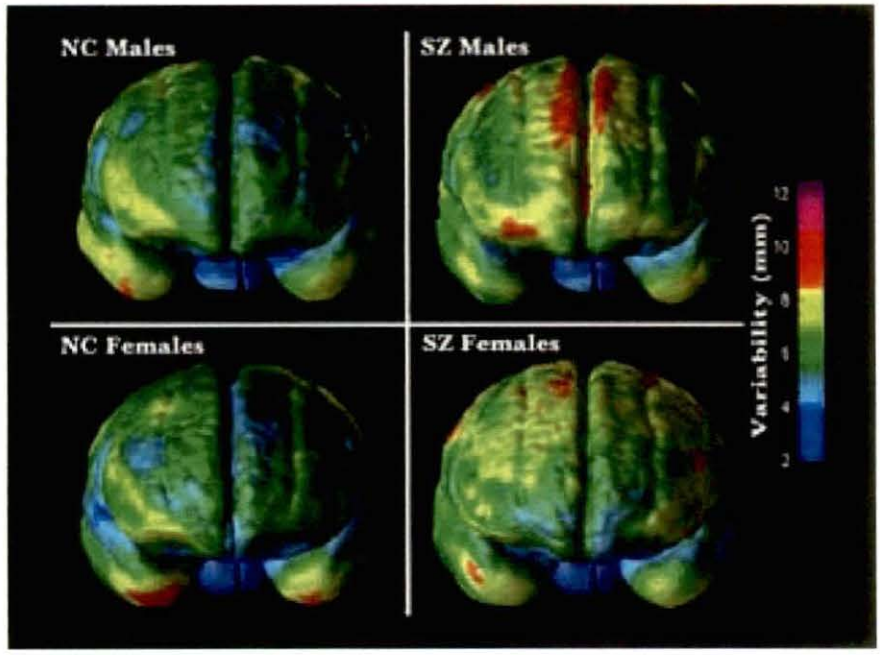

Figure 3: Frontal composite variability and cortical surface variability maps of normal and schizophrenia brains by gender.

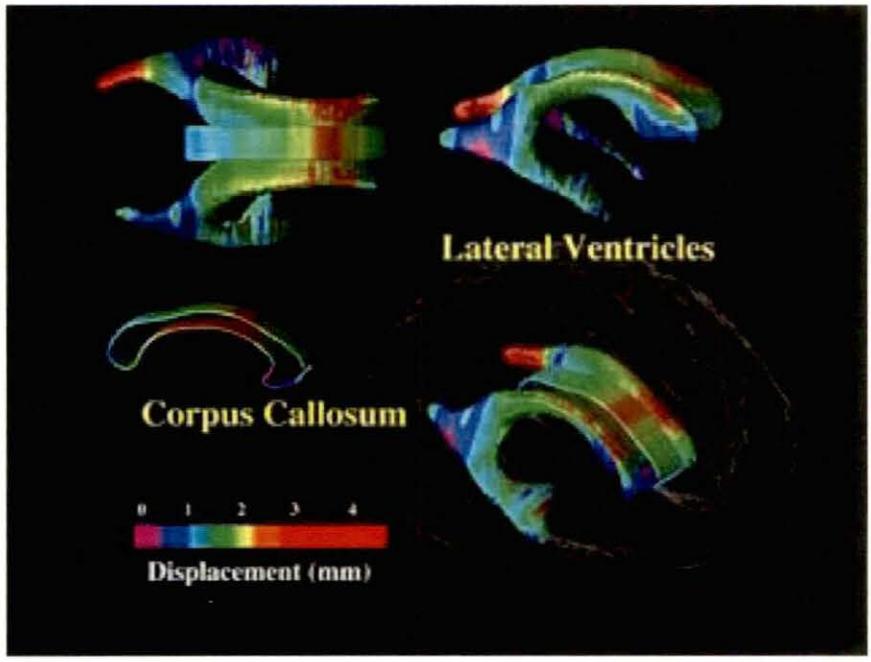

Figure 4: 3D average surface representation and variability maps of the lateral ventricles.

Variability maps are similar in both groups with highest variability in the posterior horns (NC = normal controls, SZ = schizophrenic patients). Increases in LH ventricle length and volume were determined. The color bar encodes the root mean square magnitude of variability in millimeters.

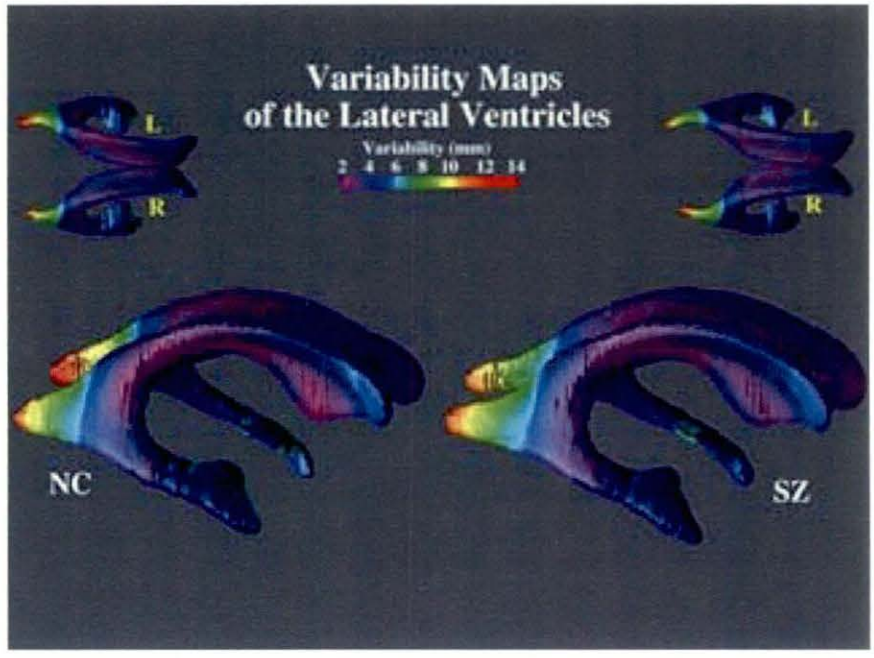

Figure 5: Displacements of the lateral ventricles and corpus callosum.

Displacement maps show the magnitude of displacement (mm) between schizophrenic patients and normal controls as represented by the color bar for the lateral ventricles and corpus callosum. A significant vertical displacement of the lateral ventricles in schizophrenic patients reflects a bilateral increase in ventricular volume, and corresponds to the displacement of the corpus callosum.

Some facts have been established about the neuropathology of schizophrenia. A 2006 meta-analysis of MRI studies found that whole brain and hippocampal volume are reduced and the ventricular volume is increased in schizophrenic patients with a first psychotic episode relative to healthy controls. The average volumetric changes in these studies are however close to the limit of detection by MRI methods, so it remains to be determined whether schizophrenia is a neurodegenerative process that begins at about the time of symptom onset, or whether it is better characterized as a neurodevelopmental process that produces abnormal brain volumes at an early age (Steen *et al.*, 2006).

A 2009 meta-analysis of diffusion tensor imaging studies identified two consistent locations of fractional anisotropy reduction in schizophrenia. One region, in the left frontal lobe, is traversed by white matter tracts interconnecting the frontal lobe, thalamus and cingulate gyrus. The second region in the temporal lobe, is traversed by white matter tracts interconnecting the frontal lobe, insula, hippocampus–amygdala, temporal and occipital lobe. It is suggested that two networks of white matter tracts may be affected in schizophrenia, with the potential for "disconnection" of the gray matter regions which they link (Ellison and Bullmore, 2009). During MRI studies, greater connectivity in the brain's default network and task-positive network has been observed in schizophrenic patients and may reflect excessive orientation of attention to introspection and to extrospection respectively. The greater anti-correlation between the two networks suggests excessive rivalry between the networks (Broyd *et al.*, 2008).

3.5.4 OBSTETRIC COMPLICATIONS

Many epidemiological studies have observed an association between obstetric complications during intrauterine life and schizophrenia. Dalman (1999) studied sets of risk factors representing three different etiological mechanisms that could lead to schizophrenia. These were (i) malnutrition during fetal life, (ii)extreme prematurity and (iii) hypoxia and ischemia. Malnutrition during fetal life could lead to a reduction in the supply of nutrients, such as oxygen, iodine, glucose and iron, which could impair development of the central nervous system (CNS). This may contribute to the development of schizophrenia. The study supports the theory of an association between obstetric complications and schizophrenia. There was evidence of increased

risk associated with all three etiological mechanisms. Pre-eclampsia was the strongest individual risk factor. Some of the factors that were looked at may not have been good indicators of the conditions they were defined as representing (i.e. small for gestational age to indicate malnutrition) therefore further studies need to be undertaken in this field.

Other researchers have also examined the relationship between obstetric complications and adult ventricular size. It has been found that obstetric complications appear to be predictive of increased ventricular size in adults, particularly in schizophrenia (Dalman, 1999). Other studies have reported earlier onset of schizophrenia in patients with a history of obstetric complications (Verdoux, 1997).

Obstetric insults do not equate with cerebral damage. Several variables probably interact in order for obstetric insults to lead to schizophrenia. These would include the site of any lesions, the timing of the injury and the presence of any genetic predisposition to schizophrenia.

3.5.5 VIRAL INFECTION

Evidence for the involvement of infectious diseases in the etiology of schizophrenia first came from the 'season of birth effect'. People born during the winter months appear to have a higher risk of developing schizophrenia. This is thought to be due to exposure to viruses during development. Epidemiological studies have shown high rates of schizophrenia among people whose mothers were exposed to the influenza virus while pregnant (Kirch,1993). The second trimester is a crucial period for the development of the CNS of the fetus. Disruption of brain development in this phase could cause the major structural deficits that are found in the brains of some people with schizophrenia. The mechanism of how a viral infection could lead to schizophrenia is unknown.

3.5.6 BRAIN DEVELOPMENT

There is a long latent period between early cerebral insults and the appearance of schizophrenia. The explanation for this could be due to the fact that brain

development continues throughout childhood and adolescence. Myelination continues into adolescence, and there is some evidence that the deleterious effects of damaged neurones may not become apparent until they myelinate (Bebbington and McGuffin, 1988). It has been suggested that synaptic elimination in adolescence may underlie the emergence of psychotic symptoms. Hormonal and sexual maturity during adolescence has also been thought to contribute to the onset of schizophrenia, but very few studies have been done in this respect.

All of these theories that tried to explain the long latency between cerebral insults and the appearance of schizophrenia, assumed that the structural abnormalities seen in the brains of schizophrenic people (Fig.6) can be regarded as 'vulnerability markers' which may lead to the development of schizophrenia during stress and the vulnerable time between puberty and old age (Harrison, 1999).

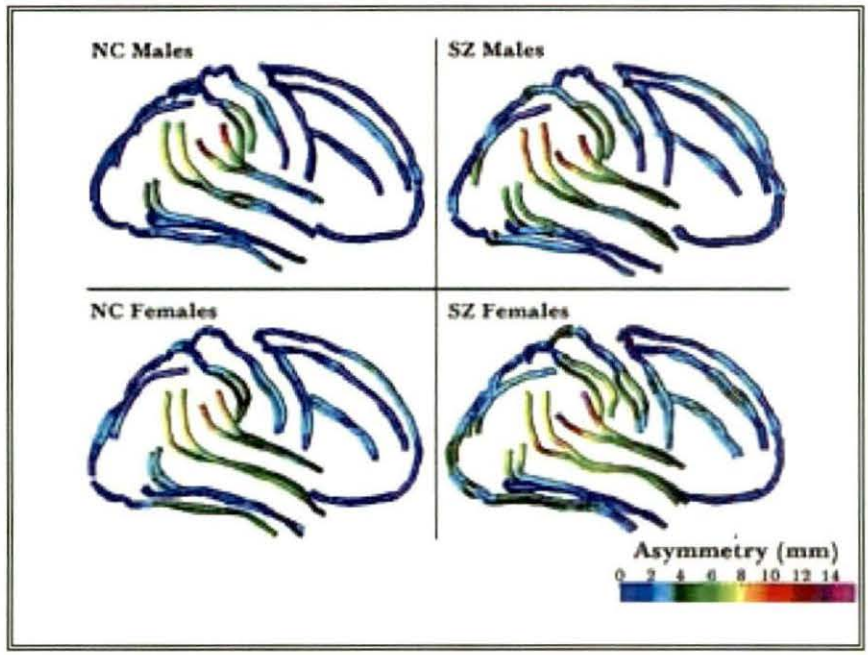

Figure 6: Asymmetry maps

Asymmetry maps were created in each group as defined by Sex and Diagnosis (NC = normal controls, SZ = schizophrenic patients). Sulcal mesh averages for each hemisphere were subtracted from a reflected version of the same structure in the other hemisphere to create displacement vectors. These maps represent in color the magnitude of average asymmetry in sulcal anatomy between the two hemispheres.

3.5.7 NEUROCHEMISTRY

The dysfunction of several neurotransmitter systems like dopamine, 5-hydroxytryptamine (5-HT) and glutamate are thought to play a part in schizophrenia.

3.5.7.1 Dopamine

Studies using neuropsychological tests and brain imaging technologies such as MRI and PET (Fig.7) to examine functional differences in brain activity have shown that differences seem to most commonly occur in the frontal lobes, hippocampus and temporal lobes (Kircher *et al.*, 2005). These differences have been linked to the neurocognitive deficits often associated with schizophrenia (Green, 2006).

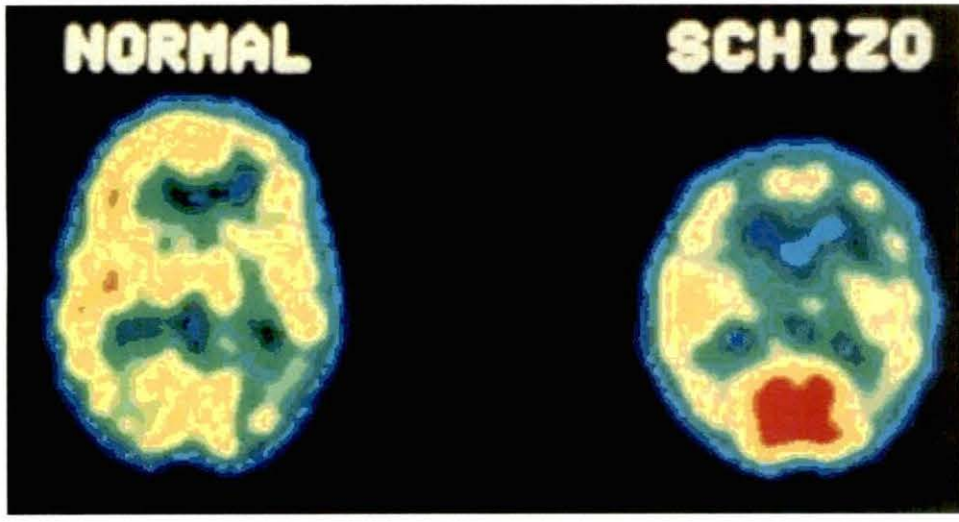

Figure 7: Positron Emission Tomography (PET) brain scans comparing a normal brain (left) with the brain of a schizophrenic (right).

Particular focus has been placed upon the function of dopamine in the mesolimbic pathway of the brain. This focus largely resulted from the accidental finding that a drug group which blocks dopamine function, known as the phenothiazines, could reduce psychotic symptoms. It is also supported by the fact that amphetamines which trigger the release of dopamine may exacerbate the psychotic symptoms in schizophrenia (Laruelle *et al.*, 1996). An influential theory, known as the Dopamine hypothesis of schizophrenia, proposed that excess activation of D2 receptors was the cause of the positive symptoms of schizophrenia. Although postulated for about 20

years based on the D2 blockade effect common to all antipsychotics, it was not until the mid-1990s that PET and SPET imaging studies provided supporting evidence. This theory is now thought to be overly simplistic as a complete explanation, partly because newer antipsychotic medication (called atypical antipsychotic medication) can be equally effective as older medication (called typical antipsychotic medication), but also affects serotonin function and may have slightly less of a dopamine blocking effect (Jones and Pilowsky, 2002).

3.5.7.2 5-hydroxytryptamine

5-hydroxytryptamine (5-HT) is thought to be involved in schizophrenia because the hallucinogen LSD is a 5-HT agonist. It has been found that in schizophrenia, there is a reduced number of 5-HT2A receptors and an increase in the number of 5-HT1A receptors in the frontal cortex (Harrison, 1999). Both of these changes were seen in the post-mortems of unmedicated patients. These changes were not seen in PET scans of younger unmedicated patients suggesting that these abnormalities may emerge during the course of the illness. Several hypothesis have been offered in order to explain the involvement of 5-HT in schizophrenia including, alterations in the trophic role of 5-HT in neurodevelopment and impaired interactions between 5-HT and dopamine.

3.5.7.3 Glutamate

Interest has also focused on the neurotransmitter glutamate and the reduced function of the N-methyl-D-asparate (NMDA) glutamate receptor in schizophrenia. The abnormally low levels of glutamate receptors found in the postmortem brain of schizophrenic patients (Konradi and Heckers, 2003). The discovery that the glutamate blocking drugs such as phencyclidine and ketamine which can mimic the symptoms and cognitive problems associated with the schizophrenic condition has added to the focus on glutamate (Lahti *et al.*, 2001). The reduced glutamate function is linked to poor performance on tests requiring frontal lobe and hippocampal function and that glutamate can affect dopamine function. This observation have suggested an important mediating (and possibly causal) role of glutamate pathways in schizophrenia (Coyle *et al.*, 2003). However other studies have observed that

glutamatergic medication failed to reduce the positive symptoms of schizophrenia (Tuominen *et al.*, 2005).

Clinically, schizophrenia is heterogeneous and this may point to heterogeneous etiology. It seems that genetics, neurodevelopmental problems, neurochemistry and abnormal connectivity, as well as psychosocial stressors probably all contribute to developing the typical clinical pictures of schizophrenia.

3.5.8 RISK FACTORS FOR SCHIZOPHRENIA

Although the twin studies have revealed the genetic predisposition to schizophrenia, the environmental factors also plays a vital role in the manifestation of the disorder. Figure 8 shows some of the schizophrenia environmental risk factors and odd ratio for each of the factors.

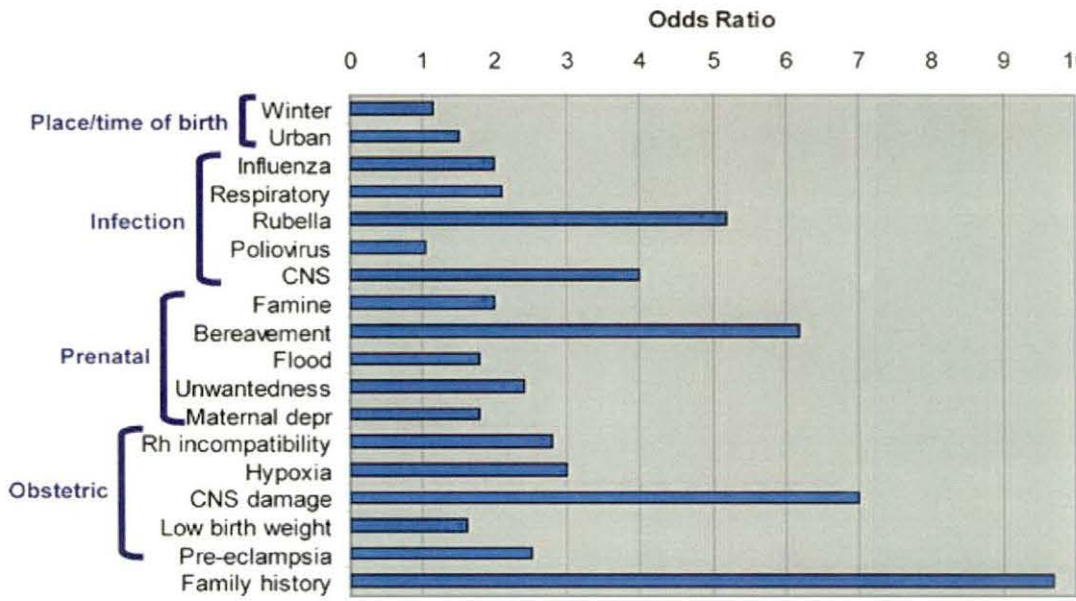

Figure 8: The risk factors for developing schizophrenia.

3.5.8.1 Season of birth

Over 40 studies have shown that individuals who later develop schizophrenia have a 5% to 15% excess of winter and spring births (Boyd *et al.*, 1986; Bradbury and Miller, 1985). Experiments in animals have shown that viruses are more likely to infect the central nervous system in conditions of cold. A time series analysis of New

York State data also reported a significant relationship between the seasonal birth patterns of schizophrenia and still births (Torrey *et al.*, 1993).

3.5.8.2 Urban birth

Two studies have shown that individuals who are born (Takei *et al.*, 1992) or who are raised (Lewis *et al.*, 1992) in cities have an increased risk for developing schizophrenia compared with those born or raised in rural areas. This is consistent with studies of psychiatric hospitalization for serious mental illnesses carried out between 1880 and 1962 that showed higher hospitalization rates for states with more urbanized populations (Torrey and Bowler, 1990).

3.5.8.3 Having older siblings

Sham *et al.* (1993) using data from a Swedish family study, reported that younger children in a family had a significantly increased risk of later developing schizophrenia if their siblings were 3 to 4 years older at the time the younger children were in utero. The researchers suggested explanation for this phenomenon was that older children are a source of viral infections, which they may transmit to their pregnant mothers, and these infections in turn may cause schizophrenia in the offspring.

3.5.8.4 Famine during pregnancy

Susser and Lin analyzed the incidence of schizophrenia among the offspring of women who were pregnant during the 1944 to 1945 war-induced severe famine in western Holland (Susser and Lin, 1992). They reported a statistically significant increase in schizophrenia among offspring who had been in the first trimester of development during the famine. Their original report found the increase for female offspring only, but subsequently research found it for both sexes (Susser and Lin, 1994). During the famine the researchers reported that "the strangest dishes were eaten," including cats and dogs. In addition to resulting in decreased intake of nutrients, famine conditions depress immune function and increase the spread of infectious diseases.

3.5.8.5 Household crowding

A study of psychiatric hospitalization rates for serious mental illnesses was carried out by Schweitzer and Su (Schweitzer and Su, 1977) in Brooklyn, New York. They utilized measures of persons per acre, buildings per acre, persons per household, and persons per room and concluded that "measures of household and family contact were found to be significantly correlated to rates of hospital utilization. If density does produce mental illness its likely mechanism of action will be routed through household contact." Similarly, King *et al.* (King *et al.*, 1982) in Northern Ireland found that prescriptions for antipsychotic medication were more frequent in areas with household crowding (persons per room). Household crowding was also common in the areas of northern Sweden (Book, 1978) and western Ireland (Torrey *et al.*, 1984) that had high reported schizophrenia prevalence rates. However, studies done to ascertain possible adult transmission of schizophrenia among siblings (Crow and Done,1986) or from psychiatric patients to psychiatric nurses (Cooper and King, 1987) have been negative. Therefore the household crowding is more likely to exert its effect in childhood than in adulthood.

3.5.8.6 Lower socioeconomic status

Studies of large cities have consistently found the prevalence rate of schizophrenia to be highest in the lowest socioeconomic class (Kohn, 1968). At least part of the explanation for this finding is that pre-schizophrenic individuals tend to drift downwards socio-economically. Other than this, it is unclear whether low socio-economical status per se is a risk factor for serious mental illnesses, or whether the correlation is due to urban birth and /or household crowding that often coexists with lower socioeconomic status.

3.5.8.7 Regional differences

Schizophrenia appears to be comparatively rare in most tropical countries and to increase in prevalence as one moves away from the equator, similar to the pattern seen in multiple sclerosis (Torrey, 1980). Areas of comparatively high prevalence have been described in diverse places such as northern Sweden (Book,1978), western

Ireland (Torrey *et al.*, 1984; Youssef *et al.*, 1991), western Croatia (Crocetti *et al.*, 1971) and some islands in Micronesia (Hezel and Wylie, 1992) and among West Indian immigrants in England (Harrison *et al.*, 1988; Wessely *et al.*, 1991). Schizophrenia prevalence rates vary from a high of 17.0 per 1,000 persons in northern Sweden (Book, 1978) to a low of 1.1 per 1,000 individuals among the rural Hutterites in the United States (Eaton and Weil, 1955). Although most studies report a prevalence in a range of 2 to 5 per 1,000 persons (Torrey, 1987). In addition to these regional differences in prevalence, a study in Ireland reported statistically significant space-time clusters of births of individuals who later developed schizophrenia (Youssef *et al.*, 1994).

3.5.8.8 Age and gender

Recent findings indicate a male excess in first-episode schizophrenia (Murray and Van Os, 1998), especially in populations with onset age 35 years (Iacono and Beiser, 1992; Jablensky, 1986; Jones *et al.*, 1998). It has been consistently reported that compared to females, males have a younger age of onset (Murray and Van Os, 1998) and are younger at the time of their first hospital admission. Larsen *et al.* (1996) also found, in a sample of 43 first-episode patients in Norway that the duration of untreated psychosis was significantly longer in males than in females, with 61% of males having duration longer than 1 year and 80% of females having duration shorter than 1 year. One variable, for example, that could contribute to the differences in age and gender patterns, at least in part, is a differential distribution of familial schizophrenia among the sample that have been studied. That is, some studies have shown that the gender difference in age of onset is not observed in familial schizophrenia but is more often found in "sporadic" cases (De Lisi *et al.*, 1994). Further examination of age and gender effects is important because differences in the timing of onset by age and/or gender might point to potential biologic clues about the etiology of schizophrenia.

3.6 SCHIZOPHRENIA AND AUTOIMMUNITY

The involvement of autoimmune process in schizophrenia is not a new concept and was first propagated by the German neuropsychiatrist Lehmann Facius in 1937 and

further popularized by Burch in the early 1960s (Burch, 1964). After analyzing the age-specific and sex-specific incidence rates and prevalence of several conditions presumed to be autoimmune in origin, Burch concluded that schizophrenia may also have an autoimmune basis. His conclusion was further strengthen by the age specific onset, sex differences and relapsing clinical course which matched well with disease such as rheumatoid arthritis. The autoimmune hypothesis was also strengthened by the finding of increased autoimmune disease in relatives of schizophrenic patients (Gilvarry *et al.*, 1996) and the inverse relationship of schizophrenia with rheumatoid arthritis (RA)(Gorwood *et al.*, 2004). While this latter association is somewhat counterintuitive, it has been hypothesized that they share a common immune etiology and that once an individual is affected by one of the diseases then they become relatively immune to the other (Torrey and Yolken, 2001). More specifically, several explanations have been proposed to explain this "negative association" at the immune system level. These explanations include factors of prostaglandin synthesis, T- and B-lymphocytes activity, serum interleukin receptor concentrations, IGF-II levels and HLA polymorphism differences (Gorwood *et al.*, 2004). The case for a dysfunction of the immunological system in schizophrenia has also been strengthened by the observation of abnormal lymphocytes in peripheral blood and bone marrow of schizophrenic patients and family members. More specifically, such lymphocytes have been described as having indented or lobulated nuclei and strong basophilic cytoplasm with perinuclear clear zones containing small vacuoles and lamellar structures (Fessel *et al.*, 1965; Hirata-Hibi and Fessel, 1964).

Witebsky and colleagues (Witebsky *et al.*, 1957) proposed criteria that could be used to determine whether a disease is actually autoimmune in origin, and these criteria were more recently refined by Rose and Bona. The criteria propose several levels of evidence such as (1) direct evidence, that is transmissibility by lymphoid cells or antibody of the characteristic lesions of the disease from human to human or human to animal or reproduction of the functional defects characteristic of the disease *in vitro*, (2) indirect evidence, that is reproduction of the autoimmune disease in experimental animals or isolation of autoantibodies or autoreactive T cells from the target organ, (3) circumstantial evidence, that is the presence of markers that are descriptive of autoimmune disease. The evidence provided in the schizophrenia literature for each level of the criteria has been described below.

3.6.1 DIRECT EVIDENCE

The most direct evidence for an autoimmune etiology of a disease is that direct transfer of T cells or antibody from a diseased to a healthy individual can induce the characteristic lesions of the disease. In the 1960s Health *et al.* (Heath *et al.*, 1967) isolated a protein that they termed 'taraxein' from the sera of actively psychotic people with schizophrenia. Taraxein was later identified as an immunoglobulin, but its specificity was not determined. When administered intravenously in monkeys, taraxein caused electroencephalographic (EEG) changes in the monkeys similar to those observed in people with schizophrenia. In addition, when taraxein was injected intravenously into healthy human volunteers it resulted in similar EEG changes and the induction of psychotic symptoms comparable to those observed in the active psychosis stage of schizophrenia.

3.6.2 INDIRECT EVIDENCE

In general, indirect evidence of autoimmunity includes such observations as the induction of autoimmunity in an animal model or the finding of autoimmune cells or antibodies in the target organ. No appropriate experiment to investigate this aspect of autoimmunity has been carried out for schizophrenia. Probably the best indirect evidence for an autoimmune basis for schizophrenia comes from studies examining systemic lupus erythematosus (SLE), a known autoimmune disease characterized by the presence of autoantibodies against double-stranded DNA. Between 14% and 75% of patients with SLE are estimated to experience neuropsychiatric symptoms, including mood and behavioural disturbances and psychotic symptoms (Kozora *et al.*, 1996; Hanly and Liang, 1997). Recently it has been shown that a subset of anti-DNA antibodies can cross–react with the NR2 subunit of the N-methyl-D-asparate (NMDA) glutamate receptor (DeGrorgio *et al.*, 2001). If the psychotic symptoms in SLE patients are the result of anti-DNA antibodies cross reacting with the glutamate receptor, then it is possible that the psychotic symptoms in some schizophrenic patients could be caused by similar reactions of antibodies with neurotransmitter receptors.

3.6.3 CIRCUMSTANTIAL EVIDENCE

Several features are common to many autoimmune diseases. These are (i) association with other autoimmune diseases in the same individual or the same family, (ii) the presence of immune cells in the affected organ, (iii) association with human leukocyte antigen (HLA), (iv) high serum level of autoantibodies, (v) alteration in the level of cytokines, (vi) deposition of antigen-antibody complexes in the affected organ and (vii) improvement of disease symptoms with immunosuppression. (Jones *et al.*, 2005).

3.6.3.1 Association with other autoimmune diseases

Epidemiological studies have shown that relatives of people with schizophrenia have an increased risk of developing several other autoimmune diseases, particularly type 1 diabetes mellitus and thyrotoxicosis (Wright *et al.*, 1996; Gilvarry *et al.*, 1996). SLE has an interesting association with schizophrenia because it arises relatively frequently in patients with schizophrenia, particularly as a result of treatment with phenothiazine or dibenzodiazepine antipsychotic agents (Gold and Sweeney, 1978; Goldman *et al.*, 1980; Wickert *et al.*, 1994). The reason for development of drug-induced SLE remains poorly understood, but it may indicate that some patients with schizophrenia have an underlying susceptibility to the development of autoimmunity.

3.6.3.2 Presence of immune cells in the target organ

Till to date there is no report of inflammatory infiltrate of mononuclear cells in the brains schizophrenic patients. There is however reports of an increased frequency of activated lymphocytes in the cerebrospinal fluid (CSF) of patients with acute schizophrenia (Nikkila *et al.*, 2001; Nikkila *et al.*, 1999). Only one study has investigated whether immunoglobulin can be detected in the brains of people with schizophrenia. Health and Krupp (1967) found that a fluorescein-tagged antihuman antibody labeled the nuclei of some neural cells in the brain tissues from 12 of 14 patients with schizophrenia, but not in the tissues from any of 19 non-schizophrenic controls.

3.6.3.3 Association with HLA

The first HLA association study of schizophrenia was reported by Cazzullo *et al.*, in 1974. More than 80 association studies have been reported since then (Bogacki *et al.*, 2005). In different ethnic population associations have been found for HLA-A*9 (Goudemand *et al.*, 1981), HLA-A*23 (Ivanyi *et al.*, 1983), HLA-A*24 (Asaka *et al.*, 1981;Wright *et al.*, 1995; Ivanyi *et al.*, 1976; Ivanyi *et al.*, 1978; Rosler *et al.*, 1980; Bogacki *et al.*, 2005), HLA-A28 (Wright *et al.*, 1995; Ivanyi *et al.*, 1976; Ivanyi *et al.*, 1978; Rosler *et al.*, 1980; Bogacki *et al.*, 2005) HLA-A*01 (Lahdelma *et al.*, 1998), HLA-A*2, HLA-A*03, HLA-A*11, HLA-B*17, HLA-B*27, HLA-B*8 and and Cw 2 (Rudduck *et al.*, 1984). However past association studies with various Class I alleles yielded inconsistent results (Nimgaonkar *et al.*, 1992) except HLA-A*9(now subdivided into A*23/A*24) (Mc.Guffin *et al.*, 1995). The reason for the inconsistencies include the differences in the diagnostic methods (Goudemand *et al.*,1981, Singer *et al.*, 1982) and typing method of HLA such as serological typing techniques (Joysey and Woolf, 1978), which have been found to be inaccurate, with 7-25% misassignment errors (Opelz *et al.*, 1991) compared with the DNA based techniques such as polymerase chain reaction (PCR) and sequence specific oligonucleotide probes (SSOP). Moreover the source of controls is not always described in sufficient detail to ensure that results are not simply due to population stratification. Significant results are not always corrected for the number of statistical tests performed (Hawi *et al.*, 1999).

In a study, chip-based mass spectrometry analysis for SNP within a 25Mb region on human chromosome 6p21 (which covers the MHC) found a significant increase in the frequency of a SNP in HLA-DOA in schizophrenia and a significant decrease in the frequency of a SNP in HLA-DRB1 (Herbon *et al.*,2003). Recently, The International Schizophrenia Consortium (2009) in the genome-wide association study also found association of schizophrenia in chromosome 6p. They have found more than 450 SNPs on chromosome 6p spanning the major histocompatibility complex. Further, the study revealed the best imputed SNP, which reached genome-wide significance was also in the MHC, 7 kilobases(kb) from NOTCH4, a gene with previously reported associations with schizophrenia (Wei and Hemmings, 2000).

However, the study didn't ascribe the association to a specific HLA allele, haplotype or region.

3.6.3.4 High serum levels of autoantibodies

In the early 1960s several investigators described a variety of antibrain antibodies in the sera of patients with schizophrenia (Heath and Krupp, 1967; Heath *et al.*, 1967; Fessel, 1962) but the consistency of these findings between different research groups has not been high. Increased level of autoantibodies in schizophrenic patients has also been observed against the brain or specific areas of the brain including the cerebrum (Shima *et al.*, 1991), septum (Heath *et al.*, 1989) and amygdale, frontal cortex, cingulated gyrus and septal area (Henneberg *et al.*,1994). However, there is no consistency in this finding. Several groups have reported significantly higher levels of antibodies to cerebral M1 colinergic muscarinic receptors (Borda *et al.*, 2002; Borda *et al.*, 2004; Tanaka *et al.*, 2003), Nicotinic acetylcholine receptors (Mukherjee *et al.*, 1994), Dopamine D2 receptors (Tanaka *et al.*, 2003; Chengappa *et al.*, 1993), astrocyte M1 and M2 muscarinic cholinergic receptors (Borda *et al.*, 2004), mu-opioid and serotonin (5-HT_{1A}) receptors (Tanaka *et al.*, 2003) in sera of schizophrenic patients. The results are suggestive of an autoimmune response directed against neurotransmitter receptors in at least some patients with schizophrenia. A number of studies have found a significantly higher frequency of non-specific autoantibodies such as circulating antinuclear antibodies in people with schizophrenia (Spivak *et al.*, 1995; Sirota *et al.*, 1993; Johnstone and Whaley, 1975). Autoantibodies to both single and double-stranded DNA have also been found to be significantly more common in people with schizophrenia compared to controls (Sirota *et al.*, 1993). This finding was not reproducible in the subsequent studies (Johnstone and Whaley, 1975).

Several possible reasons could account for the discrepancies in findings from different research groups. The heterogeneity of the disease, the medication status, the techniques used by different research groups and the diagnostic criteria used to classify patients could influence results.

3.6.3.5 Alteration in level of cytokines

The cytokine alterations in schizophrenia have been intensively investigated and reviewed by others (Gaughran, 2002; Rothermundt *et al.*, 1998, 2001; Schuld *et al.*, 2004). With regard to interleukin (IL-2), a significant negative correlation between the serum IL-2 levels and the Positive and Negative Syndrome Scale (PANSS) (Zhang *et al.*, 2002) and a positive correlation between the plasma IL-2 and homovanillic acid levels (Kim *et al.*, 2000) were observed in two studies. Additionally , Maes *et al.* (1995b) described a positive correlation between plasma soluble IL-2 receptor (sIL-2R) and transferring receptor (TfR), another marker of immune activation.

There is a discrepancy between the circulating levels of IL-2 and *ex vivo* production of this cytokine. To explain this phenomenon it was suggested that the reduced *ex vivo* IL-2 production may be a consequence of overproduction of IL-2 *in vivo* (Rothermundt *et al.*, 1998). Ganguli *et al.*, (1992) found that autoanibody-positive acutely ill schizophrenic patients had lower mitogen-stimulated IL-2 production than other patients. The only study investigating the levels of sIL-2R in the intrathecal compartment reported decreased values in the cerebrospinal fluid (CSF), but increased concentrations in serum of schizophrenic patients (Barak *et al.*, 1995). These findings indicate systemic immune activation in schizophrenia, however, more evidence in needed to support this conclusion.

Two studies demonstrated a positive association of the serum IL-6 levels with duration of illness (Ganguli *et al.*, 1994; Kim *et al.*, 2000). Another approach was presented in the study of Toyooka *et al.*, (2003) in which they measured protein and / or mRNA levels for IL-1β and IL-1 receptor antagonist (IL-1Ra) in the postmortem brain tissues of schizophrenic patients. They found decreased levels of both IL-1Ra protein and mRNA in the prefrontal cortex of the patients, whereas IL-1 levels were not altered. In the same study they found the increased serum levels of IL-1Ra in drug-free schizophrenic patients. It was suggested that the decreased IL-Ra levels in the CNS might enhance various IL-1 mediated actions in schizophrenic patients.

Interestingly, Inglot *et al.*, (1994) observed that the patients with high interferon (IFN) response to lipopolysaccharide (LPS) or phytohemagglutinin (PHA) stimulation had dominant positive symptoms of schizophrenia whereas in the patients with low IFN response, the negative symptoms prevailed. Moreover, a significant positive intercorrelation between the lowered production of IFN-γ and IL-2 were detected in the patients studied by Arolt *et al.*, (2000) during the 1-month treatment period. Preble and Torrey (1985) demonstrated that IFN-positive patients were more likely to have a recent onset or exacerbation of their illness than IFN-negative patients. Additionally, McAllister *et al.*, (1995) found that symptom exacerbation was associated with the increased CSF IL-2 levels.

3.6.3.6 Deposition of antigen-antibody complex in the affected organ

It has been proposed that schizophrenia may be caused by a covert immune complex-driven basal lamina disease of the choroids plexus (Rudin, 1980). This proposal was largely based on findings from patients with SLE and associated schizophreniform psychoses, in which immune complexes are deposited in the choroids plexus. However, in schizophrenia itself no studies have investigated whether or not such immune complex deposition occurs.

3.6.3.7 Improvement of disease symptoms with immunosuppression

Few studies have reported results of trials of immunosuppressive agents in schizophrenia. Levine and colleagues (Levine *et al.*, 1997) showed that short-term treatment with azathioprine improved the psychiatric symptomatology in a subgroup of patients with schizophrenia. Few other studies have set out to test well-defined immunosuppressive agents in schizophrenia. However, it is known that some of the antipsychotic drugs such as haloperidol and clozapine are highly immunosuppressive (Leykin *et al.*, 1997). If autoimmune responses are playing a role in the development of schizophrenia then treatment with some of the common antipsychotic drugs may act synergistically as direct antagonists of brain neurotransmitter receptors and also as inhibitors of autoimmune responses thus leading to amelioration of psychotic behaviour.

While the autoimmune hypothesis of schizophrenia remains interesting, it is still unclear precisely how the aberrant immune system observed in schizophrenic patients interferes with neuronal and glial function and how this precisely becomes expressed at the clinical level. In addition it remains incompletely understood what accounts for abnormal production of antibodies and cytokines at the molecular level. Since schizophrenia is well known to manifest as a heterogeneous illness, it may be proposed that some patients manifest aspects of autoimmune or immunological aberrations and others not. Further investigation including the newer areas of investigation of immune dysregulation may go a long way to shed the light in the etiology of this complex disorder.

3.7 MAJOR HISTOCOMPATIBILITY COMPLEX

The major histocompatibility complex (MHC) is a dense complex of genes with immunological and non-immunological functions and is present in all vertebrates. Its products play roles in intercellular recognition and in discrimination between self and non self. The MHC participates in the development of both humoral and cell mediated immune responses. While antibodies may react with antigens alone, most T cells recognize antigen only when it is combined with an MHC molecule. Furthermore, the particular set of MHC molecules expressed by an individual influences the repertoire of antigens to which that individual's T_H and T_c cells can respond because MHC molecules act as antigen-presenting structures. For this reason the MHC partly determines the response of an individual to antigens of infectious organisms and it has therefore been implicated in the susceptibility to disease and in the development of autoimmunity. The recent understanding that natural killer cells express receptors for MHC Class I antigens and the fact that the receptor-MHC interaction may lead to the inhibition or activation expands the known role of this gene family (Kuby, 2003).

3.7.1 BRIEF HISTORY OF HUMAN LEUKOCYTE ANTIGENS (HLA) - DISCOVERY AND CHARACTERIZATION

The MHC has been referred by different names in different vertebrates, such as HLA complex in humans and H-2 complex in mice. Human Leukocyte Antigens (HLA), were initially inferred to exist in 1954 when Dausset reported the observation that the

sera from 60 patients contained antibodies which agglutinated lymphocytes from certain individuals. He noted that 90% of these patients had received multiple transfusions. Dausset concluded that transfusion was responsible for creating antibodies against leukocytes as a result of an immune response towards the donor leukocytes (Dausset, 1954). In 1958 Payne and van Rood made separate observations that pregnant women formed antibodies against antigens present on foetal leukocytes. These antibodies were directed at antigens originating from the father of the foetus (Payne and Rolfs, 1958; Rood *et al.*, 1958). At the same time Dausset identified a leukocyte antigen which he named MAC, demonstrated to be present in 60% of the French population. He also showed that monozygotic twins exhibited identical agglutination patterns while dizygotic twins did not. This led him to hypothesize that leukocyte antigens are genetically controlled (Dausset, 1958). Family studies conducted by Payne and van Rood further corroborated this hypothesis (Payne and Rolfs, 1958; Rood *et al.*, 1959). In 1959 van Rood discovered additional antigens beside MAC which were designated antigens 2 and 3 (Rood *et al.*, 1959).

The complex nature of HLAs was realised early in these investigations. Researches were unable to find two antisera that would give identical agglutination patterns against a defined donor palette. In 1963 van Rood was able to define groups of antisera which gave similar but not identical agglutination patterns using 2 x 2 associations between each serum. He had defined two distinct groups of sera which he named 4a and 4b and described these as part of the system which was named Leukocyte Group FOUR (Rood and Leeuwen, 1963). In 1964 Payne *et al.*, discovered another group of antigens that differed from Group FOUR. These antigens were designated LA with two alleles LA1 and LA2 identified initially. The existence of an additional LA allele was inferred when it was observed that leukocytes of some individuals did not agglutinate with anti-LA1 nor with anti-LA2 (Payne *et al.*, 1964). The new allele LA3 was indeed identified two years later (Bodmer *et al.*, 1966). By the mid sixties, it was realized that the growing complexity of the leukocyte antigen system required a standardized approach to further investigations.

In 1965 the first HLA workshop was organized at Netherlands where it was possible for the researchers from different groups to compare their antisera on the same panel of individuals (Bruning *et al.*, 1965) (Table 7). It was discovered that antigens defined

by different groups were closely related but were not identical. It was at the second workshop held in Torino in 1967 that the many antisera from different research groups did for the first time produce identical agglutination patterns on the panel of selected individuals. The number of defined leukocyte antigens was starting to grow and it was realized that the naming of antigens required standardization, leading to the formation of the first nomenclature committee at the Torino workshop. The genes for "LA" and "FOUR" antigens were combined into a system named HL-A (human leukocyte-antigens) and newly discovered alleles that were well characterized in the workshop were given sequential numbers in the order of their discovery. The less well characterized alleles were given a provisional assignment containing the letter "w" (for workshop) followed by the appropriate allele number. Although this approach did bring some order into the naming of the antigens, it was soon realized that the nomenclature lacked clear discrimination between the two series of antigens LA and FOUR. This problem was resolved in 1975 when the HL-A became HLA (human leukocyte antigens) and locus LA was renamed A (HLA-A) and locus FOUR was renamed B (HLA-B). The provisional assignment of less well characterized alleles remained and the "w" appellation is still in the use.

Data from Torino workshop provided the first evidence that leukocyte antigens were the products of closely linked genes located on the same chromosome (Ceppellini *et al.*, 1967; Dausset *et al.*, 1967; Rood *et al.*, 1967). It was also observed that in children, both HLA-A and HLA-B loci were inherited together (Ceppellini *et al.*, 1967; Dausset *et al.*, 1970). Genetic segments containing two or more linked loci on one chromosome were called haplotypes (Ceppellini *et al.*, 1967). Although it was a feature frequently observed in family studies of HLA genes, haplotypic associations of two genes was not absolute and exceptions resulting from genetic recombination were observed (Kissmeyer-Nielsen *et al.*, 1969). Recombination results in a haplotype different from those observed in the parental chromosomes. By observing 40 recombinations in 4614 informative meioses Belvedere *et al.*, (1975) were able to estimate the physical distance between HLA-A and HLA-B loci to 0.87 centimorgans (cM). It was further observed that some HLA-A and HLA–B alleles associate more frequently then otherwise expected by chance and some associate less frequently. This phenomenon was named linkage disequilibrium.

In 1971 Lamm *et al.*, have demonstrated the linkage between HLA and phosphoglucomutase-3 (PGM3) genes and estimated the physical distance between the two genes to be approximately 20 cM (Lamm *et al.*, 1971). This finding was the starting point in the identification of the chromosome where HLA genes were located. Identification of the chromosome carrying HLA genes was achieved using hybrid mouse-human cell lines, a strategy in which the hybrid cell gradually looses human chromosomes. When several chromosomes remain and the hybrid cell is stabilized it is then cloned and the chromosomes are identified. The presence of a specific gene is tested by biochemical or immunological methods. This strategy was explored by Jongsma *et al.*, (1973) who demonstrated that PGM3 was contained in cells with human chromosome 6 and that PGM3 was absent in cells that did not contain this chromosome. More detailed information on localisation of the HLA genes was obtained from experiments with translocated chromosome 6. It was shown that HLA genes were contained on the short arm of chromosome 6, within the p21 banding region (Francke and Pellegrino, 1977).

In the 1970 Thorsby *et al.*, described an anti-serum designated anti-AJ which would react against particular A and B haplotypes in some individuals but not in other individuals with identical HLA-A and HLA-B (Thorsby *et al.*, 1970). It was hypothesized that a new locus, closely linked to HLA-A and HLA-B might exist. In 1973 this new locus -AJ was identified using the antigen capping method and shown to be expressed on leukocytes (Mayr *et al.*, 1973). This new locus was later designated HLA-C.

It was observed that leukocytes from HLA identical siblings did not stimulate each other in Mixed Leukocyte Culture (MLC) while leukocytes from HLA identical unrelated individuals did (Mempel *et al.*, 1973a; Sengar *et al.*, 1971). Yunis and Amos showed that in certain families HLA-A and HLA–B identical siblings produced a strong MLC, whereas siblings with non-identical HLA-A and HLA–B did not (Yunis and Amos, 1971). It was hypothesized that an unidentified HLA linked locus existed. The MLC test is based on the blast transformation property of leukocytes when mixed with leukocytes from different individuals. Cells marked to be stimulator cells are inactivated by radiation or mitomycin C. When mixed with the cells designated as responder cells they will stimulate them to go into blast transformation

and divide, indicating differences in HLA antigens present on the cell surface. Stimulator cells in the MLC reaction belong to the B lymphocyte population and only certain T cells may be stimulated and be responder cells (Simpson, 1976). With the MLC test it was possible to identify a "difference" between two individuals if it existed but the test could not actually identify the antigen types. This was resolved by the introduction of homozygous typing cells (HTC). These cells were known to be homozygous for determined HLA alleles and if those alleles were present on responder cells as well, no reaction should occur. Using this method it was possible to identify that novel specificities or MLC determinants do exist that stimulate the MLC reaction and were called D (Mempel *et al.*, 1973b). Primed lymphocyte typing (PLT) was also used to identify MLC determinants. This method was based on the observation that T lymphocytes, which were stimulated and then grown in culture for several days, have a much stronger secondary response when restimulated. This method developed by Sheehy *et al.*, (1975), can be directed against a single MLC determinant by using parents and children as stimulators and responders, so that only one MLC determinant in any direction is chosen in stimulation. It was later shown that determinants in classical MLC and PLT were virtually identical (Bach *et al.*, 1976).

A more efficient methodology however was required to type the D locus. This was aided by the observation that anti-HLA-A and anti-HLA-B antibodies can inhibit an MLC reaction (Grumet and Leventhal, 1970). The development of such serological methods required good controls to ensure that non D locus specific antibodies are not involved in MLC reaction inhibition. This was achieved by using HLA-A and HLA–B identical stimulator and responder cells which excluded the existence of anti-HLA-A or HLA–B antibodies. An experiment to detect D specific antibodies and confirm the existence of the D locus was performed by Leeuwen *et al.*, 1973. The study utilized the following design: a patient with a defined HLA–A and HLA–B haplotype had developed a cytotoxic antibody. This antibody was not specific to the HLA haplotype of the person that carries it therefore other individuals containing the same HLA haplotypes were selected and their cells used as stimulators and the patients cells as responders. The MLC tests were performed using normal control serum in which case all of the stimulator cells were able to stimulate the patient's responder cells. In another MLC test, the serum from the patient was used (containing the

cytotoxic antibody) and the MLC reaction was inhibited in half of the selected stimulator cells. These results were further investigated by incorporating an additional test to detect the antibody by introducing fluorescent conjugated anti-immunoglobulin which was designed to bind the anti-MLC antibody. Fluorescence was detected in about 17% of the inhibited lymphocytes which corresponded to the proportion of B lymphocytes to total lymphocytes. Serological methods were finally developed to type for the MLC determinants-D specificities which were named HLA-D antigens.

Table 7: A summary of the events occurring at HLA workshops and chronicle of the milestones of achievement in HLA research (Roitt *et al.*, 1998; Thorsby, 2009).

1964- Acceptance of cytotoxicity over agglutination
1965- Allelism of HLA antigens proposed
1967- Segregation of alleles demonstrated in families
1970- Single locus now two – HLA-A, HLA-B
1972- 60 world populations typed by 75 laboratories.
1975- Third locus, HLA-C, demonstrated
1977- HLA-D defined by Homozygous Typing Cells
1977- The serum-detected, D-related, HLA-DR defined
1984- HLA and Disease associations explored
1984- Studies of gene structure
1984- Worldwide Renal Transplantation databases
1984- Definition of MB (later to be HLA-DQ)
1987- DNA techniques with serological, biochemical and cellular methods
1987- Definition of HLA-DP and HLA-DQ
1992- Use of Polymerase Chain Reaction- e.g. for SSOP.
1996- Molecular definition of HLA-Class I
1996- Roles of HLA-G, E, DM, Tap and LMP's better understood.
2002- Molecular characterization of HLA alleles and Non HLA genes
2003- Nomenclature of KIR genes better defined
2005- New data on KIR-HLA and applications, in particular in BMTs.
2008- New data on application of HLA in clinical medicine, anthropology etc.

3.7.2 HUMAN LEUKOCYTE ANTIGEN

The HLA genes are located on chromosome 6p 21.3. Based on the structure of the antigens produced and their function there are two classes of HLA antigens, HLA Class I and Class II (Fig.9). The overall size of the HLA gene is approximately 3.5 million base pairs. Within this the HLA Class I genes and the HLA Class II genes each spread over approximately one third of this length. The remaining section known as Class III contains loci responsible for complement, hormones, intracellular peptide processing and other developmental characteristics (Sanfilippo and Amos, 1986). Thus the Class III region is not actually a part of the HLA complex but is located within the HLA region. Its components are either related to the functions of HLA antigens or are under similar control mechanisms to the HLA genes. The common HLA antigens and their molecular types are presented in table 8.

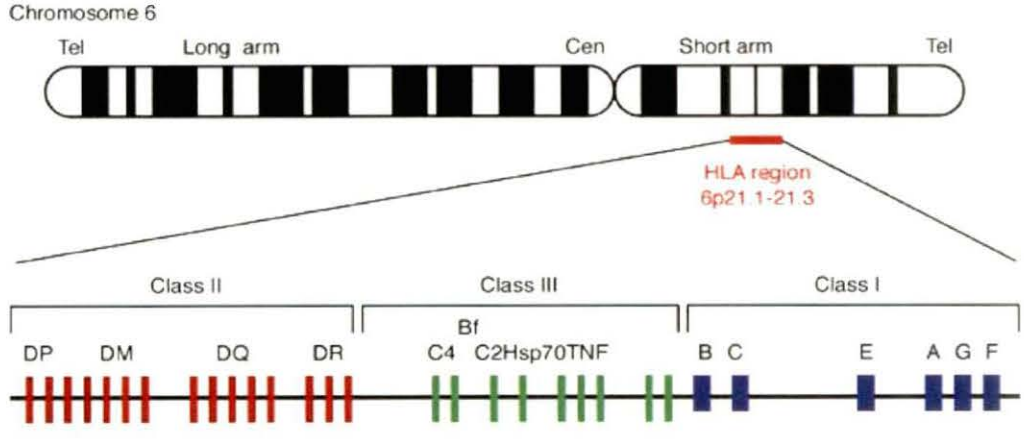

Figure 9: Gene map of the human leukocyte antigen (HLA) region.

3.7. 2.1 HLA Class I antigens

The cell surface glycopeptide antigens of the HLA-A, -B and –C series are called HLA Class I antigens (Roitt *et al.*, 1998). It is expressed on the surface of most nucleated cells of the body. Additionally, they are found in soluble form in plasma and are adsorbed onto the surface of platelets. Erythrocytes also adsorb HLA Class I antigens to varying degrees depending on the specificity (e.g. HLA-B7, A28 and B57 are recognizable on erythrocytes as so called "Bg" antigens). Immunological studies indicate that HLA-B (which is also the most polymorphic) is the most significant HLA Class I locus, followed HLA-A and HLA-C. There are other HLA Class I loci

(e.g. HLA-E,F,G,H,J,K and L) but most of these may not be important as loci for "peptide presenters".

Figure10 shows the structure of HLA Class I molecule. The HLA Class I antigens comprise a 45 Kilodalton (kD) glycopeptide heavy chain with three domains. It is non-covalently associated with β-2 microglobulin which plays an important role in the structural support of the heavy chain. The HLA Class I molecule is assembled inside the cell and ultimately sits on the cell surface with a section inserted into the lipid bilayer of the cell membrane and has a short cytoplasmic tail. The general structure of HLA Class I, HLA Class II and IgM molecules show such similarity of subunits that a common link between HLA and immunoglobulins back to some primordial cell surface receptor is likely. The full 3-dimentional structure of HLA-A Class I molecules has been determined from X-ray crystallography (Browning and Mc Michael, 1996). It has a cleft on its outermost surface which holds a peptide. If a cell becomes infected with a virus the virally induced proteins within the cell are broken down into small peptides and these are the peptides which are then inserted into this cleft during the synthesis of HLA Class I molecules. The role of HLA Class I molecules is to take these virally induced peptides to the surface of the cell and by linking to the T-Cell receptor of a Cytotoxic (CD8) T Cell (Fig.12) give clue for the presence of this virus to the immune system. The CD8 T Cell will now be "educated" and it will be able to initiate the process of killing cells which subsequently has that same viral protein/HLA Class I molecule on its surface. Due to its role in identifying cells which are changed (e.g. virally infected), HLA Class I needs to be present on all cells (Browning and Mc Michael, 1996).

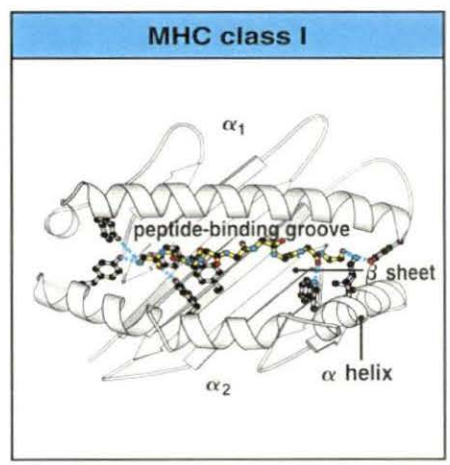

Figure 10: Detailed structure of HLA Class I molecule.

3.7.2.2 HLA Class II Antigens

Figure 11 shows the structure of HLA Class II molecule. The cell surface glycopeptide antigens of the HLA-DP, -DQ and –DR loci are termed HLA Class II (Sanfilippo and Amos, 1986). The tissue distribution of HLA Class II antigens is confined to the "immune competent" cells including B-lymphocytes, macrophages, endothelial cells and activated T-lymphocytes. The expression of HLA Class II on cells which would not normally express them is stimulated by cytokines like interferon γ and in a transplant this is associated with acute graft destruction. HLA Class II molecules consist of two chains each encoded by genes in the "HLA Complex" on Chromosome 6. HLA Class II molecule is a heterodimer consisting of two different polypeptide chains, an α chain of 33 kD and a β chain of 28 kD which associate with each other noncovalently. HLA Class II molecules are also membrane bound glycoprotein molecules like HLA Class I. HLA Class II also contains an external domain, a transmembrane segment and a cytoplasmic tail or anchor segment. The external domain contains α1 and α2 domains and bears sequence homology to the immunoglobulin-fold domain structure. Hence, HLA Class II molecules are classified as immunoglobulin superfamily. The peptide binding site of the HLA Class II molecule is composed of the α1 and β1 domains and forms the antigen binding cleft for processed antigen. The T Cells which link up to the HLA Class II molecules are Helper (CD4) T cells (Fig.12). Thus the "education" process which occurs from HLA Class II presentation involves the helper-function of setting up a general immune reaction and provides defense against the bacterial or other invasion. This role of HLA Class II in initiating a general immune response is the reason why they need only be present on "immunologically active" cells (B lymphocytes, macrophages, etc.) and not on all tissues (Browning and Mc Michael, 1996).

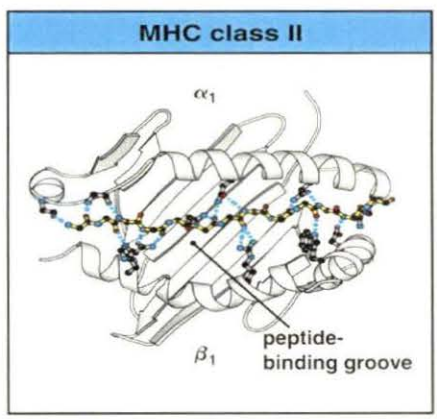

Figure 11: Detailed structure of HLA Class II molecule.

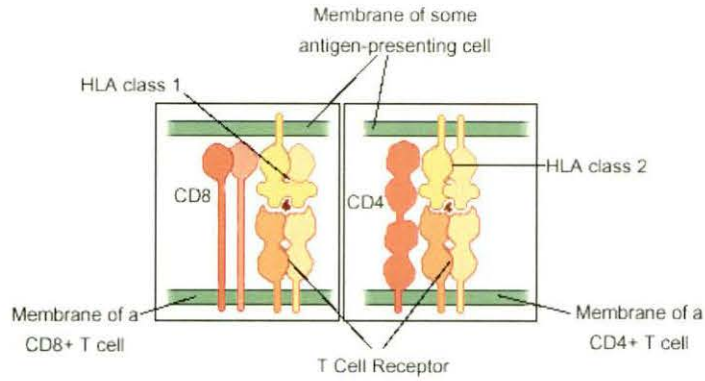

Figure 12: Class I HLA presents antigen peptide found within the cell, to CD8 cell surface protein (i.e. normally to cytotoxic T cells). Class II HLA presents antigen peptide found outside the cell, to CD4 cell surface protein (i.e. normally to helper T cells).

3.7. 2.3 HLA Class III antigens

The class III region of the MHC in humans and mice contains a heterogeneous collection of genes. These genes encode several complement components, two steroid 21-hydroxylases, two heat-shock proteins, and two cytokines (TNF-α and TNF- β). Some of these Class III gene products play a role in certain diseases. For example, mutations in the genes encoding 21-hydroxylase have been linked to congenital adrenal hyperplasia. Interestingly, the presence of a linked class III gene cluster with the HLA region is conserved in all species.

Table 8: The common HLA antigens and their molecular types expressed more frequently among the HLA-A, HLA-B, HLA-C, HLA-DR loci. (adapted from Shankarkumar, 2004)

HLA Antigens	Broad Group	No. of molecular types *	Most common alleles
HLA A			
A1		9	A*0101
A2		58	A*0201, A*0202
A3		9	A*0301
A11		13	A*1101
A23	A9	9	A*2301
A24	A9	36	A*2402
A25	A10	4	A*2501
A26	A10	18	A*2601
A29	A19	6	A*2901, A*2902
A30	A19	12	
A31	A19	8	A*3101
A32	A19	7	A*3201
A33	A19	6	A*3301

HLA Antigens	Broad Group	No. of molecular types *	Most common alleles
A34	A10	4	A*3401
A36		3	A*3601
A43		1	A*4301
A66	A10	4	A*6601
A68	A28	22	A*6801
A69	A28	1	A*6901
A74		8	A*7401
A80		1	A*8001
HLA B			
B7		31	B*0702
B8		16	B*0801
B13		10	B*1301
B14		6	B*1401, B*1402
B15		73	B*1501
B18		18	B*1801, B*1802
B27		24	B*2701, B*2702
B35		44	B*3501, B*3502
B37		5	B*3701
B38	B16	8	B*3801
B39	B16	26	B*3901
B40		44	B*4001
B41		6	B*4101
B42		4	B*4201
B44	B12	32	B*4402
B45	B12	6	B*4501
B46		2	B*4601
B47		4	B*4701
B48		7	B*4801
B49	B21	3	B*4901
B50	B21	3	B*5001
B51	B5	29	B*5101
B52	B5	4	B*5201
B53	B5	9	B*5301
B54	B22	2	B*5401
B55	B22	12	B*5501, B*5502
B56	B22	8	B*5601
B57	B17	9	B*5701
B58	B17	6	B*5801
B59		1	B*5901
B67		2	B*6701
B73		1	B*7301
B78		5	B*1517
B81		1	B*8101
B82		2	B*8201
B83		1	B*8301
HLA C			
Cw1		6	Cw*0101
Cw2		5	Cw*0202
Cw3		15	Cw*0303
Cw4		10	Cw*0401
Cw5		5	Cw*0501
Cw6		7	Cw*0602
Cw7		16	Cw*0701, Cw*0702
Cw8		9	Cw*0802
Cw12		8	Cw*1203
Cw14		5	Cw*1401
Cw15		11	Cw*1502

HLA Antigens	Broad Group	No. of molecular types *	Most common alleles
Cw16		3	Cw*1601
Cw17		3	Cw*1701
Cw18		2	Cw*1801
HLA DR			
DR1		8	DRB1*0101, 0103
DR15	DR2	13	DRB1*0501, 1502
DR16	DR2	8	DRB1*1601,1602
DR3		23	DRB1*0301
DR4		44	DRB1*0401,0404
DR11	DR5	43	DRB1*1101
DR12	DR5	8	DRB1*1201
DR13	DR6	52	DRB1*1301, 1302
DR14	DR6	43	DRB1*1401,1402
DR7		6	DRB1*0701
DR8		24	DRB1*0801,0802,0803
DR9		2	DRB1*0901
DR10		2	DRB1*1001

*The number of variants in approximate, as there will be more reported regularly

3.7.3 GENETICS OF HLA

There are a number of genetic characteristics of HLA antigens which have been described below.

3.7.3.1 Polymorphism

The polymorphism at the recognized HLA loci is extreme. It is likely that this extreme polymorphism has evolved as a mechanism for coping with all of the different peptides that the organism comes across. Therefore each HLA molecule differs slightly from each other in its amino acid sequence to gives rise to large array of HLA antigens. This difference causes a slightly different 3-dimentional structure in the peptide binding cleft. Since different peptides have different shapes and charge characteristics, it is important that the human race has a large array of different HLA antigens, each with different shaped peptide binding areas (clefts) to cope with all of these peptides. However this is not ubiquitous as the polymorphism is population specific. The frequent HLA antigens in different populations are clearly different. For example, HLA-A34, which is present in 78% of Australian Aborigines has a frequency of less than 1% in both Australian Caucasoid and Chinese. Several workers have reported HLA studies from various populations of World (Imanishi *et al.*, 1992; Clayton and Lonjou, 1997; Shankarkumar et al 1999a; Shankarkumar et al 1999b;

Mehra *et al.*,1986; Pitchappan *et al.*,1984). Thus HLA antigens are of great significance in anthropological studies. Populations with very similar HLA antigen frequencies are clearly derived from common stock. Conversely, from the point of view of transplantation it is very difficult to match HLA types between populations.

3.7.3.2 Inheritance of HLA

The normal way to present a tissue type is to list the HLA antigens as they have been detected. There is no attempt to show which parent has passed on which antigen. This way of presenting the HLA type is referred to as a Phenotype (Thomas *et al.*, 1998). When family data is available, it is possible to assign one each of the antigen at each locus to a specific grouping known as a haplotype. A haplotype is the set of HLA antigens inherited from one parent. For example a child is having HLA Phenotype HLA-A1, A3;B7, B8;Cw2, Cw4;DR15, DR4 the phenotype of his mother is HLA-A3, A69;B7, B45; Cw4, Cw9; DR15, DR17. Now it is evident from this example that the A3, B7, Cw4 and DR15 were all passed on from the mother to the child above. This group of antigens is a haplotype. Figure 13 shows the inheritance of HLA haplotypes. In the absence of genetic crossing over 2 siblings who inherit the same two HLA chromosomes (haplotypes) from their parents will be HLA identical. There is a one in four chance that this will occur and therefore in any family with more than four children at least two of them will be HLA identical. This is because there are only two possible haplotype in each parent.

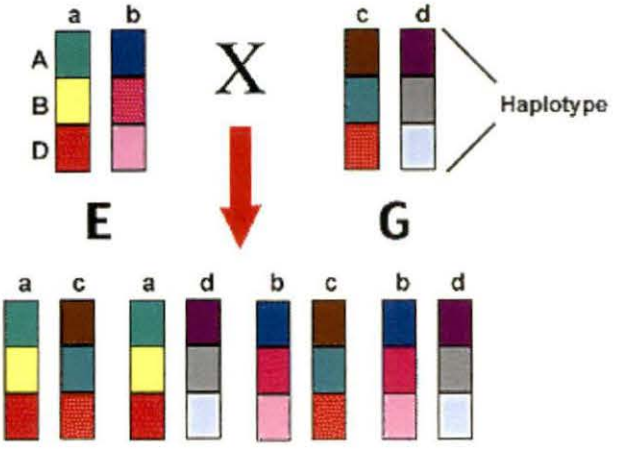

Figure 13: Inheritance of HLA haplotypes.

3.7.3.3 Linkage disequilibrium

Basic Mendelian genetics states that the frequency of alleles at one locus does not influence the frequency of alleles at another locus (Law of independent segregation). However in HLA genetics this is not true. There are a number of examples from within the HLA system that, alleles at different loci occurring together at very much higher frequencies than would be expected from their respective gene frequencies. This is termed linkage disequilibrium. The most extreme example is in Caucasians where the HLA-A1,B8,DR3 (DRB*0301), DQ2 (DQB1*0201) haplotype is so conserved that even the alleles at the complement genes (Class III) can be predicted with great accuracy. Similar haplotypes are observed in selected caste groups and tribal groups of India (Shankarkumar *et al.*, 1999a; Shankarkumar *et al.*, 1999b). Also, at HLA Class I, this phenomenon is to pronounced that the presence of specific HLA-DR alleles can be used to predict the HLA-DQ allele with a high degree of accuracy before testing. Because of linkage disequilibrium a certain combination of HLA Class I antigen, HLA Class II antigen and Class III products will be inherited together more frequently than would normally be expected. It is possible that these "sets" of alleles may be advantageous immunologically so that they have a positive selective advantage.

3.7.3.4 Cross-Reactivity

Cross-reactivity is the phenomenon whereby one antibody reacts with several different antigens, usually at the one locus (as opposed to a mixture of antibodies in the one serum) (Shankarkumar *et al.*, 1998). This is not a surprising event as it has been demonstrated that different HLA antigens share exactly the same amino acid sequence for most of their molecular structure. Antibodies bind to specific sites on these molecules and it would be expected that many different antigens would share a site (or epitope) for which a specific antibody will bind. Thus cross-reactivity is the sharing of epitopes between antigens.

The term CREG is often used to describe "Cross Reacting Groups" of antigens. It is useful to think in terms of CREG's when screening sera for antibodies as most sera found are "multi specific" and it is rare to find operationally monospecific sera. The

rarity of monospecific sera means that most serological tissue typing is done using sera detecting more than one specificity and a typing is deduced by subtraction. For example, a cell may react with a serum containing antibodies of HLA-A25, A26 and A34 and be negative for pure A26 and pure A25 antisera. In this case, HLA-A34 can be assigned, even in the absence of pure HLA-A34 antisera.

3.7.4 HLA AND DISEASE SUSCEPTIBILITY

In the 1960's, it was discovered that the mouse MHC (called H-2) controlled both the genetic susceptibility to certain leukemia's and the immune response to certain antigens. Since then innumerable reports have been published aimed to discovering the role of the HLA in the control of responsiveness and disease susceptibility (Tiwari and Terasaki, 1985).

The discovery of HLA associations with specific diseases implies that at least part of their genetic basis lies in the HLA and suggests that it may be possible to determine their etiology. Thus, in the case of insulin-dependent diabetes mellitus (IDDM) it is now known that alleles coding for an amino acid residue other than aspartate at position 57 of the DQβ chain are highly associated with IDDM (Dorman *et al.*, 1990; Khalil *et al.*, 1992; Pugliese *et al.*, 1995). However, two decades after the landmark discovery of association between HLA B27 and ankylosing spondylitis (Schlosstein *et al.*,1973; Brewerton *et al.*, 1973), the etiology of this disorder remains unknown. The same is true for Narcolepsy in that, despite finding association with HLA DR2 (Honda, 1988) the pathophysiology of this neuropsychiatric disorder is still obscure.

There are two general explanations for HLA and disease associations (Mc Devitt, 1985). Firstly, there may be a linkage disequilibrium between alleles at a particular disease associated locus and the HLA antigen associated with that disease. E.g., HLA-A*03 and Idiopathic Haemochromatosis.

Another possible explanation for these associations is that the HLA antigen itself plays a role in disease, by a method similar to one of the following models:-

i) By being a poor presenter of a certain viral or bacterial antigen.

ii) By providing a binding site on the surface of the cell for a disease provoking virus or bacterium.

iii) By providing a transport piece for the virus to allow it to enter the cell

iv) By having such a close molecular similarity to the pathogen, that the immune system fails to recognize the pathogen as foreign and so fails to mount an immune response against it.

It is most likely that all these mechanisms are involved but to a varying extent in different diseases (Throsby, 1977). In multiple sclerosis and ankylosing spondylitis cell mediated immunity is often depressed, not only in the patients but also in their parents and siblings (Kankonkar *et al.*, 2003; Shankarkumar *et al.*, 2002). Complement (C2) levels are known to be low in Systemic Lupus Erythematosus, Pulmonary Tuberclosis, Leprosy, a disease associated with HLA DR2 and DR3 (Shankarkumar *et al.*, 2003a; Shankarkumar *et al.*, 2003b; Rajalingham *et al.*, 1996; Shanmugalashmi and Pitchappan, 2002). In Gluten Enteropathy which shows a high association with HLA-DR3, a specific gene product is thought to act as an abnormal receptor for gliadin and present it as an immunogen to the body. Whatever the explanation for the long list of HLA and disease associations, it is clear that the HLA system, collaborating with other non-linked genes has an influence on our response to environmental factors which provoke the disease.

3.7.5 SCHIZOPHRENIA AND HLA

The first HLA association study of schizophrenia was reported by Cazzullo *et al.*, in 1974. More than 80 association studies have been reported since then. Table 9 shows the most frequently reported association between HLA Class I and schizophrenia. HLA and schizophrenia was first reviewed by McGuffin (1979), who commented that the MHC was a logical place in which to search for genetic markers for schizophrenia because schizophrenia was similar to diseases for which HLA association had been established in that it was familial, had a postulated autoimmune pathogenesis (Burch, 1964). These reasons, especially the autoimmune hypothesis (Wright *et al.*, 1993; Wright *et al.*, 1996), remain extant, but the following additional factors have provided the impetus for continuing investigations:

1. **Operationalised diagnostic criteria:** Several early studies did not use operationalised diagnostic criteria (Julien *et al.*, 1977; Perris *et al.*, 1979; Goudemand *et al.*, 1981; Ivanyi *et al.*, 1976, 1978) and those that did utilized several different diagnostic systems, making comparisons between studies very difficult. More recent studies have benefited from the application of DSM-IV-TR or ICD-10, and are therefore both diagnostically more reliable and more readily comparable to each other.

2. **Technical improvements in HLA serotyping:** Currently available alloantisera specify HLA antigens with a precision impossible in the 1980s, when the bulk of HLA association studies in schizophrenia were performed.

3. **The introduction of HLA genotyping:** This has effectively eliminated the inaccuracies associated with modern HLA serotyping (Mytilineos *et al.*,1990), has allowed rapid HLA typing of large numbers of samples and has revealed genetic polymorphism of the HLA to a complexity previously unimagined.

Inspite of tiring research it is evident that no common agreement regarding the association of HLA with of schizophrenia has been reached. Further research is awaited to throw light in the mechanism of association of HLA with schizophrenia. This will go a long way to find the etiology of this complex disorder.

Table 9: Most frequently reported association between HLA Class I and schizophrenia.

Investigator	Year	Ethnicity	Antigen
Eberhard *et al.*	1975	Caucasian	A9
Ivanyi *et al.*	1976	Caucasian	A28
Smeraldi *et al.*	1976	Caucasian	A10
Julien *et al.*	1977	Caucasian	A9
Ivanyi *et al.*	1977	Caucasian	Cw4
Ivanyi *et al.*	1977	Caucasian	B18
Ivanyi *et al.*	1978	Caucasian	A28
Crowe *et al.*	1979	Caucasian	Aw10 (A26 subtype)

Investigator	Year	Ethnicity	Antigen
Luchins *et al.*	1980	Caucasian	A2
Gattaz and Beckmann	1980	Caucasian	B27
Asaka *et al.*	1981	Japanese	A9 (Aw24 subtype)
Asaka *et al.*	1981	Japanese	A10 (A26 subtype)
Rosler *et al.*	1980	Caucasian	A28
Wright *et al.*	1995	Caucasian	A9 A24 (sub specificity of A9)
Blackwood *et al.*	1996	Caucasian	B35 Cw5
Bogacki *et al.*	2005	Caucasian	A24, A28
Debnath *et al.*	2005	Indian (Bengali)	A3

3.8 CYTOKINES

The development of an effective immune response involves lymphoid cells, inflammatory cells and hematopoietic cells. The complex interactions among these cells are mediated by a group of proteins collectively designated cytokines to denote their role in cell-to-cell communication (cyto-, "cell", from the Greek kinein, "to move"). Cytokines are low-molecular-weight regulatory proteins or glycoproteins secreted by white blood cells and various other cells in the body in response to a number of stimuli. These proteins assists in regulating the development of immune effector cells and some cytokines possess direct effector functions of their own.

The term cytokine encompasses broad category of protein factors, those secreted by lymphocytes are called lymphokines and those secreted by monocytes and macrophages are called monokines. There are some low molecular weight cytokines, which are specifically called as chemokines, which play an important role in inflammation. Both lymphocyte and mononuclear phagocytes produce cytokines such as colony-stimulating factors (CSFs), which stimulate the growth and differentiation of immature leukocytes in the bone marrow, providing a source of additional leukocytes to replace the cells that are consumed during inflammatory reactions. Many of the cytokines are made by certain populations of blood leukocytes (e.g., T

cells or monocytes) and act on other leukocyte populations (e.g., monocytes , neutrophils or eosinophils), these molecules are called interleukins (IL). This term should not be construed to imply that cytokines are only synthesized by or only act upon white blood cells. However, the term "interleukin" has been useful because as new cytokines are molecularly characterized they may be assigned a designated interleukin number (e.g., IL-1, IL-2) to assure that there is an unambiguous shared nomenclature among investigators. Some cytokines and their functions have been mentioned the table 10.

Table 10: Selected immune cytokines and their activities.

Cytokine	Producing Cell	Target Cell	Function**
GM-CSF	Th cells	progenitor cells	growth and differentiation of monocytes and DC
IL-1α IL-1β	monocytes macrophages B cells DC	Th cells	co-stimulation
		B cells	maturation and proliferation
		NK cells	activation
		various	inflammation, acute phase response, fever
IL-2	Th1 cells	activated T and B cells, NK cells	growth, proliferation, activation
IL-3	Th cells NK cells	stem cells	growth and differentiation
		mast cells	growth and histamine release
IL-4	Th2 cells	activated B cells	proliferation and differentiation IgG_1 and IgE synthesis
		macrophages	MHC Class II
		T cells	proliferation
IL-5	Th2 cells	activated B cells	proliferation and differentiation IgA synthesis
IL-6	monocytes macrophages Th2 cells stromal cells	activated B cells	differentiation into plasma cells
		plasma cells	antibody secretion
		stem cells	differentiation
		various	acute phase response
IL-7	marrow stroma thymus stroma	stem cells	differentiation into progenitor B and T cells
IL-8	macrophages endothelial cells	neutrophils	chemotaxis

Cytokine	Producing Cell	Target Cell	Function**
IL-10	Th2 cells	macrophages	*cytokine production*
		B cells	activation
IL-12	Macrophages B cells	activated Tc cells	differentiation into CTL (with IL-2)
		NK cells	activation
IFN-α	leukocytes	various	*viral replication* MHC I expression
IFN-β	fibroblasts	various	*viral replication* MHC I expression
IFN-γ	Th1 cells, Tc cells, NK cells	various	*Viral replication*
		macrophages	MHC expression
		activated B cells	Ig class switch to IgG_{2a}
		Th2 cells	*proliferation*
		macrophages	pathogen elimination
MIP-1α	macrophages	monocytes, T cells	chemotaxis
MIP-1β	lymphocytes	monocytes, T cells	chemotaxis
TGF-β	T cells, monocytes	monocytes, macrophages	chemotaxis
		activated macrophages	IL-1 synthesis
		activated B cells	IgA synthesis
		various	*proliferation*
TNFα	macrophages, mast cells, NK cells	macrophages	CAM and cytokine expression
		tumor cells	cell death
TNF-β	Th1 and Tc cells	phagocytes	phagocytosis, NO production
		tumor cells	cell death

*** CTL: cytotoxic T lymphocytes; DC: dendritic cells; GM-CSF: Granulocyte-Monocyte Colony Stimulating Factor; IL: interleukin; IFN: Interferon; TGF: Tumor Growth Factor; TNF: Tumor Necrosis Factor.**

**** Italicized activities are inhibited.**

3.8.1 TH1/TH2 SYSTEMS

Th1- and Th2-system are originally defined on the basis of their cytokine profiles and effector functions. They are effective against intracellular and extra-cellular pathogens (Mosmann and Coffman, 1989). The balance between both Th-subsets is thought to be pivotal in determining the outcome of an immune response towards an infectious organism (Breytenbach *et al.*, 2001) and is therefore critical for host defense and the

pathogenesis of immune-mediated diseases (Agnello *et al.*, 2003; McGuirk and Mills, 2002).

Th1 cells mainly produce IFN-γ, IL-2, TNF-α and IL-12 while Th2 lymphocytes predominantly release IL-4, IL-6, IL-10 and IL-13. However, both TNF-α and IL-10 can be secreted by Th1 and Th2 cells (Romagnani, 1999; Katsikis *et al.*, 1995). The development of Th1 and Th2 cells from a common undifferentiated precursor is regulated by the interactions of peptide antigen with the T cell receptor (TCR), cytokine signaling, actions of co-stimulatory molecules, induction of transcription factors and antigen dose (Agnello *et al.*, 2003; Rothoeft *et al.*, 2003; Farrar *et al.*, 2002; Ben Sasson *et al.*, 2001; Murphy *et al.*, 2000; Ausubel *et al.*, 1997; Carballido *et al.*, 1997; Kuchroo *et al.*, 1995; Prabhu Das *et al.*, 1995). IL-4 activates the Janus kinase 1 (JAK1) and JAK3, leading to activation of the signal transducer and activator of transcription 6 (STAT6), whereas IL-12 activates JAK1 and TYK2 conducting to STAT4 activation (Santana and Rosenstein, 2003). STAT6 and STAT4 are essential for the development of Th1 and Th2 correspondingly (Anderson *et al.*, 2003). IL-4 drives the development of the Th2-system. IL- 12 is in most cases not necessary for maintaining Th1 responses once Th1 responses are induced (Gazzinelli *et al.*, 1994). Instead, IFN-γR signaling is required for Th1 further differentiation (Tau *et al.*, 2000). Binding of IFN-γ to its receptor IFN-γR activates JAK1 and JAK2, leading to phosphrylation of STAT1 (Bach *et al.*, 1997). In addition to STAT4 and STAT6, there are some other transcription factors specific for Th1/Th2 systems. They are GATA3 and c-Maf in Th2 cells as well as ERM and T-bet in Th1 cells (Murphy *et al.*, 2000). IFN-γ and IL-4 were characterized as the key cytokines of the Th1 and Th2 system due to their roles in the differentiation and development of the Th1/Th2 system. The ratio between both major cytokines is thought to implicate the balance between both Th1/Th2 systems (Giannakoulas *et al.*, 2004; Li *et al.*, 2003; Sakami *et al.*, 2002). Recently the IFN-γ/IL-10 ratio is regarded as an indicator of Th1/Th2 balance in various viral infections (Avery and Hoover, 2004; McElhaney *et al.*, 2004; Zhang *et al.*, 2000).

The Th1 system induces cell-mediated immune responses and is associated with inflammation and tissue destruction that leads to organ-specific autoimmune diseases

(Chen *et al.*, 2000; Golding and Scott, 1995). On the contrary, the Th2 system promotes humoral immune responses, allergic reactions to environmental antigens as well as anti-inflammatory activities (Chen *et al.*, 2000; Mosmann and Sad, 1996). Activation of Th2 cells may inhibit the central nervous system (CNS) inflammation and limit the noxious effects of Th1-mediated immunity (Chen *et al.*, 2000; Racke *et al.*, 1994).

3.8.2 INTERLEUKIN-2 (IL-2) AND INTERLEUKIN-6 (IL-6) AND THEIR PRINCIPAL BIOLOGICAL FUNCTIONS

3.8.2.1 IL-2 (T cell growth factor)

IL-2 was first described as "T-cell growth factor". It is a protein of 133 amino acids with a molecular weight of 15 kDa (Malek, 2003). The main secretory source of IL-2 is the T-helper cell, particularly naïve T cells and Th1 cells. The human IL-2 gene contains four exons and maps to human chromosome 4q26-28 (Sykora *et al.*, 1984). Figure 14 shows the crystal structure of human IL-2.

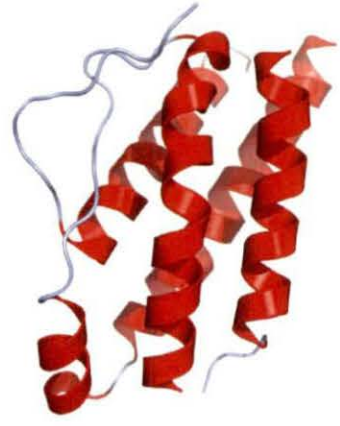

Figure 14: Crystal structure of human interleukin-2 (IL-2).

3.8.2.1.1 Interleukin-2 Receptor (IL-2R)

Three different types of IL-2Rs with high, intermediate and low affinity are distinguished. They are expressed differentially and independently. The high-affinity IL-2R consists of subunits IL-2Rα (p55), IL-2Rβ (p75) and a γ chain (64 kDa). The

intermediate-affinity IL- 2R comprises IL-2Rβ and γ chain, while the low-affinity IL-2R contains solely IL-2Rα. IL-2Rα functions as a T-cell activation (TAC) antigen, IL-2Rβ as the ligand binding domains and γ chain as a signaling component. The γ-subunit is required for the generation of high and intermediate affinity IL-2R, but does not bind IL-2 by itself (Minami *et al.*, 1993). The genes encoding these three subunits map to human chromosome 10p14-15, 22q11.2-12 and Xq13 respectively. Besides, activated lymphocytes continuously secrete a 42 kDa/55 kDa fragment of the TAC antigen, a soluble IL-2 receptor (sIL-2R), which circulates in the serum and plasma (Miska and Mahmoud, 1993; Pizzolo *et al.*, 1992). Brain IL-2Rs are enriched in the hippocampal formation. This area critical for the acquisition and consolidation of spatial learning and memory (Petitto *et al.*, 1999).

3.8.2.1.2 Interleukin-2 secreting cells

IL-2 is produced mainly by activation of CD4+ T-cells (de Waal *et al.*, 1993; Ferrer *et al.*,1992). Resting cells do not produce IL-2. There are detectable levels of IL-2-like material in the hippocampus, striatum and frontal cortex. However, specific IL-2 binding sites were observed only in the hippocampus (de Waal *et al.*, 1993; Ferrer *et al.*, 1992; Araujo *et al.*,1989). IL-2 is a growth factor for all subpopulations of T-lymphocytes (Abbas, 2003). It is an antigen-nonspecific proliferation factor for T-cells that induces cell cycle progression in resting cells and thus allows clonal expansion of activated T-lymphocytes (Malek, 2003). This effect is modulated by hormones such as prolactin (Moreno *et al.*, 1998). In addition, IL-2 mediates multiple biological processes including growth and differentiation of B cells, generation of lymphokine-activated killer cells and augmentation of NK cells (Wustrow, 1991). In the CNS, IL-2 stimulates the growth of oligodendroglial cells *in vitro* (Benveniste and Merrill, 1986), modulates N-methyl-D-aspartate receptors (NMDA-R) of native mesolimbic neurons (Ye *et al.*, 2001) and influences mesocorticolimbic dopamine release (Ye *et al.*, 2001). IL-2 damages the blood-brain-barrier (BBB) and the integrity of the endothelium of brain vessel (Ellison *et al.*, 1987). However, it does not cross the BBB via a saturable transport system.

3.8.2.2 IL-6 (neuro-endo-immunological mediator)

IL-6, also called IFN-β2/B-cell stimulatory factor 2/hepatocyte stimulating factor (Ferguson-Smith *et al.*, 1988) is a 26 kDa protein with 185 amino acids (Conti *et al.*, 2002). The human IL-6 gene contains five exons and maps to human chromosome 7p15-21 (Ferguson-Smith *et al.*, 1988). The IL-6R is a protein of 80 kDa (Fujisawa *et al.*, 2002). IL-6R consists of 2 chains, IL-6Rα and IL-6Rβ. The IL-6/IL-6R complex associates with a 130-kDa23 transmembrane glycoprotein (gp130). Glycoprotein 130 is involved in signal transduction.The IL-6R is expressed on various cell types such as lymphocytes, monocytes, fibroblasts,vascular endothelial cells and pituitary cells (Barton, 1997). In addition, soluble IL6 receptor (sIL-6R) enhances the effect of IL-6 (Schobitz *et al.*, 1995). Figure 15 shows the crystal structure of human Interleukin-6.

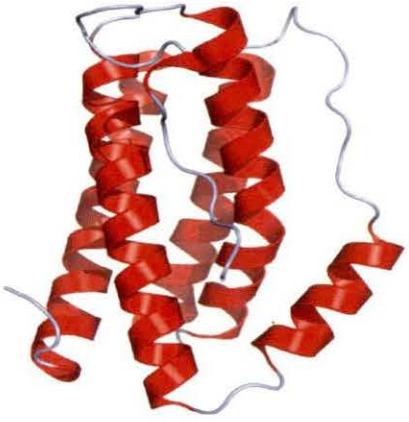

Figure 15: Crystal structure of human Interleukin-6.

3.8.2.2.1 IL-6 secreting cells

Many different cell types produce IL-6. The main sources *in vivo* are stimulated monocytes/macrophages, fibroblasts and endothelial cells (Coil *et al.*, 2004; Dalal *et al.*, 2003; Ng *et al.*, 2003; Soderquist *et al.*, 1998; Yachie *et al.*, 1990). Additionally, T-cells, B lymphocytes, eosinophils, mast cells, astrocytes and microglia also produce IL-6 after stimulation (Azzolina *et al.*, 2003; Delgado *et al.*, 2003; Inoue, 2002; Diehl and Rincon, 2002; Hoenstein *et al.*, 2001; Lorentz *et al.*, 2000; Frei *et al.*, 1989). IL-6 mRNA was found to be generally low in the brain (Schobitz *et al.*, 1993). It is present in the hippocampal formation with highest signal in the dentate gyrus, habenular nucleus, piriform cortex, hypothalamus and striatum (Chen *et al.*, 2003; Gadient and Otten, 1994; Schobitz *et al.*, 1992).

3.8.2.2.2 Biological activities of IL-6

IL-6 is involved in regulating a wide variety of immune functions, such as B- and cytotoxic T-cell differentiation, induction of IL-2 production and IL-2R expression in T cells, T cell growth, acute-phase reactions and hematopoiesis (Hirano, 1998; Taga and Kishimoto, 1997).

Recently, Diehl and Rincón (Diehl and Rincon, 2002) suggested that APC IL-6 promotes Th2 differentiation and simultaneously inhibits Th1 polarization through IL-12 independent molecular mechanisms. IL-6 activates transcription mediated by the transcription factor Nuclear Factor of Activated T cells (NFAT), leading to IL-4 production by naïve CD4+ T cells and their differentiation into effector Th2 cells. The induction of Th2 differentiation by IL-6 is dependent upon endogenous IL-4. In addition, IL-6 binds to IL-6Rα, leading to the dimerization of gp130/IL-6Rβ (Brakenhoff *et al.*, 1995). Dimerization of gp130 by IL-6 causes the activation of two signaling pathways: (1) the JAK/STAT pathway and (2) the CCAAT/enhancer binding protein (C/EBP) pathway (Weihua *et al.*, 2000; Heinrich *et al.*, 1998). IL-6 inhibits Th1 differentiation via the JAK/STAT1 pathway by inducing the suppressor of cytokine signaling 1 (SOCS1) expression (Siewert *et al.*, 1999). IL-6 upregulates SOCS1 expression in activated CD4+ T cells, thereby interfering with signal transducer and activator of transcription 1 (STAT1) phosphorylation induced by IFN-γ. Inhibition of IFN-γR-mediated signals by IL-6 prevents auto-regulation of IFN-γ gene expression by IFN-γ during CD4+ T cell activation, thus preventing Th1 differentiation. This pathway is IL-4- and IL-12-independent (Diehl and Rincon, 2002). Furthermore, IL-6 exerts distinct effects on the CNS such as activation of the hypothalamic-pituitary-adrenal axis (HPA), reduction of food intake, induction of fever and neuronal growth (Godbout and Johnson, 2004; Path *et al.*, 2000). IL-6 induces nerve growth factor (NGF) in astrocytes, enhances NGF-stimulated astrocyte proliferation (Levison *et al.*, 2000; Marz *et al.*, 1999; Schafer *et al.*, 1999; Kossmann *et al.*, 1996), promotes survival of the mesencephalic catecholaminergic and septal cholinergic neurons *in vitro* (Kushima and Hatanaka, 1992; Hama *et al.*, 1991) and attenuates the neurotoxic effects of NMDA onstriatal cholinergic neurons (Toulmond *et al.*, 1992).

3.8.3 CYTOKINES AND SCHIZOPHRENIA

The cytokine alterations in schizophrenia have been intensively investigated and reviewed by others (Gaughran, 2002; Rothermundt *et al.*, 1998; Rothermundt *et al.*, 2001; Schuld *et al.*, 2004). The most momentous findings in this respect are summarized in table 11. Additionally, some interesting observations concerning cytokines and schizophrenia are described below.

With regard to interleukin (IL)-2, a significant negative correlation between the serum IL-2 levels and the positive subscale P of the Positive and Negative Syndrome Scale (PANSS) (Zhang *et al.*, 2002) and a positive correlation between the plasma IL-2 and homovanillic acid levels (Kim *et al.*, 2000) were observed in two studies. Additionally, Maes *et al.*, (1995b) described a positive correlation between plasma soluble IL-2 receptor (sIL-2R) and transferring receptor (TfR), another marker of immune activation.

As has been observed, there is a discrepancy between the circulating levels of IL-2 and its *ex vivo* production. To explain this phenomenon, it was suggested that the reduced *ex vivo* IL-2 production may be a consequence of overproduction of IL-2 *in vivo* (Rothermundt *et al.*, 1998). Ganguli *et al.* (1992) found that autoantibody-positive acutely ill schizophrenic patients had lower mitogen-stimulated IL-2 production than other patients.

The only study investigating the levels of sIL-2R in the intrathecal compartment reported decreased values in the cerebrospinal fluid (CSF), but increased concentrations in serum of schizophrenic patients (Barak *et al.*, 1995). These findings indicate systemic immune activation in schizophrenia. However, more evidence is needed to support this conclusion.

Two studies demonstrated a positive association of the serum IL-6 levels with duration of illness (Ganguli *et al.*, 1994; Kim *et al.*, 2000). Another approach was presented in the study by Toyooka *et al.*, (2003) in which they measured protein and /or mRNA levels for IL-1β and IL-1 receptor antagonist (Il-1Ra) in the postmortem brain tissues of schizophrenic patients. They found decreased levels of both IL-1Ra protein and mRNA in the prefrontal cortex of the patients, whereas IL-1 levels were

not altered. In the same study they found the increased serum levels of IL-1Ra in drug-free schizophrenic patients. It was suggested that the decreased IL-1Ra levels in the CNS might enhance various IL-1 mediated actions in schizophrenic patients.

Interestingly, Inglot *et al.*, (1994) observed that the patients with high interferon (IFN) response to lipopolysaccharide (LPS) or phytohemagglutinin (PHA) stimulation had dominant positive symptoms of schizophrenia whereas in the patients with low IFN response, the negative symptoms prevailed. Moreover, a significant positive inter-correlation between the lowered production of INF-γ and IL-2 were detected in the patients studied by Arolt *et al.*, (2000) during the 1-month treatment period. Preble and Torrey (1985) demonstrated that IFN-positive were more likely than IFN-negative patients to have a recent onset or exacerbation of their illness and to be on low-dose or no medication. Additionally, McAllister *et al.*, (1995) found that symptom exacerbation was associated with the increased CSF IL-2 levels.

Table 11: Cytokine profiles of schizophrenic patients as reported by the various authors.

Cytokine	Level	Plasma / serum level	Ex vivo production	CSF
IL-2	Increase	Zhang *et al.* (2002), McAllister *et al.* (1995), Kim et al. (1998)	Cazzullo *et al.* (2001), Cazzullo *et al.* (1998), O'Donnel *et al.* (1996)	McAllister *et al.* (1995), Licinio *et al.* (1993)
	Decrease	Theodoropoulou *et al.* (2001)	Xu *et al.* (1994) Kim *et al* (1998) Rothermundt et al. (1998), Ganguli *et al.* (1992), Hornberg *et al.* (1995), Bessler *et al.* (1995), Bessler *et al.* (1995), Arolt *et al.* (2000), Ganguli *et al.* (1995), Ganguli *et al.* (1989), Villemain *et al.* (1987), Yang *et al.* (1994), Villemain *et al.* (1989)	Not reported
	Normal	Barak *et al.* (1995),Gattaz *et al.* (1992), Xu *et al.* (1994)	Rothermundt *et al.* (2000)	Barak *et al.* (1995), Rapaport *et al.* (1997), el-Mallakh *et al.* (1993)

Cytokine	Level	Plasma / serum level	Ex vivo production	CSF
sIL-2R	Increase	Barak *et al.* (1995), Maes *et al.* (1994), Akiyama (1999), Rapaport and Lohr (1994), Gaughran *et al.* (1998), Rapaport *et al.* (1989)	Not investigated	Not reported
	Decrease	Not reported	Not reported	Barak *et al.* (1995)
	Normal	Haack *et al.* (1999), Muller *et al.* (1997), Erbagci *et al.* (2001)	Not reported	Not reported
IL-6	Increase	Zhang *et al.* (2002), Maes *et al.* (1994), Akiyama (1999), Naudin *et al.* (1996), Frommberger *et al.* (1997), Ganguli *et al.* (1994), van Kammen *et al.* (1999), Maes *et al.* (1995a)	Not reported	Not reported
	Decrease	Not reported	Not reported	Not reported
	Normal	Haack *et al.* (1999), Erbagci *et al.* (2001), Baker *et al.* (1996), Katila *et al.* (1994a), Cazzullo *et al.* (2001)	Kim *et al.* (1998), Hornberg *et al.* (1995)	van Kammen *et al.* (1999), Katila *et al.* (1994b)
sIL-6R	Increase	Maes *et al.* (1997)	Not investigated	Not investigated
	Decrease	Maes *et al.* (1994)	Not reported	Not reported
	Normal	Muller *et al.* (1997)	Not reported	Not reported
IL-1	Increase	Theodoropoulou *et al.* (2001), Katila *et al.* (1994a)	Sirota *et al.* (1995)	Not reported
	Decrease	Barak et al. (1995) Rothermundt *et al.*	Not reported	Barak *et al.* (1995)
	Normal	(2000),Erbagci *et al.* (2001), Kim *et al.* (1998), Baker *et al.* (1996), Katila *et al.* (1994b)	Bessler *et al.* (1995), Kim *et al.* (1998)	el-Mallakh *et al.* (1993)
IL-1Ra	Increase	Akiyama (1999),Maes et al. (2000) Maes *et al.* (1997),Toyooka *et al.* (2003)	Not investigated	Not investigated

Cytokine	Level	Plasma / serum level	Ex vivo production	CSF
	Decrease	Not reported	Not reported	Not reported
	Normal	Haack *et al.* (1999)	Not reported	Not reported
IFN	Increase	Preble and Torrey (1985), Kim *et al.* (2004)	Cazzullo *et al.* (2001), Cazzullo *et al.* (2002)	Not investigated
	Decrease	Not reported	Rothermundt *et al.* (1998), Rothermundt *et al.* (2000), Arolt *et al.* (1997), Katila *et al.* (1989), Moises *et al.* (1985), Naidenova *et al.* (1988), Wilke *et al.* (1996), Inglot *et al.* (1994)	Not reported
	Normal	Gattaz *et al.* (1992), Rimon *et al.* (1985), Becker *et al.* (1990)	Not reported	Not reported
TNF-α	Increase	Theodoropoulou *et al.* (2001)	Not reported	Not investigated
	Decrease	Not reported	Not reported	Not reported
	Normal	Haack *et al.* (1999), Erbagci *et al.* (2001), Baker *et al.* (1996), Kudoh *et al.* (2001), Schattner *et al.* (1996)	Schattner *et al.* (1996)	Not reported
IL-10	Increase	Maes *et al.* (2002)	Not reported	Not investigated
	Decrease	Not reported	Not reported	Not reported
	Normal	Not reported	Rothermundt *et al.* (1998), Cazzullo *et al.* (2001), Cazzullo *et al.* (1998)	Not reported

3.8.3.1 Th2 shift hypothesis in schizophrenia

In vivo and *in vitro* studies have shown that schizophrenia may be associated with an imbalance in cytokines network, suggesting suppression of some immune functions and activation of others. To explain the phenomenon of immunosuppression and immune activation in schizophrenic patients, Muller *et al.*, (1999) put forward 'Th2 hypothesis', which states that the Th1-Th2 balance is shifted to Th2 in schizophrenia

(Muller *et al.*, 1999). The key characteristics of the Th-1 system are the production of interleukin-2 and interferon-γ, which have been reported to be decreased *in vitro* (Ganguli *et al.*,1989; Ganguli *et al.*,1995; Villemain *et al.*,1989; Bessler *et al.*,1995; Arolt *et al.*, 2000) and increased *in vivo* (Kim *et al.*, 2000).On the contrary, Th-2 system in schizophrenia is characterized by activation , which is characterized by increased IL-6, sIL-6R (Maes *et al.*, 1995a; Ganguli *et al.*,1995; Frommberger *et al.*, 1997; Lin *et al.*, 1998; Muller *et al.*, 1997; Van Kammen *et al.*, 1999; Na and Kim, 2007) and IL-10 levels (Cazzullo *et al.*, 1998a; Cazzullo *et al.*, 1998b), as well as increase IL-4 levels in the CSF of juvenile schizophrenic patients (Mittleman *et al.*,1997; Na and Kim, 2007) . Empirical evidence in support of the Th1/Th2 imbalance has been inconsistent. Some authors could not replicate the previously reported findings (Baker, 1996), or the findings are in conflict with each other, for example, increased *in vitro* IL-2 or interferon (IFN)-γ production or decreased IL-2 serum levels was recently found in schizophrenia (O'Donnell *et al.*, 1996; Cazzullo *et al.*, 2001; Theodoropoulou *et al.*, 2001). Recently Potvin *et al.*, 2008 in their quantitative review refuted the current hypothesis of a Th2 slant in schizophrenia.

3.9 C-REACTIVE PROTEIN

Human C-reactive protein (CRP) can be classified under two partially overlapping groups of proteins. On the basis of its best known biological property i.e., the striking increase of its plasma concentration during infection and inflammation, CRP is categorized as an acute-phase protein. On the basis of its structure and Ca++ dependent binding specificities, CRP is classified as pentraxin (Pepys and Hirschfield, 2003) . The name CRP is derived from the fact that this protein has the capacity to precipitate the somatic C-carbohydrate of *pneumococcus*. Acute phase proteins constitute a heterogeneous group of proteins of hepatic origin (Pepys and Hirschfield, 2003) that share the property of increased plasma concentration during infection and/or tissue injury. CRP is the most characteristic human acute-protein, since its plasma concentration raises by several hundred folds within 24-48 hours from tissue injury. These high levels persist for the duration of the acute-phase response, returning to the normal low concentrations with restoration of tissue structure and function.

3.9.1 HISTORY

Tillett and Francis first discovered C-reactive protein in 1930 as a substance in the serum of patients with acute inflammation that reacted with the C polysaccharide of *pneumococcus* (Tillett and Francis, 1930). Initially, CRP was thought to be a pathogenic secretion, as it was elevated in people with a variety of illnesses, including cancer. 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 and Hirschfield, 2003).

3.9.2 BIOCHEMISTRY

CRP is a normal alpha globulin composed of 224-residue protein with a monomer molar mass of 25106 Da (Pepys and Hirschfield, 2003). It has an annular pentameric disc in shape and a member of the small pentraxins family (Fig.16). Native CRP is a bit different, as it has 10 subunits making two pentameric discs, with an overall molecular mass of 25106 Da.

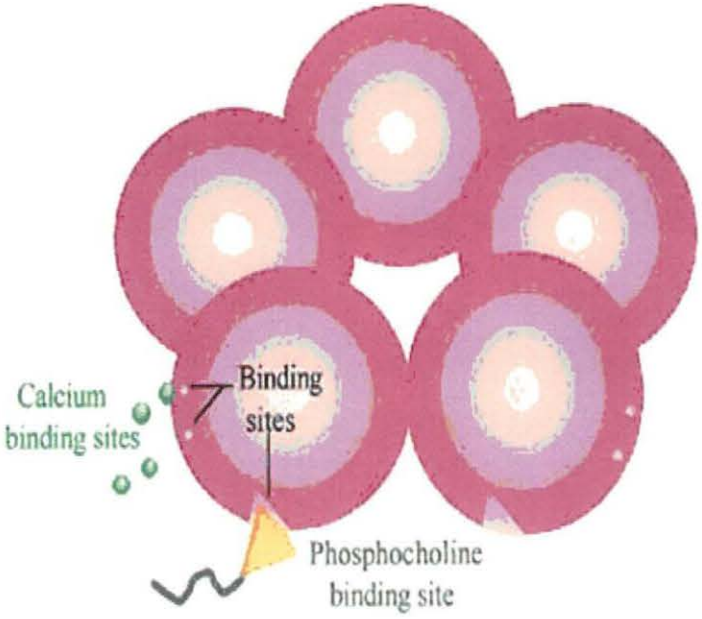

Figure 16: Pentamer structure of CRP, including calcium and phosphocholine binding sites. These sites enable CRP to recognize and bind to a variety of microorganisms, cellular debris, and nuclear material from damaged cells.

3.9.3 FUNCTION

The level of CRP rises dramatically during inflammatory process rising upto 50,000 fold in acute inflammation, such as infection (Fig.17). It rises above normal limits within 6 hours and peaks at 48hours. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by macrophages as well as adipocytes (Pepys and Hirschfield, 2003; Lau *et al.*, 2005). CRP binds to phosphocholine 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 another important role in innate immunity, as an early defense system against infections. The half-life of CRP 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 and Hirschfield, 2003).

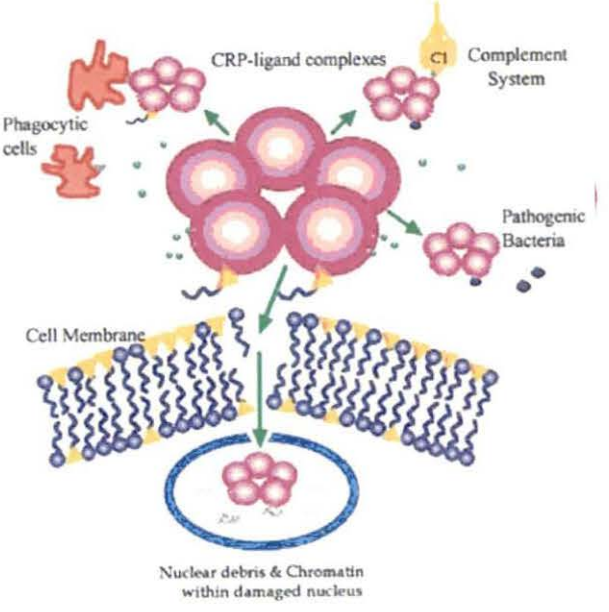

Figure 17: ***Key functions of CRP within the innate immune system include the ability to (1) recognize and bind to phosphocholine exposed in damaged cell walls and found in many bacteria, fungi, and parasites; (2) act like an opsonin, marking bacteria, damaged cell walls, and nuclear debris for phagocytosis; (3) bind to C1, the first component of the classical pathway of the complement system that triggers phagocytic activity; and (4) bind to polymorphonuclear leukocytes (PMNs) and monocytes, which stimulate the production of inflammatory cytokines.***

Elevated CRP levels are usually observed in a variety of infections and inflammatory conditions where there is tissue destruction (Fig.18). Elevated CRP is known to be the risk factor for the cardiovascular diseases, diabetes and other metabolic dysfunctions (Bassuk *et al.*, 2004; Pfutzner and Forst, 2006). In addition, it is also known to be associated with the depression (Ford and Erlinger, 2004) and cognitive impairment (Yaffe *et al.*, 2003).CRP is also useful in helping diagnose autoimmune conditions such as vasculitis, systemic lupus erythematosus, inflammatory bowel disease and rheumatoid arthritis. However, because autoimmune disorders tend to wax and wane the level CRP will not be elevated if patients with these disorders are not in an active disease state (Szalai *et al.*, 1999).The CRP test is also a generalized test that doesn't indicate the cause or site of inflammation. In chronic autoimmune disorders the CRP level is helpful in gauging a patient's response to therapy or to tell if disease flares are present. However, in terms of diagnosing autoimmune conditions a low CRP level can't be used to rule out specific diseases (Szalai *et al.*,1999).

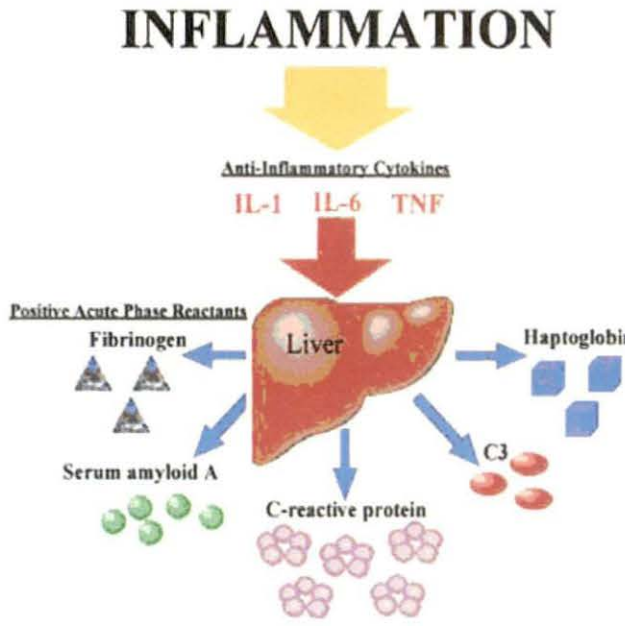

Figure 18: Stimulation and synthesis of positive acute-phase reactants during inflammation. Inflammation caused by infection or tissue damage stimulates the circulating inflammation-associated cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor (TNF)-α. These cytokines stimulate hepatocytes to increase the synthesis and release of positive acute-phase proteins, including CRP. IL-6 is the major cytokine stimulus for CRP production.

3.9.4 C-REACTIVE PROTEIN AND SCHIZOPHRENIA

As schizophrenia is thought to be manifested by some inflammatory process CRP study is implicated in this respect. Few studies have been carried out to investigate the association of CRP and schizophrenia. In one study, elevated serum levels of CRP was found in patients who showed more severe clinical symptoms of schizophrenia as reflected by the PANSS total score (Fan *et al.*, 2007). In another study, the elevated serum levels of C-reactive protein in schizophrenia are found to be associated with the severity of cognitive impairment but not of psychiatric symptoms (Dickerson *et al.*, 2007). Further studies are awaited to throw the light in the role of inflammatory process in the manifestation of schizophrenia.

3.10 LYMPHOCYTES

T cells belong to a group of white blood cells known as lymphocytes which plays a central role in cell-mediated immunity They can be distinguished from other lymphocyte types (e.g., B cells and natural killer cells) by the presence of a special receptor on their cell surface called T cell receptors (TCR). There are two defined types of TCR, one is a heterodimer of two disulphide-linked polypeptides (α and β), the other is structurally similar but consists of γ and δ polypeptides and CD3 complex Together they form the T-cell receptor complex (TCR-CD3 complex). Approximately 90-95% of blood T cells are αβ T cells and the remaining 5-10% are γδ T cells. αβ T cells are further distinguished by their expression of CD4 or CD8 marker.

3.10.1 CD4+ AND CD8+ CELLS

αβ T cells are subdivided into two distinct non-overlapping populations. A subset which carries the CD4 marker and mainly 'helps' or 'induces' immune response (TH) and a subset which carries the CD8 marker and is predominantly cytotoxic (Tc). CD4+ T cells recognize their specific antigens in association with the major histocompatibility complex (MHC) class II molecules. On the other hand CD8+ T cells recognize antigens in association with MHC class I molecules. Thus, the presence of CD4 or CD8 limits the type of cell with which the T cell can interact. A small proportion of αβ T cells express neither CD4 nor CD8 and these 'double

negative' T cells might have a regulatory function. Similarly, most circulating γδ cells are 'double negative', although a few of them are CD8+. By contrast, most γδ T cells in tissue express CD8.

3.10.2 FUNCTION OF CD4+ AND CD8+ CELLS

CD4+ T cells bind an epitope consisting of an antigen fragment lying in the groove of a class II histocompatibility molecule (Fig.19). CD4+ T cells are essential for both the cell-mediated and antibody-mediated branches of the immune system. The CD4+ cells bind to antigen presented by antigen-presenting cells (APCs) like phagocytic macrophages and dendritic cells. The T cells then release lymphokines that attract other cells to the area. The result is inflammation that is the accumulation of cells and molecules that attempt to wall off and destroy the antigenic material (an abscess is one example, the rash following exposure to poison ivy is another).The CD4+ cells, called helper T cells bind to antigen presented by B cells. The result is the development of clones of plasma cells secreting antibodies against the antigenic material.

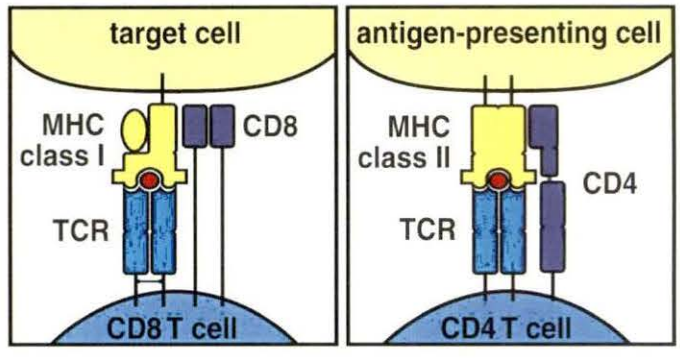

Figure 19: Binding interactions between CD8 T cells and target (virus-infected) cells and between CD4 T cells and professional antigen presenting cell.

The best understood $CD8^+$ T cells are cytotoxic T lymphocytes (CTLs). They secrete molecules that destroy the cell to which they have bound. This is a very useful function if the target cell is infected with a virus because the cell is usually destroyed before it can release a fresh crop of viruses that are able to infect other cells. In general, the role of the $CD8^+$ T cells is to monitor all the cells of the body, ready to destroy any that express foreign antigen fragments in their class I molecules.

Changes in T helper/inducer (CD4) and T cytotoxic/suppressor (CD8) cells are related to a variety of illnesses (Riddell *et al.*, 1993). CD4 cells facilitate both humoral and cell-mediated immune processes. In AIDS patients, large declines in CD4 cell counts usually result in both a general decline in immune function and a vulnerability to opportunistic infections. Indeed, higher CD4 cell counts are thought to be consistent with good health. In contrast, CD8 cells act to shut off CD4 cell activity when sufficient antibodies have been produced. The low CD4/CD8 ratios are associated with immunodeficiency (Reinherz and Schlossman, 1980). T-lymphocyte subset ratio supply valuable information about the state of the cellular immune system. An increase in CD8 lymphocytes suppresses cellular immunity, whereas a decrease could cause excessive functioning. The appropriate CD4/CD8 lymphocyte ratio is expected to be 2:1. Ratios below 1:1 indicate serious disorder of the immune system (Kouttab *et al.*, 1989). It has also been suggested that changes in the T-lymphocyte ratio reflect changes in the metabolism of central nervous system cells and that they could be used as neural markers in the analysis of psychiatric disorders (Gladkevich *et al.*, 2004).

3.10.3 CD4+ AND CD8+ CELLS AND SCHIZOPHRENIA

Advances in immunologic techniques as well as a deepening understanding of lymphocyte function have opened the way towards the quantitation of specific, functionally distinct lymphocyte subsets. Initially, these studies focused on thymus-derived (T lymphocyte) subsets. Reductions in T-cell percentage or number in schizophrenia were found by several investigators using a resetting procedure (Nyland *et al.*, 1980; Coffey *et al.*, 1983). With the development of automated cell counting methods using fluorescent-labeled monoclonal antibodies, elevations in total T lymphocytes as well as CD4 (helper) cells were observed by several investigators (DeLisi *et al.*, 1982; Henneberg *et al.*, 1990; Muller *et al.*, 1993; Masserini *et al.*, 1990). DeLisi *et al.*, (1982) and Cazullo *et al.*, (1998a) also documented an increase in the CD8 (suppressor) subset. In contrast to these results, Villemain *et al.*, (1989) observed no differences in the percentages of either CD4 or CD8 lymphocytes between unmedicated schizophrenic subjects and normal control subjects. Recently Zhang *et al.*, (2002) have found the lower CD4 cell percentage in Chinese patients. Using flow-cytometry, Mazzarello *et al.*, (2004) found lower CD8 lymphocyte level in schizophrenia patients than in controls. Rudolf *et al.*, (2004) on the other hand did

not find any differences in the T-lymphocyte subset ratios of schizophrenic patients and controls. This finding also corroborated with the recent finding by Craddock *et al.*, (2007). Moreover, some studies observed an increase in total T-lymphocytes (De Lisi *et al.*, 1982). Pirildar *et al.*, (2001) reported that there were no differences between the schizophrenic patients and the control in terms of CD4 and CD8 lymphocyte percentage.

It has been suggested that T-lymphocyte level and T-lymphocyte subset ratios could be laboratory markers for treatment response in schizophrenia (Muller *et al.*, 1993). Zhang *et al.*, (2006) have shown that post-treatment clinical improvement in schizophrenia is associated with an increase in the CD4-lymphocyte level. Despite of these findings there is no general agreement regarding the role of T-lymphocytes in schizophrenia and awaits further research.