MORPHOLOGICAL SUBTYPES OF ALZHEIMER’S DISEASE

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ABSTRACT

Initiating a computerized population-based registry of Alzheimer’s disease (AD), the IMAGE Project has developed a multimatrix model to investigate the disease. Part of the IMAGE Project 1, the neuropathological study, is designed to correlate clinical, neuropsychological and neuropathological features of AD for characterization of subtypes. This thesis reports mainly the morphometrical studies associated with project 1.

The study, based on a) brain autopsy, b) standardized histopathology, and c) quantitative morphometry, shows heterogeneity in pathophenotypes of AD. Four morphological subgroups have been presently recognized, by their characteristic histological abnormalities, and the densities, the distribution, and progression patterns of their lesions. The heterogeneity in pathophenotypes indicates that AD is not a disease with a single cause, but rather a syndrome with multiple elements involved in etiology and pathogenesis. These lead to different pathological features, and correspondingly, similar, but distinguishable clinical expressions.
Le projet IMAGE est constitué d'un registre informatisé de patients atteints de la maladie d'Alzheimer (MA) dans la région du Saguenay–Lac St-Jean. Son principal but est de développer un modèle multidimensionnel de la MA basé sur un suivi longitudinal multidisciplinaire. Le volet neuropathologique en constitue une des dimensions, dont nous rapportons ici les résultats d'une analyse morphométrique.

Une hétérogénéité phenotypique marquée fut observée, de laquelle se dégagerent 4 groupes principaux, basés sur une analyse statistique des indexes de dégénérescence neurofibrillaire et de plaques séniles. Les groupes montraient aussi une progression différenciée des indexes selon la durée, indiquant entre autres, que les formes préséniles de la MA évoluent plus rapidement que les formes séniles. Dans leur ensemble, les données structurelles suggèrent que la MA s'exprime d'une façon hétérogène sur les plans cliniques, neuropsychologiques et vraisemblablement aussi dans leur transmission génétique.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ACR</td>
<td>Alkaline Congo Red</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>BBB</td>
<td>blood brain barrier</td>
</tr>
<tr>
<td>CAA</td>
<td>cerebral amyloid angiopathy</td>
</tr>
<tr>
<td>CERAD</td>
<td>Consortium to Establish a Registry for Alzheimer’s disease</td>
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<tr>
<td>ChAT</td>
<td>choline acetyltransferase</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
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<tr>
<td>CVD</td>
<td>cerebrovascular dementia</td>
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<tr>
<td>DAT</td>
<td>dementia of Alzheimer type</td>
</tr>
<tr>
<td>DLBD</td>
<td>diffuse Lewy body disease</td>
</tr>
<tr>
<td>DS</td>
<td>Down’s syndrome</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GVD</td>
<td>granulovacuolar degeneration</td>
</tr>
<tr>
<td>HE</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>IMAGE</td>
<td>French acronym, Investigations de la Maladie d Alzheimer</td>
</tr>
<tr>
<td>LB</td>
<td>Lewy body</td>
</tr>
<tr>
<td>LBD</td>
<td>Lewy body dementia</td>
</tr>
<tr>
<td>LC</td>
<td>Locus Coeruleus</td>
</tr>
<tr>
<td>MB</td>
<td>modified Bielschowsky</td>
</tr>
<tr>
<td>MI</td>
<td>multiple infarcts</td>
</tr>
<tr>
<td>MID</td>
<td>multiple infarct dementia</td>
</tr>
<tr>
<td>nBM</td>
<td>nucleus basalis of Meynert</td>
</tr>
<tr>
<td>NFT</td>
<td>neurofibrillary tangle</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
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<tr>
<td>SD</td>
<td>senile dementia</td>
</tr>
<tr>
<td>SDAT</td>
<td>senile dementia of Alzheimer type</td>
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<tr>
<td>SN</td>
<td>substantia nigra</td>
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<tr>
<td>SP</td>
<td>senile plaque</td>
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INTRODUCTION

Alzheimer's disease (AD) is a chronic, degenerative, dementing illness of unknown etiology. Its incidence rises with increasing age, and there is presently no method of prevention or cure (Molsa, 1985; Sulkava, 1985).

A simple test for the diagnosis of AD is not yet available. There is no experimental model to facilitate study, and no biological marker has yet been identified in human patients. Heterogeneous expression and overlap with aging and some other conditions further complicate the identification. As a result, AD can not currently be diagnosed by a single method, and is defined by a combination of clinical and pathological observations.

Demonstration of AD requires postmortem evaluation of the brain, and documentation of specific lesions associated with the disease. However, there is considerable variation in the frequency and distribution of these lesions from one patient to another. It is also difficult to distinguish between structural changes in the brain related to normal aging, and those found in borderline, or early stages of AD.

Quantitative analysis of pathological lesions in the brain might provide reliable criteria to accurately
distinguish between normal aging and early AD and between different variants of AD or combinations of AD with other disorders. Such analysis is the topic of this thesis, and it is hoped that accurate classification of subgroups of AD might, in addition, contribute information of use in defining the etiology of the disease.

Many hypotheses regarding the etiology of AD have been advanced (Blass, 1984; Struble, 1985; Kilpatrick, 1983; Master, 1985; Wong, 1985). These include age-related changes, genetic factors, metabolic abnormalities, infectious agents, neurotoxins, head trauma, amyloid deposition, lack of trophic factors, and systemic abnormalities (Eisdorfer, 1978). Unfortunately, there are more questions and contradictions than answers in almost every domain of current studies. Factors which make it difficult to discover the true nature of AD, include: a) the lack of disease definition and a suitable animal model; b) the great variability in the manifestation of the disease, which implies heterogeneity and subtypes; c) controversial findings in detecting genetic factors. Therefore, an interdisciplinary approach, with a longitudinal design, on a distinct human population, seems at the present time to offer the best opportunity to understand the nature and development of this disease.

The IMAGE project (French acronym, Investigations de la
Maladie d’Alzheimer: Génétique et Epidémiologie), a multimatrix model for the study of AD (Gauvreau, 1989) has established a registry system for Alzheimer cases in a well-defined population, and created 8 disciplinary research matrices: clinico-pathology and neuropsychology, epidemiology, population genetics, molecular genetics, caretaking, physiology, socio-geography, and environmental toxicology. The targeted population is in the Saguenay-Lac-Saint-Jean (SLSJ), Quebec. The area is located between the longitudes of 70° and 75°, and latitudes 48° and 49° north, and covers about 225 kilometres from west to east and 100 kilometres from north to south. The total population of SLSJ is about 290,000, with an estimated 25,000 at risk of developing AD, and at least 600 available for autopsy during a 5-year study. The area was relatively isolated from the rest of Quebec until the end of the 19th century, because of the lack of roads and long freezing period of the river. A computerized data-base of all the parish registries including information on place and date of birth and death, residential and professional histories of the constituents, as well as geographical and socio-economic characteristics, has been established in the area. Therefore, unlike studies published so far on AD, which were mostly compartmentalized within research subspecialities and based on limited clinical series with controls selected only on the basis of age and sex, the IMAGE project possesses a well-defined population for the study. An assortment of controls
are automatically provided by the same population, and it is possible to trace family pedigrees with at least 3 generations of AD cases. The area is geographically recognizable and can be assessed in regard to possible environmental factors contributing to the development of the disease. Therefore a collaboration across research subspecialities studying this population should provide optimal conditions for advancing current understanding of the pathogenesis of AD.

The study of the correlations between clinical, neuropsychological and neuropathological features of AD for characterization of subtypes constitutes project 1, a basic part of the IMAGE project. The aims of project 1 are a) to establish a population-based registry of AD cases; b) to establish and validate a diagnostic procedure for AD; c) to identify and define subtypes of AD. This thesis deals with the neuropathological work from Project 1, aimed at identifying and defining subgroups by variations in the type and extent of lesions within the brain.
2. REVIEW OF BASIC CONCEPTS

2.1. BACKGROUND AND HISTORICAL FACTORS

2.1.1. Naming of the Disease

In 1907, Alois Alzheimer, a German physician, reported a case of a 51-year-old woman with a 4-year history of progressive memory loss, personality changes, language disturbances and apraxia. At autopsy using new silver stains, Alzheimer noted that cortical neurons contained numerous neurofibrillary tangles (NFTs). The cortex also showed diffusely distributed "miliary foci", lesions first described by Blocq and Marinesco in the brain of an elderly patient with epilepsy in 1892, and later, by Redlich in two senile dementia cases in 1898. These lesions were more extensively described by Fisher in 1907, who concluded that they were a specific finding in senile dementia (Luigi, 1986). A few years later, the term "senile plaque (SP)" replaced "miliary foci". In 1910, Emil Kraepelin, a psychiatric nosologist, codified the dementia with the salient clinical and pathological features described by Alzheimer as a separate entity in the 8th edition of his authoritative textbook "Psychiatrie: Ein Lehrbuch fur Studierend und Arzte". "Alzheimer's Disease (AD)" as suggested by Kraepelin, was thereafter accepted as a pre-senile dementia with onset before the age of 65 and characterized by microscopic findings of NFTs and SPs.
2.1.2. The Confusion in Semantics and Concepts of the Disease

There is a long history in which human mental decline in the form of senile dementia has been observed over the ages. In the time of Hippocrates, incompetent behaviour in the elderly was recognized, and probably considered a natural part of aging, because he did not include it among his mental disorders. The term "dementia" was first introduced by A.C. Celsus, a Roman writer on medical subjects in his book "De medicina" (Alexander, 1966). In 1797, Pinel gave the first medical definition for the term "dementia", and later his student, Esquirol, differentiated between 3 types of dementia, acute, chronic and senile. He defined the senile form as one that was established slowly and caused by advanced age (Lipowski, 1980). In 1837, J. Prichard, an English psychiatrist, also described the slow, stepwise progression of dementia in the elderly.

Brain atrophy was definitively described by Wilks in 1864, as a constant pathological feature of senile dementia (Clouston, 1911), which, thereafter, was considered an organic mental disorder or an organic brain syndrome. The definition of senile dementia was essentially completed by including Redlich's SP (1898), Alzheimer's NFT (1906), and Simchowicz's Granulovacuolar degeneration (GVD) (1910).
Since senile dementia (SD) was shown to have the pathological features that Alzheimer described in his case report, it seems questionable to distinguish the eponym "AD" from senile dementia, but there is a historical explanation. Two European neuropathology schools were involved in these important discoveries: Alzheimer worked at the Laboratory of Anatomy of the Psychiatric and Neurologic Clinic directed by Kraepelin in Munich, and Fischer worked at the German Psychiatric Clinic directed by Pick in Prague. They contributed respectively to describing one of the two pathological features of AD: NFT and SP, but each played down the significance of the other's finding, and rivalled for the eponym (Bonfiglio, 1908). Pick's school refused to recognize Alzheimer's finding as a specific entity. In addition, scientific rivalry and nosologic uncertainty existed even in the same school, such that Perusini, a fellow of Alzheimer, questioned giving Alzheimer's name to a classification of mental disease. Kraepelin had great success and interest in clinical classification, especially in psychiatric syndromes by age of onset. However only two cases of presenile dementia had been published, one by Alzheimer, and the second in 1908, by Bonfiglio, also from Kraepelin's school. It therefore appears that Kraepelin named AD in 1910 and distinguished it from SD, as a premature academic favour to the fellow from the school he was directing. Because of Kraepelin's great reputation and authority, the dogma he created still endures.
Interestingly, opinions for and against separating AD from SD according to onset before or after age 65, are still growing. Presently, the "separatists", more popular among European investigators, insist that AD is different from SD. AD has a rather homogeneous pattern, earlier onset, usually in the fifties or sixties, more malignant clinical progression, almost total impairment of psychic functions at the end phase, larger numbers of NFTs and SPs, and more severe neurotransmitter deficiency. AD may also have a familial risk (Bondareff, 1981, 1983; McDonald, 1969; Rossor, 1984). The "unionists", much favoured by American counterparts, argue that AD and SD should be considered a single disorder and therefore, have a unique name, since they show the same types of neuropathological changes, and the differences reported are mostly based on relatively small sample size, and are not qualitative (Kartzman, 1976; Terry, 1978; McKhann, 1984; Jorm, 1985).

As the views on AD developed, the confusion in semantics and concepts between AD and SD continued, whether the consideration was based on nomenclature, epidemiology, clinical features, pathology, etiology, diagnosis, treatment, prognosis, or prevention. The issue is still extensively debated and is affecting clinical practice, research, and social response to these disorders.
2.1.3. Terminology to be employed in this thesis

In addition to the above nosological uncertainty between AD and SD, "benign senescent forgetfulness" (BSF) (Kral, 1978) and "primary degenerative dementia" (PDD) (Lipowski, 1981) have been adopted to refer to different clinical patterns of the age-associated cognitive decline. The designation BSF implies an invariably benign course, however the patient may actually deteriorate over time. The term PDD frequently refers to SD or SD of Alzheimer type (SDAT), which is a more circumscribed term than "SD", by implying the presence of Alzheimer-type neuropathological changes. The terms presenile and senile dementia, further confounded by the terminology "presenile dementia and SD, simple type", suggested by the World Health Organization and International Classification of Diseases (1977), strongly imply a dichotomy between dementia occurring in the "presenium" (i.e. before age 65) and that occurring in the "senium" (i.e after age 65). However, these distinctions are not convincing but controversial (Clayton, 1981; Jorm, 1985; Luigi, 1986). Sometimes they refer to not only AD and SDAT, but also a broader class of dementia, including Pick's dementia and multi-infarct dementia (MID).

To avoid the confusion currently existing in the key terms denoting the disease entities and clinical syndromes, the terminology used in this thesis is defined in a manner
consistent with the usage preferred by the majority of investigators and consistent with the advancement of present understanding of the disease.

"SD" is used to refer to dementia, with onset late in life, generally after age 65, the origin of which may be AD, MID or possibly, other pathology.

"Presenile dementia" is used to refer to the dementia syndrome, with onset before age 65, which is due to various pathological processes including, but not limited to, AD.

"AD" is used to denote the disorder characterized by age-associated cognitive decline of gradual onset and progressive course, with Alzheimer-type neuropathological brain changes. No distinct age of onset is implied. "AD" is used in its traditional way to refer to presenile dementia with Alzheimer-type pathology, only when the issue of heterogeneity of AD is discussed, and when it is being compared with senile dementia with Alzheimer-type neuropathological changes.

"SDAT" refers to AD with age of onset after 65, and is used only when the issue of heterogeneity of AD is discussed, and when it is being compared with presenile dementia with Alzheimer-type neuropathological changes.
"Dementia of Alzheimer type" (DAT) refers to "probable" AD (McKhann, 1984), in which the disorder is diagnosed by clinical research criteria, but not neuropathologically verified.

2.1.4. Heterogeneity of AD

Some investigators have documented specific morphological patterns in brain lesions associated with DAT, which were certainly far beyond the neuropathological features that were initially described by Alzheimer (1906), and extended by the other pioneers, Fisher (1907), Kraepelin (1910) and Simchowicz (1910).

Bondareff (1981; 1983; 1987) suggested that AD be divided into two subgroups, according to their pathological features: AD-1, with late onset, was associated with a less severe loss of nLC neurons; AD-2, with early onset, was associated with greater nLC neuronal loss, greater loss of Choline acetyltransferase (ChAT) activity and norepinephrine from the cerebral cortex, and greater numbers of NFT and SP. More support for this separation appeared later, when AD-2 was found to be more often familial (Heston, 1983), and more progressive (Selzer, 1983), with a greater loss of somatostatin in frontal or temporal cortex, with greater loss of gamma-aminobutyric acid (GABA) in the temporal
lobe (Rossor, 1982), and greater neuronal loss in the nucleus basalis of Meynert (nbM) (Mann, 1984; Whitehouse, 1982, 1986).

The certainty of this subtyping, however, is still being questioned. There are contradictory findings in clinical and psychological studies which indicate that the differences in symptoms (McDonald, 1969; Seltzer, 1983) are closely related to the duration, rather than the age of onset of the disease (Heyman, 1984; Constantinidis, 1978). The neuropathological findings are also questionable, since DAT with later-onset, so-called AD-1 also involves a decrease of ChAT activity, as well as reduced somatostatin in all areas of the temporal cortex (Rossor, 1981; 1984), and a neuronal loss in the nbM (Wilcock, 1983). This could support the interpretation that patients with late-onset AD have fewer pathological changes, because they were still in an early stage of the disorder when they died, being more susceptible to other illnesses.

Okasaki (1961) first suggested an association between diffusely distributed LBs with SN changes indicating idiopathic PD, and the cortical changes typical of AD. Such cases show mild to moderate densities of NFT and SP, which may overlap with levels seen in non-demented aging. Most reports support the association between AD and PD (Boller, 1980; 1985; Gaspar, 1984; Chui, 1986; Hamill, 1988), although a few do not (Gibb, 1985; 1990). Recently, there has been a proposal to
define a specific subtype of AD, Alzheimer changes with LBs (Hansen, 1989; 1990). The criteria for such a subtype are still being debated, since various categories of this pathological entity have been reported. Examples include "LB dementia without Alzheimer changes", "Young adult-form dementia with NFT changes and LBs", "LB dementia with SPs only", and "AD with LBs, mainly in brain stem" (Sima, 1986; Popovitch, 1987; Dickson, 1987). Based on their clinical and pathological findings, some investigators suggest that this pathological entity belongs under PD, some admit it as a sub-type of AD, and some consider it a distinct entity (Forno, 1978; Gibb, 1985; 1990; Byrne, 1989; Leverenz, 1986; Hansen, 1990).

Jorm (1985) proposed criteria for establishing the subtypes of AD, and created a diagrammatical representative model. This was based on the structural model suggested by Jöreskog (1978) and focused on distinguishing between qualitative and quantitative differences. Chui (1987) also proposed a scheme to demonstrate that clinical heterogeneity in AD could be classified.

The uncertainty about these proposed morphological subtypes and the difficulty in interpreting their significance remain, mainly because of a) the sporadic sampling source, b) the small sample size, c) the restriction in performing longitudinal tracing, and d) the lack of multidisciplinary
collaboration. The IMAGE project, as described above, is able to avoid or to compensate for these shortcomings by its unique source of sampling, well-defined population registry, longitudinal design, and multidisciplinary approach. This permits much greater reliability in categorizing the heterogeneous pathological features and revealing etiological and pathogenic factors in relation to the morphological subgroupings.

2.2.CURRENT CONCEPTS

2.2.1. Epidemiology of the Disease.

According to the US Department of Health, Education and Welfare (1978) and Statistics Canada (1984), the percent of elderly people in the population is rising dramatically. It is presently estimated that 4% to 5% of the US population over 65 years old have severe dementia and 10% have mild to moderate impairment (Tery, 1983). These numbers are positively correlated with age. For example, in the 65-70 year-old group the prevalence of severe dementia is about 1%, but in those over 80, the prevalence rises to 15%-20%, reaching a maximum of 40%-50% around the age of 90 (Grunberg, 1961; Gottfries, 1985). At this rate, when the life expectancy extends into the late nineties by the year 2040, a large portion of our population will develop dementia at some point in their lives (Hagnell, 1981; Seegmiller, 1989).
AD is presently recognized as the most frequent cause of dementia in adults, accounting for 50% to 70%, while MID accounts for 7%-15%, and Parkinson's disease (PD) for 8% (Tomlinson, 1970; Larson, 1984; Nordberg, 1989; Robert, 1990). Although the figures are not precise (Jorm, 1987; Mortimer, 1983), it is estimated that AD affects 2.5 to 3 million Americans, accounts for 1/2 of the US nursing home population, and causes 120,000 deaths per year (Reisberg, 1983; Light, 1989). Gautrin (1990) very conservatively estimated the Canadian age-specific prevalence rates for AD over the period 1986-2031: 1% for age 65-74 years, 4% for age 75-85% and 10.5% for age over 85. AD now ranks as the 4th most common cause of death in developed nations (Reisberg, 1983; Seegmiller, 1989; Francis, 1989).

Comparisons of dementia across populations and cultures, although rare and controversial, have demonstrated large differences. Unlike the US and western Europe, Russia, Japan and China repeatedly claim to have higher rates for MID than for AD (Gavrilova, 1977; Karasawa, 1982; Li, 1989). China was reported to have the lowest rates, claiming that only 1.86% of aged people were demented, and 0.07-0.66% of them had AD (Yang, 1988; Kuang, 1984). However the most recent Chinese study carried out in cooperation with two American universities (Zhang, 1990) showed that the prevalence of dementia in people aged 65 and older was 4.6%, and AD
accounted for 65% of the demented. These values are within the range, although at the lower part, of those rates reported in Western countries.

2.2.2. Clinical and Pathological Features

The initial symptoms of AD are often so subtle that the onset and details of the early course are difficult to establish. The most common initial symptoms are impaired memory, difficulty with problem solving, and failure to respond to the environment with customary speed and accuracy. Many psychotic and neurological changes may emerge through the illness (Reisberg, 1983; Spinnler, 1987; Pirozzlo, 1989). As the disease progresses, the memory loss and disorientation become more severe, perception disorders arise, and focal cortical signs, such as aphasia and apraxia appear. Eventually, the patient loses insight, no longer attempts to compensate for the impaired mental function, and deteriorates to a stage of complete helplessness.

Because of the symptomatic heterogeneity, several subgroups of AD have been proposed, such as Mayeux's (1985) four groups: benign, myoclonic, typical and extrapyramidal. Other categories include presenile and senile forms (Roth, 1978; Seltzer, 1983), familial and sporadic forms (Sjogren, 1952; Folstein, 1981), and benign and malignant
The brains of AD patients vary in appearance from those with normal dimensions or mild atrophy to those with marked atrophy and ventricular dilatation. The atrophy, although general, is usually more apparent in the basal and medial limbic portion of the temporal lobe, the frontal lobe and post-central parietal region. The primary projection areas such as the sensorimotor cortex and calcarine gyrus, the brain stem, cerebellum and spinal cord are largely preserved, although the substantia nigra (SN) may be a bit pale. Some additional changes, such as arteriolosclerosis, atherosclerosis and frank infarctions are seen, usually in older patients (Brun, 1976; Miller, 1977).

Microscopically, the changes are usually confined to the cerebral cortex and the limbic grey matter, and have not been noted in the spinal cord. Two histological hallmarks, the NFT and the SP, although not specific to AD, are compulsory for the pathological diagnosis. NFTs, demonstrated by silver stain, are thickened and tortuous fibrils in the neuronal cytoplasm, which consist of filament bundles. These are either single and straight or, more commonly, paired helical filaments (PHF), 10 nm wide, with a periodicity of 80 nm. They are most frequent in large pyramidal neurons of the hippocampus, parahippocampal gyrus and of layers III and V in

SPs, demonstrated by silver or thioflavin S stains, usually are discrete spherical structures with diameters from a few to 200μ. They contain abnormal distended unmyelinated neurites, mainly axons, which also contain PHF, typically surrounding a central core of amyloid and inorganic material such as aluminosilicates, and are often accompanied by astrocytes, microglia and macrophages. Several types of SP are reported, which might actually represent stages in an evolution of SPs (Struble, 1987; Kidd, 1964; Burger, 1973; Candy, 1986; Wisniewski, 1985; Dickson, 1988; Abraham, 1988). SPs are most frequent in the cortex, and are found in greatest number in Layers II and III (Pearson, 1985; Tomlinson, 1989).

The nature and evolution of NFTs and SPs remain debatable. Because of the insolubility of the NFT, its composition has been demonstrated only by immunohistochemistry. It contains neurofilament proteins, microtubule-associated proteins, such as tau and MAP2, Aβ protein, ubiquitin and beta-amyloid protein (Anderton, 1982; Iqbal, 1984; Glenner, 1984; Yen, 1987). Based on its appearance in other disorders, such as Down's syndrome, pugilistic dementia, severe hemispheric infarction, and even in young patients with leukodystrophy or subacute sclerosing panencephalitis (Harada, 1988; Hauw, 1989; Kato, 1988; Tomlinson 1970), the NFT
is supposed to be a nonspecific response of the neuroskeleton to chronic cellular injury (Shaw, 1988; Bergeron, 1989). The aggregation of abnormally phosphorylated tau in NFTs may be the earliest stage of its formation (Delaere, 1989; Iqbal, 1986).

The typical SP consists of multiple types of neuronal fibers, including cholinergic, noradrenergic and many peptidergic fibres (De Souza, 1986; Donald, 1986; Struble, 1982; Rossor, 1986), and a β-amyloid core. The amyloid precursor is coded for by a gene on chromosome 21 (Glenner, 1984; Tanzi, 1987). The amyloid is hypothesized to accumulate from a serum precursor which results in both cerebral amyloid angiopathy (CAA) and formation of the SP core (Wong, 1985; Glenner, 1981, 1983; Duyckaerts, 1987). Many problems related to this hypothesis, including the unidentified trigger of SP and NFT formation, and the obscure relationship between SPs and NFTs and their contribution to the dementia, remain either unexplained or debatable.

Other pathological features have been reported, although their natures and roles in the disease are not known yet. These include GVD, which consists, microscopically, of one or more vacuoles 3-5μ in diameter with a core of argyrophilic granules 0.5-1.5μ in diameter, mostly found in hippocampal pyramidal neurons (Tomlinson, 1972; Ball, 1977; Price, 1986).
Hirano bodies (HB) are rod-like eosinophilic inclusions 3μ in length, which are mostly seen in the neurites and perikarya of hippocampal neurons and contain some filaments. These differ morphologically from PHF neurofilaments, or microtubules (Hirano, 1968; Ogata, 1972; Gibson, 1977; Tomonaga, 1974).

Selective losses of neuronal populations and decreased activities of their transmitter systems have been reported in AD. Affected areas may include the nucleus basalis of Meynert (nvM) and its acetylcholinergic projections (Davies, 1979; Hedreen, 1984; McGeer, 1984; Perry, 1983), the Locus Coeruleus (LC) and its noradrenergic projections (Bondareff, 1982; Forno, 1978; Mann, 1980), and the brain stem raphe nuclei and their serotoninergic projections (Curcio, 1984; Tomlinson, 1982; D'Amato, 1987).

2.2.3. Clinical and Pathological Diagnosis

The clinical and pathological features are not specific in AD. They vary in magnitude or stage and they overlap with those found in "normal" aging individuals and in other dementing illnesses (Mölsä, 1984, 19852; Brown, 1984; Ball, 1980; Berg, 1985; Henderson, 1986). Therefore, no standardized and definite diagnostic criteria are available yet. The clinical diagnosis of AD can only be made at "probable" or "possible" levels (McKhann, 1984; Tarn, 1986), and is confirmed by
neuropathology in 43% to 90% of cases (Suldava, 1983; Neary, 1986; Wade, 1987; Joachim, 1988). Having no "gold standard", the neuropathological diagnosis is by no means absolute, and requires clinical confirmation (McKhann, 1984; Growdon, 1984; Tarn, 1986). The criteria for AD diagnosis recommended by the National Institute of Neurological and Communicative Disorders and Stroke and the AD and Related Disorders Association (NINCDS-ADRDA) (McKhann, 1984) are the most widely used at present and are still being validated.

The diagnosis of definite AD depends on the neuropathological confirmation, but the NINCDS-ADRDA work group was limited to qualitative criteria. Quantitative morphometric criteria have been promulgated by Tomlinson (1976) and his Newcastle group, Khachaturian (1985) and his American panel, and Ball (1988) and his team in Western Ontario, in order to differentiate normal aging from AD and other disorders. These studies varied in samples taken, staining techniques employed, pathological lesions targeted, and the morphometry and quantitative standards used for the diagnosis.

The first such analysis was performed by a British team (Blessed, 1968; Tomlinson, 1976), and focused on the quantitative study of NFTs and SPs in samples stained by silver-impregnation, from 4 lobes, as well as GVD from the hippocampus. They did find SPs, NFTs and GVD occurred with
significantly greater frequency in the demented population than in the non-demented controls. For example, the average SP count in demented elderly (14.7 per field) differed greatly from that of the controls (3.3 per field, p<0.001), and large numbers of NFTs were invariably associated with dementia. They did not devise a combination of quantitative parameters to distinguish between AD and "normal elderly" with severe lesions.

An American team (Khachaturian, 1985) emphasized the quantitative measurement of SPs in 3 neocortical areas and some subcortical areas, stained by silver or thioflavin, and suggested minimum quantitative criteria required for diagnosing AD, dependent upon the patient's age at death. The number of NFT or SP per field required are: 2-5, for patients less than 50; >8, for patients of 50-65; >10, for patients of 66-75; >15, for patients over 75. This method seems to be practical to apply in uncomplicated cases. However, the authors did not specify criteria to be used during coexistence of other pathological entities, such as PD. Also, their quantitative adjustment by the age of death is questionable, since it is possible that duration of the disease and/or the age of onset, rather than the age at death, has more effect on the severity of the lesions.

A Canadian group from the University of Western Ontario
(Ball, 1988) promulgated quantitative standards, by multivariate analysis of lesions in the hippocampus, in order to differentiate AD from normal aging. At least 20 tangle-bearing neurons per mm$^3$ and/or at least 55 nucleolated nerve cells with GVD per mm$^3$ and/or less than 5,600 nucleolated neurons per mm$^3$ of pyramidal cortex, were required for the diagnosis. The quantitative criteria were reported to be very sensitive. Explaining and further developing this data was restricted by the relatively small sample size of both controls and disease groups, and the relatively isolated samples.

All of these investigators found quantitative morphological differences between AD and the non-demented elderly, and tried to determine the morphological demarcations of normal aging and the very early stage of AD. They had difficulty in establishing minimal diagnostic criteria for AD, and therefore stressed the necessity of multidisciplinary collaboration and large, longitudinal studies.

A comprehensive and longitudinal survey on a well-defined population, with a collaborating network of investigators from the various specialities in neurobiology should avoid these limitations, and provide a better chance to discover relevant information on every aspect of the development of AD. Correlations between all research aspects
can be used to formulate significant conclusions about the etiology of AD and the interplay among the different pathogenic factors. Recently the Consortium to Establish a Registry for AD (CERAD) (Morris, 1989; Mirra, 1991) has organized an extensive network in the United States. Their neuropathological team is focusing on developing a practical and standardized protocol, not to define each case definitively, but to provide a simple, easily understood, and uniform approach that would indicate levels of diagnostic certainty, reduce subjective interpretation, and assure a common language. No work focusing on morphological subtypes has been published yet.

Presently there are very few such organized projects going on in the world, but none of them has, as IMAGE does, a unique source of sampling: a computerized population-based registry of AD patients in a relatively well-defined area. This can permit forward or backward inferences to be made between the different matrices and yield more informative results by correlating data from different disciplines. Working in the neuropathological matrix of the IMAGE project, our results can be used: a) to standardize the neuropathological and morphometric protocol, making it reliable and comparable with those of NINCDS-ADRDA and CERAD, b) to detect all related morphological changes and to probe the evolution of lesions in the brain, c) to define
morphological subgroups which may reveal etiological subgroups, when correlated with those from neurology and neuropsychology, d) to serve as a reference for other research within the IMAGE framework, particularly, the epidemiological and genetic studies.

2.2.4 Etiological and Pathogenic Factors:

A variety of factors have been reported to contribute to the development of AD, but few of them are securely established as relevant (Henderson, 1988, 1990). Age, gender, and a first-degree relative with dementia have been correlated with AD incidence. Many other factors, including aluminum deposition, a transmissible agent, neurotoxins, head trauma, metabolic abnormalities, immunologic deficit, and lack of trophic factors have been hypothesized.

Age:

There is agreement in all surveys that AD increases with age. According to Jorm (1987), the rate doubles every 4.5 years from 60 to 90 years of age. The clinical, neuropathological and neurochemical features of AD overlap with those of normal aging (Braody, 1955; Roth, 1967; Hubbard, 1981), therefore, some suggested that AD, or specifically, the senile form of AD (Whitehouse, 1983), is an exaggeration of the normal aging process (Hubbard, 1981; McGeer, 1984). However, it is generally
accepted that AD is not an invariable component of aging (Berg, 1985; Price, 1986). The obvious relation to age suggests that: a) there is accumulation of some toxin, or b) there is a time dependent effect following the exposure to the toxin or infectious agent, or c) there is an age-related decrease in a biological process necessary for normal neuronal function, and this decrease may accelerated by some environmental factor or genetic vulnerability.

Sex:

It has been noted that the rates of AD are significantly higher in women than in men (Akesson, 1969; Hagnell, 1983; Sulkava, 1985; Jorm, 1987), which is still confirmed even when allowance is made for the sex-bias due to their longer survival (Kokmen, 1980). Factors associated with being female that promote the development of AD have not been ascertained, however, and even the correlation has been questioned by some investigators (Kaneko, 1975).

Genetic hypothesis:

Increased prevalence of dementia among the first- and second-degree relatives of AD patients has been reported (Akesson, 1969; Breitner, 1984; Nee, 1983; Heyman, 1984; Rocca, 1986; Amaducci, 1986). However, approximately 60% of AD cases did not show any familial aggregation (Heston, 1981;
Heyman, 1983; Amaducci, 1986). Based on the age-dependent penetrance, which may fully penetrate at the age of 90 (Folstern, 1984; Huston, 1981), this could be explained either by inadequate information about family history or by premature death before the age of maximal risk. Twin studies indicate higher concordance rates in monozygotic than in dizygotic twins (Kallmann, 1949, 1953; Embry, 1985), although this data is presently incomplete (Nee, 1987; St. George-Hyslop, 1989), since the unaffected of twin pairs may develop the disease later in life.

Some investigators have suggested polygenic/multifactorial heritability in AD (Sjögren, 1952; Whalley, 1982; Anderson, 1989). Most favour an autosomal dominant pattern (Feldman, 1963; Heston, 1981; Breitner, 1984; Goudsmit, 1981; Nee, 1983). Genetic hypotheses in AD are supported by the similarities between AD and Down’s syndrome (DS), a trisomy 21. Patients with DS invariably develop the neuropathological features of AD (Ellis, 1974; Burger, 1973; Ball, 1980; Wisniewski, 1985 Casanova, 1985). However, not all authors feel the genetic contribution to AD is significant (Nee, 1983; Amaducci, 1986; St Georg-Hyslop, 1987). Parental age and birth order have been proposed as risk factors (Cohen, 1982; Whalley, 1982), but these have been disputed by the findings of Corkin (1983), English (1985) and Braekeleer (1988). Knowing the association between AD and DS, researchers are encouraged to
search for a genetic defect on chromosome 21 as a possible cause of AD, however, the findings are inconclusive (Cook, 1978; Brun, 1978; Peterson, 1985). Transcriptional, translational and posttranslational abnormalities have been postulated (Mann, 1981; Crapper, 1979; Sajdel-Sulkowska, 1984; Heston, 1986).

Molecular genetic approaches, using DNA probes together with the delineation of amyloid deposits, show that a common component is shared by NFTs, SPs and CAA in AD and DS (Wong, 1985; Masters, 1985a, b; Glenner, 1984). However the findings are still difficult to interpret (Merz, 1983). A DNA sequence involved in the development of AD does map to chromosome 21, but outside of the DS region (St. George-Hyslop, 1987; Robakis, 1987), and it is neither identical with nor closely linked to the $A_4$ locus, a major subunit of the $\beta$-amyloid (Tanzi, 1987a; Van Broeckhoven, 1987). A gene encoding $A_4$ maps on chromosome 21 (Kang, 1987; Goldgaber, 1987; Tanzi, 1987b), but its messenger RNA is distributed to areas similar, but not limited to areas of pathological change in AD (Bahmanger, 1987; Tanzi, 1987b). Duplication of this gene is found in DS but not in sporadic or familial AD (Tanzi, 1987c; Podlisny, 1987; Furuya, 1988). Early-onset AD has been explained as an autosomal dominant trait with at least 1 age-dependent but highly penetrant locus on chromosome 21 (Haines, 1991), or with mutations in the gene for the precursor
protein of the amyloid (APP) (Goate, 1991), and it has been suggested that loci on both chromosomes 21 and 19 are also involved in late-onset AD (Pericak-Vance, 1991), although the inheritance modes are unclear and environmental factors can not be ruled out.

Because of the different patterns in clinical phenotype, and inheritance, and the complexity of the chromosomal studies, genetic heterogeneity is expected to exist in AD (Chui, 1986; Bird, 1989; Anderson, 1989; Haines, 1991).

Amyloid deposition:

Cerebral amyloid angiopathy (CAA), an age-related change (Creasey, 1985; Ferszt, 1983), appears to be most severe in AD and occurs in as many as 92% of affected brains (Vinters, 1983; Kurucz, 1981; Glenner, 1985). Immunocytochemical and biochemical evidence suggest that the amyloid core of the SP, CAA amyloid, and possibly even NFT proteins in both AD and DS are nearly identical (Johnson, 1981; Allsop, 1986; Glenner, 1984). The vascular protein shows a striking similarity, in terms of molecular weight and amino acid composition and sequence, to peptides isolated from amyloid core of SPs and from NFTs (Allsop, 1983; Master, 1985), which leads to an interesting hypothesis, that a circulating amyloid precursor passes through injured cerebral capillaries, penetrating the blood-brain barrier (BBB). This could result in initial deposition.
of amyloid in the vessel walls as CAA, and subsequent deposition in the neuropil, either in the original form or after processing by microvascular endothelium, leading to the formation of NFTs and SPs (Wong, 1985; Glenner, 1979).

However, some evidence and unexplained questions do not support the hypothesis. For instance, CAA is found in elderly brains in the absence of AD. Also the hippocampal formation, an area that is severely altered in AD (Ball, 1985; Hooper, 1976), is relatively spared from CAA, even in severe cases (Vinter, 1985; Mandybru, 1975; Morimatsu, 1975). SPs may occur in the absence of CAA and CAA may occur without SPs (Miyakaswa, 1979). Furthermore, the reverse relation among CAA, SPs and NFTs can also be hypothesized as the β-protein might be deposited first in the neuronal soma, next in neurites and plaque core, and finally in the vascular wall (Doebler, 1988; Masters, 1985).

Environmental factors:

The infectious hypothesis: There is an overlap in the clinical and pathological features of AD and Creutzfeldt-Jakob disease (CJD), a transmissible subacute spongiform encephalopathy (Bernoulli, 1979; Brown, 1982; Boellard, 1980; Flament-Durand, 1979). This has prompted the search for an infectious agent in AD, which is supported by the finding that the olfactory forebrain, a region prone to severe
alteration in AD (Ferreyra-moyano, 1989; Ball, 1980) is also a target for the herpes simplex virus. Moreover, NFTs have been associated with several viral illnesses, including herpes simplex encephalitis, subacute sclerosing panencephalitis, and rabies (Wisniewski, 1979). The finding that aggregates of infectious proteinaceous particles from scrapie, called "prions" (Prusiner, 1984) and CJD share tinctorial and structural properties with amyloid has also aroused much attention (McDermott, 1978; Prusiner, 1984). The scrapie-associated fibrils are neurofilament-like atypical viruses, which may interfere with slow axonal transport and lead to amyloid accumulation (Gajdusek, 1985). Preliminary reports indicate the possible transmission of disease, morphologically similar to spongiform encephalopathy, to monkeys inoculated with brain tissues from familial AD patients (Gajdusek, 1977; Rewcastle, 1978).

However, unlike the spongiform encephalopathies, AD has not been transmitted to experimental animals. Prion protein antiserum does not react to extracts of AD brain or the amyloid core of SPs (Bockman, 1987; Kitamoto, 1986). Moreover, the amino acid sequence of human prion protein differs from that of the β-protein in AD and DS, and the prion protein maps to chromosome 20, not chromosome 21 (Liao, 1986; Sparkes, 1986). Although Cook (1978) claimed that familial AD, like CJD is an infection, it seems that the infectious etiology of AD has not
been demonstrated with a strong degree of certainty.

Neurotoxins: Several environmental toxins have been suggested to contribute to the etiology of AD, including aluminum and silicon (Crapper, 1973; Nikaido, 1972), two of the earth’s most ubiquitous elements. Some studies demonstrate an increased aluminum and calcium concentration in NFT-bearing neurons of the hippocampus (Perry, 1985). Aluminum silicate is found in the amyloid core of SPs. The formation of NFTs can be elicited by intracerebral application of aluminum (Crapper, 1973; McLachlan, 1986), although the ultrastructure and distribution of these is different from NFTs in AD. Dialysis dementia (Alfrey, 1976; McDermott, 1977, 1979) is associated with high concentrations of aluminum in the dialysate fluid. Lastly, in support of some role for aluminum in AD, an amino acid sequence identical to that of β-amyloid in AD has been derived from the aluminum-containing tangles of Guam-Parkinsonian dementia (Guiroy, 1987; Perl, 1982).

In contrast, attempts to treat AD with agents that chelate aluminum have not been successful (Whitehouse, 1985). Some investigators (Markesbery, 1981) have not found an increased level of aluminum in AD. It is not clear, whether the alterations in brain levels of aluminum or other metals reported in AD (Ehmann, 1982) represent a primary etiologic factor or a secondary accumulation or depletion after the
initial pathophysiological events. Many other compounds, including those found in numerous industries, cigarette smoke, and coffee, as well as moderate alcohol consumption and use of analgesics have been suggested to play some role in AD, but findings are controversial (Amaducci, 1986; Chandra, 1986; Heyman, 1984).

Head trauma: The documentation of posttraumatic dementia and the finding that trauma may cause NFTs (Rudelli, 1982; Corsellis, 1973), have implicated head injury as a possible risk factor for AD. Epidemiological studies have indicated an increased risk of developing AD after significant head trauma in some reports (Heyman, 1984; Mortimer, 1985; Shalat, 1986), but not in others (Bharucha, 1983; Amaducci, 1986; Chandra, 1987). Interestingly, dementia is found in professional boxers (pugilistic dementia), and there may be a latent period of years between the end of their active career and the development of dementia, which is characterized neuropathologically by widespread NFTs (Corsellis, 1973, 1978). The proposed mechanisms by which head trauma could initiate AD include damage to the BBB permitting the leucocytes to come in contact with brain antigens and to trigger an autoimmune response (Mortimer, 1985). In contrast, trauma may directly cause axonal damage, with cytoskeletal accumulation of abnormal structural proteins (Henderson, 1989).
Metabolic abnormalities:

Protein synthesis and metabolism are disturbed in cortices of AD patients (Sajdel-Sulkowska, 1983). Amounts of mRNA, total protein and cytoplasmic RNA are abnormal (Mann, 1985; Doebler, 1987, 1988). Accompanying morphological changes, such as dendritic bluntness in dentate gyrus granule cells and the significant atrophy of neocortical and hippocampal neurons, may reflect deafferentation and decreases in many RNA species (Mann, 1985; Flood, 1987; Doebler, 1987; Coleman, 1987).

A variety of other metabolic abnormalities have also been reported, which has led to the suggestion that AD may be a generalized disorder in which several systems are affected by common metabolic defects (Blass, 1984), including those arising from carbohydrate metabolism. Abnormalities reported in nonneuronal tissues include altered oxidative metabolism and calcium homeostasis in cultured skin cells or lymphocytes, and membrane abnormalities in red blood cells and in platelets (Zubenko, 1987; Baker, 1988; Balin, 1988; Deary, 1987). The specificity of these changes is not known yet. Some suggest that a genetic defect leads to abnormalities in cellular metabolism that causes the premature degeneration and death of vulnerable cells in the AD brain (Blass, 1990).

Immune hypothesis:
Indications that the immune system may be impaired in old age have led to the search for an immunological deficit in AD. Abnormalities in both humoral and cellular immune activity have been reported in AD patients, including a decrease in specific immunoglobulins (Eisdorfer, 1978). Although not consistently confirmed (Miller, 1981), a decrease of natural killer cell activities (Kraus, 1984), and the presence of antibrain antibodies in serum of AD patients does support the possibility that AD is an autoimmune disorder (Nandy, 1978). However, increased immunosuppression has also been found (Miller, 1981). Case control studies linking AD with alterations in the immune system, found an association with a family history of lymphoma, lymphosarcoma, or Hodgkin’s disease (Heston, 1981) but no association with allergies, arthritis, asthma, tonsillectomy, or leukaemia (Bharucha, 1983; Heyman, 1984; Amaducci, 1986; Chandra, 1986).

Neurotrophic factors:

Depletion or impairment of neurotrophic hormones could be a causative factor in AD (Apple, 1981). These neurotrophic hormones are produced or stored in hippocampal or cortical tissues, and normally exert effects in retrograde fashion on projections originating in the basal nuclei or medial septal regions (Appel, 1981; Ojika, 1983). For example, nerve growth factor is transported from the cortex to neurons in the basal forebrain, where the ChAT activity is subsequently
increased (Seiler, 1984; Gnahn, 1983). A hippocampal peptide that specifically enhances survival and cholinergic activity of medial septal neurons in vitro has also been reported (Appel, 1984). According to this hypothesis, when the neurotrophic factor is not sufficient, forebrain cholinergic neurons show structural abnormalities and then degenerate. It seems this hypothesis is still far from explaining the nature of AD.

2.2.5. Key Questions and Considerations in the Study of AD

Significant progress has been made in refining diagnostic criteria for and in approaching the nature of AD, however, no definitive diagnostic test is available yet, and the etiology of the disease remains an enigma. Controversy and uncertainty exist in all discussions of the pathogenesis. Some of the key questions and considerations regarding the neuropathology of AD include:

First, the definition of AD is still uncertain, and the diagnostic criteria currently used are neither standardized nor reliable, because of the lack of a biological marker. Second, the expression of AD is enormously variable, and heterogeneity is reported in almost every study. It is not known whether these variations are qualitative subtypes representing different basic etiologies or whether they
represent different quantitative expressions of the same
disease process. Third, the considerable overlap of AD with
aging and with other diseases, suggests that AD may not be a
single disease, like Huntington’s disease, but may represent
a response of the human brain to a number of specific
etiological factors. There may be a highly complex interplay
of two conditions: an age-related pathological process, like
atherosclerosis, which occurs to a limited extent in virtually
all humans over time, and expression of some genetic
predisposition, triggered by different environmental factors,
which breaks down the usual control mechanisms and leads to a
premature and accelerated brain degeneration.

To resolve some of the conceptual obscurity would require
a comprehensive approach to the study of AD. This would
ideally include large sample size, careful choice of controls,
estensive longitudinal design, standardized procedures and
multidisciplinary investigating (Spinnle,1987; Henderson,1986;
Gauvreau,1989; Khachaturian,1989). The IMAGE project, which
this thesis is a part of, incorporates a multimatrix model and
a longitudinal design on a well-defined population, and
therefore appears eminently suited for investigating the
interaction of genetic and environmental factors in the
development of AD.
3. AIMS OF THE PROJECT

Within the IMAGE project, a neuropathological core is required to provide a reliable morphological and morphometric data base which can eventually be correlated with the epidemiology and molecular biology of AD. Three specific aims are pursued:

1) It is necessary to first develop a standardized technical protocol and to validate the neuropathological diagnostic criteria for AD, by comparing results from AD and nondemented controls. Since there is currently no systematic Canadian approach to this problem, the morphometric protocol is standardized along the lines suggested by CERAD and the Institute for Basic Research in Developmental Disabilities, so that our results could be compared with those of leading American centers.

2) Our phase of the project will identify morphological subtypes of AD, which will then be matched with neurological and neuropsychological ones for better understanding of phenotypical subtypes, and further exploration of possible etiological subtypes.

3) We will try to differentiate between morphological alterations in the brain during normal aging, and the earliest phase of AD, and possibly to define the evolution and staging of AD pathology. This would require a significant number of cases where the patient died of another cause during an early phase of AD.
4. WORKING HYPOTHESIS

Marked variability in expression of AD has been reported by many investigators (Mayeux, 1985; Bondareff, 1987; Iversen, 1987; Bird, 1989; Friedland, 1988; Anderson, 1989). We propose that there is a combination of different etiological factors contributing to the neurodegeneration in AD. The variable interaction of these factors could produce the heterogeneous expressions of AD, which are distinguishable and measurable both morphologically and symptomatically.

Investigating the clinical and biological heterogeneity may reveal homogeneous subgroups, and correlations among these subgroups might indicate presumptive etiological categories. We further propose that morphological subgroups may indicate the evolitional patterns of brain lesions, and correspond with the symptoms, in contrast to morphological patterns of normal aging. The morphological subgrouping could also provide some basis for possible treatment.
5. RATIONALE

In summary, important factors to be considered in the design of a study on AD include: a) the disease definition is ambiguous, and the clinical and pathological diagnosis is not absolutely reliable, b) heterogeneity in disease expression is reported in almost every domain of the studies, c) multiple etiological factors, along with genetic predisposition and aging seem involved in the development of the disease, d) no experimental model is available yet. Therefore a multidisciplinary and longitudinal approach on a well defined population should provide the best chance for revealing various etiological factors and assessing their contribution in the pathogenesis. The IMAGE project is so designed. It has a well documented target population in which to investigate the evolutorial pattern of the disease. The interaction of genetic and environmental factors can be examined through the very large families with affected members from different generations, who are sometimes separated geographically. A neuropathological study within the IMAGE project would be able to define morphological subgroups and disease evolution, which could be meaningfully correlated with neurological or neuropsychological characteristics.
6. EXPERIMENTAL DESIGN

6.1. MATERIAL

The pathological studies use human brain specimens from individuals with "probable or possible" DAT (Mckhann, 1984) provided by the IMAGE project. Control specimens from non-demented individuals are collected by collaborating with the Douglas Hospital Research Centre Brain Bank. The criteria used by IMAGE (Gauvreau, 1989) to screen the brain specimens and to submit them for neuropathological study are mainly adopted from those widely used and recommended by NINCDS-ADRSA (Mckhann, 1984). Currently 135 brains provided by the IMAGE project, and 49 brains collected by the Douglas Hospital Research Centre Brain Bank have been involved in the study.

Because of the restriction in the budget, and the difficulty in collecting control brains, we were not able to have all control brains come from the same targeted population. It would have made the morphometry more powerful and informative, if we had been able to do so.

6.2. METHODS

The major methods employed in the study are 1) autopsy on the brains 2) histological staining of the brain specimens following a specific protocol designed for maximum
standardization with other on-going major studies; 3) quantitative morphometry on selected brain specimens. Most autopsies on these brains were carried out by Dr. Cholette at Chicoutimi General Hospital, and the neuropathological study was supervised by Dr. Robitaille at the Montreal Neurological Institute.

6.2.1. Brain Autopsy Techniques

Within the IMAGE project, brain autopsies were performed as soon as possible after death, and the exact time postmortem was recorded. The major procedures within the specific protocol were as follows:

1) External examination of the brain which involved dissecting the arachnoid on the midline and along both sylvian fissures to inspect the vasculature.

2) Dissection of the brain, which included the following steps: a) One hemisphere was cut into 1 cm thick coronal sections rostro-caudally, fast frozen in isopentene cooled on dry ice, and then kept in air-tight bags at -70°C; b) The other hemisphere was placed in a solution of 10% buffered formalin for fixation. If one hemisphere exhibited greater gross pathology it was selected for fixation, otherwise, sides were chosen alternately for freezing or formalin fixation from specimen to specimen, in order to allow for morphometric analysis of lateral differences.
3) Fixation of the brain, by immersion of specimens in formalin for at least 5 weeks, in order to stabilize brain volume and weight (Leibnitz, 1971).

4) Division of the brain, according to the following sequence: a) a coronal section was made 1 cm anterior to the optic chiasm, and another, 1 cm anterior to the insertion of the pineal gland; b) the block in the middle of the three was cut into 2 halves; c) 1 cm thick cross-sections were made on the other two blocks rostro-caudally; d) the cerebellum and brain stem were cut into 0.5 cm thick slabs.

6.2.2. Histological Techniques

Portions of the fixed brain were processed using the following techniques:

1) Sections were selected from the vulnerable areas of the brain in AD (Blessed, 1968; Wilcox, 1982), and isocortical association areas, in order to correlate our data with the neuropsychological studies. The list of sections was as follows:

   a) The hippocampus including Ammon's horn, the parahippocampal and fusiform gyri (Brodmann's areas: 20, 28, 34, 35, 36), was represented by 4 sequential coronal sections from the rostro-caudal axis of the posterior aspect of Ammon's horn, localized by its presence beneath the lateral geniculate
b) Frontal lobes including orbital area (Brodmann’s areas: 9, 10, 45, and 11, 12) were represented by 3 coronal sections.

c) Parietal lobes including superior and inferior lobules (Brodmann’s areas: 7, 40, 19 and 49) were represented by 3 coronal sections.

d) Thalamus, midbrain, cerebellar cortex and dentate nucleus were represented by 1 section each. Additional sections were taken, if lesions were observed lying outside of the 13 areas routinely sampled in the protocol.

2) Sections were obtained from the selected areas by dissection in situ with a sharp scalpel, instead of a large Virchow knife, to avoid excessive architectural distortions. These were paraffin-wax-embedded, and were later cut into 8 micron histological sections.

3) Sequential histological sections from each wax block were stained with Hematoxylin and Eosin (HE), Modified Bielschowsky (MB) and Alkaline Congo Red (ACR) respectively. Other stains, such as Holder and Bodian were added, when required. The sections were stained according to standard procedures (McManus, 1960; Adams, 1965). Some considerations in choosing the stains were:

a) The HE stain, which has been widely used in
neuropathology, was employed for basic purposes: to detect major lesions, to exclude those dementing conditions other than AD, and to serve as a reference for analysis of the sections processed by other stains.

b) The MB stain, as we have experienced, and as suggested by others (Khachaturian, 1985; Yamamoto, 1986), was found to be more sensitive for demonstrating NFTs and SPs than Sevier-Munger and Thioflavin S preparations. Previous studies have often found poor agreement between the clinical and morphological diagnosis (Tarn, 1988; Huppert, 1986), due to the lack of satisfactory standardized processing and staining techniques used among neuropathologists. The problem can be minimized by use of the MB stain which will detect maximum densities of the NFTs and SPs, as well as the early changes in non-demented individuals (Tomlinson BE, 1980; Katzman R, 1988; Wisniewski H, 1989). This range of sensitivity is essential for further quantitative morphometry.

c) The ACR stain is considered superior for demonstrating amyloid-laden vessels and amyloid deposits in the tissue when compared to other stains, such as HE, periodic acid-Schiff, toluidine blue, crystal violet and thioflavin S or T (Stokes, 1973; Cooper, 1974; Puchtler, 1962). Use of the ACR stain would make both qualitative and quantitative analysis possible, so that sections stained by ACR could be used as a quality control for analysis of the sections processed by MB stains and for future morphometry. Positive
detection of CAA was defined as yellow-green birefringence under polarized light.

6.2.3. Morphometric Techniques

The morphometric analyses were made on the section samples prepared with MB stain by two investigators (a third joined, commencing on specimen PI 01-88) in a blind fashion. Results used for the final analysis were chosen by the chief investigator, Dr. Robitaille. In the majority of cases there were no significant differences between results determined by various investigators. Where they differed, the samples were reexamined and an agreement was reached and confirmed by the chief. The major processes and criteria were as follows:

a) All slides were first thoroughly screened in order to identify the areas with maximal pathological changes, and to examine intraneuronal neurofibrillary degeneration and SPs under 10 x eyepiece graticules calibrated with a Leitz micrometric rule.

b) The densities of NFTs and SPs were determined in regions with maximal pathological changes, within hippocampal sector 1, subiculum, pre- or para-subiculum, fusiform gyrus, frontal cortex, cingulate gyrus, and parietal cortex. Based on preliminary findings, an adjustment in the protocol was thought necessary, to distinguish between the lateral frontal and orbital frontal cortex, and inferior and superior
parietal cortex, beginning with specimen PI01-89. These locations are illustrated in Figure 1.1.

c) The intraneuronal NFTs were counted under a 25 x objective within 3 separate 0.3cm quadrants in 3 adjacent high power fields. The SPs were counted under a 10x objective within 2 adjacent 0.5cm wide quadrants.

d) The NFT/SP ratios in the 3 areas of hippocampus and 6 areas of iso-cortex were determined, and all data from each specific area, were recorded in the IMAGE morphometric data sheets.

e) The minimal diagnostic criteria for AD were set at 20 NFTs/mm$^3$ and 10 SPs/mm$^2$, found in at least one of the hippocampal and isocortical sections (Ball, 1988).
7. PROCESSING AND RESULTS

7.1. Autopsy and Histopathology

Presently we have examined 135 abnormal brains with "probable" or "possible" AD (McKhann, 1984; Gauvreau, 1989) collected during 4 years by the IMAGE network, and 49 control brains from non-demented individuals without neurological problems. The brain autopsies, the histological preparations and the pathological diagnoses were performed on all these specimens according to the IMAGE protocol briefly described above. General information on the samples obtained from the IMAGE project is itemized in Table 1. Table 2 provides comparable data on the control brains.

The pathological status of the 135 abnormal brains can be summarized as follows:

AD was confirmed in 112 of the samples (Figure 2.1). Of the remaining 23 brains which were rejected from the AD category, 11 were classified as cases of cerebrovascular dementia (CVD) based on the following criteria (De Reuck, 1980; Fields, 1986; Cummings, 1991). NFTs and SPs were either absent or so infrequent as to be below the diagnostic level for AD; no pathological changes associated with any other neurological disorder were evident; and significant cerebrovascular changes were present. These included small vessel arteriosclerosis,
atherosclerosis, multiple infarcts (MI) of varying size in cortical and or subcortical areas, and/or white matter degeneration. The remaining 12 samples were defined as "others", and consisted of 1, Down's syndrome; 1, progressive supra-nuclear palsy; 1, adult polyglucosan body axonopathy; 1, brain tumour; 1, Wernicke-Korsakoff disease; 1, Corticodentatonigral degeneration with neuronal achromasia; 1, Multiple Sclerosis; 1, non-communication hydrocephalus; 2, non-specific senile changes, and 2, Parkinsonian dementia. Those samples defined as others or CVD were excluded from the morphological study of AD according to the IMAGE protocol.

The abnormal morphological features found in the 112 AD brains showed extensive variation, both quantitatively and qualitatively. All brains contained the characteristic markers, NFTs and SPs, but heterogeneity was seen in the following aspects:

a) presence or absence of additional abnormalities, such as LBs, or vascular pathology, mainly MI;

b) the densities of the NFTs and SPs;

c) the distribution patterns of NFTs and SPs, and the ratio of NFT/SP;

e) the development of NFTs and SPs, in terms of the progression in the hippocampus and neocortex throughout the duration of the disease.
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Std: 14.3 3.5 7.5 154.3 6.1

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f:80 L:63
Total: 135 Unkn:2

unkn: unknown
std: standard deviation
N/A: information was not available.
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<td>1160</td>
<td>7</td>
<td>Pneumonia</td>
</tr>
<tr>
<td>DH-476</td>
<td>m</td>
<td>73</td>
<td>1160</td>
<td>7</td>
<td>COPD</td>
</tr>
<tr>
<td>DH-488</td>
<td>f</td>
<td>86</td>
<td>1145</td>
<td>6</td>
<td>Fracture femur</td>
</tr>
<tr>
<td>DH-562</td>
<td>f</td>
<td>67</td>
<td>1600</td>
<td>11</td>
<td>Hypert.heart dis</td>
</tr>
<tr>
<td>DH-563</td>
<td>m</td>
<td>67</td>
<td>1140</td>
<td>6</td>
<td>Myoc.infarct</td>
</tr>
<tr>
<td>DH-564</td>
<td>m</td>
<td>61</td>
<td>1305</td>
<td>9</td>
<td>Myoc.infarct</td>
</tr>
<tr>
<td>DH-582</td>
<td>f</td>
<td>80</td>
<td>1235</td>
<td>11</td>
<td>Fracture hip</td>
</tr>
<tr>
<td>DH-616</td>
<td>f</td>
<td>86</td>
<td>1060</td>
<td>5</td>
<td>Fracture hip</td>
</tr>
<tr>
<td>DH-622</td>
<td>m</td>
<td>66</td>
<td>1295</td>
<td>7</td>
<td>GI bleeding</td>
</tr>
<tr>
<td>DH-627</td>
<td>m</td>
<td>78</td>
<td>1545</td>
<td>10</td>
<td>Emphysema</td>
</tr>
</tbody>
</table>
Average 67.79 1328.1 10.01
Std. 11.58 120.16 6.33
Total 49 M:29 F:20

N/A: Information was not available.
Std.: standard deviation
Coronal sections of the human brain, from anterior to posterior, are illustrated in diagrams 1 to 4, respectively. The relevant areas sampled are: a, orbital frontal cortex, b, lateral frontal cortex, c, cingulate gyrus, d and e, hippocampi, f, superior parietal cortex, g, lateral parietal cortex, and h, fusiform gyrus.
Fig. 1.2

Distribution of the Samples
according to the age of death

The ages of IMAGE samples and the controls

<table>
<thead>
<tr>
<th></th>
<th>IMAGE</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>135</td>
<td>49</td>
</tr>
<tr>
<td>Sex</td>
<td>m:55 f:80</td>
<td>m:29 f:20</td>
</tr>
<tr>
<td>Average Age of Death</td>
<td>77.68 std.7.4</td>
<td>67.79 std.11.5</td>
</tr>
<tr>
<td>T-test for the two</td>
<td>t=0.08 p&gt;0.05</td>
<td>no significant difference</td>
</tr>
</tbody>
</table>
Fig. 2.1  Pathological Diagnosis
135 brains /w "Probable/Possible" AD

Others(12) (8.9%)
CVD(11) (8.1%)
AD(112) (83.0%)

Fig. 2.2  Morphological Subgrouping
112 brains /w pathol. confirmed AD

AD subgroup 4(17) (15.2%)
AD subgroup 3(26) (23.2%)
AD subgroup 1(55) (49.1%)
AD subgroup 2(14) (12.5%)
Based on these findings, we primarily divided the AD brains into 4 subgroups as follows: a) Brains which showed a varying number of NFTs and SPs distributed diffusely, with a predilection for the hippocampus, orbital frontal and inferior parietal lobes and fusiform gyrus were defined as subgroup 1. b) Brains which showed a low density of NFTs, mostly confined to the hippocampus, along with a high density of SPs, particularly in the neocortex, were defined as subgroup 2. c) Brains showing a moderate density of NFTs and SPs in the hippocampus, with sub-threshold lesions in some other sampled areas, plus LBs of varying intensity detected in cortices and/or SN were defined as subgroup 3. d) Brains showing the same morphological pattern of NFTs and SPs as those of subgroup 1, with a significant vascular component, such as extensive cerebrovascular atherosclerosis, hyalin arteriosclerosis, and/or multiple infarcts of varying size in the cortex and/or subcortex were defined as subgroup 4. In the few cases where a sample showed characteristics of more than one subgroup, (for example, 3 brains had predominant SPs in the neocortex, which would indicate subgroup 2, as well as significant vascular pathologies, which would indicate subgroup 4), we considered SPs to be of primary importance and the vascular component as a relatively distinct and concomitant process, and tentatively put these under subgroup 2 for the morphometric statistics. The proportion of samples in each subgroup is illustrated in Figure 2.2, and additional
information is listed in Table 3.

7.2. MORPHOMETRY

In order to further analyze these four morphological subgroups defined primarily by major pathological features, morphometry was performed on all 112 AD brains, 11 brains with CVD, and all 49 controls, according to the IMAGE morphometry protocol described above. Lesions in 9 representative areas from each brain were counted, then the statistical analysis was performed for each proposed AD subgroup, CVD and controls. We calculated:

a) the densities of NFTs and SPs in the 3 hippocampal and 6 cortical areas,

b) the ratios of NFTs/SPs in the hippocampus and neocortical areas,

c) the development of NFTs and SPs in the hippocampus and neocortex with increasing duration of the disease,

d) the average accumulating rate of the lesions after the onset of the dementias.

Comparisons of the data among the 4 subgroups and between the AD subgroups and the CVD or the controls were based on the two-tailed t-test. The "r", Pearson correlation coefficient of the correlation between the lesions and the duration(year) of the disease, and the p value were calculated with the statistical analysis system(SAS program, SAS institute, NC,US, 1986).
Table 3. GENERAL INFORMATION ON THE 4 SUBGROUPS and CVD

<table>
<thead>
<tr>
<th></th>
<th>Subgroup 1</th>
<th>Subgroup 2</th>
<th>Subgroup 3</th>
<th>Subgroup 4</th>
<th>CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>55</td>
<td>14</td>
<td>26</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Age of Onset(yr)</td>
<td>Avg. 69.33</td>
<td>75.46</td>
<td>70.5</td>
<td>75.01</td>
<td>71.09</td>
</tr>
<tr>
<td></td>
<td>Std. 7.71</td>
<td>7.24</td>
<td>7.6</td>
<td>5.4</td>
<td>7.9</td>
</tr>
<tr>
<td>Duration(yr)</td>
<td>Avg. 6.53</td>
<td>6.74</td>
<td>6.5</td>
<td>6.0</td>
<td>4.47</td>
</tr>
<tr>
<td></td>
<td>Std. 3.8</td>
<td>3.7</td>
<td>3.8</td>
<td>3.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Brain wgt(g)</td>
<td>Avg. 1073.18</td>
<td>1104.0</td>
<td>1123.3</td>
<td>1117.4</td>
<td>1089.63</td>
</tr>
<tr>
<td></td>
<td>Std. 147</td>
<td>187</td>
<td>157</td>
<td>156</td>
<td>115</td>
</tr>
<tr>
<td>Post-mortem Delay(hr)</td>
<td>Avg. 8.75</td>
<td>8.85</td>
<td>9.97</td>
<td>8.9</td>
<td>8.5</td>
</tr>
<tr>
<td></td>
<td>Std. 6.3</td>
<td>4.6</td>
<td>7.1</td>
<td>5.1</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Avg.: average, Std: standard deviation, wgt: weight, CVD: cerebrovascular dementia
Legend for Graphs 3.1-3.6: Mean densities of NFTs and SPs are shown for the following brain areas, from left to right, hippocampal sector 1, subiculum, parasubiculum, fusiform gyrus, lateral frontal, orbital frontal lobes, cingulate gyrus, superior parietal and lateral parietal lobes.
Fig. 3.1 The Distribution Pattern of NFTs & SPs
in Subgroup 1 ("classic")

The Distribution Pattern of NFTs & SPs
in Subgroup 2 (SPs predominate)
Fig. 3.3 The distribution pattern of NFT & SP in Subgroup 3 (with LBs)

Fig. 3.4 The Distribution Pattern of NFT&SP in Subgroup 4 (with MI)
Fig. 3.5  The Distribution Pattern of NFTs & SPs

![Graph showing the distribution pattern of NFTs & SPs in CVD.]

Fig. 3.6  The Distribution Patterns of NFTs & SPs

![Graph showing the distribution patterns of NFTs & SPs in the controls.]

Legend:
- NFT (Left)
- SP (Right)
Fig. 4  Mean Densities of NFT&SP

4 subgroups, vascul. and the controls

Mean Density $n/mm^2(NT)$

<table>
<thead>
<tr>
<th>subgroup1</th>
<th>subgroup2</th>
<th>CVD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n: 55</td>
<td>n: 14</td>
<td>n: 11</td>
<td>n: 49</td>
</tr>
</tbody>
</table>

Values were calculated from 9 representative areas within the hippocampus and neocortex. The exact value of each mean density and p value can be found in Tables 4 and 5.
The distributions, mean densities and ratio of NFT/SP

The NFTs and SPs in the 9 representative areas of each brain were counted. Mean densities and the ratios were calculated and the results were graphed for each proposed subgroup (Figures 3.1 to 3.6, 4, Tables 4 and 5).

The results showed that:

a) There were great differences between the histological features of brains with AD and non-demented control brains (p<0.001) or brains with CVD (p<0.001), in terms of the mean densities of NFTs and SPs.

b) There was a common feature shared by all 4 subgroups: the hippocampus, and the inferior parietal and orbital frontal lobes had the highest density of NFTs, while neocortices, particularly, inferior parietal, orbital frontal lobes and fusiform, as well as parasubiculum possessed the highest density of SPs.

c) There were significant differences between the 4 morphological subgroups, in terms of the mean densities, the ratio of the lesions, and the vulnerable areas in the brains.

--- subgroup 1 and subgroup 4 showed the same distribution pattern of both NFTs and SPs. Although mean densities of the lesions were lower in subgroup 4, this difference was not significant.

--- subgroup 2 had a distribution pattern of SPs that was similar to subgroups 1 and 4, but a significantly lower mean
NFT density \((p < 0.001\) and \(p < 0.005\)), with the ratio of NFT/SP being significantly lower \((p < 0.05)\) than that of the other three.

--- subgroup 3 showed a significantly lower mean NFT density than that of subgroups 1 \((p < 0.001)\), and 4 \((p < 0.01)\), as well as a significantly lower mean SP density, than that of subgroup 1 \((p < 0.005)\), subgroup 2 \((p < 0.005)\) or subgroup 4 \((p < 0.01)\).

7.2.2. The progression of NFTs and SPs

The brain samples in each subgroup were then analyzed with respect to the duration of the disease, by comparing average values from clusters of 3 consecutive years, or 4 consecutive years when the sample size was too small. The mean densities of the lesions in the hippocampus and neocortical areas were divided by the respective average durations, in order to examine the development of the lesions with time after the onset of the dementia. In addition, the mean densities of the lesions in hippocampal and neocortical areas of each morphological subgroup were divided by the average duration for that subgroup as a whole to compare the accumulation rates of the lesions after the onset of the disease.

Great variety in development of the lesions was found among the 4 subgroups, and also between the AD subgroups and CVD or the controls (Figures 5.1 to 5.7, 6, Tables 4 and 5).
Fig. 5.1 The development of NFT&SP in Subgroup 1

Fig. 5.2 The development of NFT&SP in Subgroup 2
Fig. 5.3 The development of NFT&SP in Subgroup 3

Fig. 5.4 The development of NFT&SP in Subgroup 3a
Fig. 5.5 The development of NFT&SP in Subgroup 4

![Graph showing the development of NFT&SP in different durations]

Fig. 5.6 The development of NFT&SP in CVD

![Graph showing the development of NFT&SP in different durations]
Fig. 5.7

Development of NFT & SP in the Controls

different age group (yrs)

Fig. 6

The average accumulating rates

of NFT and SP

Mean NFT/mm³ or SP/mm² developed over yr

Subgroup1  Subgroup2  Subgroup3  Subgroup4  Vascular Dementia  Control

In each pair of columns: NFT(L) SP(R)

Hippocampus  Cortices
The results demonstrated that:

a) Subgroups 1 and 4 had a similar progression pattern and similar highest average accumulation of both lesions in the hippocampus and neocortex, (Figures 5.1, 5.5, 6), although the latter was a bit less aggressive.

b) Subgroup 2 showed an asynchronous progression of the two lesions, with little change in NFT density over the years in both hippocampus and neocortex, and the lowest average accumulating rate of NFTs (Figures 5.2, 6), but a large increase in the density of SPs, with time, and an average SP accumulating rate similar to that seen in subgroup 1 (Figures 5.2, 6, Table 4).

c) Subgroup 3 had a heterogeneous progression and lower average accumulating rate for both lesions than subgroups 1 and 4 (p<0.05). When subgroup 3 was further subdivided by excluding samples with LBs seen only in SN, the remaining samples having LBs in both SN and cortex showed a similar progression with time for both NFTs and SPs in both hippocampus and neocortex, resembling subgroups 1 and 4 (Figures 5.3, 5.4, 6, Table 4).

d) In the CVD group both lesions were nonaggressive throughout the entire duration, while the controls showed a small increase in lesion density with time. The average accumulating rates in both CVD and the control were significantly lower than in the AD subgroups (Figures 5.6, 5.7 6, Table 5).
When samples with classical Alzheimer pathology, ie, subgroup 1, were further divided by onset of dementia before (presenile) or after (senile) age 65, there were two differences between them. The samples with onset after age 65 showed a continual decrease in the average accumulating rates with time, while those with earlier onset showed an initial rise, then a decline (Figure 7). Late onset dementia also had a higher ratio of females to males (Figure 8).

When all 112 AD samples were classified by age of onset of dementia, all four subgroups were found in the senile category, while only subgroups 1 and 3 were found before the age of 65 (Figures 9).

7.2.3. Summary of the morphology

The main morphological features of each subgroup, CVD and the control have been listed in Tables 4 and 5. Analysis of the classical morphological features of AD revealed a heterogeneous expression in our brain samples, as listed and graphed above. We detected 4 subgroups, which are morphologically distinct from each other. Their lesions differ in a) characteristic histological pattern, b) mean densities, c) distribution and predilection, and d) progression.

Subgroup 1 has the classical pathological features of AD,
as described previously. It is morphologically distinguished from others by its wide distribution of NFTs and SPs with varying density, in both hippocampus and neocortex. It has the highest mean densities of both lesions. NFTs are most prominent in the hippocampus, inferior parietal and orbital frontal lobes, and SPs have highest densities in the parasubiculum, inferior parietal and orbital frontal lobes and the fusiform gyrus. This subgroup also has the fastest average accumulation rate of both NFTs and SPs in every vulnerable brain area sampled, and the numbers of both lesions increase in proportion to the duration of the disease. All our samples with pre-senile onset DAT fall in this group.

Subgroup 2 distances itself morphologically from others by the great predominance of SPs, particularly in the neocortex. It has a mean density and topographic distribution of SPs similar to subgroup 1, but a much lower mean density of NFTs in all sampled areas. Although the hippocampus also shows the greatest involvement of NFTs, the progression of NFTs is not significant, in terms of the average accumulating rate in both hippocampus and neocortex, nor is it consistent with the duration of disease. In contrast, the progression of SPs is as fast as that in subgroups 1 or 4 in both hippocampus and neocortices, and consistent with the duration of the disease. All in this group had a senile onset of DAT.
Subgroup 3 characterizes itself morphologically by the LBs present in SN and neocortices, mainly in the mesolimbocortical system, as well as the low indices of NFTs and SPs, which are little above the diagnostic level in most sampled areas. Mean densities of NFTs are significantly lower than in subgroups 1 or 4, and SP densities are significantly lower than in all other 3 subgroups. The progression of both NFTs and SPs is slow, with significantly slower average accumulating rates in both hippocampus and neocortices; however it is consistent with the duration of the disease. Excluding the samples with LBs present in SN only, but not in neocortices, leaves samples with LBs present in both areas, and this population shows a homogeneous progression pattern for both lesions, similar to subgroups 1 and 4. Progression was also consistent with the duration of the disease.

Subgroup 4 differentiates itself morphologically from others mainly by its vascular component, including multiple infarcts in the cortex, lacunae in subcortical areas, and rarefaction in white matter with paravascular gliosis. Other pathological features are quite similar to that of subgroup 1, including the mean densities, the topographic distribution, the evolution of NFTs and SPs, the ratio of NFT/SP, and the progression pattern of the lesions in terms of the average accumulating rates in hippocampus and neocortex. However, progression was inconsistent with the duration of the disease.
<table>
<thead>
<tr>
<th>Table 4. SUMMARY OF THE MORPHOLOGY OF THE 4 SUBGROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subgroup</strong></td>
</tr>
<tr>
<td><strong>Number</strong></td>
</tr>
<tr>
<td><strong>PATHOLOGICAL CHARACTERISTICS</strong></td>
</tr>
<tr>
<td>NFT &amp; SP</td>
</tr>
<tr>
<td><strong>DISTRIBUTION</strong></td>
</tr>
<tr>
<td>NFT</td>
</tr>
<tr>
<td>SP</td>
</tr>
<tr>
<td><strong>MEAN DENSITIES</strong></td>
</tr>
<tr>
<td>NFT</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>SP</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>EARLIEST &amp; MOST INVOLVED AREAS</strong></td>
</tr>
<tr>
<td>NFT</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>SP</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>PROGRESSION</strong></td>
</tr>
<tr>
<td>Average accumulating rate (NFT/mm² or SP/mm² per year)</td>
</tr>
<tr>
<td>NFT (mean)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>(hip; cort)</td>
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<tr>
<td>SP (mean)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>(hip; cort)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Ratio NFT/SP</strong></td>
</tr>
<tr>
<td>hip:</td>
</tr>
<tr>
<td></td>
</tr>
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<td>cort:</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>CORRELATION WITH THE DURATION</strong></td>
</tr>
<tr>
<td>NFT</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>SP</td>
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</table>

Table 5. SUMMARY OF THE MORPHOMETRY OF CVD AND THE CONTROL

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<th>CONTROL</th>
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<tr>
<td></td>
<td>11</td>
<td>49</td>
</tr>
</tbody>
</table>

**MEAN DENSITIES**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>NFT/mm³</td>
<td>3.41 std.2.1</td>
<td>1.49 std.1.09</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001(1,2,3,4)</td>
<td>p&lt;0.001(1,2,3,4)</td>
</tr>
<tr>
<td>SP/mm³</td>
<td>4.45 std.3.3</td>
<td>2.8 std.4.23</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001(1,2,3,4)</td>
<td>p&lt;0.001(1,2,3,4)</td>
</tr>
</tbody>
</table>

**EARLIEST & MOST INVOLVED AREAS**

<table>
<thead>
<tr>
<th></th>
<th>CVD</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFT</td>
<td>hip,</td>
<td>hip, ip, of</td>
</tr>
<tr>
<td>SP</td>
<td>hip,</td>
<td>ps, ip,</td>
</tr>
</tbody>
</table>

**PROGRESSION**

Average accumulating rate (n/mm³, or n/mm³ per yr)

<table>
<thead>
<tr>
<th></th>
<th>CVD</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFT (/mm³)</td>
<td>1.67 std.0.7</td>
<td>0.025 std.0.04</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001(1,2,3,4)</td>
<td>p&lt;0.001(1,2,3,4)</td>
</tr>
<tr>
<td>SP (/mm³)</td>
<td>1.34 std.1.2</td>
<td>0.01 std.0.03</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001(1,2,3,4)</td>
<td>p&lt;0.001(1,2,3,4)</td>
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</tbody>
</table>

**CORRELATION WITH THE DURATION**

<table>
<thead>
<tr>
<th></th>
<th>CVD</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFT</td>
<td>-, r=0.1 p&gt;0.05</td>
<td>+, r=0.73 p&lt;0.001</td>
</tr>
<tr>
<td>SP</td>
<td>-, r=0.12 p&gt;0.05</td>
<td>+, r=0.93 p&lt;0.001</td>
</tr>
</tbody>
</table>

ip: inferior parietal, of: orbital frontal, hip: hippocampus,
ps: parasubiculum, std.: standard deviation, correlation: two-tailed, (): the subgroup with which the data was compared, p: two-tailed, t-test. CVD: cerebral vascular dementia, r: Pearson correlation coefficient.
Fig. 7
Higher Female Prevalence
in Subgroup 1 with Same Onset

Fig. 8
Morphologic Subgroups in Samples
with Present or Same Onset
Presenile onset: n=15, average age of onset: 56.2 yrs old, average duration of the disease: 6.3 yrs.

Senile onset: n=40, average age of onset: 72.5 yrs old, average duration of the disease: 6.31 yrs.
8. ANALYSIS AND DISCUSSION

8.1. GENERAL CONSIDERATIONS

Heterogeneity of AD has been repeatedly suggested, and evidence documenting different subtypes is important, as it may lead to the consideration of ways in which such subtypes represent different basic etiologies. Subtypes could result from different diseases, or could reflect multiple risk factors in the development of AD. Knowledge of subtypes could eventually be used to construct different treatment strategies, or to monitor disease progression and indicate relevant therapy.

The subtypes of AD, which have been repeatedly reported and recognized by a few researchers, can be summarized in regard to the following categories: a) the age of dementia onset, with presenile or senile forms (Seltzer, 1981; Roth, 1978); b) the progression of the disease, with benign and malignant forms (Seltzer, 1983; Mayeux, 1985); c) the neurological or neuropsychological features, such as symmetrical or lateralized (Moossy, 1989; Grady, 1986), associated with or without myoclonus, depression, Parkinsonism, or other disorders (Mayeux, 1985); d) the inheritance, such as familial or sporadic forms (Folstein, 1981; Heston, 1981); e) metabolism, with various patterns or lateral
asymmetry of regional glucose use (Duara, 1986; Chase, 1984; Grady, 1986, 1987); f) neuropathological features, with various morphological criteria regarding selective neuronal loss or location of lesions (Brun, 1981; Bondareff, 1987) and various neurochemical criteria regarding selective neurotransmitter abnormalities (Perry, 1986; Rossor, 1988) and/or different immunocytochemical findings (Kosik, 1986 Selkow, 1986).

g) brain imaging, with asymmetry of lateral ventricle enlargement and/or varied rates of longitudinal change (Luxenberg, 1987).

Among these proposed forms of heterogeneity, there are some correlations between the subgroups found by different research specialities (Mayeux, 1985; Bondareff, 1987; Rossor, 1988), which seems to imply the existence of meaningful subtypes. However most of these studies have one or more limitations. Sample sizes are generally small. Individual samples are infrequent, and obtained at random from a highly variable population. There are no reliable and standardized protocols which are universally accepted for clinical, neuropsychological and neuropathological assessment of AD. The results of different research projects are mainly compartmentalized within each research speciality, and are often uninterpretable, or contradictory to those of other specialities, due to lack of collaboration and limited sampling. All of these problems produce considerable
difficulty in interpretation of the data and in further application of the results.

The morphological picture of AD can be briefly summarized. There is invariably a severe, probably primary involvement of the hippocampus and adjoining entorhinal area of the cortex, and anatomically correlated association cortices. Lesions have a highly regional and laminar distribution with clustering of the NFTs, which implies that pathological processes spread along well-defined groups of connections. There is also great variability, with regard to the intensity, the predilection, the progression pattern and other related changes. These and other research findings support the view that AD is not generated by any single presently proposed etiology.

8.2. CONSIDERATIONS IN CHOOSING THE MORPHOLOGICAL VARIABLES.

The first step in constructing morphological subgroups is to choose variables which can identity relatively homogeneous subpopulations. We considered several criteria in choosing the morphological variables for the primary subgrouping. The variables should be relatively specific to AD, or be associated with DAT (Selkoe, 1986; Henderson, 1989; Tomlinson, 1989; Davies, 1991). They should be common and comparably easy to detect and measure. They should be distinct from each
other in the morphological patterns they present. They should possess the ability to link certain clinical and pathological phenomena, which are involved in AD. Lastly, they should have the potential to implicate etiology or shape diagnostic or therapeutic strategy.

Therefore, we defined subgroups primarily by the morphological hallmarks of NFT and SP, and their characteristic distribution patterns and also related these to LBs and the vascular components. We then characterized these proposed subgroups by quantitative morphometry, calculating the densities, the selective topographic distributions, the developing rates and the evolution features of the lesions, for each group as a whole, as well as for the hippocampus and neocortex in each group, when sample size was sufficient.

The variables we chose for the four subgroups allow several important comparisons to be made. Firstly, the relative role of the two lesions can be examined by comparing subgroup 1, the "classical" AD, with high densities of both NFTs and SPs, to subgroup 2, with a relative predominance of SPs. Secondly, the relationship between AD and the two other major causes of dementia in the elderly can be evaluated, as subgroups 3 and 4 contain an overlap with PD and CVD, respectively, while subgroup 1 represents classical AD alone.
8.3. SIGNIFICANCE OF THE MORPHOLOGICAL SUBGROUPS

8.3.1. General considerations

No previous data derived from a multidisciplinary longitudinal project and specifically aimed at subgrouping the morphological features in AD has been published. Focusing on the morphological subgroups, we think that heterogeneous pathologies could be due to qualitative subtypes of the same disease, and future correlation between pathological and symptomatological subgroups could indicate different etiologies.

The subgroups could also represent independent disorders that share some common etiologies, to differing extents. Lastly, subgroups may simply reflect the developmental stages of the disorder, and unless pathology can be matched with symptomatology, the data can not be logically interpreted. Only when one of the causative influences is dichotomous, such as an autosomal dominant gene, rather than continuous or polygenic (Jorm, 1985), can a bimodal distribution of the symptomatology indicate a specific etiology.

A larger number of samples and further refinement by matching clinical and psychological subgroups will be needed in order to construct relatively homogeneous subtypes from our
data. These may in future indicate etiological and pathogenic factors. However, the significance of the subgrouping is already apparent, and even without these further analyses, the subtyping may be meaningful in terms of diagnostic or therapeutic applications.

8.3.2. Subgroup 1

Subgroup 1 represents the largest proportion (49.1%) of the samples of 112 pathologically and morphologically confirmed AD. The pathological picture mainly reflects the traditional findings (Alzheimer, 1906; Fisher, 1907; Kraepelin, 1910; Simchowicz, 1910), which are still accepted by most as the diagnostic criteria for AD (Brun, 1981; Khachaturian, 1985; Ball, 1988; Tomlinson, 1989; Mirra, 1991), although the specificity and sensitivity are being challenged. All 15 samples of traditionally labelled "AD with presenile onset", as well as 40 samples with onset later than age 65, fall in this subgroup.

Subgroup 1 showed the worst involvement of all vulnerable areas, in terms of the mean densities of both NFTs and SPs. It had the fastest development of both NFTs and SPs in both hippocampus and neocortices, extensively distributed SPs with fairly mature forms, and very disordered cortical cytoarchitecture with extensive gliosis in severe cases. These
findings, and the positive correlation between the densities of both lesions and the duration of the disease, constitute the distinct features of this morphological subgroup. Although the exact correlation between dementia and the densities of NFTs and SPs is still debated (Wilcock, 1982; Terry, 1987; Katzman, 1988; Hauw, 1989), the disease is nevertheless characterized by both the severity and the topographic distribution of these lesions (Terry, 1987; Henderson, 1989). Our data confirmed that NFTs and SPs could be used to quantitatively differentiate AD from non-demented aging and CVD.

8.3.2.1. Characteristics of NFTs and SPs

The presence of these pathological changes does not only have a diagnostic use, but also is fundamentally linked to the pathogenic process in AD. The real natures of both NFTs and SPs are not clear, but it is generally accepted that the NFTs represent an accumulation within the perikaryon and nerve terminals of proteinaceous filamentous material. This reduces the capacity for protein synthesis and interrupts axoplasmic flow (Sumpter, 1986; Kowall, 1987). Seen through the electronmicroscope, NFTs consist of clusters of unbranched fibrillary structures twisted into a helix, referred to as paired helical filaments (PHF) (Kidd, 1963; Hauw, 1989). Immunohistochemically, they possess some antigenic determinants shared by normal cytoskeletal elements and other
determinants not found in normal neuronal tissue. They contain neurofilament proteins, microtubule-associated proteins, such as tau and MAP2, A68 protein identified by the Alz-50 antibody, ubiquitin, and A4(β-amyloid) protein (Anderton, 1982; Iqbal, 1984; Glenner, 1984; Miller, 1986; Yen, 1987; Mori, 1987).

The SPs are discrete round or oval structures consisting of abnormal distended unmyelinated neurites with an amyloid core and inorganic material (Wisniewski, 1985; Kidd, 1963; Terry, 1964; Candy, 1986). They also contain PHF, as well as astrocytes, microglia and macrophages. In addition to these typical features, we have also noticed several different variants of SP morphologically. Some have a very compact amyloid core only, some are devoid of encompassing neurites, some have a cluster of a few abnormal neurites and reactive cells with no distinct central core, and others, have a fine network of delicate fibrillar or granular material, and seem to be devoid of the amyloid core or dystrophic processes. These variants are compatible with descriptions mentioned in other reports (Burger, 1973; Wisniewski, 1973; Dickson, 1988; Abraham, 1988) as "burnt-out", "immature", "very early" or "diffuse" SP respectively. Different types of SPs may be seen in the same brain sample, as reported by Braak (1989), and may be sensitive to different immunohistological or histochemical staining (Yamaguchi, 1988; 1989, Tomlinson, 1989).
These structural lesions are neither diffuse nor random. Regional and laminar selectivity and specificity are apparent. As mentioned by others (Hooper, 1976; Pearson, 1985; Hyman, 1986; Roth, 1986; Lewis, 1987) and demonstrated by our results, NFTs leave some large neurons unaffected, such as Purkinje cells of the cerebellum and giant Betz cells of the motor cortex. They mainly affect larger pyramidal neurons in neocortices, as well as hippocampi, parahippocampal areas and some subcortical structures, with a particular intensity in layer II, III and IV of the entorhinal cortex and layer III and V in the neocortex. Some of these areas are probably the earliest, and most severely involved. SPs are mainly seen in the same areas with greatest numbers in layers II and III of association cortices.

In the medial temporal lobe, selectivity and specificity are shown in that NFT-bearing neurons, consistent with conspicuous neuronal loss, are constantly found in the perforant pathway. This is a major input from the association cortex, transmitted at the entorhinal cortex into the hippocampal formation (Steward, 1976; Van Hoesen, 1976, 1982), and giving rise to the major hippocampal output (Van Hoesen, 1982; Rosene, 1977). The terminal zone of the perforant pathway, the molecular layer of the dentate gyrus (Hyman, 1986) and the output of the hippocampus, subicular complex, via the fimbria-fornix system, is severely populated by SPs. The morphological
changes are considered to severely disconnect the hippocampus from the rest of the brain, and are consistent with the neurobiological finding, a significant depletion of glutamate, the major neurotransmitter of the association cortex pyramidal neurons and the perforant pathway (Hardy, 1987; Hyman, 1987). Given the crucial role of these structures in memory (Hyman, 1986, 1987; Damasio, 1984; Murray, 1983), the morphological isolation of the hippocampus by depriving it of input and output constitutes a major mechanism of memory impairment.

In the neocortex, NFTs and SPs have a predilection for the association cortex. In contrast, the primary motor and sensory areas are almost totally spared. Regional and laminar vulnerability are evident, as NFT-bearing neurons are mainly those giving rise to axons forwarding to other association areas or feeding back to the sources of cortical and subcortical afferents (Pearson, 1985; Parnavelas, 1983). These morphological patterns are correlated with deficits (Davies, 1979; Hedreen, 1984; Forno, 1978; Mann, 1980; Curcio, 1984; D’Amato, 1987) in related neurotransmitter systems, and the neuronal loss in their origins. For example, the cholinergic system, which plays a role in memory functions (Bartus, 1982; Coyle, 1983) and its origin, nbM; the noradrenergic system, which plays a role in vigilance and selective attention (Mason, 1980), and its origin, LC; the serotonergic system
and its origin, brain stem raphe nuclei. Such findings imply that symptoms may result from cortical deafferentation, as association cortices are progressively deprived of cortical and subcortical input.

8.3.2.2. Cerebral amyloid angiopathy

With regard to the etiology, many proposed hypotheses, as described previously, do not win support from the morphological patterns. The CAA hypothesis suggests that deposition of a systemic amyloid precursor in cerebral blood vessels leads to an alteration in the BBB and initiates a toxic effect, the deposition of amyloid in the neuropil and neuritic plaque formation. This concept is based mainly on evidence that: a) amyloid in SP core, CAA, and even in NFT, is almost identical immunocytochemically (Coria, 1988; Johnson, 1981; Allsop, 1986; Glenner, 1984), b) CAA appears to be most severe in AD (Vinter, 1983; Kurucz, 1981; Glenner, 1985; 1986). c) LC, a structure suggested to exert major controls on BBB integrity and cerebral blood flow (Raichle, 1975; Bates, 1977; Harik, 1984) is consistently affected in AD. However, this hypothesis does not explain: a) the regional and laminar selectivity and specificity of the morphological lesions, b) the greater number of SPs in some cases of AD without concomitant CAA (Terry, 1985; Mountjoy, 1982), c) the poor spatial correlation between SPs and vascular amyloid, in particular, the lack of CAA involvement in the hippocampal
formation, which is of great importance in maintaining cognitive and memory functions (Hyman, 1986, 1987; Damasio, 1984; Murray, 1983; Barr, 1988) and is always severely involved by the morphological lesions in AD.

8.3.2.3. Aging versus AD

Aging has been suggested as the etiological factor in AD, since a close relationship between the two has been repeatedly found (Jorm, 1987; Braody, 1955; Roth, 1967; Kral, 1978; Hubbard, 1981). The suggestion that AD, especially, the senile form, may not be a "disease" at all, but a natural part of the aging process (McGeer, 1984; Hubbard, 1981; Whitehouse, 1983; Drachman, 1983; Randall, 1991; Mann, 1985), leads to a corollary, that if the differences are only quantitative, "we all potentially, with some escapees, could develop the disease by 120 years of age" (Randall, 1991). We think this is too conjectural, since differences (Berg, 1985; Price, 1986; Hauw, 1989) do exist, some of which reflect fundamentally separate pathological or physiological processes. For example (Hagnell, 1981; Berg, 1985; Coleman, 1987), a) lack of an increased amount of lipofuscin in the brains of AD patients implies a lack of correlation with the aging process, b) there is a loss of dendritic arborization and compensatory response in AD, but a continued expansion in normal aging, implying a different process, c) lack of further increase in prevalence of AD beyond the age of 90 implies a pathological process different from
aging. In addition, our data show significant differences between AD and aging in the extent, and the progression pattern of the pathologies. There are also differences in brain atrophy, neuronal loss, neurometabolic changes, cerebral blood flow, and other age-related histological changes, as well as the neuropsychology and clinical neurophysiology (Davis, 1977; Tomlinson, 1968, 1982; Ball, 1977; Price, 1986; Coleman, 1987; McGeer, 1976, 1981; Creasy, 1985; Anderson, 1983; Mountjoy, 1983).

Our results highlight the contrast between aging and this disease. Major differences include: a) the morphological picture of non-demented control brains is very different from that seen in AD, in terms of the extent, and the severity of the histological changes, b) lesions that form in the controls are not selective for certain brain areas, as seen in AD, c) accumulation of both lesions is so low in the controls compared to AD (over several hundred times lower) that it seems impossible that the extent, the severity and selectivity of changes in those controls would ever be able to compare with AD. If "normal aging" were the only causative biological process, without other etiological factors actively operating, even extreme longevity would not produce the extensive lesions found in AD.

However, a difficulty remains in practically
distinguishing the elderly with some of the structural changes associated with AD and the early stage of AD. Confusion arises from the findings that some very old persons without clinical diagnosis of DAT may have certain pathological changes in their brains, which are histological hallmarks for AD, particularly, a relatively high density of SPs (Blessed, 1968; Drachman, 1982; Crystal, 1988). Also, some with a clinical diagnosis of DAT may have fewer lesions than the threshold which is usually required for diagnosis (Bowen, 1981; Risse, 1990; Ball, 1985; Wisniewski, 1989). Quantitative pathological criteria were put forward, as described, by a British team (Blessed, 1968; Tomlinson, 1976), a Canadian group (Ball, 1988), and an American group (Khachaturian, 1985). It seems that no matter how the quantitative procedures are combined, or adjusted to the age, the problem remains in dealing with some particular cases, and judgment relies on clinical observation and personal preference of the investigator. Nevertheless, generally speaking, morphological differences between normal aging and AD do exist, and are significant enough to define most cases. However, the criteria can not be a clear-cut all-or-none parameter, but a range adjusted to the age.

One other aspect to consider is that SPs themselves may differ in structure (Wisniewski, 1973; Dickson, 1988; Abraham, 1988). Whether SPs in brains of the elderly who do not
suffer from overt neurological or psychiatric disease derive from the aging process, and are fundamentally different from lesions that actually reflect an early stage of a pathological process (Mann, 1987; Morris, 1991), can not be answered, until the etiology and specific biological markers are ascertained. Alz-50 antibody may be useful in this regard (Davies, 1991) as it can distinguish the plaques from normal tissue, and could facilitate further characterization.

In summary, morphological findings as well as various other considerations (Berg, 1985; Ulrich, 1985; Mann, 1987; Price, 1986; Coleman, 1987; McGeer, 1976, 1981; Creasy, 1985; Anderson, 1983; Mountjoy, 1983; Morris, 1991) do not support the hypothesis that AD is an invariable outcome of aging alone, but rather that it is due to an age-related pathological processes. The fact that overlaps are seen between aging and AD implies that: a) AD happens to share some biological changes with aging, without a causative relation in between. An organ like the brain and a cell like the neuron have limited ways in which they can react, and several distinct etiological factors may produce, by final common pathways, the same pathological and symptomatological expressions (Berg, 1985; Chui, 1987). b) There is an accumulation of some toxin, or a time dependent effect following the exposure to the toxin or infectious agent. c) There is a decrease in certain biological processes necessary for normal neuronal
function, which is also part of the aging process. This can be triggered by age-dependent gene(s) expression, or exaggerated by the interplay of environmental agents and genetic predisposition.

As for the issue of differences between presenile-onset and senile-onset DAT (McDonald, 1969; Bondareff, 1981; 1983; Seltzer, 1983; Roth, 1978), we did not find such differences in the regional and laminar distribution, the extent, the mean densities, and the progression of the lesions in the hippocampus and neocortex with time. However in some aspects differences did exist, which indicates that different pathogenic factors were contributing. A more heterogeneous morphological expression was seen in samples with senile onset, and the average accumulating rates of the two lesions were different. These findings suggest that:

a) In senile-onset DAT NFTs and SPs had been accumulating in the brains for a long time, and had probably reached the maximal level at the time dementia became apparent. b) The symptomatic expression, ie, dementia, of the morphological lesions did not only depend on the anatomical distribution and quantity of the lesions, but also on the development rate, ie, how aggressive they were in the brain. This property cannot be detected directly, and it may account for the notably poor correlation (Ball, 1977; Crystal, 1988; Morris, 1991), between the lesions and the symptoms. c) Interestingly there was a
possibility, which needs further study, that different kinds of lesions exist with respect to the rate of formation and involution of their structures. These could have a similar appearance, when seen at the terminal stage after autopsy, but differ in the underlying pathogenesis, and cause the separate clinical categories of senile or presenile onset. Therefore, the factors contributing to different "tolerance" of the morphological lesions, with early or late clinical onset of dementia, could be different, both genetically and environmentally. Until the interplay among these factors is clarified, the argument will continue between the "separatists" and the "unionists" with regard to age-related categories of dementia.

8.3.3. Subgroup 2

Subgroup 2 represents a small proportion (12.5%) of the samples, with clinically diagnosed "probable or possible" AD. These cases were of senile-onset DAT, with symptoms beginning at an average age of 75.46 years. The average duration of the disease before death was 6.74 years. In addition to various pathological changes usually associated with AD, this group was distinguishable from others by the great predominance of SPs, particularly in the neocortex. Topography and mean density of SPs resembled that of subgroup 1, but in contrast, this group had a significantly lower density of NFTs in all
sampled areas, with significantly low NFT/SP ratios in both hippocampus and neocortices. Strikingly, NFTs were sometimes very rare in the neocortex. This phenomenon has also been noted in some cases by other investigators (Terry, 1987; Katzman, 1988; Probst, 1989). The progression of SPs was similar in subgroups 1 and 2, in terms of the regional selectivity, the average accumulating rate, and the positive correlation with the duration of the disease. NFT progression was very slight in almost every sampled area, compared with subgroup 1 or 4, and was not consistent with the duration of the disease.

How should we account for this, mainly neocortical, absence or low intensity and lower progression of NFTs in some DAT with senile onset? Terry (1982, 1987) reported a similar finding in 30% of elderly patients, and decided against the possibility that it was a variant of normal aging or a disease different from AD. He suggested it be considered a variant of SDAT, and Katzman (1988) later supported this view. Tomlinson (1980, 1989) was hesitant in recognizing this variant, and referred to it only as "probably SDAT", being bothered mainly by a) the questionable accuracy and integrity of the diagnostic criteria, b) the very low incidence found by himself (7%) and European investigators, and c) the evidence that some of these cases clinically differ from those with both NFTs and SPs in the paucity of some typical symptoms, such as aphasia, apraxia, and agnosia (Constantinidis, 1978).
There are some considerations suggested by our morphological data: a) It does not support the concept that this subgroup represents normal aging or even an early stage of the disease, since the intensities of both lesions are much higher than in the controls. There are no differences from subgroup 1, or "typical" Alzheimer pathology (Terry, 1987; Katzman, 1988), in the duration of the disease, or in the intensity, the distribution or the progression patterns of SPs. It appears to represent the same fundamental disease process, but differs in the severity and progression of NFTs. b) Our data does not support the view that NFTs may appear at an earlier stage of the disease and then disappear gradually, since hippocampal NFTs are typically located and remain visible in severe cases. c) Our results do not support the suggestion that NFTs and SPs are always pathologically related, as proposed by Tomlinson (1989), who felt that formation of one of these lesions would initiate production of the second. d) Our findings suggest that the pathogenesis may be more complicated in these cases than in the classical subgroup, since the uncoupled development of NFTs and SPs could be caused by heterogeneous contribution of etiological factors. It also raises the question of whether the many results from other aspects of AD research in neurochemistry, neuroimmunology, and even in morphology, are complete and meaningful, since they are mostly derived from work conducted only on samples with both NFTs and SPs.
Furthermore, what we generally call SPs may vary in structure and may be selectively visualized by different staining. In addition to the morphological differences we have seen, other variations have been reported. A diffuse type of SP was more sensitive to β-protein immunostain and MB stain, than to Congo Red stain (Braak, 1989) or Bodian stain, and consisted of mainly amyloid, but no neuritic components (Yamaguchi, 1988; Morris, 1989). This diffuse SP also showed a different laminar distribution from "primitive" or "mature" forms of neuritic plaques (Braak, 1989). The most interesting finding is that some SPs fail to react with antibody against PHF or Tau (microtubule-associated protein) in the absence of NFTs in the neocortex (Probst, 1989). This further implies that the presence of tau might not be a prerequisite, as suggested (Bancher, 1989; Flament, 1989) for the formation of abnormal filaments, and that the SPs may occur independently of PHF pathology.

It will be interesting to see in the future, through the IMAGE network, if there is a neuropsychological or clinical subgroup with distinguishable variables, which matches with this neuropathological subgroup, since almost all reported research on AD has been based only on the cases with both NFTs and SPs. Important clues about the possible etiology and pathogenesis may lie in such findings.
Subgroup 3 represents 23.2% of our AD samples. Most of these had senile onset DAT, with an average onset at 70.5 years and an average duration of 6.5 years. The morphological picture is characterized by low densities and slow progression of both NFTs and SPs, as well as the presence of LBs in cortices and/or SN. The LBs are widely distributed in varying frequencies with a predilection for temporal, anterior frontal, insular cortices, cingulate gyrus, and particularly the deeper layers of the cerebral cortex, which coincides with the mesolimbocortical dopaminergic pathway (Gibb, 1985; Richard, 1986; Yagishita, 1980; Kosaka, 1984; Perry, 1990). The mean density of NFTs is significantly lower than that of subgroup 1 or subgroup 4, and the mean density of SPs is the lowest among the 4 subgroups. The progression rates of both NFTs and SPs are slow, compared with subgroup 1, in both hippocampus and neocortices, but both are consistent with the duration of the disease. When the samples with LBs restricted to SN were excluded, the remaining samples with LBs in both SN and neocortices (3a) showed a much more homogeneous pattern. Progression of both NFTs and SPs was similar to subgroup 1, although slower. Some of the samples with short duration showed predominant SPs and a progression pattern resembling that seen in subgroup 2.
8.3.4.1. AD versus PD

The major and complex issue reflected by this subgroup is the question of overlap between AD and PD, which has been found to occur more frequently than expected on the basis of individual prevalence of these diseases with age (Chui, 1986; Boller, 1980; 1985; Gaspar, 1984; Jorm, 1985; Hamill, 1988), in all but one study (Gibb, 1990). Idiopathic PD is a relatively well-defined entity with typical neuropathological lesions, neuronal loss, mainly of dopaminergic cells, gliosis and LB formation, located in the SN (Selby, 1968; Forno, 1981; Perry, 1986; Gibb, 1989). Clinically, PD is characterized by motor symptoms, but dementia frequently occurs as well (30-56%) (Lieberman, 1979; Hakim, 1979; Brown, 1984; Huber, 1986; Oyebode, 1986). There is some disagreement (Stern, 1986), but most reports indicate (Boller, 1980) that PD dementia is similar in its qualitative aspects, course and progression to Alzheimer's dementia, and is found either with (Hakim, 1979; Boller, 1980; Jellinger, 1990) or without (Perry, 1985; Nakano, 1983; Ball, 1984; Sima, 1986) significant Alzheimer-type pathology.

On the other hand, AD patients are also found to have a high incidence (up to 67%, Molsa, 1984) of PD-like symptoms, mainly, extrapyramidal features resembling PD, and some of the neuropathological features of PD are also seen (Pearce, 1974; Forno, 1978; Gaspar, 1984; Molsa, 1984;
Suzanne, 1989), mainly LBs in both SN and neocortex. The distribution of LBs is greater in the mesolimbocortical dopaminergic pathway (Gibb, 1985; Yagishita, 1980; Torack, 1986; Perry, 1990), which has been shown to play an important role in maintaining cognitive function and regulating emotional behaviour (Thierry, 1978; Stevens, 1978; Weiberger, 1988).

In these reports, cases were classified as either AD, PD, a variant subgroup, or a combination of the two. Okasaki (1961) first suggested that this was a distinct clinico-pathological entity and proposed the name, LB disease (LBD) (Kosaka, 1984), for this disorder with clinical features characterized by Parkinsonian symptoms and Alzheimer's dementia. In some cases, one feature may predominate, which has led to further classification into three types (Yoshimura, 1983): juvenile type, dementia plus occasional extrapyramidal symptoms, and Shy-Drager syndrome. These are all characterized by mild NFTs and SPs with cortical LBs of varying intensity. Pathologically these cases have also been divided into three types (Kosaka, 1980, 1984; Yoshimura, 1983) according to the distribution patterns of LBs, being diffuse (DLBD), transitional, and brain stem LBD. However there is no correlation between the pathological and clinical patterns.

Other attempts to match the pathology to either the
dementia or the extrapyramidal signs have led to contradictory conclusions. The dementia was (Hansen, 1989, 1990; Bergeron, 1989) or was not (Sima, 1986; Suzanne, 1989) caused by Alzheimer’s changes. The dementia was (Whitehouse, 1983; Rinne, 1989) or was not (Chui, 1986) caused by subcortical neuronal loss, and it was (Kosaka, 1984; Torack, 1986; Popovitch, 1987; Dickson, 1987; Burkhardt, 1988; Byrne, 1990; Perry, 1990), or was not caused by LBs (Gibb, 1990; Xuereb, 1990). Extrapyramidal signs were (Gibb, 1990), or were not (Morris, 1989) caused by SN degeneration. Lack of detailed neuropsychiatric or clinical assessments, and insufficient quantitative neuropathological data on both cortical and subcortical regions, have contributed to this confusion. The arguments about a "new disease", (Kosaka, 1984; Sima, 1986; Perry, 1990) a subset of AD, (Hansen, 1989, 1990; Bergeron, 1989) or a subset of PD, (Gibb, 1985, 1986, 1990; Xuereb, 1990) are still heated. This is not surprising, considering the partial, small, isolated sampling, the difficulty confirming a definite diagnosis of AD, and the problem that even the definition of PD is not "definite" (Calne, 1989).

Generally speaking, clinically these cases have both Parkinsonian motor symptoms and Alzheimer’s dementia. They may have only a single sign or symptom of each disease, or more extensive and complex combinations. Pathologically the brains vary from typical Parkinson’s lesions plus mild senile changes
(Chui, 1985; Rinne, 1989; Gibb, 1990), mainly SN neuronal loss and LBs with very low densities of NFTs and SPs, to typical Alzheimer's changes plus mild SN degeneration (Brun, 1976; Morris, 1989). In between there are brains with diffusely distributed LBs without significant NFTs and SPs (Sima, 1986; Yoshimura, 1988; Douglas, 1986), those with the co-existence of significant Parkinson's pathology, cortical LBs and Alzheimer's pathology (Kosaka, 1984; Torack, 1986; Hansen, 1989; 1990), and those with diffusely distributed LBs concomitant with predominant SPs (Forno, 1978; Lennox, 1989). It is very likely that these confusing reports reflect different combinations of one or more dynamic pathological processes, which could produce any variant of the above. Lesions that occur earlier and/or progress more rapidly may be expressed clinically as an initial and/or outstanding symptom or sign. Therefore, it is reasonable to suggest that there are several etiological factors, one or more of which are shared by PD and AD.

Our morphological data support this suggestion, especially when the samples with LBs confined to SN are excluded, leaving a relatively homogeneous subpopulation 3a. The formation of LBs in both SN and cortex therefore appears to be associated with a distinct pathological process, rather than coexistence of the two diseases. In contrast, the excluded samples may be due to coexistence, and constitute
what Gibb(1985,1986,1990) defined as a subset of PD or a combination of PD and AD.

Some recent findings also support our idea. For example, LBs contain epitopes shared by NFTs and SPs(Probst,1989; Galloway,1988) such as, MAP1, MAP2, and ubiquitin. Another microtubule-associated protein, tau was shown in LBs when these lesions were present in both SN and cortex, but not when they were found only in SN(Galloway,1989). This implies that the LBs in the two conditions may differ in pathogenesis.

Whether we should consider this subgroup 3a an independent disease, senile dementia of LB type, or DLBD (Kosaka,1980,1984; Richard,1986; Perry,1990) is beyond what we can discuss here, since it requires a precise definition of PD and AD, as well as knowledge of their etiologies and specific biological markers. Presently it depends on the investigator’s personal interest and philosophical inclination, since there seems to be no consistency in the history of the nomenclature(Calne,1989).

With the fairly homogenous morphological pattern in subgroup 3a, we would expect a matched clinical or neuropsychological subgroup. The classical triad of tremor, akinesia and rigidity with a pure subcortical dementia (Albert,1974; Cummings,1984) is associated with PD
Although the concept of subcortical dementia itself is being criticized (Whitehouse, 1986). A typical three-phase cortical dementia is generally characteristic of AD (Steven, 1989; Lindy, 1991). In subgroup 3a we would not expect either of these classical patterns, but some mixture of the two, varying from typical Parkinsonism with subsequent appearance of dementia, through any variation of Parkinsonism with any phase of DAT, to typical DAT with subsequent appearance of Parkinsonism. The initial, if definable, and outstanding sign or symptom would depend upon the initial and more progressive pathology, and correspond in general with the pathological and morphological data.

8.3.5. Subgroup 4

Subgroup 4 contains 15.2% of our 112 AD brains. These had a senile onset of dementia, beginning at an average age of 75.01 years, and died an average of 6 years later. Morphologically they resemble subgroup 1, in terms of the topographic distribution, the extent and severity, and the progression pattern of both NFTs and SPs, except there is no consistency with the duration of the disease. The major histological difference is the concomitant marked vascular pathology in this subtype.

The vascular pathologies in this subgroup are mainly: a)
hyalin arteriolosclerosis or atherosclerosis and/or b) multiple infarcts, usually seen as brownish and puckered scars of 0.5-2mm in diameter, located mainly in the cortex, including frontal, temporal, parietal, and occipital lobes, and/or c) lacunas, usually seen as small cavitary infarcts with ragged margins less than 1mm in diameter, located mainly in the basal ganglia, thalamus, subthalamic nucleus, internal capsule, and white matter. These have a cumulative volume greater than approximately 50ml, which has been suggested as a minimal requirement (Roth, 1967) for dementia to become manifest. These samples also have white matter changes, usually seen as extensive diffuse rarefaction with patchy accentuation.

8.3.5.1. AD versus CVD

The concept of cerebrovascular dementia (CVD) has been evolving, and the relationship between cerebrovascular disease and dementia is still difficult to assess. The exact frequency, the role of different vascular pathologies in causing the dementia, the clinical evaluation, and the criteria for diagnosis are controversial. In North America CVD is considered to be the second most common cause of dementia in the elderly with a frequency of 35-39% (Roman, 1987; Sulkava, 1985), and it may be the first in Japan, China and Russia (Gavrilova, 1977; Karasawa, 1982; Li, 1989). Some investigators feel this is an overestimation, and suggest
rates from 1% to 12% (Smith, 1981; Wade, 1987; Joachim, 1988; Del Ser, 1990). The controversy is probably due to inconsistencies in the definition of dementia and in the histopathological criteria employed.

It is now generally accepted that dementia of vascular etiology is not caused by progressive chronic ischemia from cerebral arteriosclerosis, but by the accumulation of deficits from multiple infarcts (MI) (Hachinski, 1974; William, 1986; Cumming, 1991), thus the name multi-infarct dementia (MID) (Hachinski, 1974). The MI mainly result from thrombi in extracranial arteries and the heart, and from cerebral atherosclerosis (Hachinski, 1974; William, 1986).

It has been known for a long time that AD and MID overlap both clinically and neuropathologically (Corsellis, 1962; Roth, 1967; Erkinjuntti, 1988). The Ischemia Scale of Hachinski (Folstein, 1978) was introduced to clinically differentiate MID from AD. It considers the mode of onset and deterioration, the concomitant psychiatric and local neurological symptoms and signs, and the histories of hypertension, atherosclerosis and stroke, but it is not universally recognized as valid (Rosen, 1980; Wade, 1987).

Three major varieties of MID have been suggested (Rogers, 1984; Bono, 1989): type I, cortical form with major infarcts
probably associated with thromboembolic events from large artery disease; type II, subcortical form with multiple lacunar infarcts probably associated with occlusion of penetrating branches of large cerebral arteries; type III, leuko-araiosis, demyelination of subcortical white matter, alone or combined with pericapsular lacunar infarcts, probably associated with alterations of the small perforating medullary arteries. Combination of types II and III constitutes Binswanger's encephalopathy. The minimal cumulative volume of brain destruction is 50 to 60ml in MID, and the important locations of infarct are well recognized as the temporal, frontal and occipital lobes, corpus callosum, basal ganglia, and thalamus (Tomlinson, 1970; 1976; Roth, 1967).

Difficulty still remains in the clinical differentiation between MID and AD, and in the neuropathology defining the mixed situation. In other words, we are not absolutely sure at what stage MI contributes to the dementia. There is no agreement among neuropathologists on the present clinical and neuropathological criteria, which are still largely empirical, and require further refining with prospective methodology and large sampling (Robitaille, personal communication, 1991).

There has been a recent and frustrating revival of an old debate on the role of chronic ischemia in CVD. After Parnetti (1989) argued that most cases of CVD are due to
subcortical damage, Hachinski (1991) himself shifted to a middle position. He still refutes chronic ischemia theory, and insists that MI is probably the main mechanism of CVD. However he admits that deep white matter damage through hypotensive episodes affecting the watershed zones is also important.

More radical opinions simply go back to the old theory that CVD is caused by chronic ischemia. A recent critical review (Munoz, 1991) concluded that the most common pathological feature in CVD is extensive white matter degeneration associated with arteriosclerosis. The argument is based on chemical alterations that occur in CSF and postmortem membrane lipids, and on changes in computed tomography. The volume and the location of the MI are not considered to be important. The underlying cerebrovascular hypoperfusion due to atherosclerosis is felt to be responsible for brain damage with myelin loss and/ or lacunas, finally leading to the dementia (Wallin, 1991).

All these recent reports actually clarify nothing, but stir up more confusion and controversy on this issue. Further understanding would require a common set of basic definitions and operative descriptions, and a large and multidisciplinary study. Because IMAGE provides such an opportunity, we were able to evaluate some aspects of CVD. Some of our
considerations in this regard are as follows:

a) Although the Alzheimer changes in subgroups 4 and 1 are similar, the brain destruction from vascular pathologies in subgroup 4 is so obvious, that we can not simply overlook this component in the production of dementia. Furthermore the inconsistency between the progression of both AD lesions and the duration of the disease, shown by our data, would imply that there were more pathological processes underlying the dementia in subgroup 4 than in subgroup 1.

b) Brain lesions multiply, rather than add to, previous impairment, particularly when they occur over a short period of time. Because multiple infarcts or hemodynamic alterations have a greater global effect than the sum of individual lesions, their clinical expression may be different from dementia without vascular pathologies.

c) The subgrouping was expected to facilitate the treatment, since most of the vascular pathological changes are associated with treatable conditions such as severe hypertension and recurrent emboli from extracranial sources. Although it would not be easy to recognize this mixed type clinically, the patients should be given the benefit of the doubt, before being given up as having hopeless AD. Furthermore, during clinical trials of new therapeutic agents to treat AD (Gamzu, 1990; Gauthier, 1991), it would be of great benefit to recognize this morphological subgroup and its clinical expression to properly evaluate the potential merit
of treatment.

d) Appropriate treatment may arrest, or slow the rate of
deterioration in dementia. Examples include the helpful
effect of anticoagulant therapy in DAT (Walsh, 1978, Klerman,
1990), and preliminary trials on Cyclandelate, a vasodilator,
and on Sulfomucopolysaccharides, which reduce blood viscosity,
show improved cognitive function in some patients
(Blakemore, 1987; Passari, 1989).

The etiology and pathogenesis leading to this mixed type
of dementia have been implicated indirectly by a few reports:

1) NFTs may be a consequence of neuronal injury after
axonotomy and deaffereertation (Shaw, 1988) or occur after a
massive cerebral infarct (Kato, 1988);

2) Demented patients with CVD have a greater intensity of
SPs than the nondemented (Del Ser, 1990);

3) There is irregularity, and altered density of
capillaries in AD (Perlmutter, 1990), which implies that a
vascular component may be an integral part of AD;

4) Increased prevalences of myocardial infarction and
atherosclerotic cardiovascular disease are found in AD
patients (Gambert, 1991); in fact there is a mutual
predisposition between AD and cardiovascular disorders

5) A pathogenic role of CAA in AD is highly probable,
particularly in this mixed condition, given its close
association with NFTs and SPs (Vinters, 1983; Coria, 1988; Davies, 1991), and with cortical microinfarcts and white matter degeneration (Okazaki, 1979; Gray, 1985).

8.5. FURTHER DEVELOPMENT

The functional consequences, indications for therapy and implications of etiology, for the 4 morphological subgroups will be clarified or redefined, when the subgroups are further tested in the IMAGE framework. Statistical matching with the clinical and neuropsychological categories will be done, after the morphometry is expanded to include more samples and additional parameters such as CAA. Therefore, some questions remain at this point in the study. For example, a certain degree of overlap exists among the subgroups. Is this due to some variables being redundant or missing? Does the nature of the variances between the subgroups represent qualitative or quantitative differences, which may redefine AD or separate it into different diseases? Robert (1990) philosophically postulated that we should not be surprised to find many additional changes as we study different parameters, and not try to encompass them all in one theory. Instead, we should establish valid core positions, from which to evaluate both general phenomena and the cellular and molecular events, as well as to keep the investigators "talking to each other". This is the strategy of IMAGE.
As the sample size grows within the IMAGE network, the comparisons of morphological variables will be made between presenile and senile onset groups, and between familial and sporadic groups. Morphometry for CAA with the help of β-amyloid antibody staining (Glenner, 1984; Allsop, 1986) will be added, in order to see the correlation between CAA, SPs and NFTs. A cause and effect relationship linking these changes has been suggested (Wong, 1985; Glenner, 1985; Tanzi, 1987) and opposed (Van Broeckhoven, 1987; Schellenberg, 1988; Bergeron, 1987), and qualitative subgroups regarding Alzheimer’s changes with and without CAA have also been suggested (Chui, 1987; Bergeron, 1987).

It has been reported (Braak, 1986; 1988) that cortical neuropil threads result from dendritic sprouting of cortical NFT-bearing neurons. To see whether subgroup 2 are devoid of the threads using immunocytochemistry and electronmicroscopy, would be an interesting way to further define the pathogenesis and to differentiate AD from normal aging.

The morphological variables and subgroups will be redefined statistically, and it will be determined if the clinical or neuropsychological subgroups match, in order to define etiologically and pathogenically homogeneous subgroups.
9. CONCLUSION

Present results on the pathology and morphometry from 135 brains with "probable or possible" AD in the IMAGE project have revealed 4 different subgroups. Morphologically the AD subgroups are clearly distinguished from the non-demented aging group, and mutually distinct by a) major pathological alterations which are recognized as diagnostic hallmarks of AD, b) coexisting alterations which are pathogenically associated with DAT, c) regional and laminar distribution patterns of the lesions, d) the severity of the lesions, e) the progression patterns of the lesions and their correlation with the duration of the disease. The morphological subgroups would be expected to match with clinical counterparts, and then potentially indicate etiological factors. Presently, the extensive heterogeneous and incomplete pathological picture, which defines AD, can not be well explained by any single etiology. AD is more likely to be a syndrome with multiple etiologies leading to heterogeneous pathological features, and correspondingly, to similar, but possibly distinguishable clinical manifestations. It is even possible that, at least, for therapeutic purposes, separate diseases may be defined in the future.
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