

**GENETICS OF PARKINSON'S DISEASE, ALZHEIMER'S DISEASE,
AND THE LINK BETWEEN THEM:
A GENETIC EPIDEMIOLOGICAL APPROACH**

by

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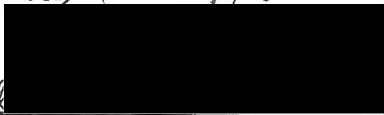
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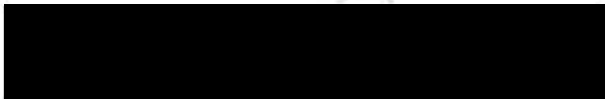
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ABSTRACT

The two most common age-related neurodegenerative diseases are Alzheimer's disease (AD) and Parkinson's disease (PD), whose underlying causes are still unknown. Both are complex genetic diseases with clinically indistinguishable subtypes. The overall objectives of this thesis project were three-fold.

- 1) To identify genetic risk factors for AD and PD. We discovered that age at onset of AD is associated with the A2 allele of the human leukocyte antigen (HLA-A2). HLA-A2 was the second gene identified for common late-onset AD, the first was apolipoprotein E (APOE). We found that the APOE-associated risk of AD is influenced by gender. We discovered that APOE modulates age at onset of PD in a manner similar to its effect on onset of AD. These analyses were performed using novel methodology that we developed for genetic analysis of age at onset.
- 2) To dissect the genetic complexity of AD and PD by identifying specific subtypes associated with specific genetic risk factors. Our studies using linkage and mutation analysis have helped define three genetic subtypes within the rare autosomal dominant form of PD: kindreds that are linked to the α -synuclein gene, those that are linked to a susceptibility locus on chromosome 2, and those that are not linked to α -synuclein or chromosome 2, indicating the existence of an additional as yet unidentified PD gene(s). We performed complex segregation analysis to determine if transmission of common PD is consistent with Mendelian inheritance and to test hypotheses regarding mode of inheritance. We found that the familial clustering of PD was best explained by non-Mendelian transmission of a rare familial factor that influences age at onset. The results of segregation analysis suggest that common PD

is not caused by dominant mutations with incomplete penetrance, and thus may represent another disease subtype distinct from the rare hereditary forms.

- 3) To identify genetic risk factors that may be associated with both AD and PD and may explain the observed clinical and neuropathological overlap between the two disorders. Our observation that APOE modulates age at onset of PD similar to its effect on the onset of AD suggested that APOE may be a common link between these disorders. We found no evidence in familial PD for linkage to α -synuclein, which is a component of Lewy bodies in PD and amyloid plaques in AD. Nor did we find any evidence in familial PD for linkage to *tau*, in which mutations are known to cause a spectrum of phenotypes including dementia and parkinsonism.

CHAPTER 1

INTRODUCTION

THESIS RATIONALE

Alzheimer's disease (AD) is a progressive cognitive disorder that affects 2-10% of the population over the age 65 (Evans et al., 1989). Parkinson's disease (PD) is a progressive movement disorder that affects 1-2% of the population over the age of 60, and many who are younger (Tanner, 1992). Both diseases progress to the point of causing significant disability such that a patient is unable to function independently. AD and PD have a devastating impact on the lives of the patients and their families. AD and PD are the two most common age-related neurodegenerative diseases, whose underlying causes are still largely unknown. AD and PD have a complex genetic basis due partly to the presence of various clinically indistinguishable subtypes. To tease out AD and PD into their individual disease subtypes with specific etiologies, a comprehensive approach that combines clinical neurology, neuropathology, molecular genetics and genetic epidemiology is needed.

The overall objective of this thesis project was to identify genetic risk factors for AD and PD, to attempt to dissect their genetic complexity by identifying specific subtypes associated with specific genetic risk factors, and to identify genetic risk factors that may be associated with both AD and PD and may explain the observed clinical and neuropathological overlap between the two disorders. The identification of these genetic risk factors will increase our understanding of the pathogenesis of these complex disorders and will enhance our understanding of the key mechanisms in neurodegeneration. This thesis has utilized data from clinical neurology and neuropathology, and the tools of molecular genetics and genetic epidemiology to accomplish this task.

During the course of this thesis project, novel and unexpected findings were unraveled, adding new twists to the complexity inherent in AD and PD. One of the most important lessons that I learned was the novel concept that a disease can have a genetic component that is not due to the presence of genes that influence disease causation or susceptibility but rather due to the presence of genes that influence onset and progression of a disease. This concept may be applicable to other complex disorders with late and variable onset.

The subjects for this thesis have been a great resource; majority of whom were recruited from the Oregon Alzheimer's Disease Center and the Parkinson's Disease Center of Oregon. Subsets of the subjects were recruited in collaborations with the Seattle Alzheimer's Disease Center and several other Alzheimer's Disease Centers in United States. For all the studies, I had some, although limited involvement in subject ascertainment and clinical aspects of the studies. This experience has given me a first-hand appreciation of the complexities in establishing the phenotype and of the amount of effort required for subject ascertainment. However, I spent majority of my time on the molecular genetics and statistical aspects of the studies. Having been involved in all aspects of each study from study design and patient recruitment to data generation, analysis and interpretation of the results, has given me an understanding of what it takes to carry out genetic studies of complex diseases.

A Road Map to this Thesis

This thesis is primarily composed of published studies. Here, I will briefly describe the rationale behind each study by describing the research problem, the goal, the implications of the results and my contributions to each study.

“Gender Difference in Apolipoprotein E-Associated Risk for Familial Alzheimer Disease: A Possible Clue to the Higher Incidence of Alzheimer Disease in Women”

At the time of this study, the effect of gender on the risk of late-onset AD was the subject of controversy. Prevalence studies had reported significantly higher rates of AD in women than men (Aronson et al., 1990; Rocca et al., 1991; Bachman et al., 1992), while population-incidence studies had been inconsistent, with some studies reporting significantly higher rates in women, and some studies reporting no difference (Schoenberg et al., 1987; Kokmen et al., 1988; Bachman et al., 1993). It was not clear whether the increased prevalence of AD in women was reflective of a gender difference in disease mechanism or an artifact due to longer survival by women. In a study of late-onset familial AD kindreds, to avoid confounding by longer survival of women, age-adjusted odds ratios were estimated and age for age, women were found to have a 2-3 fold increased risk of AD (Payami et al., 1996a). The results supported the role of gender as an independent risk factor for familial AD.

This study was carried out to explore whether the gender-specific difference in the risk of familial AD is a function of apolipoprotein E (APOE) genotypes. APOE- ϵ 4 allele is a risk factor for AD with ϵ 4-carriers having increased risk and earlier onset of AD. In an earlier preliminary study of familial late-onset AD kindreds, a significantly higher risk of AD was observed for ϵ 4 heterozygous women than ϵ 4 heterozygous men (Payami et al., 1994c). I confirmed the effect of gender on the APOE-associated risk for familial late-onset AD, by analyzing an independent data set and estimating gender- and genotype-specific odds ratios. The results indicated that having one ϵ 4 allele was associated with

increased risk of AD in women but not in men. A direct comparison revealed a significant 2-3 fold higher risk of AD in $\epsilon 4$ heterozygous women than $\epsilon 4$ heterozygous men.

The gender difference in APOE-associated risk for AD has now been confirmed by several studies including a meta-analysis of a data set containing more than 15,000 AD patients and controls contributed by 40 AD research teams from around the world (Farrer et al., 1997; Palumbo et al., 1997; Martinez et al., 1998). The increased risk of AD in $\epsilon 4$ heterozygous women poses a major public health impact, since 20% of middle-aged Americans are $\epsilon 4$ heterozygous (Wilson et al., 1994). The increased risk of AD in women may be due to loss of estrogen after menopause. Estrogen promotes the growth of cholinergic neurons, synapse formation and may interact with APOE (Toran-Allerand et al., 1992; Honjo et al., 1995). Estrogen use has been reported to be associated with a significant delay in the onset of AD and reduced risk of AD in $\epsilon 4$ heterozygous women (Tang et al., 1996). The gender difference in the APOE-associated risk of AD may also be related to the observed gender difference on the influence of APOE on the distribution of lipids (Reilly et al., 1994). Thus, the gender difference in the APOE-associated risk of AD may result from reduced efficacy of neuronal repair in women influenced by the effects of APOE genotypes and estrogen loss, resulting in a faster deterioration and earlier onset in women.

My contributions: The hypothesis of a gender difference in the APOE-associated risk of AD was developed by my thesis advisor prior to my joining her group. This study consisted of two independent data sets. The first data set was analyzed prior to my involvement. I alone worked on the second data set and was involved in its study design by prioritizing the families according to the completeness of data and availability of

autopsy documentation. My thesis advisor and I then chose to study the top 15 kindreds that had autopsy documentation and at least 3 affected individuals with known ages at onset to minimize confounding due to missing data. I APOE-genotyped 90 individuals from 15 autopsy-confirmed kindreds, and did all of the data analysis. My thesis advisor wrote the manuscript. I contributed to the writing of the results section and was involved in the discussion of the manuscript. I participated in responding to the reviewer's comments and performed additional analyses on both data sets.

“Evidence for association of HLA-A2 allele with onset age of Alzheimer's disease”

Although late-onset AD is associated with the APOE- ϵ 4 allele, several lines of evidence have suggested the existence of additional, as yet unidentified susceptibility genes for AD (Corder et al., 1993; Payami et al., 1993; Bennett et al., 1995; Farrer et al., 1995; Duara et al., 1996; Jarvik et al., 1996; Li et al., 1996). Prior to the discovery of the APOE associated risk of AD, several studies had explored the possible association of AD with the human leukocyte antigen (HLA), the major histocompatibility complex in humans. The results of the HLA association studies had been inconsistent (reviewed by Payami et al., 1991); however, an increased frequency of the HLA-A2 allele in men with early-onset AD was observed by two studies (Small and Matsuyama, 1986; Payami et al., 1991). The effect of HLA-A2 on the onset of AD had not been examined previously. The knowledge that genes such as APOE can have a significant effect on age at onset prompted this study to test the hypothesis that HLA-A2 is associated with earlier onset of AD. In this study, the HLA-A2 allele was found to be associated with a significant 3-year shift to earlier

onset of AD. The results suggest that HLA-A2, or a closely linked gene modulates age at onset of AD.

The earlier onset of AD in HLA-A2 carriers has been confirmed by two other groups (Combarros et al., 1998; Ballerini et al., 1999). The association between AD and HLA-A2 may reflect the presence of an immune/inflammatory response mechanism for AD. This is supported by the presence of immune-associated proteins and cells such as activated microglia (Aisen and Davis, 1994; McGeer et al., 1994), and the delayed onset of AD in patients taking nonsteroidal anti-inflammatory drugs (Breitner et al., 1995). Alternatively AD may be associated with a gene in linkage disequilibrium with A2. In addition to HLA, more than 30 genes have been mapped to chromosome 6p21.3, including tumor necrosis factor-alpha, heat shock proteins, and the haemochromatosis gene. Linkage disequilibrium in this region has long been recognized. Further studies are needed to investigate the possibility of a closely linked gene and the role of HLA molecules in the pathogenesis of AD.

My contributions: Although the hypothesis that HLA-A2 may affect age at onset of AD was developed by my thesis advisor, we had various discussions regarding this hypothesis. This study consisted of 2 independent data sets. I was involved in the study design and selection of the second data set. Because the first data set consisted of subjects from several AD centers selected by different methods, my thesis advisor and I decided to confirm our results in consecutive AD patients from the Oregon AD center with the diagnosis of probable or definite AD. I analyzed the data from the first and the second data set, and participated in the discussion of the results. My thesis advisor wrote the

manuscript. I contributed to the writing of the methods and the results sections, and the overall content of the manuscript.

“Modulation of the Age at Onset of Parkinson’s Disease by Apolipoprotein E Genotypes”

AD and PD are common neurodegenerative diseases with clinical and neuropathological overlap. PD patients frequently develop dementia (Marder et al., 1995; Tison et al., 1995), and AD patients often exhibit parkinsonian signs (Funkenstein et al., 1993; Merello et al., 1994). Neuropathological studies have shown the frequent occurrence of coincidental AD pathology in brains of PD patients (Hughes et al., 1993), and an increased frequency of Lewy bodies in brains of AD patients (Hulette et al., 1995). PD is frequently observed among first-degree relatives of AD patients (Hofman et al., 1989), and dementia is often observed among first-degree relatives of PD patients (Katzman and Kawas, 1994). The familial clustering and the clinical and neuropathological overlap between AD and PD may reflect the existence of a common neurodegenerative pathway and common susceptibility genes.

AD is associated with APOE, and a common association of APOE with AD and PD may explain the clustering of AD and PD in individuals and families. APOE is associated with AD at two levels: 1) $\epsilon 4$ frequency is higher in patients compared to controls, 2) patients with $\epsilon 4$ have earlier onset than patients without $\epsilon 4$. Several studies had examined APOE allele frequencies in PD patients and did not detect a significant difference in PD compared to controls (Arai et al., 1994; Marder et al., 1994; Rubinsztein et al., 1994; Koller et al., 1995; Whitehead et al., 1996). No one had examined age at onset of PD as a

function of APOE alleles. The observation that PD patients with a positive family history have significantly earlier age at onset than PD patients without a family history (Bonifati et al., 1995; Payami et al., 1995), suggests the possibility of a genetic influence on the age at onset of PD. Given the overlap between AD and PD and the effect of APOE alleles on onset of AD, we tested the hypothesis that APOE genotypes influence age at onset of PD. In this preliminary study, PD patients with the $\epsilon 4$ allele had the earliest onset, $\epsilon 3$ homozygous patients had an intermediate onset and patients with the $\epsilon 2$ allele had the latest onset; the same pattern seen in AD. The results indicate that APOE genotypes modulate the age at onset of PD in a manner similar to their effect on the age at onset of AD.

If confirmed, the common effect of APOE on the age at onset of AD and PD supports prior evidence that APOE may play a general role in neurodegeneration, perhaps through its involvement in neuronal repair (Poirier, 1994), rather than being a specific marker for AD. The causes of AD and PD are still largely unknown. If $\epsilon 4$ -carriers had a reduced capacity of neuronal repair in response to injury, they may develop a neurodegenerative disease earlier than those more capable at neuronal repair. Furthermore, this study provided added support for the concept that genetic factors can modulate age at onset of a disease. This concept may be applicable to complex disorders with late and variable onset, however these factors may not be detected by case-control association studies that examine only allele frequency differences.

My contributions: The hypothesis that APOE may affect age at onset of PD was developed jointly by my thesis advisor and myself. I was involved in the design of the study, recruitment of subjects and collection of clinical information. I APOE-genotyped

105 Oregon patients and analyzed the data from both the Oregon and Washington patients. I wrote the manuscript with the help of my thesis advisor since this was my first paper. I responded to the reviewer's comments with her help.

"Analysis of the α -synuclein G209A mutation in familial Parkinson's disease"

A major advance in the genetics of PD was the report of linkage between PD and a region on chromosome 4q21-23 in a large kindred of Italian descent with autosomal dominant early-onset PD (Polymeropoulos et al., 1996), and the subsequent identification of a missense mutation (G209A) in the α -synuclein gene located within the candidate region, that segregated with PD phenotype (Polymeropoulos et al., 1997). The authors suggested that the α -synuclein gene may play a role in a large proportion of early-onset familial PD cases. Immediately after the initial linkage report, I analyzed several of our most informative families for linkage to the proposed candidate region. I excluded the candidate region including and surrounding the α -synuclein gene, suggesting that α -synuclein is not the gene responsible for familial PD in these kindreds. After the identification of the missense mutation, I screened the affected members from 40 familial PD kindreds for the mutation. No one had the mutation. Our study was the first published report demonstrating that α -synuclein and the G209A mutation are not a common cause of PD in families of Northern European origin.

Several other studies have screened for the presence of the G209A mutation in familial PD kindreds and non-familial cases, and have reported the absence of this mutation (Munoz et al., 1997; Bennett and Nicholl, 1998; Chan et al., 1998a; Chan et al., 1998b; Farrer et al., 1998; Group, 1998; Vaughan et al., 1998). In addition, some studies have

sequenced the coding region (Chan et al., 1998a; Farrer et al., 1998; Group, 1998; Kruger et al., 1998; Vaughan et al., 1998), and only one missense mutation that segregated with PD was identified in one family (Kruger et al., 1998). Linkage to this region was also examined by a few groups who excluded the candidate region including and surrounding the α -synuclein gene (Gasser et al., 1997; Scott et al., 1997). Therefore, the collective data from linkage and sequence analysis suggest that α -synuclein mutations are rare causes of PD. However, α -synuclein may play a role in the pathogenesis of PD due to its presence in Lewy bodies in substantia nigra of PD patients (Spillantini et al., 1997). Further investigations are needed to elucidate the role of α -synuclein in the pathogenesis of PD.

My contributions: After linkage of PD to chromosome 4q was reported, I carried out a linkage study to determine whether several of our large familial PD kindreds were linked to this region. I genotyped over 100 individuals from our 9 largest kindreds for the 4 markers identified by the linkage report, and performed the linkage analysis. I also screened 65 affected members from 40 PD kindreds for the α -synuclein G209A mutation. I wrote the manuscript and responded to the reviewer's comments.

“Segregation Analysis of Parkinson Disease”

Approximately 10-30% of all PD patients have a positive family history (Payami et al., 1994a; Bonifati et al., 1995; Uitti et al., 1997), and in a rare subset, the mode of inheritance is autosomal dominant (Maraganore et al., 1991; Lazzarini et al., 1994; Waters and Miller, 1994; Denson and Wszolek, 1995; Markopoulou et al., 1995; Golbe et al., 1996). But the majority of the families show no discernible inheritance pattern. There have

been several hypotheses regarding the mode of inheritance of PD such as the possibility that mutation of a single gene may account for PD, in familial PD as well as non-familial PD (Golbe et al., 1990; Duvoisin and Golbe, 1995); the presence of subtypes with different modes of inheritance, including an autosomal dominant essential tremor-related parkinsonism and an autosomal recessive familial akineto-rigid syndrome (Barbeau and Roy, 1984); a multifactorial inheritance involving a combination of genetic and environmental factors (Kondo et al., 1973; Semchuk et al., 1993); and a possible role for mitochondria and mitochondrial DNA (mtDNA) in the pathogenesis of PD (reviewed by Schapira, 1995). Despite the interest in deciphering the mode of inheritance of PD, none of the studies has performed a formal segregation analysis to address the question properly in a randomly selected clinic or population-based sample. The aim of our study was to test hypotheses regarding the familial clustering of PD, to assess the likelihoods of genetic and environmental models, and to determine if transmission of PD is consistent with Mendelian inheritance. The hypotheses of a non-transmissible environmental factor, no major gene (sporadic), and all Mendelian inheritance (dominant, recessive, additive, decreasing) were rejected. In this data set, familial clustering of PD was best explained by transmission of a rare familial factor whose transmission probabilities deviated from those expected by Mendelian transmission. In addition, this factor was found to influence age at onset of PD.

The observed non-Mendelian transmission may be reflective of genetic heterogeneity in PD, gene-environment interaction, or a mitochondrial component to PD. The other intriguing finding was that the factor(s) exert its effect by influencing age at onset rather than susceptibility. In this context, a factor affecting susceptibility is assumed to exert its affect by increasing vulnerability to the cause of the disease whereas a factor affecting age

at onset is assumed to influence age at onset distribution without affecting susceptibility to disease (i.e., increasing vulnerability to the cause of the disease). If confirmed, both of these findings have immediate implications in gene-mapping studies, currently underway in several laboratories which usually assume that the familial clustering of PD is due to a gene, inherited in a Mendelian fashion which affects susceptibility to PD. The results of our study have also long-term implications in understanding the pathogenesis of PD, its treatment and prevention, by encouraging the search for genetic and environmental factors that influence the age at onset of PD. Their identification will lead to a better understanding of their role in the pathogenesis of PD and development of strategies to delay the onset of PD.

My contributions: In consultation with my thesis advisor, I performed complex segregation analysis to test the hypothesis that transmission of PD is consistent with Mendelian inheritance and to test several genetic and environmental hypotheses regarding the familial clustering of PD. I built the data file that consisted of more than 1000 individuals, and performed all of the analyses. Although Todd Taylor had performed some of the analyses as part of a class we were taking, I had to repeat those and carry out additional analyses for the manuscript. I wrote the manuscript and received help from my thesis advisor regarding the discussion section of the manuscript.

“Exclusion of the FTDP-17 locus on chromosome 17 for Parkinson’s disease”

Despite the recent advances in genetics of PD, the cause of PD and its selective neuronal degeneration is still unknown. Several neurodegenerative disorders with motor, cognitive and behavioral disturbances have been mapped to chromosome 17q, and are

collectively called “frontotemporal dementia and parkinsonism linked to chromosome 17” (FTDP-17) (Foster et al., 1997). Recently, mutations in *tau* were identified as the cause of FTDP-17 (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998). The spectrum of clinical manifestations and pathological findings in FTDP-17 overlap with clinical and pathological features of other neurodegenerative disorders such as dementia and PD, suggesting the possible existence of a common pathway in neurodegeneration. This overlap may also reflect the presence of mutations in the same locus that lead to other neurodegenerative disorders that are clinically related to FTDP-17. Therefore, it has been suggested that it would be worthwhile to test other familial neurological disorders for linkage to the candidate region on chromosome 17. The goal of our study was to determine whether some cases of familial PD are caused by mutations within *tau*. Currently, there are no reported studies of *tau* in familial PD. We examined 8 of our familial PD kindreds for linkage to the candidate region on chromosome 17 including and surrounding *tau*. We found no evidence for linkage and excluded the 6cM candidate region which suggests that in our families, PD is not caused by mutations within *tau*. In this study, the candidate regions of chromosomes 4, 2, and 17 were excluded in one autosomal dominant kindred, suggesting the existence of an additional as yet unidentified PD gene.

My contributions: In consultation with my thesis advisor, I examined the role of *tau* in our largest familial PD kindreds. Our collaborator in Sweden provided the genotypes for the 4 markers used. In addition to study design, I performed the linkage analysis and wrote the manuscript.

“Genetic variation of the CYP2D6 gene and Parkinson’s disease”

The most extensively studied candidate gene for PD is CYP2D6, a member of the cytochrome P450 gene family. CYP2D6 codes for debrisoquine 4-hydroxylase, the enzyme involved in the metabolism of over 25 drugs (Landi et al., 1996). Inactivating mutations in this gene (predominantly alleles A, B and D) result in the poor metabolizer phenotype, which is reportedly associated with PD (Barbeau et al., 1985). Two initial studies noted that the frequency of the inactivating mutations, particularly the B allele, was higher in PD patients than in controls (Armstrong et al., 1992; Smith et al., 1992). There have been numerous studies since, with inconsistent results. A meta-analysis utilizing 11 independent published data sets found that the poor metabolizer phenotype was significantly associated with increased risk of PD, but the association with the B allele did not reach significance ($p=0.06$) (McCann et al., 1997). One study has reported higher frequency of the B allele in familial PD than non-familial PD patients (Bordet et al., 1994). Increased frequency of the B allele in young-onset PD than late-onset PD patients was reported by one group (Agundez et al., 1995); however, this finding was not replicated in 2 subsequent studies (Diederich et al., 1996; Sandy et al., 1996). Our study had two objectives: 1) to examine the role of CYP2D6-B allele as a susceptibility allele for PD, and 2) to determine whether carriers of the B allele have an earlier onset. We did not find any significant association between the B allele and susceptibility to, or age at onset of PD.

However, when grouped by age at onset, we observed considerable fluctuation in the frequency of the B allele (low in early and late-onset, high in mid-onset) suggesting that CYP2D6-B allele may be associated with a subset of cases, and if so, may help define a disease subtype. The inconclusive results obtained by the various studies may possibly

reflect the association of the B allele with a specific subtype of PD and this association can be obscured in heterogeneous samples.

My contributions: In consultation with my thesis advisor, I examined the role of CYP2D6-B allele as a susceptibility allele for PD, and tested the hypothesis that the B allele may be associated with earlier onset of PD. I designed the study, genotyped the 189 patients and analyzed the data.

“Genetic Epidemiology of Parkinson Disease”

Haydeh and I were invited to write this review paper on PD. In this paper, we have summarized the results of various lines of research and provided the reader with a perspective as to what has been accomplished by PD research studies and what future directions research studies will be heading to. I am enclosing this paper as the last chapter to provide the reader with a complete picture of PD and how the studies in this thesis have contributed to our current understanding of PD.

My contributions: I conducted a comprehensive literature search on PD. I read the relevant papers and summarized their findings. My thesis advisor, the first author, wrote the manuscript and used the summaries as the basis for the paper. We then discussed the overall content and worked on the final versions together.

BACKGROUND ON ALZHEIMER'S DISEASE

Clinical and Pathological Description

Alzheimer's disease (AD), an age-related neurodegenerative disease, is the most common cause of dementia among the elderly. The prevalence and incidence of AD increase with advancing age. The incidence of AD is estimated to double every 5 years from 3.5 per 1,000 in individuals aged 65-69 years to 72.8 per 1,000 in individuals aged 85-89 years (Bachman et al., 1993). AD currently afflicts approximately 4 million Americans, more women than men, and is a major public health concern.

AD was originally described by Alois Alzheimer who reported a case of presenile dementia in a 51-year old woman at a Psychiatry meeting in 1906. Alzheimer had examined the patient for several days and concluded that the patient suffered from progressive cognitive impairment, speech and perception problems, hallucinations, delusions, and psychosocial incompetence, which were all symptoms of senile dementia but occurring at an early age (O'Brien, 1996). This condition received its name when Emil Kraepelin mentioned his colleagues' description of this female patient as "this Alzheimer's disease" in his influential psychiatry textbook published in 1910. Ironically, it appears that this original patient might now be classified as having a different dementia based on the neuropathology reports, which were recently found and reviewed by others (O'Brien, 1996).

AD is characterized by a global cognitive decline including progressive loss of memory, orientation and reasoning. Symptoms begin insidiously and an individual becomes increasingly forgetful. Within a few years, the forgetfulness increases and other

cognitive impairments appear such as difficulties with oral and written language, calculation, judgement, and ability to deal with complex tasks. Other common manifestations include psychosis, depression, agitation and anxiety. Eventually, a patient with AD is unable to function independently (Berg and Morris, 1994).

The neuropathological hallmarks include extracellular amyloid plaques, intracellular neurofibrillary tangles (NFTs), and loss of synapses and neurons (Cummings et al., 1998). The major component of the amyloid plaques is the A β peptide, a highly hydrophobic peptide ranging from 39 to 43 amino acids in length. In addition, other proteins including tau, α 1-antichymotrypsin and APOE are found to be present inside amyloid plaques. Whether A β is neurotoxic to neurons is not clear since regions such as the cerebellum which contains many diffuse plaques is unaffected in AD (Robakis, 1994). NFTs occur intraneuronally and consist mainly of paired helical filaments containing a hyperphosphorylated form of the microtubule-associated protein tau. NFTs can lead to the death of the neurons in which they occur, however, neuronal death is also observed in regions with few NFTs (Terry et al., 1994). In AD brains, neuronal loss of predominantly the neurons in the hippocampus, cortex and basal forebrain is observed along with decreased pre-synaptic terminal density (Terry et al., 1994).

In AD, there is a major and consistent cholinergic deficiency due to loss of cholinergic innervation of the cerebral cortex and decreased levels of acetylcholine (ACh) which is synthesized by choline acetyltransferase (CAT). The nucleus basalis of Meynert in the basal forebrain is a major source of CAT and is one of the regions affected in AD leading to a marked and consistent deficit in synthesis of CAT and ACh in AD (Cummings et al.,

1998). In addition to the cholinergic deficit, other neurotransmitters are affected in AD including serotonin, GABA, somatostatin, and norepinephrine (Cummings et al., 1998).

General Risk Factors for AD

In addition to the genetic risk factors (discussed below in detail), there are several other risk factors for AD. Age is a major risk factor for AD. The prevalence and incidence of AD are estimated to double approximately every 5 years after the age of 60 (Bachman et al., 1993), with the overall prevalence of AD reaching approximately 10% after the age of 65. The increased risk of AD associated with advancing age may be the result of neurobiologic changes such as loss of neurons, loss of synapses, decrease in cortical ACh, amyloid plaques and NFTs that are observed in normal aging (Cummings et al., 1998). A positive family history is a consistent risk factor for AD. Having an affected first-degree relative is associated with a four-fold increased risk for AD, and a total life-time risk of 23.4 to 48.8% (reviewed by Levy-Lahad and Bird, 1996). There are more women with AD than men and the female gender is associated with increased risk of AD. In a study of late-onset familial AD kindreds, age for age, women were found to have a 2-3 fold increased risk of AD (Payami et al., 1996a). In addition, the association between AD and APOE is modified by gender (discussed in more detail later). There is an inverse relationship between education and risk of AD with higher education levels being associated with reduced risk and delayed onset of AD (Mayeux, 1996). Lower intelligence levels are also associated with increased risk of AD independent of education (Mayeux, 1996). Individuals with smaller head and brain sizes have an increased risk of AD even after adjustments for possible confounding factors such as weight, height, education, gender and APOE genotype (reviewed by Cummings et al., 1998). A history of previous

head injury has been found to be associated with increased risk of AD by some studies (reviewed by Mayeux et al., 1995b). One study found that the effect of head injury on risk of AD may be limited to those with the APOE- ϵ 4 allele implying a synergistic relationship between head injury and APOE (Mayeux et al., 1995b). A prior history of depression has been found to increase the risk of AD, however it is not known whether depression is an early symptom or a sign of impending disease (Mayeux, 1996). Several studies have found an inverse relationship between smoking and AD with smoking conferring some protection against AD (reviewed by Slooter and van Duijn, 1997).

Classification of AD based on Age at Onset

AD is categorized as early onset (age at onset <60 years) and late onset (age at onset $\geq$ 60 years). This age at onset classification of AD has proved to be the key to the success in determining the genetics of this complex and heterogeneous disease.

Genetics of Early-Onset Autosomal Dominant AD

For early-onset familial AD, a rare form characterized by autosomal dominant inheritance, three causative genes have been identified: the amyloid precursor protein gene (APP) on chromosome 21 (Goate et al., 1991), the presenilin 1 gene (PS1) on chromosome 14 (Sherrington et al., 1995), and the presenilin 2 gene (PS2) on chromosome 1 (Levy-Lahad et al., 1995).

The APP gene, located on chromosome 21, was the first gene in which mutations were found to cause early-onset familial AD (Goate et al., 1991). APP was a candidate gene because 1) Down syndrome patients (trisomy 21) develop clinical and pathological features of AD (Wisniewski et al., 1985); and 2) APP was identified as the precursor protein from which A β is derived (Kang et al., 1987). The APP gene codes for a 695- to

770- amino acid precursor protein, that is proteolytically cleaved to form A β , a 39- to 43- amino acid peptide and a major component of the amyloid plaque (Kang et al., 1987; Lemaire et al., 1989). APP mutations are a rare cause of early-onset familial AD, accounting for perhaps 5% of cases (Goate, 1998). The role of APP mutations in the development of amyloid plaques and pathogenesis of AD remains to be determined. It has been suggested that APP mutations may result in aberrant APP processing and may ultimately lead to AD, through increased production of A β 42 than A β 40. A β 42 peptides have been shown to be far more prone to aggregation and are therefore postulated to be more deleterious (reviewed by Goate, 1998).

The PS1 gene, located on chromosome 14, was the second gene in which mutations were found to cause early-onset familial AD. The PS1 gene was identified by a genome-wide scan where linkage was established to a locus on chromosome 14 in a group of 9 early-onset familial AD kindreds (Schellenberg et al., 1992). The PS1 gene was cloned as a novel gene which encodes a 463-amino acid integral membrane protein with six to nine predicted transmembrane (TM) domains (Sherrington et al., 1995). PS1 mutations account for approximately 50% of early-onset familial AD cases. The PS2 gene was cloned by virtue of its homology to PS1, and encodes a 448-amino acid integral membrane protein that is 67% identical to PS1 (Levy-Lahad et al., 1995). To date, only three PS2 mutations have been described, one in the Volga German kindreds, one in an Italian kindred, and one in a non-familial AD patient from the Netherlands which may be a rare polymorphism with or without pathogenic effects (Renbaum and Levy-Lahad, 1998). Studies of PS1 knockout mice have indicated that PS1 is required for normal neurogenesis and neuronal survival (Shen et al., 1997; Price and Sisodia, 1998). In transfected cell lines

expressing mutant forms of PS1, an increase in A β 42 relative to A β 40 has been observed (Borchelt et al., 1996). Brains of patients carrying PS1 mutations, also predominantly show deposition of the A β 42 form of the peptide (Mann et al., 1996). In cells co-transfected with PS2 mutations and APP, increased total A β production was observed with a 5- to 10-fold increase in A β 42 levels (Citron et al., 1997; Tomita et al., 1997). It appears that PS1 and PS2 affect APP processing, although their roles in the pathogenesis of AD warrant further investigations.

Genetics of Common AD

The common forms of AD, including early-onset non-familial AD and late-onset AD are associated with the APOE gene on chromosome 19. APOE was identified as an A β -binding protein in cerebral spinal fluid, and a strong allelic association between AD and the APOE- ϵ 4 allele was found in late-onset familial AD kindreds (Strittmatter et al., 1993). However the mechanism of action of APOE is still unknown. APOE is the principal apolipoprotein in the brain and is involved in the transport of lipids and cholesterol (Beffert et al., 1998). APOE has three common alleles: ϵ 2, ϵ 3, and ϵ 4. These alleles differ from each other by one or two amino acids: ϵ 3 has cysteine at residue 112 and arginine at 158, ϵ 4 has arginine at both residues and ϵ 2 has cysteine at both residues. APOE was previously shown to be associated with cardiovascular disease (reviewed by Siest et al., 1995). Numerous studies have shown that the ϵ 4 allele is associated with increased risk of AD, and earlier age at onset of AD (Mayeux et al., 1993; Payami et al., 1993; Poirier et al., 1993; Saunders et al., 1993; van Duijn et al., 1994; Zubenko et al., 1994). The association of APOE with risk of AD originally identified in Caucasians, has now been

replicated in various ethnic groups including African-Americans, Hispanics and Japanese (Maestre et al., 1995; Farrer et al., 1997). The age at onset of AD has been reported to be related to APOE- ϵ 4 dose, whereby each additional ϵ 4 allele shifts the onset of AD to an earlier age. Some studies have observed a reduced frequency of the APOE- ϵ 2 allele in subjects with late-onset AD, suggesting that ϵ 2 may have a protective effect (Corder et al., 1994; West et al., 1994).

The role of gender in the risk of AD had been controversial. A difference in the ϵ 4 effect had been observed between men and women (Payami et al., 1994c). In women, having one ϵ 4 allele shifted the onset to a younger age, similar to having two ϵ 4 alleles. In men, however, having two ϵ 4 alleles shifted the onset to a younger age, while those with one ϵ 4 did not differ from those without ϵ 4. This gender difference in the APOE-associated risk of AD was later replicated in an independent sample of familial AD, where a direct comparison of ϵ 4 heterozygous men and women revealed a significant 2-fold increased risk in women (see Chapter 2). The gender difference in APOE-associated risk for AD has been confirmed independently in a meta-analysis of a data set containing more than 15,000 AD patients and controls contributed by 40 AD research teams from around the world (Farrer et al., 1997).

The role of APOE in the pathogenesis of AD is still unknown. Research studies have focused on the following 3 mechanisms of action for APOE: 1) The effect of APOE on amyloid plaques, 2) the effect of APOE on NFTs, and 3) the effect of APOE on neuronal repair. Several groups have examined the interaction between APOE and A β . APOE- ϵ 4 was shown to bind more rapidly to A β compared to APOE- ϵ 3, but the opposite trend was

observed by another group (reviewed by Schellenberg, 1995). Neuropathological studies have demonstrated that $\epsilon 4/\epsilon 4$ patients had significantly increased amyloid plaque density compared to $\epsilon 3/\epsilon 3$ patients (reviewed by Beffert et al., 1998). Several groups have studied the effect of APOE on the formation of NFTs by binding to tau, the major component of NFTs. It was shown that APOE- $\epsilon 3$ could bind tau more tightly than APOE- $\epsilon 4$ and possibly prevent tau from being phosphorylated and forming NFTs (reviewed by Schellenberg, 1995). The role of APOE in the formation of A β and NFTs requires further investigation *in vivo*. APOE is the principal apolipoprotein in the brain involved in the transport of lipids and cholesterol, which are needed for neuronal repair such as axonal regeneration and remyelination. It was shown in Rabbit dorsal root ganglion neurons that APOE- $\epsilon 3$ complex (with other proteins such as lipoprotein receptors) increased neurite extension and decreased the amount of branching, while APOE- $\epsilon 4$ complex decreased both extension and branching (Nathan et al., 1994). Similarly, in a central nervous system (CNS)-derived neuronal cell line, APOE- $\epsilon 3$ but not APOE- $\epsilon 4$ was found to increase neurite extension (reviewed by Beffert et al., 1998). It has been suggested that APOE- $\epsilon 4$ may be potentially detrimental to neurons by inhibiting regeneration and thereby contributing to the pathogenesis of the disease. This hypothesis was supported by the report that AD patients carrying one or two $\epsilon 4$ alleles had more severe neuronal degeneration in several brain areas than patients lacking the $\epsilon 4$ allele (Arendt et al., 1997). In APOE deficient mice, synaptic degeneration and neuronal death along with impairments in cognitive performance evaluated by the Morris water maze occurred as they aged (reviewed by Beffert et al., 1998).

The genetic risk of AD is not explained completely by APOE since the presence of $\epsilon 4$ is neither necessary nor sufficient for development of AD. Approximately 35 to 50% of AD patients lack $\epsilon 4$, yet they develop AD (Levy-Lahad and Bird, 1996). Several lines of evidence suggest the existence of additional as yet unidentified susceptibility genes for AD (Corder et al., 1993; Payami et al., 1993; Bennett et al., 1995; Farrer et al., 1995; Duara et al., 1996; Jarvik et al., 1996; Li et al., 1996). Therefore, on-going research studies have attempted to identify these additional genetic risk factors, and several groups have focused on genes whose products may have a biological interaction with APOE. The candidate genes examined to date, include $\alpha 1$ -antichymotrypsin (Kamboh et al., 1995; Haines et al., 1996; Muller et al., 1996; Talbot et al., 1996; Helisalmi et al., 1997; Murphy et al., 1997; Yoshiiwa et al., 1997; Ezquerra et al., 1998; Itabashi et al., 1998; Lamb et al., 1998; Nacmias et al., 1998; Yamanaka et al., 1998), the very low-density lipoprotein receptor (Okuiizumi et al., 1995; Chung et al., 1996; Okuiizumi et al., 1996; Pritchard et al., 1996; Brookes et al., 1997; Chen et al., 1998), the low-density lipoprotein receptor-related protein (Kang et al., 1997; Hollenbach et al., 1998; Kamboh et al., 1998; Lambert et al., 1998; Woodward et al., 1998), butyrylcholinesterase (Lehmann et al., 1997; Brindle et al., 1998; Crawford et al., 1998; Russ et al., 1998; Singleton et al., 1998), transferrin (Namekata et al., 1997), and bleomycin hydrolase (Montoya et al., 1998). Although some groups have reported an association between risk of AD and these candidate genes, the results have not been consistent. In addition to candidate gene studies, several groups have carried out genome-wide searches. While suggestive evidence for linkage has been reported for regions on chromosomes 4, 6, 12 and 20 (Pericak-Vance et al., 1998), several studies have reported linkage to a locus on chromosome 12 (Pericak-Vance et al., 1998;

Rogaeva et al., 1998; Wu et al., 1998). However, the estimated location of this locus varies among studies.

In addition to searching for specific genetic causes, one study has searched for a more general mechanism that may ultimately lead to neuronal degeneration. This study found evidence for a defect operating possibly at the transcriptional level or at the post-translational editing of RNA, occurring in non-familial early- and late-onset forms of AD (van Leeuwen et al., 1998). They examined APP and ubiquitin-B (Ubi-B) transcripts, which contain a GAGAG motif and found that both transcripts, can be modified by dinucleotide deletions (Δ GA) leading to +1 frameshift mutations. They observed immunoreactivity for APP⁺¹ and Ubi-B⁺¹ proteins in brains of early- and late-onset AD patients but not in brains of young controls or PD patients. The authors suggest that the accumulation of +1 proteins and the consequent lack of functional proteins may lead to cellular disturbances leading to degeneration of cells in which they occur (van Leeuwen et al., 1998). Although the results are intriguing, the role of these mutant proteins in development of AD is not clear. The frameshift mutation in APP transcripts results in an early stop signal which in turn results in a shortened mutant protein. It is not clear whether A β fragments can be derived from the shortened mutant proteins.

Mitochondria have been suspected to play a role in AD. A defect in the electron transport chain in AD has been reported, specifically a deficiency in cytochrome C oxidase (COX) was observed in mitochondria isolated from platelet and brain samples of AD patients (Mutisya et al., 1994; Parker et al., 1994). Davis et al. (1997) reported the identification of missense mutations in mitochondrial COX1 and COX2 genes, which segregated at higher frequency in AD patients than patients with other neurodegenerative

or metabolic diseases. The mutations were associated with decreased COX activity and increased production of reactive oxygen species. However, the mutations were later shown to be of nuclear origin arising from nuclear-embedded mtDNA pseudogenes (Hirano et al., 1997; Wallace et al., 1997; Davis and Parker, 1998), and it is unlikely that they played any role in the etiology of AD. Several other mtDNA mutations have been examined in AD, but to date, no mtDNA mutation has been shown unequivocally to be associated with AD (Corral-Debrinski et al., 1994; Hamblet and Castora, 1997; Hutchin et al., 1997; Zsurka et al., 1998).

BACKGROUND ON PARKINSON'S DISEASE

Clinical and Pathological Description

Idiopathic Parkinson's disease (PD), the second most common age-related neurodegenerative disease after AD, is a progressive movement disorder that affects 1-2% of the population over the age of 60, and many who are younger (Tanner, 1992). The incidence and prevalence of PD rise almost exponentially with age. Prevalence studies have estimated an increase in cases of PD from 3-47 per 100,000 for before the age of 50, to 2179-3096 per 100,000 for above age 80 (Schoenberg et al., 1985; Tanner, 1992; Mayeux et al., 1995a).

PD was first described by James Parkinson who published a small monograph entitled "An Essay on the Shaking Palsy" in 1817. He had seen six cases over a period of some time and described the disorder as follows: "Shaking Pulsy (Paralysis Agitans): Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forwards, and to pass from a walking to a running pace; the senses and intellect being uninjured." Jean Marie Charcot later added the muscular rigidity to the list of manifestations and insisted that the shaking pulsy should be named Parkinson's disease (Duvoisin, 1991).

The clinical symptoms of PD include resting tremor, rigidity, bradykinesia (the slowness of bodily movements) and postural instability (Duvoisin, 1991). The tremor usually affects the hands and feet and it tends to occur when the affected limb is at rest. The rigidity results from the loss of the ability to relax by the affected muscles, which seem to be in a state of contraction even at rest. Bradykinesia reflects the hesitancy to initiate a

new movement or activity, slowness in its execution, rapid fatiguing and a lack of spontaneity and reduced performance of the automatic movements such as the swing of the arms while walking.

The neuropathological features include neuronal loss in the pars compacta of the substantia nigra and the presence of Lewy bodies (intracytoplasmic neuronal inclusions) (Hughes et al., 1992). The neurons of the substantia nigra produce and store dopamine. The clinical symptoms of PD manifest when 80% or more striatal dopamine is lost (Hornykiewicz and Kish, 1986), presumably as a result of substantial neuronal loss in substantia nigra. The cause of this selective neuronal loss is not known.

General Risk Factors for PD

The relative importance of genetic versus environmental factors in the pathogenesis of PD has been the subject of controversy for over a century, with scientific opinion shifting from one extreme to the other several times. In addition to the genetic risk factors (discussed below in detail), there are several other risk factors for PD including age, family history of PD and environmental agents.

Advancing age is a major risk factor for PD, since prevalence and incidence of PD rise almost exponentially with age in all populations (Tanner and Goldman, 1996). The reasons for this correlation with age are not known but possible explanations include an age-related neuronal vulnerability or a time-dependent disease mechanism. It is also not known if the incidence continues to rise at very high ages, eventually reaching 1.0 if one lived long enough.

A positive family history is a consistent risk factor for PD. A familial component to PD was first proposed by Gowers in 1888 (Gowers, 1888). Although many groups provided

further evidence for a familial component, they were dismissed in the 1980's because they were affected by selection bias, diagnostic uncertainties and methodological limitations (Duvoisin, 1987). Also in the 1980's, twin studies failed to show a higher concordance rate in monozygotic twins than in dizygotic twins (Ward et al., 1983; Marsden, 1987; Marttila et al., 1988; Vieregge et al., 1992). It was later suggested that the results of twin studies may have been seriously affected by the variability in age at onset and preclinical disease in twin pairs (Johnson et al., 1990). Subsequent studies that utilized measures to detect preclinical disease, observed an increase in the monozygotic concordance rate (Burn et al., 1992). In the 1990's, several carefully designed family studies consistently and unequivocally demonstrated familial aggregation in PD (Butterfield et al., 1993; Semchuk et al., 1993; Payami et al., 1994a; Bonifati et al., 1995; Vieregge and Heberlein, 1995; De Michele et al., 1996; Marder et al., 1996; Uitti et al., 1997). All of the studies, regardless of the location of the study or the ethnic origin of the subjects, concluded that the risk to first-degree relatives of patients was significantly higher than the risk to first-degree relatives of controls.

The role of environmental agents received a great deal of attention when a synthetic compound, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was found to induce parkinsonism (Langston et al., 1983). MPTP was a side product and contaminant of a "designer drug" that was sold as an injectable synthetic heroin in 1970's, and some of the individuals who injected MPTP developed parkinsonism within days of exposure (Langston et al., 1983). A metabolite of MPTP, MPP⁺, is a neurotoxin that inhibits complex I of the oxidative phosphorylation system, causing energy depletion and oxidative stress leading to neuronal death (Tipton and Singer, 1993). Due to the striking similarities

between MPTP-induced parkinsonism and idiopathic PD, it has been suggested that similar compounds may be involved in the pathogenesis of PD. Environmental studies have linked various aspects of rural living to an increased risk of PD. An association between PD and at least one of the proposed exposures - rural residence, farming, well water drinking, or herbicide/pesticide exposure - has been observed by several groups (Tanner and Goldman, 1996). Both retrospective and prospective studies have found an inverse relationship between smoking and PD with lower rates of PD among smokers (reviewed by Tanner and Goldman, 1996). Nicotine was found to have a protective effect against induced neuronal loss in substantia nigra in animal studies (Janson et al., 1992; Prasad et al., 1994).

Mode of Inheritance of PD

Approximately 10-30% of all PD patients have a positive family history (Butterfield et al., 1993; Payami et al., 1994a; Bonifati et al., 1995; De Michele et al., 1995; Vieregge and Heberlein, 1995; Uitti et al., 1997). In a subset of familial PD kindreds, PD follows a Mendelian autosomal dominant inheritance pattern (Golbe et al., 1993; Waters and Miller, 1994; Denson and Wszolek, 1995; Markopoulou et al., 1995; Golbe et al., 1996; Morrison et al., 1996). However, the majority of familial cases show no clear inheritance pattern. There have been several hypotheses regarding the mode of inheritance of PD. One group has suggested that mutation of a single gene may account for PD in familial PD, as well as in non-familial PD (Golbe et al., 1990; Duvoisin and Golbe, 1995). Barbeau and Roy (1984) have suggested the existence of two familial subtypes based on clinical features: an autosomal dominant essential-tremor related parkinsonism and an autosomal recessive familial akineto-rigid syndrome. A multifactorial inheritance involving a combination of

genetic and environmental factors has been proposed for PD (Kondo et al., 1973; Semchuk et al., 1993). None of these studies has performed a formal segregation analysis to address the question properly in a randomly selected clinic or population-based sample.

Genetics of Autosomal Dominant PD

The rare autosomal dominant PD kindreds have been the focus of gene mapping studies and have resulted in the identification of two loci. The first locus was identified by a genome-wide search in 1996, when linkage between PD and a region on chromosome 4q21-23 was reported in a large kindred of Italian descent with autosomal dominant early-onset PD (Polymeropoulos et al., 1996). Subsequently, a missense mutation was identified in the α -synuclein gene which segregated with PD in the same kindred and three of the five Greek kindreds studied (Polymeropoulos et al., 1997). The mutation was a single base pair change from guanine (G) to adenine (A) at position 209 (resulting in an alanine to threonine substitution). An additional different mutation, a guanine (G) to cytosine (C) substitution at position 88 (resulting in an alanine to proline substitution) that segregated with PD was found in one family (Kruger et al., 1998). To date, over 530 PD subjects have been screened for the G209A mutation, including 141 familial PD kindreds, and the mutation was absent (Munoz et al., 1997; Bennett and Nicholl, 1998; Chan et al., 1998a; Chan et al., 1998b; Farrer et al., 1998; Group, 1998; Kruger et al., 1998; Vaughan et al., 1998; Zarepari et al., 1998a). Sequence analysis of the coding regions of α -synuclein have not revealed other mutations (Chan et al., 1998a; Farrer et al., 1998; Group, 1998; Kruger et al., 1998; Vaughan et al., 1998). Linkage to the candidate region including and surrounding the α -synuclein gene was excluded by several groups (Gasser et al., 1997; Scott et al., 1997; Zarepari et al., 1998a). The collective results from linkage and

sequence analysis suggest that α -synuclein mutations are rare causes of PD, even familial PD. Nevertheless, α -synuclein may have a central role in the pathogenesis of PD, since the presence of α -synuclein in Lewy bodies was recently demonstrated (Spillantini et al., 1997). The Lewy bodies in substantia nigra from patients with PD stained with antibodies raised to amino acid residues 11-34 and to residues 116-131 of human α -synuclein. Interestingly, α -synuclein was originally discovered as a component of the amyloid plaques in the brains of patients with AD, and is the second most abundant component of the amyloid plaques (Ueda et al., 1993). Although the function of the protein is unknown, it has been postulated that α -synuclein may be central to the neurodegenerative process (Spillantini et al., 1997).

The second locus was recently implicated when evidence for a PD susceptibility gene on chromosome 2p13 was reported in several familial PD kindreds with autosomal dominant inheritance (Gasser et al., 1998). A common haplotype was identified that segregated with PD in two unrelated families, suggesting the possibility of a founder mutation. This haplotype was present in 25 family members, 10 of whom had PD, yielding a penetrance estimate of 40%. Efforts are now focused on confirming the initial linkage report and to identify and clone the disease gene.

Genetics of Common PD

For the common forms of PD, genetic research studies have extensively used allelic association studies to test for involvement of candidate genes with potential functional significance in the pathogenesis of PD. The candidate genes for PD may be classified into four categories: genes that regulate metabolism of xenobiotics and may therefore determine ones susceptibility to toxins, genes involved in dopamine metabolism and

transport as a possible source of dopamine deficiency in PD, genes that are associated with neurodegeneration in other disorders, and mitochondrial mutations as the possible source of the complex I deficiency in PD.

The most widely studied candidate gene for PD is CYP2D6, a member of the cytochrome P450 gene family, which codes for debrisoquine 4-hydroxylase, the enzyme involved in the metabolism of over 25 drugs (Landi et al., 1996). Inactivating mutations in this gene (predominantly alleles A, B and D) result in the poor metabolizer phenotype. Barbeau et al (1985) originally reported an association between the poor metabolizer phenotype and PD, based on pharmacokinetic studies in 1985. In 1992, Armstrong et al (1992) and Smith et al (1992) noted that the frequency of the inactivating mutations, particularly the B allele, was higher in PD patients than in controls. There have been numerous studies since, with inconsistent results (Kurth and Kurth, 1993; Mazzetti et al., 1994; Plante-Bordeneuve et al., 1994; Rempfer et al., 1994; Agundez et al., 1995; Akhmedova et al., 1995; Saitoh et al., 1995; Bordet et al., 1996a; Bordet et al., 1996b; Diederich et al., 1996; Gasser et al., 1996a; Lucotte et al., 1996; Sandy et al., 1996; Bandmann et al., 1997). McCann et al (1997) have performed a meta-analysis utilizing 11 independent published data sets. In the pooled data, the poor metabolizer phenotype was present in 7.4% of patients as compared to 5.4% of controls, yielding a modest, but statistically significant odds ratio of 1.47.

Monoamine oxidase (MAO) A and B are candidate genes for PD because they encode enzymes that metabolize dopamine (Oreland, 1991) and MPTP (Tipton and Singer, 1993). A number of groups have examined possible association of MAO polymorphisms with PD, but the results have been inconsistent. With respect to MAO-B, four studies found

evidence for association with PD (Kurth et al., 1993; Hotamisligil et al., 1994; Nanko et al., 1996; Costa et al., 1997), two did not (Ho et al., 1995; Plante-Bordeneuve et al., 1997). With respect to MAO-A, two studies found an association (Hotamisligil et al., 1994; Nanko et al., 1996), two did not (Kurth et al., 1993; Plante-Bordeneuve et al., 1997).

Few studies have examined the genes that govern dopamine synthesis and transport in relation to PD susceptibility. Plante-Bordeneuve et al (1997) have reported a significant distortion in the dopamine receptor D2 allele frequencies in both sporadic and familial PD, as compared to controls. Le Couteur et al (1997) has found an allelic association, and a possible effect on age at onset, with the dopamine transporter gene in Caucasian PD patients. But two other studies, one in Caucasian patients and one in Chinese patients, did not find any association with dopamine transporter gene (Leighton et al., 1997; Plante-Bordeneuve et al., 1997).

There is considerable phenotypic overlap between idiopathic PD and several other neurodegenerative disorders, such as AD, essential tremor, frontotemporal dementia with parkinsonism, and dopa-responsive dystonia. The etiologies of these disorders are probably distinct, each being caused by disease-specific genes or environmental insults that damage specific populations of neurons. The mechanisms of response to neuronal damage, however, are probably genetically determined and may not be disease specific. Hence, genes that control the response mechanisms may be associated with more than one neurodegenerative disease, and may be responsible for some of the overlap in their phenotypes.

Approximately 30% of PD patients develop dementia (Marder et al., 1995; Tison et al., 1995; Hughes, 1997), and 30% of AD patients develop parkinsonian signs (Funkenstein et al., 1993; Merello et al., 1994). Moreover, brains from PD patients often display coincidental AD pathology (Hughes et al., 1993), and similarly, brains from AD patients frequently contain Lewy bodies (Hulette et al., 1995). Soon after the discovery of the association of APOE with AD, several investigators questioned if APOE was a link between the two disorders. Numerous studies have examined $\epsilon 4$ frequency in PD; few have tested an association with age at onset. The frequency of $\epsilon 4$ was not elevated in PD, or in PD-dementia in most studies (Arai et al., 1994; Benjamin et al., 1994; Han et al., 1994; Hardy et al., 1994; Harrington et al., 1994; Marder et al., 1994, 1994 #383; Rubinsztein et al., 1994; Koller et al., 1995; Martinoli et al., 1995; Poduslo et al., 1995; Whitehead et al., 1996; Group, 1997; Zarepari et al., 1997; Inzelberg et al., 1998). Thus, $\epsilon 4$ does not seem to be associated with susceptibility to PD or to PD-dementia. But APOE may have an effect on age at onset. Whitehead et al (1996) examined age at onset of PD in 189 subjects, all with onset at or before age 55. While there was no $\epsilon 4$ effect on onset age, they reported $\epsilon 2$ -carriers as having significantly delayed onset.

A mitochondrial component to PD has been the focus of several investigations. As summarized in the review by Schapira (1997), the results of many studies suggest that there is a specific ~35% complex I deficiency in PD nigra. This defect is restricted to substantia nigra and does not affect any other parts of PD brain. Several laboratories have detected a modest complex I deficiency in platelets from PD patients as well. Recently, Swerdlow et al (1996) demonstrated that the complex I deficiency in PD is linked to mtDNA by showing that the complex I defect followed the transfer of mtDNA when

mtDNA from PD patients were fused into rho⁰ cells, which have a nucleus but no mtDNA. There have been several reports of slight frequency increases of various point mutations, deletions and insertions in mtDNA in PD versus controls, none of which has been replicated (Schapira, 1997). The exclusive maternal transmission of mtDNA would lead to an excess of maternal transmission in familial PD, if inherited mtDNA mutation were involved in PD. Most studies, however, do not find that (Maraganore et al., 1991; Zweig et al., 1992; Lazzarini et al., 1994; Bonifati et al., 1995; De Michele et al., 1995; Plante-Bordeneuve et al., 1995; Wooten et al., 1997).

CHAPTER 2

GENDER DIFFERENCE IN APOLIPOPROTEIN E-ASSOCIATED RISK FOR FAMILIAL ALZHEIMER DISEASE: A POSSIBLE CLUE TO THE HIGHER INCIDENCE OF ALZHEIMER DISEASE IN WOMEN

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ABSTRACT

Late-onset Alzheimer disease (AD) is associated with the Apolipoprotein E (APOE)- $\epsilon 4$ allele. In late-onset familial AD, women have a significantly higher risk of developing the disease than do men. The aim of this study was to determine whether the gender difference in familial AD is a function of APOE genotype. We studied 58 late-onset familial AD kindreds. Kaplan-Meier survival analysis was used to assess genotype-specific distributions of age at onset. Odds ratios were estimated by logistic regression with adjustment for age and by conditional logistic regression with stratification on families. All methods detected a significant gender difference for the $\epsilon 4$ heterozygous genotype. In women, $\epsilon 4$ heterozygotes had higher risk than those without $\epsilon 4$; there was no significant difference between $\epsilon 4$ heterozygotes and $\epsilon 4$ homozygotes. In men, $\epsilon 4$ heterozygotes had lower risk than $\epsilon 4$ homozygotes; there was no significant difference between $\epsilon 4$ heterozygotes and those without $\epsilon 4$. A direct comparison of $\epsilon 4$ heterozygous men and women revealed a significant two-fold increased risk in women. We confirmed these results in 15 autopsy-confirmed AD kindreds from the National Cell Repository at Indiana University Alzheimer Disease Center. These observations are consistent with the increased incidence of familial AD in women and may be a critical clue to the role of gender in the pathogenesis of AD.

INTRODUCTION

Late-onset Alzheimer's disease (AD) affects an estimated 3-10% of individuals over age 65 years (Evans et al., 1989; Bachman et al., 1992) and 25-50% of all cases are familial (≥ 2 affected first-degree relatives) (Fitch et al., 1988; Hofman et al., 1989;

Edwards et al., 1991; Payami et al., 1994b). The established risk factors for AD are age, family history of dementia and presence of allele $\epsilon 4$ of the Apolipoprotein E (APOE) gene (Katzman, 1994). APOE has three major alleles: $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, with relative frequencies of .08, .77, and .15 in Caucasians (Davignon et al., 1988b). Allele $\epsilon 4$ is associated with both sporadic and familial AD but the association is stronger with familial AD (Corder et al., 1993; Payami et al., 1993; Saunders et al., 1993).

Whether gender is an independent risk factor for AD has been controversial. Prevalence studies have generally shown higher rates in women than in men (Aronson et al., 1990; Rocca et al., 1991; Bachman et al., 1992) but population-incidence studies have been inconsistent, some showing a significantly higher rate in women and others finding no gender difference (Schoenberg et al., 1987; Kokmen et al., 1988; Katzman et al., 1989; Bachman et al., 1993). The increased prevalence rate in women may in part be due to longer survival of women after onset of dementia, but may also be indicative of a gender difference in the risk of AD. The controversy in incidence results may be due to heterogeneity. Studies that have examined the incidence of AD in relatives of random patients have consistently found higher risk or earlier age at onset in women than in men (Breitner et al., 1988; van Duijn et al., 1993; Rao et al., 1994; Silverman et al., 1994), which in the face of inconsistent population incidence results, suggest that gender may be a risk factor only in familial AD. We recently tested this hypothesis in two independent samples of well-characterized familial AD kindreds and found significantly higher age-specific risk and earlier age at onset for women than men in both data sets (Payami et al., 1996a), supporting the notion that gender is an independent risk factor for familial AD.

The aim of the present study was to determine if the increased risk to women in familial AD is a function of APOE genotype. In an earlier study of familial AD, we noted a significantly higher risk for $\epsilon 4$ heterozygous women than $\epsilon 4$ heterozygous men (Payami et al., 1994c). This finding has been controversial because in another study of familial AD the increased risk to $\epsilon 4$ heterozygous women was not statistically significant (Corder et al., 1995a). These studies were considered preliminary because the method used in both cases treated family members as unrelated individuals. In this paper, we investigated the effect of gender on the risk of AD in a larger data set than previously reported. We estimated gender- and genotype-specific odds ratios, taking into account family relationship of the subjects and longer survival of women. To confirm the results, we analyzed an independent data set consisting of autopsy-confirmed kindreds from the National Cell Repository (NCR) at the Indiana University Alzheimer Disease Center.

SUBJECTS AND METHODS

The Oregon/Washington/Minnesota data included 58 late-onset familial AD kindreds, recruited and evaluated by the Oregon and Washington Alzheimer Disease Centers and the University of Minnesota research group. The kindreds were selected for having at least two first-degree relatives with AD and family average age-at-onset greater than 60 years. The 58 kindreds comprised 29 nuclear families and 29 extended families. All families were Caucasian, from mixed European origin. The NINCDS-ADRDA guidelines (McKhann et al., 1984) were used for clinical diagnosis. Fifty of the 58 families had at least one autopsy-confirmed case of AD. Every participant or legal guardian signed informed consent in the presence of a witness. Age of onset was defined as the age when

memory loss was first noticed by relatives. Subjects were tested for all the known AD genes. No evidence was found for the presence of a pathologic mutation in the amyloid precursor protein gene on chromosome 21 (Kamino et al., 1992), or in STM2 on chromosome 1 (Levy-Lahad et al., 1995). When the entire family group was considered, no evidence was found for linkage to S182 (AD gene on chromosome 14); however, there was evidence for linkage in a subgroup of families of intermediate age at onset (60-70 years) (Schellenberg et al., 1994). Thus, some of the families may have a mutation in S182.

APOE genotypes were determined using the method described by Hixon and Vernier (1990). The observed genotype frequencies were compared to the random (Hardy-Weinberg) expectations by a χ^2 test. Allele frequencies were estimated by allele counting. Age-at-onset distributions were plotted using Kaplan-Meier survival analysis and the difference between the curves was tested by log rank statistics. Logistic regression analysis was used to estimate odds ratios after adjusting for age. Unconditional logistic regression can give biased estimates because it does not take family relationship into account. To account for the family unit, conditional logistic analysis was used, stratifying on families. This method gives unbiased estimates when the sampling unit is not the individual, such as matched-pairs or families (Kleinbaum, 1994). However, it does not distinguish nuclear families from extended families. Thus, although it may be the best method available, it is not ideal. SPSS software (release 5, 1992) was used for Kaplan-Meier survival analysis and for unconditional logistic regression analysis. LogXact-Turbo computer program (CYTEL Co. 1993) was used for conditional logistic regression.

In all analyses, age was specified as age at last contact or at death for the unaffected individuals, and age at onset for the affected individuals. We used age at onset instead of age at last contact or death for the patients because we wished to evaluate the effect of gender on the onset of AD and to avoid possible confounding by longer survival of women after disease onset.

The NCR data included kindreds that were ascertained by various groups and pooled by the NCR at Indiana University. Because of confidentiality, we are not aware of the sites where or the methods by which these families were originally ascertained. Only the Oregon families that have been placed in the NCR pool were identified and excluded to avoid overlap with the Oregon data. The 47 families that were sent to us were chosen by the NCR staff for being the largest and best characterized kindreds in the NCR database. To minimize confounding due to missing data, before genotyping, we prioritized the families according to the completeness of data and availability of autopsy documentation. We chose the top 15 kindreds that had autopsy documentation and at least 3 affected individuals with known ages at onset. The kindreds were Caucasian and the average age at onset in each kindred was >60 years. The 15 kindreds were composed of 11 nuclear families and 4 extended families. We genotyped every available sample from sibships with affected members, using a method similar to the method described by Hixon and Vernier (1990). The NCR data were analyzed separately using the Kaplan-Meier survival analysis and logistic regression as described above.

RESULTS

The Oregon/Washington/Minnesota data. APOE genotypes were available for 182 women (101 affected and 81 unaffected) and 113 men (60 affected and 53 unaffected). Genotypes were grouped according to the number of the $\epsilon 4$ alleles present: $\epsilon 4$ homozygous ($\epsilon 4/\epsilon 4$), $\epsilon 4$ heterozygous ($\epsilon 4/-$ including $\epsilon 4/\epsilon 3$ and $\epsilon 4/\epsilon 2$) and no $\epsilon 4$ ($\epsilon -/\epsilon -$ including $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 2$ and $\epsilon 2/\epsilon 2$). The average ages at onset for the $\epsilon 4/\epsilon 4$, $\epsilon 4/-$ and $\epsilon -/\epsilon -$ subjects were 69.8 ± 5.1 , 71.0 ± 6.8 , and 73.6 ± 8.6 for women and 67.3 ± 8.3 , 69.6 ± 8.1 and 68.6 ± 8.2 for men. The average ages of the $\epsilon 4/\epsilon 4$, $\epsilon 4/-$ and $\epsilon -/\epsilon -$ unaffected subjects were 63.8 ± 13.4 , 67.7 ± 15.4 , and 59.1 ± 17.4 for women and 63.5 ± 15.7 , 67.0 ± 12.7 , and 62.6 ± 22.6 for men.

The APOE genotype and allele frequencies are shown in Table 1. Considering all family members who were sampled (affected and unaffected), men and women had similar APOE genotype frequencies. Considering affected individuals only, men and women had significantly different genotype frequencies ($P=.01$), although the significance level is probably inflated because some of the subjects are related. The difference appeared to be due to an excess of the $\epsilon 4/\epsilon -$ genotype in women, because when compared to the Hardy-Weinberg expectations, the observed genotype frequency distribution for affected women was significantly distorted ($p < .001$) with an excess of $\epsilon 4/\epsilon -$ and deficiencies of $\epsilon 4/\epsilon 4$ and $\epsilon -/\epsilon -$, while the observed distribution for affected men was in close agreement with the Hardy-Weinberg expectations. Despite the apparent difference in the genotype frequency distributions, allele frequencies were similar in affected men and affected women.

Age-adjusted odds ratios of being affected were estimated with logistic regression (Table 2). In women, $\epsilon 4/\epsilon 4$ and $\epsilon 4/\epsilon -$ had higher risk than $\epsilon -/\epsilon -$; $\epsilon 4/\epsilon 4$ and $\epsilon 4/\epsilon -$ were

similar. In men, $\epsilon 4/\epsilon 4$ had significantly higher risk than $\epsilon 4/\epsilon -$ and $\epsilon -/\epsilon -$; $\epsilon 4/\epsilon -$ and $\epsilon -/\epsilon -$ were similar. For men and women with similar genotypes, $\epsilon 4/\epsilon -$ women had significantly higher risk than $\epsilon 4/\epsilon -$ men; there was no significant difference between $\epsilon 4/\epsilon 4$ men and women or between $\epsilon -/\epsilon -$ men and women.

Since these data represent data from families and family unit might alter the risk status, we fit a conditional logistic model with stratification on families. In this analysis, gender was a significant risk factor for the $\epsilon 4/\epsilon -$ genotype (odds ratio for women versus men = 2.64, $P = .02$, 95% confidence interval [CI]: [1.19, 5.88]), but not for the $\epsilon 4/\epsilon 4$ ($P = .10$) or the $\epsilon -/\epsilon -$ genotype ($P = .31$).

The Kaplan-Meier curves (Figure 1) exhibited the same trends as was seen with odds ratios. In women, the curve for $\epsilon 4/\epsilon -$ was significantly different from $\epsilon -/\epsilon -$ ($P = .02$) but not from $\epsilon 4/\epsilon 4$. In men, the curve for $\epsilon 4/\epsilon -$ was significantly different from $\epsilon 4/\epsilon 4$ ($P = .01$) but not from $\epsilon -/\epsilon -$. In contrast to Kaplan-Meier curves, the difference in lifetime cumulative risks was not statistically significant.

The NCR data. APOE genotypes were available for 56 women (38 affected and 18 unaffected) and 34 men (18 affected and 16 unaffected). The average ages at onset for the $\epsilon 4/\epsilon 4$, $\epsilon 4/-$ and $\epsilon -/\epsilon -$ subjects were 69.3 ± 8.1 , 72.2 ± 7.4 , and 73.1 ± 11.4 for women and 64.8 ± 6.8 , 76.2 ± 4.5 and 72.0 ± 8.2 for men. The average ages of the $\epsilon 4/\epsilon 4$, $\epsilon 4/-$ and $\epsilon -/\epsilon -$ unaffected subjects were 72.0 ± 9.0 , 66.9 ± 9.6 , and 76.1 ± 7.3 for women and 77.0 ± 0 , 72.0 ± 7.8 , and 67.7 ± 9.5 for men.

When all family members who were genotyped were considered, there was no significant difference in the genotype frequencies of men and women ($\epsilon 4/\epsilon 4$, $\epsilon 4/\epsilon -$, $\epsilon -/\epsilon -$:

.21, .47, .32 vs. .11, .62, .27). When only the affected individuals were considered, men and women had different genotype frequencies (.33, .5, .17 vs. .08, .74, .18, $P=.05$). Genotype frequencies differed significantly from the Hardy-Weinberg expectations in both affected men and affected women, but in different directions: in affected women, there was an excess of $\epsilon 4$ heterozygotes, whereas in affected men there was an excess of $\epsilon 4$ homozygotes.

The NCR data showed the same pattern of gender difference as our data. Using logistic regression adjusting for age, $\epsilon 4/\epsilon -$ women had significantly higher risk than $\epsilon 4/\epsilon -$ men (OR=6.88, 95% CI (1.52-31.19, $P=.01$)). There was no significant difference between $\epsilon 4/\epsilon 4$ men and women (odds ratio=.26, 95% CI (.01-4.6, $P=.36$)) or between $\epsilon -/\epsilon -$ men and women (odds ratio=2.39, 95% CI (.41-13.87, $P=.33$)). The sample size of the NCR data was too small to allow stratification on families. When Kaplan-Meier survival analysis was used (Figure 2), women showed a significant difference between $\epsilon 4/\epsilon -$ and $\epsilon -/\epsilon -$ ($P=.02$), but not between $\epsilon 4/\epsilon -$ and $\epsilon 4/\epsilon 4$. Men, on the other hand, showed a significant difference between $\epsilon 4/\epsilon -$ and $\epsilon 4/\epsilon 4$ ($P=.004$), but not between $\epsilon 4/\epsilon -$ and $\epsilon -/\epsilon -$.

DISCUSSION

The purpose of this study was to assess the influence of gender on APOE- associated risk in late-onset familial AD. We studied 58 kindreds that were ascertained by us, and for confirmation, an additional 15 autopsy-proven kindreds from the NCR. Currently, there is no ideal method for estimating risk ratios from family data. For this reason, we analyzed the data with the three most commonly used methods: Kaplan-Meier survival analysis,

unconditional logistic regression adjusting for age, and conditional logistic regression stratifying on families. The results were qualitatively the same for all analyses; every method gave statistically significant evidence for a gender difference for the $\epsilon 4$ heterozygous genotype.

The present study includes two data sets with familial late-onset AD. In both data sets, $\epsilon 4$ heterozygous women appeared to have significantly higher risk than $\epsilon 4$ heterozygous men. A third data set with familial late-onset AD was analyzed by Corder et al (1995a). Although their initial study did not show a significant difference, they recently reported different patterns of risk for men and women in relation to APOE $\epsilon 4$ (Corder et al., 1995b).

Our study included large kindreds that were selected for having multiple affected individuals; thus the results cannot be generalized to smaller families or to sporadic AD. A number of studies of sporadic AD have questioned the gender effect on the APOE genotype. Tsai et al (1994) combined the $\epsilon 4/\epsilon 4$ and $\epsilon 4/\epsilon -$ genotypes and found no difference between men and women. The two studies that investigated the $\epsilon 4/-$ genotype separately detected a gender difference. Poirier et al (1993) studied Caucasian subjects and found a higher frequency of the $\epsilon 4$ heterozygous genotype in women than in men, which is consistent with our results. They also detected a gender difference in the frequency of the $\epsilon 4$ allele, which we did not. Yasuda et al (1995) studied Japanese subjects, and found a higher risk for $\epsilon 4$ heterozygous women than $\epsilon 4$ heterozygous men. In their study, the $\epsilon 4$ allele frequency was similar in men and women.

Corder et al (1995b) have suggested that the higher prevalence of AD in women may be a result of longer survival in affected women. The gender difference in our studies

cannot be attributed to differences in survival after the onset of dementia. When adjusting for the ages of the subjects, we used current age or age at death for the asymptomatic individuals, but for the affected individuals we used their ages at onset. As a result, affected individuals were effectively eliminated from further consideration once they became demented and disease duration did not enter the analyses. This strategy enabled us to study the effect of gender on the onset of dementia and to avoid confounding by longer survival of women after disease onset.

One possible explanation for our results is that gender plays a role in the pathogenesis of familial AD and influences the risk of developing the disease. The role of gender in AD has been controversial. Community-based incidence studies have been inconsistent, but family studies have generally shown higher age-specific risks for female relatives of patients than male relatives, suggesting that gender is an independent risk factor for familial AD (Breitner et al., 1988; van Duijn et al., 1993; Rao et al., 1994; Silverman et al., 1994; Payami et al., 1996a). The present findings are consistent with the previous family studies, and suggest that gender may interact with APOE in determining susceptibility to AD.

A recent study has demonstrated an interaction between APOE genotype, gender, age and cholesterol levels in AD, suggesting that some aspects of cholesterol transport may be involved in the pathogenesis of this disease (Jarvik et al., 1995a). APOE plays a major role in lipid transport and metabolism. Recently, it has been proposed that the link between AD and APOE involves a dysfunction of lipid-transport system in hippocampal response to injury (Poirier, 1994). Gender is known to affect the influence of APOE on the distribution of plasma lipids and apolipoproteins (Reilly et al., 1994), and thus may also affect the efficacy of APOE in redistributing myelin cholesterol during nerve repair.

A number of studies have implicated estrogen in cognitive health and AD. It has been suggested that estrogen has a protective effect against age-related cognitive decline (Honjo et al., 1989; Henderson et al., 1991; Barrett-Conner and Kritiz-Silverstein, 1993), and estrogen use after menopause may enhance memory function in older women (Kampen and Sherwin, 1994; Robinson et al., 1994). Although controversial, estrogen replacement therapy may reduce the risk of AD in women (Brenner et al., 1994; Paganini-Hill and Henderson, 1994). It is possible that the gender difference observed here is related to hormonal changes that women experience at menopause.

$\epsilon 4$ is a major risk factor for AD, but it is neither necessary nor sufficient for the development of the disease (Corder et al., 1993; Payami et al., 1993). It is now clear that factors other than $\epsilon 4$ are involved in the etiology of AD (Corder et al., 1993; Payami et al., 1993; Bennett et al., 1995). It is possible that the added susceptibility due to the other risks (e.g. estrogen depletion) is inconsequential in $\epsilon 4$ homozygotes, who are already highly susceptible, but is relatively substantial in $\epsilon 4$ heterozygotes and can increase their risk to the level of $\epsilon 4$ homozygotes.

Another possible explanation for the results is that the data were skewed by incomplete sampling of the family members. This may seem unlikely since two independent data sets yielded similar results, but it is conceivable that a similar bias existed in both our data and the NCR data. Despite efforts to sample every affected and unaffected individual in each sibship, a considerable number of family members were deceased and their genotypes could not be reconstructed. Further, some of the currently unaffected subjects may eventually develop AD; thus follow-up of the younger subjects may alter the results.

In light of the reported association of APOE with longevity (Kervinen et al., 1994; Schachter et al., 1994), stroke (Pedro-Botet et al., 1992; Couderc et al., 1993), and possibly heart disease (Payne et al., 1992; van Bockxmeer and Mamotte, 1992; Wilson et al., 1994), we questioned whether our results were seriously compromised by competing risks and the missing genotypes of the deceased relatives. First, although the APOE profile of the population changes with age, middle-aged and octogenarian men and women have similar genotype frequencies (Davignon et al., 1988a; Wilson et al., 1994). Thus the difference that we see in men and women with AD cannot be attributed to a gender/APOE effect on longevity. In our AD kindreds, there was no difference in genotype frequencies in men and women, except for those who had developed AD, which suggests that the gender difference is specific to AD.

Next, we wondered whether the missing genotypes represented death from heart disease, stroke, or another common cause that might be influenced by an APOE/gender interaction. In the 21 Oregon kindreds that we examined, the missing genotypes were due to death from cancer (8 women, 9 men), heart disease (4 women, 9 men), AD (7 women, 4 men), stroke (3 women, 3 men), other causes combined (4 women, 11 men), and unknown causes (6 women, 2 men). In light of the diverse causes of death, and the infrequency of each, it is difficult to attribute the gender difference in AD to any individual cause. However, nearly 30% of the deaths were due to stroke and heart disease combined. Thus the possibility remains that the data were skewed by the missing genotypes of the deceased individuals.

Finally, we reasoned that if the increased risk to $\epsilon 4$ heterozygous women is real, then given the high frequency of this genotype in AD kindreds, women should exhibit a higher

risk than men when APOE genotype is not considered. Alternatively, if the results are due to missing genotypes, there should not be a gender difference when all family members are accounted for. We assessed the gender specific risk of AD in the Oregon families, regardless of APOE and with virtually no missing data, and for confirmation, in families from the NCR. In both data sets, we found significantly elevated age-specific risk in women than in men (Payami et al., 1996a). In short, we found no evidence that our results are caused by selection bias. Nevertheless, we cannot rule out the possibility that the data were skewed by the missing data. Resolution of this issue requires large prospective studies that take gender and family history of AD into account and are well-controlled for competing risk factors.

If confirmed, an increased risk to $\epsilon 4$ heterozygous women can have a major public health impact. Although $\epsilon 4$ homozygosity confers the highest risk of AD in both men and women, this genotype is relatively rare. Only 3% of middle aged Americans (ages 40-77) and 0.4% of Canadian octogenarians are reportedly $\epsilon 4$ homozygous, as compared to 20% of middle-aged and 16% of octogenarians who are $\epsilon 4$ heterozygous (Davignon et al., 1988a; Wilson et al., 1994). Therefore, in light of the high prevalence of $\epsilon 4$ heterozygosity, a two-fold difference in risk between men and women with this genotype could have a major effect on the gender-specific incidence of the disease in the population.

In conclusion, we have found evidence for a gender difference in the APOE associated risk for familial AD and confirmed it in an independent data set. The results may reflect a gender difference in the etiology of AD or in survival, due to competing risks. This study raises some critical questions that have wide implications in basic research, public health

and genetic counseling. The role of gender in AD, and its possible interaction with APOE, warrants further investigation.

	Total	$\epsilon 4/\epsilon 4$	$\epsilon 4/\epsilon 3$	$\epsilon 4/\epsilon 2$	$\epsilon 3/\epsilon 3$	$\epsilon 3/\epsilon 2$	$\epsilon 2/\epsilon 2$
All women	182	.16	.52	.04	.23	.05	0
All men	113	.24	.47	.04	.22	.03	0
Affected women	101	.16	.64	.04	.14	.02	0
Affected men	60	.35	.44	.03	.18	0	0
Unaffected women	81	.16	.37	.04	.34	.09	0
Unaffected men	53	.11	.51	.06	.26	.06	0

	Total	$\epsilon 4/\epsilon 4$	$\epsilon 4/\epsilon -$	$\epsilon -/\epsilon -$
All women	182	.16	.56	.28
All men	113	.24	.51	.25
Affected women	101	.16	.68	.16
Affected men	60	.35	.47	.18
Unaffected women	81	.16	.41	.43
Unaffected men	53	.11	.57	.32

	Total	$\epsilon 4$	$\epsilon 3$	$\epsilon 2$
All women	364	.44	.51	.05
All men	226	.50	.47	.03
Affected women	202	.50	.47	.03
Affected men	120	.58	.40	.02
Unaffected women	162	.36	.58	.06
Unaffected men	106	.40	.55	.05

Table 1. APOE genotype and allele frequencies.

	Odds ratio	95% CI	P
Women			
$\epsilon 4/\epsilon 4$ (29) vs. $\epsilon -/\epsilon -$ (50)	2.53	.95 - 6.75	.06
$\epsilon 4/\epsilon -$ (103) vs. $\epsilon -/\epsilon -$ (50)	3.71	1.74 - 7.90	.0007
$\epsilon 4/\epsilon 4$ (29) vs. $\epsilon 4/\epsilon -$ (103)	.68	.29 - 1.62	.4
Men			
$\epsilon 4/\epsilon 4$ (27) vs. $\epsilon -/\epsilon -$ (28)	5.43	1.64 - 17.97	.006
$\epsilon 4/\epsilon -$ (58) vs. $\epsilon -/\epsilon -$ (28)	1.36	.54 - 3.46	.52
$\epsilon 4/\epsilon 4$ (27) vs. $\epsilon 4/\epsilon -$ (58)	3.98	1.39 - 11.44	.01
Women versus Men			
$\epsilon 4/\epsilon 4$ women (29) vs. $\epsilon 4/\epsilon 4$ men (27)	.32	.09 - 1.07	.06
$\epsilon 4/\epsilon -$ women (103) vs. $\epsilon 4/\epsilon -$ men (58)	2.11	1.08 - 4.10	.03
$\epsilon -/\epsilon -$ women (50) vs. $\epsilon -/\epsilon -$ men (28)	.76	.27 - 2.10	.6

Table 2. Odds ratio of developing AD, using unconditional logistic regression, adjusting for age. Sample sizes are shown in parentheses.

Oregon/Washington/Minnesota Families

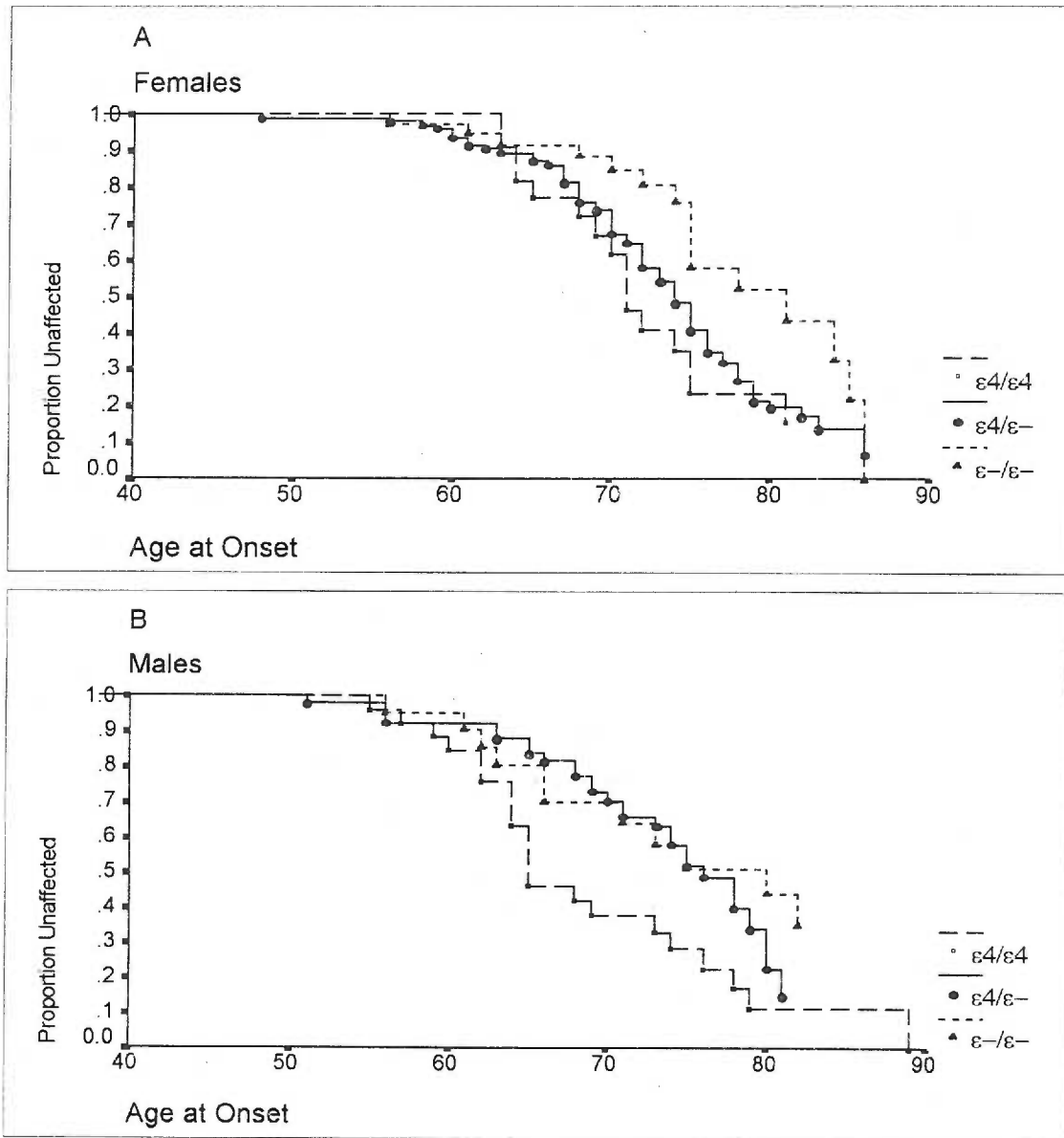


Figure 1 Genotype-specific distribution of age at onset in Oregon/Washington/Minnesota families. A) Differences in age at onset curves in women: $\epsilon 4/\epsilon 4$ (N=25) vs. $\epsilon -/\epsilon -$ (N=45), $P=0.02$. $\epsilon 4/\epsilon 4$ (N=25) vs. $\epsilon 4/\epsilon -$ (N=91), $P=0.53$. $\epsilon 4/\epsilon -$ (N=91) vs. $\epsilon -/\epsilon -$ (N=45), $P=0.02$. B) Differences in age at onset curves in men: $\epsilon 4/\epsilon 4$ (N=22) vs. $\epsilon -/\epsilon -$ (N=26), $P=0.01$. $\epsilon 4/\epsilon 4$ (N=22) vs. $\epsilon 4/\epsilon -$ (N=42), $P=0.01$. $\epsilon 4/\epsilon -$ (N=42) vs. $\epsilon -/\epsilon -$ (N=26), $P=0.40$.

NCR Families

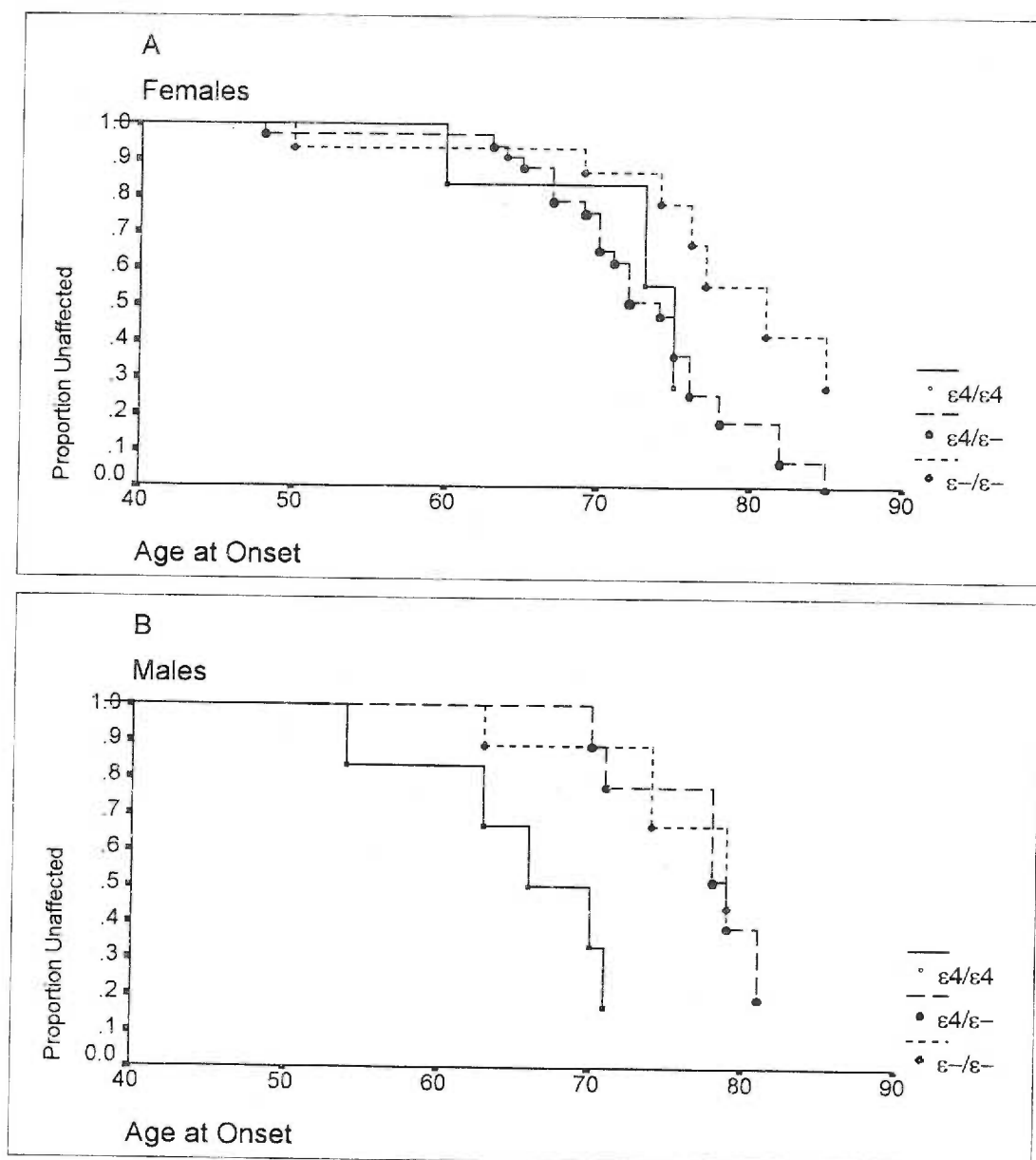


Figure 2. Genotype-specific distribution of age at onset in NCR families. A) Differences in age at onset curves in women. $\epsilon 4/\epsilon 4$ (N=6) vs. $\epsilon -/\epsilon -$ (N=15), $P=0.35$. $\epsilon 4/\epsilon 4$ (N=6) vs. $\epsilon 4/\epsilon -$ (N=35), $P=0.67$. $\epsilon 4/\epsilon -$ (N=35) vs. $\epsilon -/\epsilon -$ (N=15), $P=0.01$. B) Differences in age at onset curves in men. $\epsilon 4/\epsilon 4$ (N=6) vs. $\epsilon -/\epsilon -$ (N=11), $P=0.02$. $\epsilon 4/\epsilon 4$ (N=6) vs. $\epsilon 4/\epsilon -$ (N=13), $P=0.004$. $\epsilon 4/\epsilon -$ (N=13) vs. $\epsilon -/\epsilon -$ (N=11), $P=0.98$.

CHAPTER 3

EVIDENCE FOR ASSOCIATION OF HLA-A2 ALLELE WITH ONSET AGE OF ALZHEIMER'S DISEASE

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ABSTRACT

Our earlier studies had suggested a possible association between the human leukocyte antigen (HLA) allele A2 and Alzheimer disease (AD). In the present study, we tested the hypothesis that A2 is associated with earlier AD onset. We performed two independent studies: a collaborative study with 111 patients and a confirmatory study with 96 patients. We found similar patterns of reduced age at onset as a function of A2, in both data sets. Overall, A2 was associated with a significant three-year shift to earlier onset. The effects of A2 and $\epsilon 4$ on age at onset appeared additive. Our results suggest A2, or a closely linked gene, modulates onset age of AD. Association with A2 would suggest an immune/inflammatory response mechanism for AD.

INTRODUCTION

Alzheimer disease (AD) is a common neurodegenerative disorder characterized by a progressive loss of memory and cognitive function. AD is genetically heterogeneous. Early-onset familial AD, a rare form characterized by onset before age 60 years and an autosomal dominant mode of inheritance, is linked to the amyloid precursor protein (APP) gene on chromosome 21 (Kamino et al., 1992), the presenilin I (PSI) gene on chromosome 14 (Schellenberg et al., 1994), and the PSII gene on chromosome 1 (Levy-Lahad et al., 1995). The common forms of AD, including early-onset AD without autosomal dominant inheritance and late-onset AD, are associated with the apolipoprotein E (APOE) gene on chromosome 19. The APOE- $\epsilon 4$ allele is associated with earlier onset of dementia (Corder et al., 1993). Several lines of evidence suggest the existence of

additional, as yet unidentified, AD genes (Corder et al., 1993; Payami et al., 1993; Bennett et al., 1995; Farrer et al., 1995; Duara et al., 1996; Jarvik et al., 1996).

Before any of the currently recognized genetic markers for AD were discovered, several studies examined the possible association of AD with the human leukocyte antigens (HLA). HLA, the major histocompatibility complex in humans, consists of several highly polymorphic and tightly linked genes on chromosome 6 (Parham and Ohta, 1996). HLA genes regulate the immune response. AD brains are characterized by the presence of immune-associated proteins and cells such as activated microglia that express high levels of HLA molecules (McGeer et al., 1994). Further, nonsteroidal anti-inflammatory drugs may delay the onset of AD (Breitner et al., 1995). Thus, AD may involve an immune/inflammatory response mechanism mediated by HLA.

HLA association studies with AD have been inconsistent (reviewed by Payami et al., 1991). HLA studies are plagued with spurious findings because each study examines numerous alleles and, by chance alone, one in 20 will show a statistically significant association. While some of the findings were probably spurious, some may have been real, and the inconsistencies may have been due to disease heterogeneity, which at the time was not recognized. Small and Matsuyama reported increased frequency of the A2 allele in men with early-onset (<60 yrs) AD (1986). A2, the most common allele at the HLA-A locus, is present in 40 to 60% of Caucasians (Imanishi et al., 1992). Payami et al. also found significantly elevated A2 frequency in men with early-onset AD (1991). A third study did not replicate this finding (Small et al., 1991). The effect of A2 on AD onset has not been examined.

The recent understanding that genes, like APOE, can have a significant influence on age at onset, and the evidence for the existence of additional AD genes, prompted us to carry out the present study. The aim of this study was to test the hypothesis that A2 is associated with earlier onset of AD. We did not type the patients for any other HLA alleles, thus spurious results that often plague HLA association studies were of no concern. We performed all comparisons within the patient population, to avoid possible confounding by the selection of controls. The study was designed as a collaborative effort and included patients from four AD research centers. To confirm the results, we subsequently studied an independent series of consecutive patients from one AD center.

METHODS

The collaborative study included 111 unrelated, Caucasian subjects with the clinical diagnosis of probable AD (McKhann et al., 1984), or neuropathological diagnosis of definite AD (Mirra et al., 1991). The subjects were from four sources: probands from 47 late-onset familial AD kindreds ascertained at University of Washington, Oregon Health Sciences University and University of Minnesota (WA/OR/MI data) (Payami et al., 1996b); 42 patients from University of California at Los Angeles, West Los Angeles Veterans Medical Center and Harbor/UCLA Medical Center (WLAVA/UCLA data) (Jarvik et al., 1995b); 16 patients referred by family members or physicians for diagnosis and management of dementia at Southern Illinois University (SIU data); and 6 patients chosen randomly for another study at University of California, San Diego (UCSD data). Every participant or legal guardian signed informed consent in the presence of a witness. Subjects who were in previously published HLA studies were excluded. Positive family

history was defined as having a first degree relative with AD or progressive memory loss. Age at onset was defined as the age when memory loss was first noticed by relatives.

HLA-A2 typing was done by PCR (Fernandez et al., 1992) for 88 subjects and by the serological technique (Terasaki et al., 1978) for 23 subjects. A subset of subjects were typed by serology and PCR, and the results were the same. APOE genotypes were determined using the method described by Hixon and Vernier (1990). A subset of WA/OR/MI families had been tested for the known pathologic mutations in A β P, PSI and PSII. Other subjects were not tested. Thus, some of the patients may have had a mutation in one of these genes.

Prior to data analysis, we classified the patients into three age at onset categories: early-onset (<60 years), mid-onset (60-75) and late-onset (>75). Based on the prior observations, the primary hypothesis of this study was that A2 is associated with reduced age at onset in the early-onset subtype of AD, hence the cut-off at age 60. The cut-off at age 75 was based on evidence that ϵ 4 frequency drops dramatically after age 75 (Rebeck et al., 1994; Yu et al., 1994; Corder et al., 1995b; Sobel et al., 1995; Corder et al., 1996). Therefore, if A2 is associated with late-onset AD, its effect may be masked by a strong ϵ 4 effect in the mid-onset range, and may be more readily detectable after age 75.

We used the *t*-test to compare the mean age at onset of A2+ and A2- subjects, and the analysis of variance (ANOVA) to examine age at onset as a function of A2 phenotype and APOE genotype. To test for independence between A2 and ϵ 4, the observed joint frequencies of A2+ and A2- phenotype with ϵ 4 ϵ 4, ϵ 4 ϵ -, ϵ - ϵ - genotypes were compared to the expected frequencies under the assumption of random association, using the Chi-square test. We also examined the effects of gender and family history using the analytical

methods outlined above, and corrected the results for multiple comparisons. Statistical analyses were carried out with SPSS software (version 6, 1993).

The confirmatory study. After completion of the collaborative study, we collected a second data set that included 96 consecutive AD patients from the Oregon Alzheimer Disease Center (OADC) with the diagnosis of probable or definite AD (McKhann et al., 1984; Mirra et al., 1991). We do not know if any of these subjects had an APP, PSI or PSII mutation. Subjects were A2-typed using the HLA-A low resolution PCR-SSP (Sequence Specific Primers) typing kit from DYNAL Inc. (Oslo, Norway). APOE genotyping and data analyses were done using the same methods as those used for the collaborative study.

RESULTS

The collaborative study. The characteristics of the subjects are shown in Table 1. We pooled the collaborative data after a test of heterogeneity showed no significant difference in A2 frequencies. There were, however, clear differences in the individual data sets in the ratio of familial to sporadic cases and in ages at onset, which reflect differences in patient selection. (This concern was the impetus for using a consecutive patient series from one center for the confirmatory study.) Since A2 homozygotes and A2 heterozygotes could not be distinguished, subjects were classified as A2+ or A2-. HLA-A and APOE are on different chromosomes. Thus, unless there is an interaction, their joint frequencies should be randomly distributed. The observed frequencies were in close agreement with random expectations, providing no evidence for an interaction. $\epsilon 4$ frequency was elevated, as expected.

Table 2 shows the difference in mean age at onset as a function of A2. In early-onset AD, A2 was associated with a significant 2.8 years earlier onset, consistent with our hypothesis. There was no difference in mid-onset cases. A non-significant 2.0-year difference was present in late-onset AD. Considering all patients (onset, 40-90 years), mean onset age was 2.6 years earlier in A2+ subjects than A2- subjects, but this difference was not statistically significant. Figure 1 shows the overall age at onset distribution for A2+ and A2- patients:

The overall A2 phenotype frequency in patients was 0.48, which was within the range for the general Caucasian population (Imanishi et al., 1992). If A2 is associated with earlier age at onset, one would expect the frequency of A2 to be high in patients with earliest onset and low in patients with latest onset. A2 frequency was 0.83 in patients with onset age <50 years, and 0.43 in those with onset $\geq$ 80 years. The A2 frequency was relatively constant (0.48 to 0.45) in the intermediate onset range of 50 to 79 years.

The confirmatory study. Although the primary hypothesis was confirmed, we were concerned about the methods by which the subjects were selected for the collaborative data and the possibility of ascertainment bias. Also, we wondered about the reproducibility of the A2 effect in the late-onset group. Thus we opted to repeat the study in a series of 96 consecutively ascertained AD patients (Table 1). Mean age at onset in this group was 70.2 ± 11.1 years. The joint frequencies of A2 and $\epsilon 4$ were randomly distributed with no evidence for interaction.

The confirmatory data yielded the same pattern as the collaborative data (Figure 1 and Table 2). In early-onset AD, there was a significant 4.3-year earlier onset in A2+ subjects, consistent with the original hypothesis. There was no difference in mid-onset AD. In late-

onset AD, mean onset age was 2.9 years earlier in A2+ subjects. The difference in late-onset AD was statistically significant, confirming the trend seen in the collaborative study. Overall, considering all patients' onset ages 40 to 90 years, A2 was associated with a non-significant 2.4-year earlier onset, a trend similar to the collaborative data.

The overall A2 phenotype frequency was 0.35, which is within the lower end of the range for Caucasians. Similar to the collaborative data, the A2 frequency was highest in patients with earliest onset (0.75 for onset <50 years) and lowest in those with latest onset (0.33 for onset $\geq$ 80 years). The A2 frequency was relatively constant (0.43 to 0.40) in the intermediate onset range of 50 to 79 years.

The combined data. It was intriguing that the two data sets produced similar results despite the obvious differences in the two samples. The patients in the confirmatory study were significantly older at onset than the patients in the collaborative study (mean onset age, 70.2 ± 11.1 vs. 65.2 ± 10.5 , $p < 0.001$), and interestingly, their A2 frequency was also lower (0.35 vs. 0.48, not significant). The two patient groups may in fact represent the two ends of the same distribution where A2 is enriched in early-onset patients and depleted in late-onset patients. The subject selection for the collaborative study favored familial and early-onset patients and had a higher A2 frequency, whereas the confirmatory study included clinic patients with later onset and lower A2 frequency. Since the two data sets produced similar results (i.e., independent evidence for association of A2 with AD onset), we pooled them to increase the sample size, in order to assess the effects of APOE, family history and gender in relation to A2.

As anticipated, once data were pooled, the differences in mean age at onset for A2+ versus A2- became more significant in early-onset AD (3.2-year mean difference,

$p < 0.005$) and in late-onset AD (2.6-year mean difference, $p < 0.01$), but not in the mid-onset AD (-0.8-year mean difference, not significant). When all subjects were considered, regardless of age at onset, the mean onset age was 3.1 years earlier in A2+ subjects than in A2- subjects, and the difference was statistically significant ($p < 0.05$).

Figure 2 shows the mean ages at onset as a function of A2 and $\epsilon 4$. Based on ANOVA there was no evidence for interaction between $\epsilon 4$ and A2 on onset age. As shown in Figure 2, age at onset was earliest in patients who were A2+ and $\epsilon 4$ homozygous (60.4 ± 8.9 years), intermediate in those who were either A2+ without $\epsilon 4$ (65.8 ± 12.1 years) or $\epsilon 4$ homozygous without A2 (65.7 ± 7.4 years), and latest in those who had neither A2 nor $\epsilon 4$ (70.4 ± 12.1 years). $\epsilon 4$ heterozygotes had intermediate age at onset and there was no A2 effect on age at onset in this group. Within $\epsilon 4$ homozygotes, A2+ subjects had a 5.3-year earlier onset than A2- subjects (60.4 ± 8.9 vs. 65.7 ± 7.4). Within $\epsilon 4$ heterozygotes, the difference was 0.4 year (68.3 ± 10.5 vs. 68.7 ± 10.1). Among those without $\epsilon 4$, A2+ subjects had 4.6 year earlier onset (65.8 ± 12.1 vs. 70.4 ± 12.1). Note however that (a) the differences were not significant after correction for multiple comparisons, and (b) we were able to take dosage into account for $\epsilon 4$ but not for A2, because we were unable to distinguish A2 homozygotes from heterozygotes.

A2 frequency did not vary significantly by gender or family history. There was however a larger difference in age at onset as a function of A2 in non-familial than in familial AD. In familial AD, A2 was associated with a non-significant 1.3-year earlier onset (66.6 ± 11.6 vs. 67.9 ± 9.4 , not significant). In non-familial AD, the difference was 5.8 years (62.8 ± 10.1 vs. 68.6 ± 12.2 , not significant after correction). The A2-effect may

appear stronger in non-familial cases because they may be less complicated by the presence of other genes. If so, it was of interest to examine the A2 effect in subjects who had neither family history nor $\epsilon 4$. In that group (N=31) A2 was associated with a 7.6-year earlier onset (62.7 ± 9.2 vs. 70.3 ± 12.3 , not significant after correction).

DISCUSSION

Previous studies, although inconsistent, had suggested that HLA-A2, or a closely linked gene, may be associated with early-onset AD (Small and Matsuyama, 1986; Payami et al., 1991; Small et al., 1991). Earlier studies had relied solely on differences in allele frequencies between patients and controls. With the recent understanding that susceptibility genes (such as APOE) can modulate age at onset, we questioned if the presence of A2 was associated with earlier disease onset. An association with earlier disease onset can lead to elevated frequency of the marker in early-onset patients. At the same time, failure to detect an increased allele frequency does not negate the possibility that the marker modulates age at onset (Payami et al., 1997). In the present study, we found that A2+ subjects had significantly earlier age at onset than A2- subjects, and that A2 frequency was elevated in early-onset patients. These findings are consistent with the previously published reports of elevated A2 frequency in early-onset AD. Further, the present results were replicated in a second, independent data set, making it less likely that the A2 effect in AD is due to chance alone. If confirmed, our data suggest A2, or a gene in linkage disequilibrium with A2, modulates age at onset of AD.

We examined the A2 effect in early- (<60 years), mid- (60 to 75 years), and late-onset (>75 years) AD separately. The cut-off at 60 was based on earlier evidence for association

of A2 with early-onset AD (Small and Matsuyama, 1986; Payami et al., 1991). The cut-off at 75 years was based on the evidence from APOE studies showing a diminishing effect of $\epsilon 4$ after age 75 (Rebeck et al., 1994; Yu et al., 1994; Corder et al., 1995b; Sobel et al., 1995; Corder et al., 1996). It was therefore of interest to see if an A2 effect would be more apparent after age 75 when the $\epsilon 4$ effect is relatively weak. In early-onset AD, A2 was associated with significantly earlier onset, both in the collaborative data and the confirmatory data. In mid-onset AD, there was no difference in either data set. In late-onset AD, the evidence for association of A2 with earlier onset reappeared in both data sets.

The lack of evidence for an A2 effect in the mid-onset range is intriguing, especially in light of the fact that two data sets, with different patient populations, showed similar trends. It is possible that A2 is not associated with mid-onset AD. Alternatively, an A2 effect may be present in all AD but is not detectable in the mid-onset range for a variety of related reasons. Suppose presence of A2 shifts the onset age to 3 years earlier; say for example, age at onset ranges from 43 to 89 years for A2- subjects and from 40 to 86 for A2+ subjects. The shift involves the entire distribution, but when measured by the difference in mean age at onset between A2+ and A2- subjects, it will be detectable only at the two ends of the distribution and not in the mid-range. This is the pattern seen in the age at onset distributions in both the Collaborative and Confirmatory data (Figure 1). The ability to detect an A2 effect in the mid-range may be further compromised by other confounding factors. We originally created the mid-range because other studies had shown a dramatic drop in $\epsilon 4$ frequency after age 75 (Rebeck et al., 1994; Yu et al., 1994; Corder et al., 1995b; Sobel et al., 1995; Corder et al., 1996). In the present data, $\epsilon 4$ was present

in 57% of early-onset patients, 75% of mid-onset patients, and 48% of late-onset patients. Thus, the ability to detect an A2 effect in the mid range may have been masked by the strong $\epsilon 4$ effect. Disease heterogeneity can also affect the ability to detect associations. AD is a heterogeneous disorder and, if early- and late-onset patients represent distinct etiologies, mid-onset patients would include the most heterogeneous group of patients and may be the most difficult to decipher.

Overall, 42% of the AD patients had A2, which is within the reported range for Caucasians (Imanishi et al., 1992). In the AD patients, however, A2 frequency varied as a function of onset age. It was highest in patients with early onset AD and declined with increasing age at onset. Previous studies of HLA and longevity, which have compared subjects from 10 to 100 years old, have shown that the population frequency of A2 does not vary with increasing age (Blackwelder et al., 1982; Proust et al., 1982; Lagaay et al., 1991; Rea and Middleton, 1994).

We cannot directly compare the relative effects of A2 and $\epsilon 4$ because we cannot separate A2 homozygotes from A2 heterozygotes. If we combine $\epsilon 4$ homozygotes with $\epsilon 4$ heterozygotes, this study shows only a 1-year earlier onset in $\epsilon 4+$ patients than $\epsilon 4-$ patients, as compared to a 3-year earlier onset in A2+ patients than A2- patients. This comparison, however, is crude because it does not take into account dosage, which is critical for $\epsilon 4$, and may also be critical for A2. If we consider $\epsilon 4$ genotype (dosage) jointly with the A2 phenotype (ignoring dosage), age at onset was earliest in patients who were A2+ and $\epsilon 4$ homozygous (mean, 60.4 years), and latest in those who lacked A2 and $\epsilon 4$ (70.4 years). Subjects who had A2 but no $\epsilon 4$ had the same mean onset age (65.8 years) as those who had two $\epsilon 4$ s and no A2 (65.7 years). These data suggest that A2 and $\epsilon 4$ have

additive effects on onset of AD. We found no distortions in the joint frequencies of APOE and A2, nor did we find any evidence from ANOVA for an interaction between A2 and $\epsilon 4$ on onset age. On the other hand, we detected an A2 effect in $\epsilon 4$ homozygotes and those without $\epsilon 4$, but not in $\epsilon 4$ heterozygotes, which may suggest a possible interaction between the two markers.

Previous studies suggested a possible gender and family history effect on the association of A2 with early-onset AD (Small and Matsuyama, 1986; Payami et al., 1991). Here, we did not find a gender difference. The A2 effect on age at onset was more pronounced in non-familial AD than in familial AD. The fact that both the previous study and the present study detected a stronger A2 effect in non-familial AD may be due to an increased ability to detect an association in the absence of other genetic factors. In fact, the strongest A2 effect was seen in subjects who had neither family history nor $\epsilon 4$, among whom A2+ subjects had a 7.6-year earlier onset than A2- subjects.

An association with HLA-A2 would suggest an immune/inflammatory response mechanism for AD, which is consistent with studies that have shown activated microglia in AD brains (McGeer et al., 1994), and the effect of anti-inflammatory drugs on delaying AD onset (Breitner et al., 1995). Alternatively, the association may be with a gene in close linkage to A2, whose function may or may not be immune related. In addition to HLA, more than 30 genes have been mapped to the 6p21.3 region, including tumor necrosis factor, zinc finger and heat shock proteins. Linkage disequilibrium is a strong phenomenon in this region. Thus A2 could be only a neighboring marker that is in linkage disequilibrium with the disease-associated locus. Further studies are needed to confirm these findings and to answer the question of linkage disequilibrium.

Study parameters	Collaborative study					Confirmatory
	WLA/VA/UCLA	SIU	UCSD	WA/OR/MI	Pooled	OADC
N	42	16	6	47	111*	96*
% Male	52%	25%	67%	45%	46%	42%
% Familial	40%	88%	33%	100%	72%	36%
APOE frequency						
ε4ε4	3	2	1	11	17	7
ε4ε3	11	5	3	27	46	43
ε4ε2	0	0	1	1	2	3
ε3ε3	14	9	1	7	31	31
ε3ε2	0	0	0	1	1	8
ε4	0.30	0.28	0.50	0.53	0.42	0.33
ε3	0.70	0.72	0.42	0.45	0.56	0.61
ε2	0	0	0.08	0.02	0.02	0.06
Mean Onset age ± SD						
All	65.1 ± 11.6	55.5 ± 4.4	71.3 ± 9.4	67.9 ± 9.1	65.2 ± 10.5	70.2 ± 11.1
ε4ε4	61.7 ± 4.0	55.5 ± 6.4	67	68.2 ± 6.7	65.5 ± 7.3	58.7 ± 9.2
ε4ε-	62.2 ± 11.6	56.2 ± 2.7	70.8 ± 11.2	69.3 ± 8.3	66.4 ± 9.9	70.8 ± 10.1
ε-ε-	66.6 ± 12.5	55.1 ± 5.3	78	62.8 ± 13.1	62.8 ± 11.9	73.1 ± 10.6

Table 1. Characteristics of the subjects by the contributing site. (* A2 phenotype and age at onset was known for 111 subjects in collaborative study and 96 in confirmatory study. Family history was known for 108 of 111 and 84 of 96. APOE genotype was known for 97 of 111 and 92 of 96).

	Mean onset age $\pm$ SD (N)			
	in A2+ subjects	in A2- subjects	Δ yrs	p
Collaborative study				
Early onset, <60 yrs	53.0 $\pm$ 4.7 (21)	55.8 $\pm$ 3.2 (20)	2.8	<.05
Mid-onset, 60-75 yrs	68.0 $\pm$ 4.4 (24)	67.5 $\pm$ 4.3 (26)	-0.5	NS
Late-onset, >75 yrs	80.0 $\pm$ 2.1 (8)	82.0 $\pm$ 4.1 (12)	2.0	NS
All onset 40-90 yrs	63.9 $\pm$ 10.7 (53)	66.5 $\pm$ 10.3 (58)	2.6	NS
Confirmatory study				
Early onset, <60 yrs	50.5 $\pm$ 5.3 (8)	54.8 $\pm$ 3.6 (10)	4.3	<.05
Mid-onset, 60-75 yrs	69.2 $\pm$ 4.7 (13)	67.8 $\pm$ 5.0 (29)	-1.4	NS
Late-onset, >75 yrs	79.2 $\pm$ 3.1 (13)	82.0 $\pm$ 4.6 (23)	2.9	<.05
All onset, 40-90 yrs	68.6 $\pm$ 11.9 (34)	71.0 $\pm$ 10.7 (62)	2.4	NS
Combined data				
Early onset, <60 yrs	52.3 $\pm$ 4.9 (29)	55.5 $\pm$ 3.3 (30)	3.2	<.005
Mid-onset, 60-75 yrs	68.5 $\pm$ 4.5 (37)	67.7 $\pm$ 4.6 (55)	-0.8	NS
Late-onset, >75 yrs	79.5 $\pm$ 2.7 (21)	82.0 $\pm$ 4.4 (35)	2.6	<.01
All onset, 40-90 yrs	65.7 $\pm$ 11.3 (87)	68.8 $\pm$ 10.7 (120)	3.1	<.05

Table 2. Onset age in A2+ versus A2- patients, mean difference (Δ yrs) and significance (p).

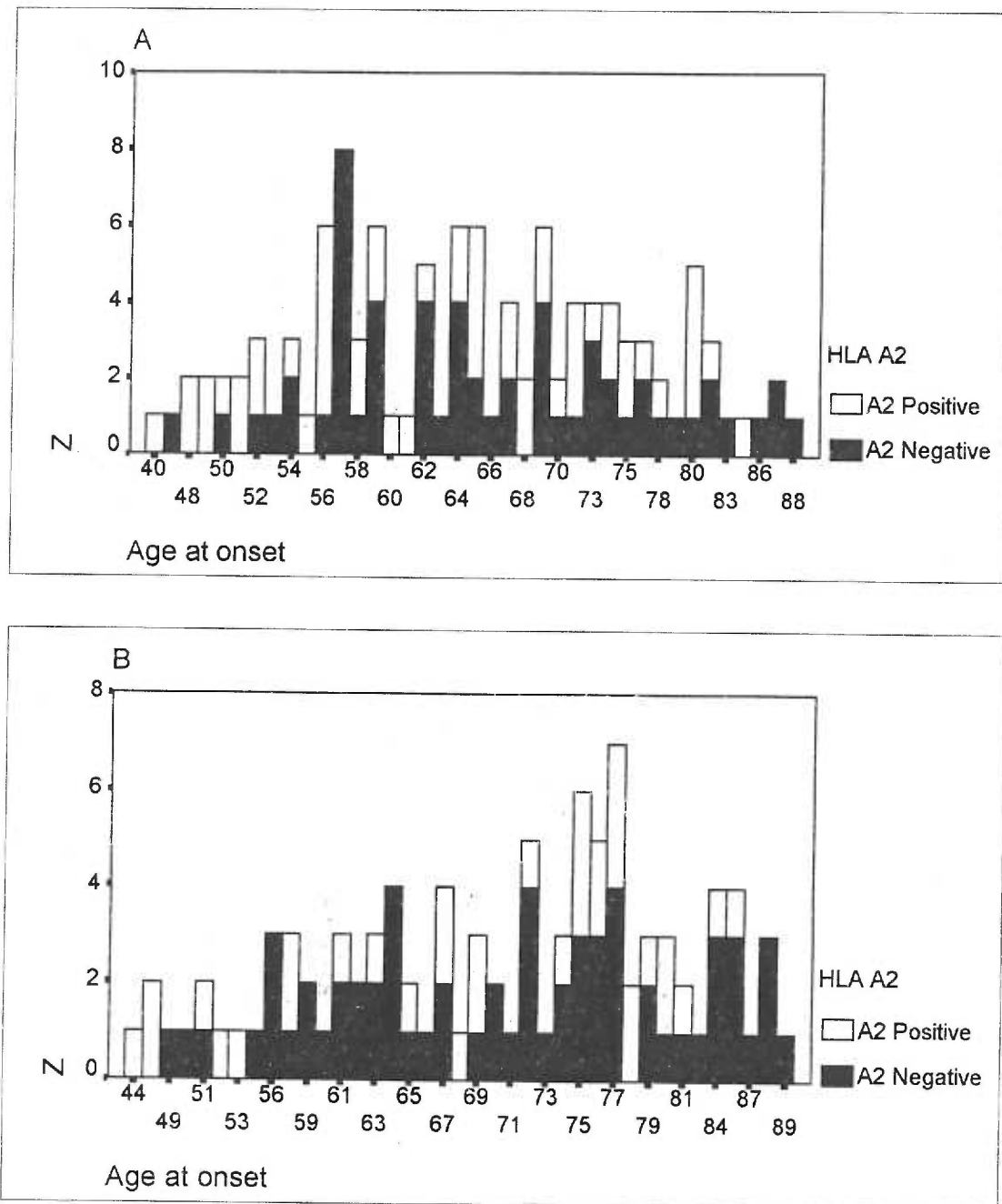


Figure 1. Age at onset distribution in A2+ and A2- patients.

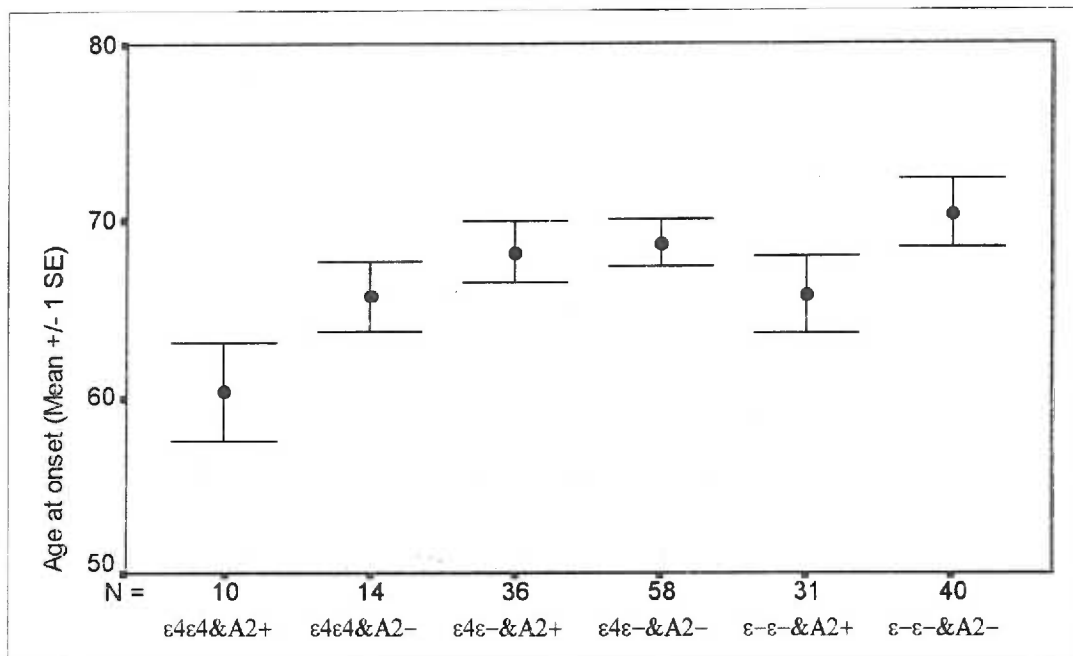


Figure 2. Age at onset as a function of $\epsilon 4$ and A2. Patients were classified by APOE genotype ($\epsilon 4\epsilon 4$, $\epsilon 4\epsilon -$, $\epsilon -\epsilon -$) and A2 phenotype (A2+, A2-). Mean age at onset (± 1 SE) were plotted for the six groups.

CHAPTER 4

MODULATION OF THE AGE AT ONSET OF PARKINSON'S DISEASE BY APOLIPOPROTEIN E GENOTYPES

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ABSTRACT

Parkinson's disease (PD) patients often develop dementia, and Alzheimer's disease (AD) patients frequently develop parkinsonian signs. The Apolipoprotein E- ϵ 4 allele is associated with increased risk and earlier onset of AD. We studied 137 unrelated, Caucasian PD patients. Those with ϵ 4 had the earliest onset (52.7 ± 9.8), ϵ 3/ ϵ 3 patients had an intermediate onset (56.1 ± 11.1), and those with ϵ 2 had the latest onset (59.1 ± 13.4). The age at onset distribution for ϵ 4/ ϵ - was significantly earlier than for ϵ 3/ ϵ 3 and ϵ 2/ ϵ 3. These preliminary results suggest that APOE genotypes modulate the age at onset of PD.

INTRODUCTION

Parkinson's disease (PD) and Alzheimer's disease (AD) are common neurodegenerative diseases. Parkinson's disease is a movement disorder whose symptoms include tremor, rigidity, and bradykinesia, and affects 1-2% of the population older than 60 years of age (Tanner, 1992). In contrast, AD which affects 2-10% of the population older than age 65 (Katzman and Kawas, 1994), is characterized by loss of memory and cognitive function. Patients with PD frequently develop dementia (Marder et al., 1995; Tison et al., 1995), and conversely, AD patients often exhibit parkinsonian features (Funkenstein et al., 1993; Merello et al., 1994). Autopsy reports show an increased frequency of Lewy bodies, the prime pathological feature of PD, in brains of patients with AD (Hulette et al., 1995), and PD patients often display coincidental AD pathology (i.e., senile plaques and neurofibrillary tangles) (Hughes et al., 1993). PD is more frequent among first-degree relatives of AD patients (Hofman et al., 1989), and similarly, dementia

is more frequent among first-degree relatives of PD patients (Katzman and Kawas, 1994). The familial clustering of PD and AD suggests that the mechanism responsible for their clinical and neuropathological overlap may have a genetic basis.

AD is genetically heterogeneous (Schellenberg, 1995). The vast majority of AD is late-onset (>60 years) and is associated with the allelic variants of the Apolipoprotein E (APOE) gene on chromosome 19. APOE has three common alleles: $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$. In most AD patient populations, $\epsilon 4$ frequency is elevated, and $\epsilon 2$ frequency is reduced (Schellenberg, 1995). $\epsilon 4$ -carriers have significantly earlier onset and the highest age-specific risk of developing AD, and $\epsilon 2$ -carriers have delayed onset and the lowest age-specific risk (Schellenberg, 1995; Payami et al., 1997). The role of APOE in the pathogenesis of AD is still unknown.

Recent studies suggest that PD also has a genetic component (Lazzarini et al., 1994; Payami et al., 1994a; Polymeropoulos et al., 1996). We have previously shown that PD patients with a family history positive for PD have significantly earlier age at onset than patients without a positive family history (Payami et al., 1995), which is suggestive of a genetic influence on the age at onset of PD. In the present study, we questioned whether APOE genotypes modulate the onset of PD in a manner similar to their effect on onset of AD, such that $\epsilon 4$ -carriers would have the earliest onset, $\epsilon 3$ homozygotes would have an intermediate onset, and $\epsilon 2$ -carriers would have the latest onset. A common association of APOE with AD and PD may explain the clustering of PD and AD in individuals and families. Several studies have shown that APOE allele frequencies are not altered in PD (Arai et al., 1994; Marder et al., 1994; Rubinsztein et al., 1994; Koller et al., 1995; Whitehead et al., 1996). However, the lack of a difference in allele frequencies does not

negate an APOE effect on age at onset (Payami et al., 1997). The aim of this study was to test the hypothesis that APOE genotypes modulate the age at onset of PD.

PATIENTS AND METHODS

The study population consisted of 105 unrelated, Caucasian PD patients recruited from the Movement Disorder Clinic at Oregon Health Sciences University in Portland, and 32 unrelated, Caucasian PD patients from the University of Washington. All patients were evaluated by a neurologist and were diagnosed with idiopathic PD, based on the presence of two or more of the cardinal features of PD (tremor, rigidity, and bradykinesia), and the lack of other signs and history that could indicate alternative diagnoses or causes. Patients were not demented at the time of PD diagnosis, however, they were not followed-up for subsequent development of dementia. All subjects signed an informed consent, approved by the Institutional Review Board.

Age at onset was defined as the age at which the patient noticed the first symptom indicative of PD. Patients with onset before 30 years of age were excluded to avoid confounding by the rare early-onset forms of PD (Tanner, 1992). Familial PD patients consisted of those who reported at least one family member diagnosed with PD. APOE genotyping was performed using a standard technique (Hixson and Vernier, 1990). Allele frequencies were estimated by allele counting. Due to the small number of patients with the $\epsilon 4/\epsilon 4$ genotype (N=4), they were combined with $\epsilon 3/\epsilon 4$ patients (N=35). The following three genotypic groups were used in analyses: $\epsilon 4/\epsilon -$ (N=39), $\epsilon 3/\epsilon 3$ (N=74), $\epsilon 2/\epsilon 3$ (N=19). The analyses were performed with and without $\epsilon 2/\epsilon 4$ patients (N=5), because it is not

known whether this genotype is high risk because of $\epsilon 4$, low risk because of $\epsilon 2$, or neutral, if $\epsilon 4$ and $\epsilon 2$ effects cancel out. Analysis of variance (ANOVA) was used to compare mean ages at onset. Kaplan-Meier survival analysis was used to plot genotype-specific age-at-onset distribution curves, and the differences between the curves were tested using log-rank statistics. ANOVA was used to examine the interaction between family history and APOE genotype. The COX proportional hazard model was used to test for possible confounding by family history and study site. All statistical analyses were performed using SPSS Software (Release 6, 1993).

RESULTS

The patient characteristics are summarized in Table 1. There were no significant differences between the Oregon and Washington patients with respect to age at onset of PD, APOE allele frequencies, or genotype-specific age at onset distributions; thus they were pooled for all subsequent analyses. APOE allele frequencies in PD patients were within the range reported by other studies (Arai et al., 1994; Marder et al., 1994; Rubinsztein et al., 1994; Koller et al., 1995; Whitehead et al., 1996).

We initially examined the age at onset of PD as a function of the $\epsilon 4$ and the $\epsilon 2$ alleles. $\epsilon 4$ -carriers had onset 4 years earlier than those who lacked $\epsilon 4$ (52.7 ± 9.8 vs. 56.7 ± 11.6 , $p=0.06$). $\epsilon 2$ -carriers had a 4.2 years later onset than those without $\epsilon 2$ (59.1 ± 13.4 vs. 54.9 ± 10.8 , $p=0.13$). The reported p -values are two-tailed. Given our *a priori* hypothesis that $\epsilon 4$ -carriers would have earlier onsets, and $\epsilon 2$ -carriers would have delayed onsets, the one-tailed p -values are 0.03, and 0.07 respectively. Inclusion of $\epsilon 2/\epsilon 4$ patients did not

qualitatively change the results. The mean age at onset of PD was 52.5 ± 11.9 years for $\epsilon 4/\epsilon 4$, 52.7 ± 9.8 years for $\epsilon 3/\epsilon 4$, 54.4 ± 9.3 years for $\epsilon 2/\epsilon 4$, 56.1 ± 11.1 years for $\epsilon 3/\epsilon 3$, and 59.1 ± 13.4 years for $\epsilon 2/\epsilon 3$ patients. This pattern is consistent with our hypothesis that $\epsilon 4$ -carriers would have the earliest onset of PD, $\epsilon 3$ homozygotes would have an intermediate onset, and $\epsilon 2$ -carriers would have the latest onset. Figure 1 demonstrates the age at onset of PD as a function of APOE genotypes. The age at onset distribution for $\epsilon 4/\epsilon -$ was significantly earlier than for $\epsilon 3/\epsilon 3$ ($p=0.048$) and $\epsilon 2/\epsilon 3$ ($p=0.003$). Although the onset distribution for $\epsilon 2/\epsilon 3$ patients was delayed compared to $\epsilon 3/\epsilon 3$ patients, the difference was not significant ($p=0.18$). The COX proportional hazard analysis showed that the effect of APOE genotypes on the age at onset of PD was not confounded by family history ($p=0.35$) or study site ($p=0.86$).

Disregarding APOE genotypes, familial PD patients had significantly earlier age at onset than non-familial PD patients (52.4 ± 11.1 vs. 58.5 ± 10.3 , $p<0.001$). Figure 2 shows mean ages at onset of PD as a function of family history of PD and APOE genotypes. Within each genotype, familial PD patients had earlier ages at onset than non-familial PD patients. ANOVA results indicated that there was no interaction between APOE genotypes and family history on the age at onset of PD ($p>0.7$). Within familial PD patients, the mean age at onset of PD was 50.9 ± 9.6 for $\epsilon 4/\epsilon -$, 52.7 ± 11.7 for $\epsilon 3/\epsilon 3$, and 54.4 ± 13.3 for $\epsilon 2/\epsilon 3$ patients. Within non-familial PD patients, the mean age at onset of PD was 55.1 ± 9.9 for $\epsilon 4/\epsilon -$, 59.1 ± 9.8 for $\epsilon 3/\epsilon 3$, and 63.3 ± 12.6 for $\epsilon 2/\epsilon 3$ patients. The trend in Figure 2 suggests that family history and APOE have additive effects on the age at

onset of PD. At the two extremes, patients with $\epsilon 4$ and family history had 12.4 years earlier onset than those with $\epsilon 2$ and no family history (50.9 ± 9.6 vs. 63.3 ± 12.6).

DISCUSSION

This study provides evidence that APOE genotypes modulate the age at onset of PD in a manner similar to their effect on onset of AD. Although $\epsilon 4$ allele frequency was not significantly increased in PD patients, similar to other studies (Arai et al., 1994; Marder et al., 1994; Rubinsztein et al., 1994; Koller et al., 1995; Whitehead et al., 1996), APOE appeared to affect the onset of PD. The trend is consistent with our hypothesis that $\epsilon 4$ -carriers would have the earliest onset, $\epsilon 3$ homozygotes would have an intermediate onset, and $\epsilon 2$ -carriers would have the latest onset. As we have previously shown (Payami et al., 1995), a positive family history of PD was associated with earlier age at onset. There was no interaction between APOE and family history on age at onset of PD, rather it appeared that they had additive effects. Patients with $\epsilon 4$ and family history had 12.4 years earlier onset than those with $\epsilon 2$ and no family history. The independent effects of APOE and family history suggest that the onset of PD is influenced by APOE and other, as yet unidentified, genes.

At Present, it is generally believed that APOE is not associated with PD because case control studies have consistently shown that APOE allele frequencies are not altered in PD (Arai et al., 1994; Marder et al., 1994; Rubinsztein et al., 1994; Koller et al., 1995; Whitehead et al., 1996). Our data, although preliminary, suggest that this conclusion may be premature. APOE allele frequencies are also not altered in the present study, despite

the significant evidence for an age at onset effect. Although controversial, some studies have shown significantly altered APOE frequencies in PD patients with dementia (Arai et al., 1994; Marder et al., 1994; Rubinsztein et al., 1994; Koller et al., 1995; Whitehead et al., 1996). In our study, none of the patients were demented at PD diagnosis. A subset of patients may have developed dementia subsequently, but at the time of this study, cognitive data were unavailable.

Two of the case-control studies mentioned have reported the age at onset of their PD subjects. One study noted no association between $\epsilon 4$ and PD onset, however, their sample size was small with only seven $\epsilon 4$ -positive patients (Rubinsztein et al., 1994). The second study examined age at onset of PD as a function of APOE alleles (Whitehead et al., 1996). The following three overlapping groups were compared: $\epsilon 2$ -positive patients (including $\epsilon 2\epsilon 2$, $\epsilon 2\epsilon 3$ and $\epsilon 2\epsilon 4$), $\epsilon 3$ -positive patients ($\epsilon 2\epsilon 3$, $\epsilon 3\epsilon 3$, and $\epsilon 3\epsilon 4$) and $\epsilon 4$ -positive patients ($\epsilon 2\epsilon 4$, $\epsilon 3\epsilon 4$, and $\epsilon 4\epsilon 4$). They found significantly delayed onset of PD in $\epsilon 2$ -positive subjects, which is consistent with our finding. They did not detect any difference between $\epsilon 3$ -positive and $\epsilon 4$ -positive subjects. Use of this alternative grouping, does not change our results.

If confirmed, our findings have two major implications. First, APOE modulates the age at onset of PD, and the pattern resembles that of the APOE effect on AD. The same effect of APOE on age at onset of AD and PD does not necessarily imply a common cause. The causes of AD and PD are unknown, and so is the role of APOE and the mechanism by which it modulates the onset of symptoms. The present study adds to the evidence that APOE may play a general role in neurodegeneration, rather than being a specific marker for AD (Schellenberg, 1995).

Second, genetic factors that modulate age at onset can go undetected in case-control association studies that rely solely on allele frequency differences. This can be an important issue for complex diseases with late and variable age at onset. AD, whose association with APOE is unquestionable, provides such an example. $\epsilon 4$ allele frequency is elevated in AD, but it drops dramatically in patients with onset ages after 75 years, implying APOE effect wanes in very-late-onset AD (Rebeck et al., 1994; Corder et al., 1995b; Sobel et al., 1995). However, a recent prospective study has shown that, although there may be no detectable allele frequency difference between demented and non-demented elderly over the age of 75, there is a significant difference in age at onset as a function of APOE genotype up to the age of 105 (Payami et al., 1997). Thus it is important to examine age at onset as a function of genotypes when studying complex disorders with late-and variable onset and not to rely solely on allele frequency differences.

	Oregon	Washington	Pooled
N	105	32	137
Age (Mean $\pm$ SD)	65.5 $\pm$ 10.9	66.1 $\pm$ 9.6	65.6 $\pm$ 10.6
Age at onset (Mean $\pm$ SD)	55.0 $\pm$ 11.3	56.9 $\pm$ 10.6	55.5 $\pm$ 11.1
Male / Female	63/42	21/11	84/53
Familial/Non-familial	61/44	7/25	68/69
ϵ^2	0.09	0.08	0.09
ϵ^3	0.73	0.76	0.74
ϵ^4	0.18	0.16	0.17

Table 1. Characteristics of subjects.

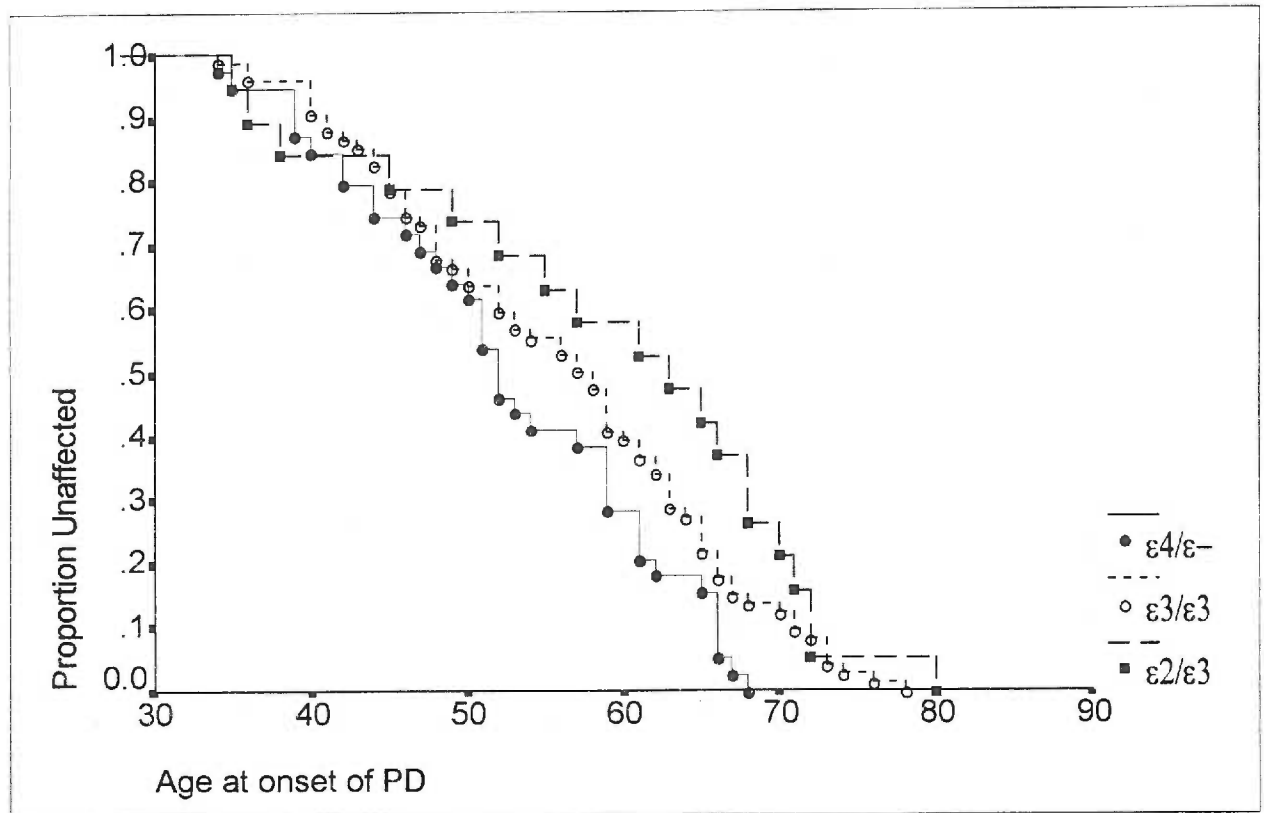


Figure 1. Age at onset of PD as a function of APOE genotypes. Kaplan-Meier age-at-onset distribution of PD in $\epsilon 4/\epsilon -$, $\epsilon 3/\epsilon 3$, and $\epsilon 2/\epsilon 3$ PD patients. The age at onset distribution for $\epsilon 4/\epsilon -$ (N=39) was significantly earlier than for $\epsilon 3/\epsilon 3$ ($p=0.048$) and $\epsilon 2/\epsilon 3$ ($p=0.003$). Although $\epsilon 3/\epsilon 3$ patients (N=74) had earlier ages at onsets than the $\epsilon 2/\epsilon 3$ patients (N=19), the difference was not significant ($p=0.18$).

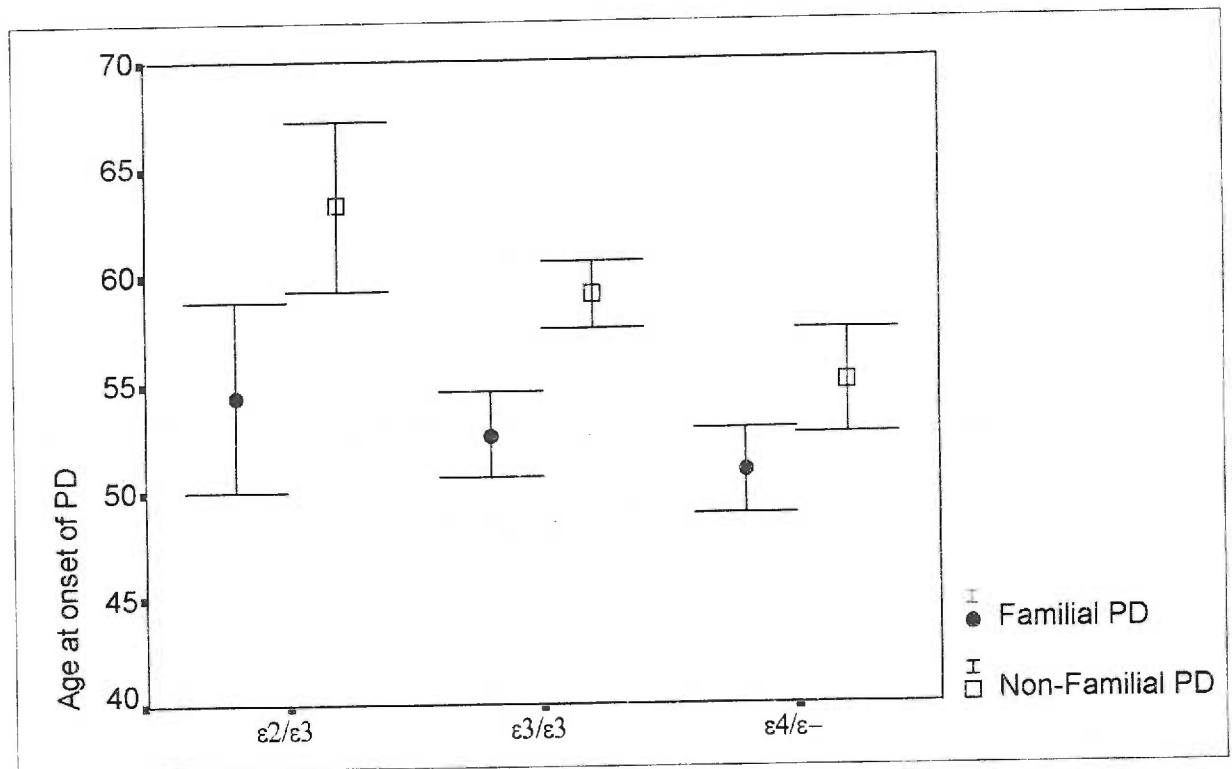


Figure 2. Age at onset of PD as a function of APOE genotypes ($\epsilon 4/\epsilon -$, $\epsilon 3/\epsilon 3$, $\epsilon 2/\epsilon 3$) and family history of PD. Mean ages at onset (± 1 SE) are plotted for the six groups.

CHAPTER 5

ANALYSIS OF THE α -SYNUCLEIN G209A MUTATION IN FAMILIAL PARKINSON'S DISEASE

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INTRODUCTION

Parkinson's disease (PD) is a movement disorder that affects 1-2% of the population over the age of 60. Recently, Polymeropoulos et al. identified a mutation in the α -synuclein gene on chromosome 4q21-23, that segregated with PD in a large Italian (Contursi) kindred, and three Greek kindreds (Polymeropoulos et al., 1997). The mutation is a base pair change from G to A at position 209 (G209A). Polymeropoulos et al. suggest that this mutation may account for a significant proportion of early-onset familial PD (Polymeropoulos et al., 1997), in which case a simple mutation screen may prove to be a highly reliable clinical tool for diagnosis of PD and for presymptomatic risk assessment.

SUBJECTS AND METHODS

We screened 65 affected members from 40 Caucasian PD kindreds, of Northern European origin, for the G209A mutation. The mean onset of PD was 51.4 ± 17.5 years in the 65 cases. In fourteen of the 40 kindreds, the mean onset age was less than 50 years. Nineteen of the 40 kindreds had 3 to 15 affected individuals, and the mean onset age was 57.9 ± 15.7 years. In seven kindreds, one or more members had onset <46 years, which was the mean in the Contursi kindred. In addition, we studied 21 smaller families with only two affected individuals, because onset was <46 years in at least one relative. The kindreds were ascertained and evaluated at Oregon Health Sciences University, and the University of Washington after giving informed consent. All 40 probands and seven relatives were diagnosed by us using the Hughes et al. criteria with one exception: family history was not taken as an exclusion criteria (Hughes et al., 1992). The remaining 18 relatives were diagnosed by community physicians. Autopsy confirmation was available on

the two subjects who have died, and in both, Lewy bodies were present in the substantia nigra. Age at onset was defined as the age at which the patient noticed the first PD symptom. The mutation analysis was carried out as described in Polymeropoulos et al (1997). We included PUC 19 DNA in all reactions, to ensure that lack of evidence for the mutation was not due to incomplete digestion.

RESULTS AND DISCUSSION

None of the subjects had the G209A mutation. The absence of this mutation in these 40 kindreds argues against it being a common cause of PD in families of Northern European origin. Also, it implies that this mutation may not be of wide utility for diagnostics in clinical practice. This finding was not surprising because the Contursi kindred is highly unusual, having 60 affected members, high penetrance (~90%), and a rapid course from onset to death despite levodopa responsiveness. The presence of the mutation in three Greek kindreds suggests that this mutation may be more prevalent in PD families of Mediterranean and Southern European origin.

To determine if PD in our kindreds is caused by another mutation in the α -synuclein gene, we carried out linkage analysis in the nine largest kindreds, with the same model and markers used to establish linkage in the Contursi kindred (Polymeropoulos et al., 1996). With the exception of two families (lodscore = 0.41 and 0.59), others showed no positive evidence for linkage. In these two families, the possibility of a mutation in the α -synuclein can not ruled out. The nine kindreds combined yielded a two-point lodscore of -13.8 (lodscore $\leq$ -2 is evidence for exclusion). Four of the nine kindreds each yielded lodscores

<-2. These results suggest α -synuclein is not the gene responsible for familial PD in these kindreds. Two previous studies had also found no evidence for linkage to this region in their collection of PD families (Gasser et al., 1997; Scott et al., 1997). Additional PD gene(s) must exist elsewhere in the genome.

CHAPTER 6

SEGREGATION ANALYSIS OF PARKINSON DISEASE

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ABSTRACT

Parkinson's disease (PD) is a prevalent movement disorder of unknown cause whose incidence rises with increasing age. Nearly 20% of PD is familial, a subset of which exhibits autosomal dominant transmission. However, in most families, the inheritance is not clear. To determine the most likely mode of inheritance of PD, we performed complex segregation analyses using kindreds of 136 PD patients randomly ascertained from a clinic population. The hypotheses of a non-transmissible environmental factor, no major gene or type (sporadic), and all Mendelian inheritance (dominant, recessive, additive, decreasing) were rejected ($p < .001$). Familial clustering of PD in this data set is best explained by a rare familial factor which a) is transmitted in a non-Mendelian fashion, and b) influences the age at onset of PD. If confirmed, our results have immediate implications in gene mapping studies which often search for genes that behave in a Mendelian fashion that affect susceptibility rather than age at onset and long term implications in understanding the pathogenesis of PD.

INTRODUCTION

Parkinson's disease (PD) is a common age-related neurological disorder affecting about 1-2% of the population over the age of 60 (Tanner and Goldman, 1996). The age at onset of PD can vary by more than 50 years, and the risk of developing PD increases with increasing age. The clinical manifestations of PD include resting tremor, muscular rigidity, bradykinesia, and postural instability. PD is characterized by a progressive neuronal degeneration of predominantly the dopaminergic neurons of the substantia nigra; the underlying cause of this selective cell death is still unknown.

The role of genetics in the etiology of PD has been debated since it was first proposed by Mjones, based on his observation of additional cases of PD among parents and siblings of patients (Mjones, 1949). The genetic hypothesis was weakened by the discovery of the association of 1-methyl-1,2,4,6-tetrahydropyridine (MPTP) and other environmental factors with PD (Tanner and Goldman, 1996), but in recent years, it has gained increasing support from several lines of studies. Several studies, performed in United States, Europe and Canada have reported increased risk of PD in relatives of PD probands compared to relatives of controls (Semchuk et al., 1993; Payami et al., 1994a; Bonifati et al., 1995; Vieregge and Heberlein, 1995; De Michele et al., 1996; Marder et al., 1996; Uitti et al., 1997). Many groups have reported PD kindreds that exhibit an autosomal dominant inheritance pattern (Golbe et al., 1990; Maraganore et al., 1991; Gasser et al., 1994; Markopoulou et al., 1995). A mutation in the α -synuclein gene on chromosome 4 has been identified, which co-segregates with PD in several autosomal dominant families (Polymeropoulos et al., 1997). However, this mutation is not responsible for the majority of the familial cases of PD (Zarepari et al., 1998a). Recently, a susceptibility locus for PD was mapped to chromosome 2p13 (Gasser et al., 1998).

The mode of inheritance of PD is not known. Overall, 10-30% of all PD are familial (Barbeau and Roy, 1984; Maraganore et al., 1991; Semchuk et al., 1993; Lazzarini et al., 1994; Payami et al., 1994a; Bonifati et al., 1995; Vieregge and Heberlein, 1995; Uitti et al., 1997). In some families, transmission appears to be autosomal dominant (Golbe et al., 1990; Maraganore et al., 1991; Gasser et al., 1994; Lazzarini et al., 1994; Bonifati et al., 1995; Markopoulou et al., 1995), but most of the families show no discernible inheritance pattern. One group has suggested that mutation of a single gene may account for all PD, in

familial as well as non-familial cases (Golbe et al., 1990; Duvoisin and Golbe, 1995). Another group has suggested the presence of subtypes with different modes of inheritance, namely, an autosomal dominant essential tremor-related parkinsonism and an autosomal recessive familial akineto-rigid syndrome (Barbeau and Roy, 1984). A multifactorial hypothesis has also been suggested, involving a combination of genetic and environmental factors (Kondo et al., 1973; Semchuk et al., 1993). Several studies have suggested a role for mitochondria and possibly mitochondrial DNA (mtDNA) in pathogenesis of PD (reviewed by Schapira, 1995). There is clearly interest in deciphering the mode of inheritance of PD; however, none of the studies to date have performed a formal segregation analysis to address the question properly in a randomly selected clinic or population-based sample. The aim of this study was to test hypotheses regarding the familial clustering of PD, to assess the likelihoods of genetic and environmental models, and to determine if transmission of PD is consistent with Mendelian inheritance.

Complex segregation analysis is an analytical tool that uses family data from randomly selected subjects to estimate the most likely mode of inheritance of a trait. It allows formal tests of hypotheses for comparing a series of environmental and genetic models as possible explanations for familial clustering of a trait. We applied complex segregation analysis to PD, using information on families of PD patients who were ascertained randomly from a movement disorder clinic. We estimated and compared the likelihoods of the hypotheses that the familial clustering of PD is due to a non-transmissible environmental factor, vs. a Mendelian gene, vs. no major gene (sporadic). We tested these hypotheses under two models: 1) the gene/factor influences age at onset of PD, and 2) it influences susceptibility to PD.

SUBJECTS AND METHODS

Subjects

We randomly ascertained unrelated PD patients from the Movement Disorder Clinic at the Oregon Health Sciences University who were seen in the clinic between 1991 and 1993 (Payami et al., 1995). The diagnosis of idiopathic PD was based on the presence of two or more cardinal features of PD (tremor, rigidity, and bradykinesia), and the lack of other signs and history that could indicate alternative causes or diagnoses. Subjects were selected regardless of their age, age at onset or family history of PD. Subjects were selected by two different schemes: a mailing to all clinic patients with a 47% success rate, and telephone recruitment of patients selected randomly by computer with a 92% success rate. Since, the two patient groups did not differ with respect to age at onset, family history, and age-specific incidence rate in their relatives, the data were pooled (Payami et al., 1995). The study group consisted of 136 Caucasian PD patients. All subjects signed an informed consent, approved by the Institutional Review Board.

Age at onset of PD was defined as the age at which the patient noticed the first symptom of PD. Family history of PD in the proband's siblings, parents, and aunts/uncles was obtained through a self-administered questionnaire. Reports of affected relatives were verified by review of medical records when available, or phone interviews with two family members. The sequential sampling method was used for constructing pedigrees for data entry into segregation analysis. Using this method, the first-degree relatives of the proband were included, and if among them, a subject was affected, then their first-degree relatives were also included in the pedigree.

Statistical methods

Complex segregation analyses were performed using the REGTL program of the Statistical Analysis for Genetic Epidemiology (SAGE) software, Release 2.2, which examines a discrete trait that follows a logistic distribution such as age at onset or disease susceptibility (SAGE, 1994). PD was defined as a dichotomous trait (affected or unaffected) with variable age at onset. The likelihood of the data was calculated using a correction factor for single ascertainment, and conditioning the likelihood of each pedigree on the proband being affected by his or her age at examination. The susceptibility parameter γ , was defined as the probability of developing PD if one lived long enough. Age at onset of PD was assumed to have a logistic distribution that depended on an age coefficient α , and a baseline risk parameter β (PD risk). The cumulative probability that an individual develops PD by age a' can be determined from $\gamma F(a') = \gamma e^{\beta + \alpha a'} / 1 + e^{\beta + \alpha a'}$.

The term “type” refers to a discrete underlying characteristic that determines one’s phenotype. Genotype is a special case of type. The term type, however, is more general and allows for any kind of discrete transmission, whether Mendelian or not. Two individuals have the same type, if and only if the expected phenotypic distribution of their offspring by a mate of a given type are identical, and this is true for every possible type of mate.

Mendelian inheritance, if present, was assumed to occur through a single autosomal locus with two alleles, A and B. The A allele is associated with the disease, and may represent multiple disease-associated alleles, if they have the same inheritance pattern. In general, A represents the risk factor. The B allele may represent multiple non-disease-

associated alleles with the same inheritance pattern. “q” denotes the frequency of A, and is estimated from the data.

In a single locus, diallelic Mendelian model, there are three possible genotypes: AA, AB, BB. Genotype frequencies in the sample are assumed to be in Hardy-Weinberg equilibrium. Transmission parameter “ τ ” refers to the probability that a parent transmits an A to an offspring, and depends solely on parental type. In a Mendelian inheritance model, the transmission probabilities are $\tau_{AA}=1$, $\tau_{AB}=0.5$ and $\tau_{BB}=0$. In the general model where all parameters are estimated from the data, the transmission probabilities are expected to deviate from 1, 0.5 and 0, unless the true inheritance pattern is Mendelian, in which case the general model should yield estimates approximating 1, 0.5 and 0.

Data were analyzed under two different regressive models for family data (Bonney, 1986). Class A assumes that sibs are similar because of common parentage. Given parental phenotypes, the phenotypes of sibs do not contribute any more information. Class D allows for effects from both parents and older sibs. The phenotype of each older sib is assumed to have an equal effect on the phenotype of the individual.

Biological Models

Data were analyzed under two different biologically plausible models. Model 1 assumes that type or genotype influences age at onset of the disease, but not susceptibility to disease (β is type or genotype specific). In this model, individuals who are susceptible follow a specific age at onset distribution, which is influenced by type or genotype. Model 2 assumes that type or genotype influences susceptibility to disease, but not age at onset (γ is type or genotype specific). In this model, all susceptible individuals follow the same age at onset distribution.

Hypotheses of Mode of Inheritance

We considered and tested seven hypotheses regarding the mode of inheritance of PD.

1) General or unrestricted model, which estimates all parameters from the data without restrictions. The general model is used as a baseline against which all other hypotheses can be tested. 2) Dominant Mendelian inheritance model, where $\beta_{AA}=\beta_{AB}>\beta_{BB}$ under model 1, or $\gamma_{AA}=\gamma_{AB}>\gamma_{BB}$ under model 2. 3) Recessive Mendelian inheritance model, where $\beta_{AA}>\beta_{AB}=\beta_{BB}$ under model 1, or $\gamma_{AA}>\gamma_{AB}=\gamma_{BB}$ under model 2. 4) Additive Mendelian inheritance model, where $\beta_{AB}=0.5(\beta_{AA}+\beta_{BB})$ under model 1, or $\gamma_{AB}=0.5(\gamma_{AA}+\gamma_{BB})$ under model 2. 5) Decreasing Mendelian inheritance model, where $\beta_{AA}\geq\beta_{AB}\geq\beta_{BB}$ under model 1, or $\gamma_{AA}\geq\gamma_{AB}\geq\gamma_{BB}$ under model 2. 6) Environmental model, which assumes that PD is due to random non-transmissible environmental factors. Transmission probabilities are independent of type and are constrained to equal the frequency of A. 7) No major gene or type (sporadic) model where $\beta_{AA}=\beta_{AB}=\beta_{BB}$ under model 1, or $\gamma_{AA}=\gamma_{AB}=\gamma_{BB}$ under model 2.

We compared the difference in the $-2\ln$ likelihood (L) of a model of interest to that of the general model using the Chi-square distribution to assess departure from the model of interest; the degrees of freedom were the differences in the number of parameters estimated. Akaike's information criterion (AIC) was used to compare non-hierarchical models (Akaike, 1974). AIC is defined as $-2\ln L + 2$ (number of parameters estimated). The model with the minimum AIC was considered the best fit to the data. The goal of these comparisons was to find the best-fitting, simplest model.

RESULTS

The data set consisted of 1060 individuals in 199 nuclear families from the 136 kindreds. A positive family history of PD among the first-degree relatives was observed in 25 of the 136 kindreds (18%), which is similar to the reports of positive family history in other clinic populations (Lazzarini et al., 1994; Bonifati et al., 1995; Uitti et al., 1997). In addition to the probands, there were 42 relatives with PD in this data set. The characteristics of the PD patients and their relatives (gender, age, and age at onset of PD) are summarized in Table 1.

The results of segregation analyses performed under REGTL model 1, class A regressive models are summarized in Table 2. The environmental model ($p < .001$) and the no major gene or type (sporadic) model ($p < .001$) were rejected as compared to the general model. Furthermore, the Mendelian models of dominant ($p < .005$), recessive ($p < .001$), additive ($p < .005$), and decreasing ($p < .001$) inheritance were rejected when compared to the general model. In the general model, the estimated transmission probability from heterozygotes was approximately half of what is expected by Mendelian inheritance ($\tau_{AB} = 0.26$ vs. 0.5). Based on the frequency estimate, the factor transmitting PD seemed to be rare in this data set ($q_A = 0.003$). The frequency of heterozygote carriers (AB) was 0.006. The results suggest that PD clusters in families, and the familial clustering is due to a rare factor that is transmitted from parent to child, but that the transmission probabilities deviate from Mendelian expectations.

The results of segregation analyses performed under REGTL model 2, class A regressive models are summarized in Table 3. The environmental model ($p < .001$) and the no major gene or type (sporadic) model ($p < .001$) were rejected as compared to the

general model. Furthermore, the Mendelian models of dominant ($p < .001$), recessive ($p < .001$), additive ($p < .001$), and decreasing ($p < .001$) inheritance were rejected in comparison to the general model. The estimated transmission probability from heterozygotes was once again less than what is expected by Mendelian inheritance ($\tau_{AB} = 0.4$ vs. 0.5) (Table 3).

Consistently better likelihoods were obtained under REGTL model 1 than model 2. Note that for any given inheritance pattern, the likelihood was better for model 1 (Table 2) than model 2 (Table 3). The results imply that model 1, which assumes that type or genotype influences the age at onset of PD but not susceptibility, is more likely in this data set.

We performed segregation analyses under class D regressive models (Table 4). Once again, the environmental model ($p < .001$), the no major gene or type (sporadic) model ($p < .001$), the Mendelian models of dominant ($p < .001$), recessive ($p < .001$), additive ($p < .001$), and decreasing ($p < .001$) inheritance were rejected as compared to the general model. Note that once again, the transmission probabilities estimated in the general model deviated from Mendelian expectations. The coefficient of older affected sibs ($\delta_{\text{affected sibs}}$) indicates that the risk of developing PD in an individual increases based on the number of older affected sibs for that individual. The coefficient of older unaffected sibs ($\delta_{\text{unaffected sibs}}$) indicates that the risk of developing PD in an individual decreases based on the number of older unaffected sibs for that individual. For example, those with an older affected sib have 1.4 times higher probability of having PD than those with no older sibs. Furthermore, they have 1.8 times higher probability of having PD than those with an older unaffected sib.

The fluctuations in the estimated τ_{AB} between the general model under class A and the general model under class D (0.87 vs. 0.26) may be due to the effects from older sibs and sample size differences. For class D models, it is necessary to know the birth order for each sibship. However, we did not know the birth order among many of the parental sibships, and since individuals with missing information were excluded, the results were based on a smaller subset of the original data set. To find out if the variation in the sample is responsible for the fluctuations in τ_{AB} , we ran the general model under class A using the smaller subset that was used for class D models. The estimated τ_{AB} was 0.67 (rather than 0.26) which is closer to 0.87 obtained under class D models.

DISCUSSION

Determination of the mode of inheritance of PD has been the focus of many previous studies. The proposed models have ranged from a single Mendelian gene accounting for all PD (Golbe et al., 1990; Duvoisin and Golbe, 1995), to polygenic or multifactorial (Kondo et al., 1973; Semchuk et al., 1993), mitochondrial (reviewed by Schapira, 1995), and environmental (reviewed by Tanner and Goldman, 1996). With the resurgence of the genetic hypothesis for PD, many studies have launched a search for the disease genes. The success of gene discovery studies, however, depend on a fundamental knowledge of the mode of inheritance. We used complex segregation analysis, the most widely used method for tests of mode of inheritance, to determine the most plausible explanation for the familial clustering of PD. Our study was the first to utilize this method for PD. We rejected all single gene Mendelian hypotheses and the non-transmissible environmental factor hypothesis. Our results suggest the existence of a rare familial factor, with non-

Mendelian transmission, that influences age at onset of PD. If confirmed, our results imply that special care must be taken in gene mapping studies to avoid, or to overcome, the confounding by genetic heterogeneity or non-Mendelian inheritance mechanisms.

Ascertainment. The method by which subjects are selected has a direct effect on the outcome of segregation analysis. Our study included a random clinic patient population which was selected regardless of family history. Previously, Lazzarini et al. (1994) and Maraganore et al. (1991) calculated segregation ratios on families that were selected for having multiple affected members. Their results were compatible with autosomal dominant inheritance with reduced penetrance. In fact, the discovery of the α -synuclein gene as an autosomal dominant cause of PD confirms their finding that a rare subtype of familial PD can be caused by a single gene (Polymeropoulos et al., 1997; Zarepari et al., 1998a). The purpose of our study was to gain a broader view of the familial clustering of PD in general. We used the single ascertainment method, where the initial sample includes randomly selected affected individuals, whose families are then ascertained and analyzed. We applied the recommended procedure to correct any bias that may result from this selection scheme. Despite the care that was taken to avoid confounding by ascertainment, we must emphasize that our sample was not a random sample of PD patients. The subjects were recruited from a specialty movement disorder clinic, and some were self-selected for having unusual features such as early age at onset. This referral bias may have affected our results such that the findings may not be applicable to all PD populations. Our results need to be confirmed in other clinic and population-based studies.

Mendelian transmission. It has been suggested that mutation of a single gene may account for all PD in familial as well as in non-familial cases (Golbe et al., 1990; Duvoisin

and Golbe, 1995). Our results are not consistent with this postulate, as all single gene Mendelian models were rejected. However, non-Mendelian mechanisms such as triplet repeat expansion and imprinting were not ruled out. Recently, autosomal dominant mutations in the α -synuclein gene were shown to be associated with PD in some families (Polymeropoulos et al., 1997). This gene did not account for PD in the kindreds that we studied (Zarepari et al., 1998a). Therefore, one gene cannot be responsible for all PD.

Two groups have proposed a dominant and a recessive subtype in PD (Barbeau and Roy, 1984; Tanaka et al., 1991). We questioned if our results could be explained by this model. Segregation analysis could potentially reject Mendelian inheritance when the sample is a mixture of two subtypes, each caused by a single Mendelian gene, but with different modes of inheritance. In our data set, one family clearly exhibits autosomal dominant inheritance. This family contributed 15 affected members to the analysis, and because of its size, it may have had a significant impact on the results. We removed this family, and repeated the analysis. Again, the general model was most compatible with the data, rejecting all Mendelian hypotheses including autosomal dominant and autosomal recessive. This result does not negate the existence of a smaller, autosomal recessive subtype in PD, but it points to a more complex etiology than only two Mendelian subtypes.

Mitochondria. Anomalies in complex 1 activity observed in substantia nigra and platelets from PD patients point to possible involvement of mitochondria in the pathogenesis of PD (Schapira, 1995). The decrease in complex 1 activity has been shown to be of mitochondrial origin, thus may have a genetic basis and arise from mutations in mtDNA (Swerdlow et al., 1996). Several studies have attempted to identify PD-specific

mtDNA mutations, however, there is currently no convincing evidence for a pathological role of mtDNA in PD (Woods, 1997). Mitochondria are transmitted exclusively from mothers, hence, if a subset of PD was caused by mitochondrial mutations, or if mitochondria was one of the factors in a multifactorial pathogenesis, one would expect to see an excess of maternal transmission in PD kindreds. Wooten et al. (1997) observed an excess of maternal transmission in selected families, but others did not (Maraganore et al., 1991; Lazzarini et al., 1994). To determine if the lack of evidence for Mendelian inheritance in our data was due to the involvement of mitochondria, we tried to use segregation analysis to estimate parental effects. However, we were unable to obtain meaningful results. The present study had 13 affected parent-child pairs, including 9 affected mothers and 4 affected fathers. In our larger collection of familial PD, 40 of the probands had PD in the maternal side and 20 on the paternal side, the difference being statistically significant. However, when we examined the age-specific incidence of PD in children of women with PD as compared to children of men with PD, there was no difference. If mitochondria were a cause of PD, the incidence rate should have been higher in children of affected mothers. It is possible that mitochondria play a role in the pathogenesis of PD, without being the actual cause. Alternatively, the excess maternal transmission in some studies may reflect a sampling bias.

Environment. Until a few years ago, PD was assumed to be environmental with little or no genetic involvement. The strongest evidence for an environmental cause is the observation that intravenous injection of MPTP causes Parkinsonism (Tanner and Goldman, 1996). Epidemiological studies have linked rural residence, farming, well-water drinking, and herbicide/pesticide exposure to an increased risk of developing PD (Tanner

and Goldman, 1996). Despite the increasing interest in a genetic etiology in recent years, it remains clear that exposure to environmental factors such as neurotoxins play a role in PD. The non-transmissible environmental factor hypothesis tested and rejected in this study assumed that exposure was random and independent of family structure. Some exposures, however, may cluster in families and appear as though they are transmitted from parents to children. This would be true with the elements of rural living, which seem to be associated with PD. It is therefore possible that the non-Mendelian transmission observed in this data set is indicative of shared exposure to an environmental factor that clusters in families.

Gene-environmental interaction. A number of studies, with varying approaches and methodologies, have suggested a multifactorial etiology for PD (Kondo et al., 1973; Semchuk et al., 1993). Recent evidence for a combined genetic and environmental etiology comes from genetic studies that implicate the presence of one or more inherited susceptibility factors as a background to the effect of environmental agents (Poirier et al., 1991; Woods, 1997). For example, several studies have shown an increased frequency of the mutant alleles of cytochrome P450 2D6 (CYP2D6) in PD patients. CYP2D6 metabolizes many xenobiotics including debrisoquine and possibly MPTP. The mutant alleles that may be associated with PD result in the poor metabolizer phenotype. These studies suggest that PD may result from an interplay of genetic susceptibility to environmental agents. Our result of non-Mendelian transmission could be explained by a multifactorial etiology, involving gene-environment interaction.

The effect of older sibs. In addition to class A models which assume a vertical transmission, we analyzed the data under class D models, which allow for vertical

transmission and an older sib effect. Two possible explanations for the observed non-Mendelian transmission in our data set are polygenic inheritance, and existence of an environmental factor that clusters in families. If either of these possibilities were operative, one would expect to find an added familial component when considering the effect of older sibs (class D). Therefore, under class D models, the cumulative risk of developing PD should increase as a function of the number of older affected sibs, and should decrease as a function of the number of older unaffected sibs. Hence, if there is no evidence for an older sib effect, polygenic inheritance and an environmental factor with familial clustering would be considered less likely. Our data suggest that the cumulative risk of developing PD increases by the number of older affected sibs, and decreases by the number of older unaffected sibs, consistent with the two above-mentioned possibilities.

Biological models: age at onset vs. susceptibility. Our results suggest that it is more likely that the putative factor manifests its effect in the form of earlier age at onset, rather than conferring greater susceptibility with no effect on age at onset. It has been shown that the familial cases of PD have significantly earlier ages at onset than the non-familial cases, (Bonifati et al., 1995; Payami et al., 1995) which is consistent with the existence of familial factors that strongly influence age at onset. The better fit of the age at onset model to our data does not negate the existence of factors that influence susceptibility to PD. We were not able to consider both models simultaneously because complex segregation analysis allows test of hypotheses assuming either an effect on age at onset or an effect on susceptibility, but not both.

Case-control allelic association studies have provided evidence for genes that affect the risk of developing PD, such as mutant CYP2D6 alleles (reviewed by Woods, 1997). It

is not known if these polymorphisms affect the susceptibility to the disease or age at onset. Depending on the magnitude of the effect on onset, the age when they exert their effect and the age structure of the study population, an age at onset modifier can have a significant impact on the age-specific incidence and prevalence of the disease. As a results, age at onset modifiers can mimic susceptibility genes in case-control studies.

In summary, the present results have immediate implications in gene mapping studies and long term implications in understanding the pathogenesis of PD, treatment and prevention. Gene mapping studies, which are currently underway for PD, usually assume that the familial clustering of PD is due to a gene, and that the gene behaves in a Mendelian fashion. Our results suggest that at least one, and possibly both of these assumptions may be incorrect. Hence, special care may be needed to overcome these potential complexities. If confirmed, the present findings will encourage the search for genes and environmental factors that affect the age at onset of PD. Identification of such factors will shed light on the pathogenesis of PD and may explain why some people develop PD in their 20's and others not until they are in their 80's, or older. By understanding the mechanism of action of these factors it may be possible to delay the onset of PD.

	PD Probands	Relatives with PD	The entire sample
N	136	42	1060
% Male	56%	57%	53%
Age at last contact or death			
Mean $\pm$ SD	70.2 $\pm$ 9.5	72.2 $\pm$ 10.8	65.9 $\pm$ 18.9
Range	(40-89)	(40-93)	(1-104)
Age at onset of PD			
Mean $\pm$ SD	57.5 $\pm$ 12.0	59.8 $\pm$ 13.2	---
Range	(29-87)	(39-84)	---

Table 1. Characteristics of PD patients and their relatives.

Parameter	Mendelian					
	General	Dominant	Recessive	Additive	Decreasing	No major type
τ_{AA}	1§	1†	1†	1†	1†	---
τ_{AB}	0.26	0.5†	0.5†	0.5†	0.5†	---
τ_{BB}	0.04	0†	0†	0†	0†	---
q_A	0.003	0.0002	0.05	0.0002	0.0002	---
α	0.12	0.07	0.08	0.07	0.07	0.08
β_{AA}	-1	-4	-4	2	-3	-6
β_{AB}	-8	-4†	-9†	-4†	-4	-6†
β_{BB}	-16	-9	-9	-9	-9	-6†
γ	1§	1§	1§	1§	1§	0.12
-2lnL	545.7	563.4	567.3	563.4	563.4	613.0
χ^2 (df)	---	17.7 (3-4)	21.6 (3-4)	17.7 (3-4)	17.7 (1-3)	67.2 (4-6)
P	---	<.005	<.001	<.005	<.001	<.001
AIC	563.7	573.4	577.3	573.4	575.4	619.0
						614.0

χ^2 =(-2lnL of the data under hypothesis) - (-2lnL of the data under the general model). (Two extreme degrees of freedom are indicated because some parameters were fixed at bounds).

† Parameter is fixed at this value and not estimated in this model.

‡ Parameter is constrained to equal the preceding parameter and is not estimated individually.

§ Parameter is fixed at bounds by the program.

Table 2. Parameter estimates from segregation analysis of PD based on the model that type or genotype influences age at onset (REGTL model 1), under class A regressive models.

Parameter	General	Mendelian				No major type	Environmental
		Dominant	Recessive	Additive	Decreasing		
τ_{AA}	1§	1†	1†	1†	1†	---	0.2
τ_{AB}	0.42	0.5†	0.5†	0.5†	0.5†	---	0.2‡
τ_{BB}	0.04	0†	0†	0†	0†	---	0.2‡
q_A	0.002	0.0007	0.098	0.006	0.08	---	0.2‡
α	0.1	0.09	0.09	0.09	0.09	0.08	0.2
β	-6.53	-5.97	-5.93	-5.97	-5.95	-5.71	-0.3
γ_{AA}	1§	1§	1§	1§	1	0.12	0.95
γ_{AB}	1§	1‡	0.054‡	0.527‡	0.096	0.12‡	0.32
γ_{BB}	0.001§	0.064	0.054	0.053	0.044	0.12‡	0.08
-2lnL	551.2	578.2	582.4	585.2	582.1	613.6	613.6
χ^2	---	27 (1-4)	31.2 (1-4)	34 (1-4)	30.9 (1-3)	62.4 (2-6)	62.4 (1-3)
P	---	<.001	<.001	<.001	<.001	<.001	<.001
AIC	569.2	588.2	592.4	595.2	594.1	619.6	625.6

Table 3. Parameter estimates from segregation analysis of PD based on the model that type or genotype influences susceptibility (REGTL model 2), under class A regressive models.

Parameter	General	Mendelian					No major type	Environmental
		Dominant	Recessive	Additive	Decreasing			
τ_{AA}	1§	1†	1†	1†	1†	---	---	0.04
τ_{AB}	0.87	0.5†	0.5†	0.5†	0.5†	---	---	0.04‡
τ_{BB}	0.05	0†	0†	0†	0†	---	---	0.04‡
q_A	0.0003	0.0002	0.05	0.0002	0.03	---	---	0.04‡
α	0.13	0.08	0.08	0.08	0.08	0.06	0.06	0.13
β_{AA}	-1	-3	-3	2	-3	-7	-7	-0.1
β_{AB}	-9	-3‡	-9‡	-3‡	-8	-7‡	-7‡	-8
β_{BB}	-24	-9	-9	-9	-11	-7‡	-7‡	-15
γ	1§	1§	1§	1§	1§	1§	1§	1§
$\delta_{\text{affected sibs}}$	0.53	-0.07	-0.24	-0.07	-0.3	0.86	0.86	1.8
$\delta_{\text{unaffected sibs}}$	-0.4	-0.15	-0.08	-0.15	-0.01	-0.3	-0.3	-0.8
-2lnL	456.2	510.1	514.2	510.1	512.5	531.6	531.6	523.6
χ^2 (df)	---	44.9 (3-4)	49.0 (3-4)	44.9 (3-4)	47.3 (1-3)	66.4 (4-6)	66.4 (4-6)	58.4 (1-3)
P	---	<.001	<.001	<.001	<.001	<.001	<.001	<.001
AIC	485.2	524.1	528.2	524.1	528.5	541.6	541.6	539.6
$\delta_{\text{affected sibs}}$: coefficient of older affected sib effect								
$\delta_{\text{unaffected sibs}}$: coefficient of older unaffected sib effect								

Table 4. Parameter estimates from segregation analysis of PD based on the model that type or genotype influences age at onset (REGTL model 1), under class D regressive models.

CHAPTER 7

EXCLUSION OF THE FTDP-17 LOCUS ON CHROMOSOME 17 FOR PARKINSON'S DISEASE

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ABSTRACT

Parkinson's disease (PD) is a prevalent movement disorder, and 10-30% of PD is familial. Several neurodegenerative disorders which are collectively called frontotemporal dementia and parkinsonism have been mapped to chromosome 17q and mutations in *tau* have been identified. The clinical and pathological overlap suggests that these related conditions may be due to mutations in *tau*. We examined linkage to the candidate region on chromosome 17 including and surrounding *tau* in 8 familial PD kindreds. We found no evidence for linkage and excluded the 6cM candidate region which suggest that in our families, PD is not caused by mutations within *tau*. We have excluded the candidate regions of chromosomes 4, 2, and 17 in one autosomal dominant kindred, suggesting the existence of an additional as yet unidentified PD gene.

INTRODUCTION

Parkinson's disease (PD) is a common, age-related movement disorder characterized by progressive neuronal degeneration of mainly the dopaminergic neurons of the substantia nigra. The cause of this selective cell death is not known. About 10-30% of all PD patients have at least one first or second degree relative with PD (Payami et al., 1994a). Within familial PD, several rare autosomal dominant kindreds have been reported (Waters and Miller, 1994; Markopoulou et al., 1995; Golbe et al., 1996), however the majority of familial PD kindreds consist of 2 to 5 affected members. The first locus for PD was mapped to chromosome 4q21-23 in a large kindred of Italian descent (Polymeropoulos et al., 1996). Subsequently, mutations in the α -synuclein gene were identified that segregated with the disease (Polymeropoulos et al., 1997; Kruger et al.,

1998). A PD susceptibility locus with low penetrance (most likely below 40%) has been mapped to chromosome 2p13 in several large European kindreds (Gasser et al., 1998). Juvenile PD characterized by onset <30 years has been mapped to chromosome 6q and mutations in the *parkin* gene have been identified (Matsumine et al., 1997; Kitada et al., 1998). Despite the recent advances in the genetics of PD, the cause (s) of most cases of PD and the underlying neuronal degeneration is still unknown.

Several neurodegenerative disorders with motor, cognitive and behavioral disturbances have been mapped to chromosome 17q, and are collectively called "frontotemporal dementia and parkinsonism linked to chromosome 17" (FTDP-17) (Foster et al., 1997). Recently, mutations in the *tau* gene associated with this disorder were identified (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998). The spectrum of clinical manifestations and pathological findings in FTDP-17 overlap with clinical and pathological features of other neurodegenerative disorders such as dementia and PD, suggesting the possible existence of a common pathway leading to neurodegeneration. This overlap may also reflect the presence of mutations in the same locus that lead to other neurodegenerative disorders that are clinically related to FTDP-17. Therefore, it has been suggested that it would be worthwhile to test other familial neurological disorders for linkage to the candidate region on chromosome 17.

The goal of our study was to determine whether some cases of familial PD are caused by mutations within *tau*, by examining our familial PD kindreds for linkage to the candidate region on chromosome 17 including and surrounding *tau*.

SUBJECTS AND METHODS

Eight familial PD kindreds of Western European descent were selected for linkage analysis. The families were ascertained through the proband, diagnosed with idiopathic PD at the Movement Disorder Clinic at Oregon Health Sciences University. A complete family history and blood samples were obtained after giving informed consent. There were a total of 23 individuals with PD, with 2 to 4 affected members per family. The mean age at onset of PD was 58.3 ± 18.5 years. In one individual (OR-4), Lewy bodies were present in the substantia nigra and diagnosis of PD was confirmed pathologically. These families were previously tested and excluded from linkage to chromosome 4q21-23, and the α -synuclein G209A mutation was absent in affected members (Zarepari et al., 1998a). The six largest families (OR-1-6) have also been tested for linkage to chromosome 2p13: a subset of the families (OR-1, 5, 6) provided positive LOD scores which are consistent with the reported linkage, OR-4 was non-informative, and linkage was excluded in OR-2 and 3.

Families were genotyped for markers D17S800, D17S579, HOX2B and D17S958 spanning the 6 cM candidate region on chromosome 17q. Genotypes were amplified using standard protocols as specified by Research Genetics (www.resgen.com). Genotypes for markers D17S800 and HOX2B were separated on a 6% denaturing polyacrylamide gel and determined manually. Genotypes for markers D17S579 and D17S958 were separated on an ABI 377 Automated sequencer and were determined using GenScanTM and GenoTyperTM software (PE Applied Biosystems).

Linkage analysis was performed using an autosomal dominant model with age-dependent penetrance. Liability classes for unaffected individuals were assigned based on age at onset of PD in these families; penetrance increased in a stepwise manner from 0 at

birth to a maximum of 75% at age 63, after which it remained constant. Two-point and multi-point linkage analyses were performed with equal allele frequencies, using FASTLINK (Schaffer et al., 1994) and VITESSE (O'Connell and Weeks, 1995), respectively. Similar results were obtained when allele frequencies were estimated from the families.

RESULTS AND DISCUSSION

The results of the two-point linkage analyses are summarized in table 1. In these families, there is no evidence for linkage to the candidate region on chromosome 17q as consistently negative LOD scores were obtained for all 4 markers. Furthermore, the entire 6cM candidate region on chromosome 17 is excluded in these PD families by multi-point linkage analysis as shown in figure 1.

The exclusion of the candidate region on chromosome 17q indicates that PD in these families is not caused by mutations within the locus responsible for FTDP-17. This locus was also excluded in a large family with dementia, parkinsonism and Lewy body pathology (Hardy et al., 1998). In this family, there were over 15 affected members, and the mean onset of PD was 33.5 ± 8.5 years. Since we have excluded the candidate regions of chromosomes 4, 2, and 17 in one autosomal dominant kindred (OR-2), the results suggest the existence of an additional as yet unidentified PD gene.

		D17S800			D17S579			HOX2B			D17S958		
Family	# of	LOD score at Theta											
Affecteds	0	0.05	0.1	0	0.05	0.1	0	0.05	0.1	0	0.05	0.1	
OR-1	3	-3.21	-0.77	-0.47	-3.00	-0.55	-0.28	-0.32	-0.25	-0.20	0.06	0.08	0.09
OR-2	4	0.70	0.63	0.55	-2.00	-0.90	-0.59	0.33	0.27	0.21	-0.02	-0.01	0.00
OR-3	4	-2.29	-0.50	-0.24	0.24	0.25	0.24	0.09	0.07	0.05	-0.77	-0.45	-0.29
OR-4	2	-0.48	-0.28	-0.17	-0.48	-0.28	-0.17	-0.48	-0.28	-0.17	-0.59	-0.39	-0.27
OR-5	3	-2.56	-0.52	-0.27	-0.16	-0.11	-0.07	-2.78	-0.62	-0.36	-0.34	-0.23	-0.16
OR-6	3	-0.08	-0.02	0.02	-0.43	-0.41	-0.37	-0.35	-0.25	-0.19	-0.30	-0.22	-0.17
OR-7	2	-0.08	-0.07	-0.05	-0.35	-0.26	-0.19	0.02	0.02	0.01	0.02	0.02	0.01
OR-8	2	-0.18	0.12	0.20	-2.18	0.00	0.17	-5.18	0.10	0.28	-0.32	0.17	0.28
Total		-8.18	-1.41	-0.43	-8.36	-2.26	-1.26	-8.67	-0.94	-0.37	-2.26	-1.03	-0.51

Table 1. Results of two-point linkage analyses for the 4 markers spanning the candidate region of chromosome 17.

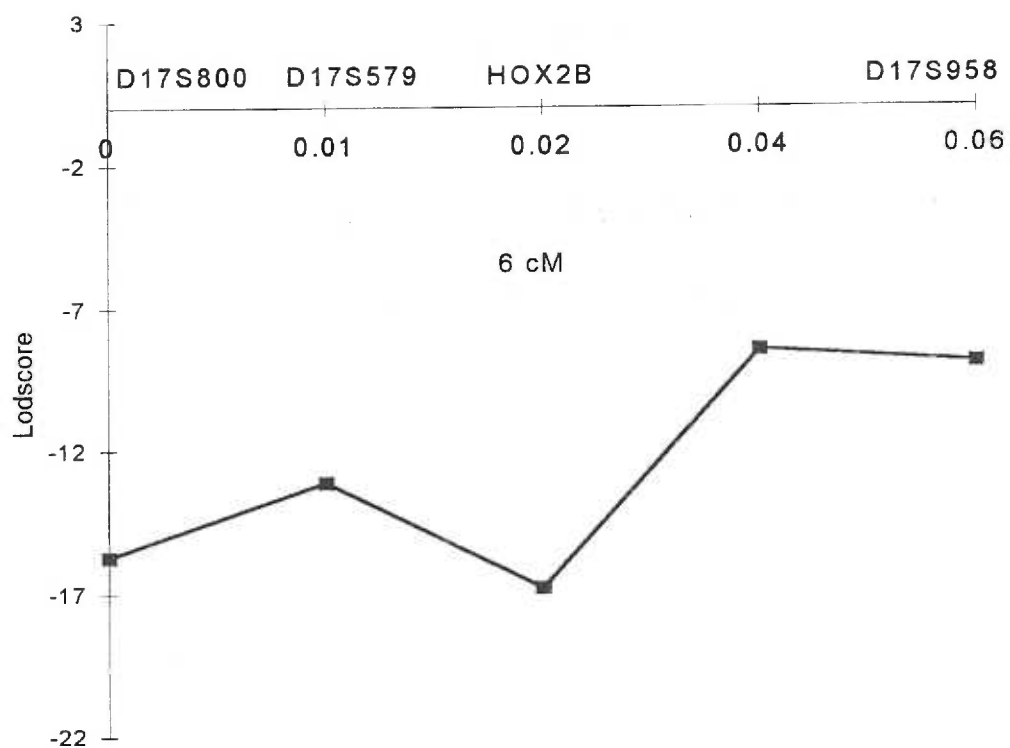


Figure 1. Results of the multi-point linkage analyses for the candidate region of chromosome 17 in the eight PD families.

CHAPTER 8

GENETIC VARIATION OF THE CYP2D6 GENE AND PARKINSON'S DISEASE

INTRODUCTION

Parkinson's disease (PD) is a progressive movement disorder that affects 1-2% of the population over the age of 60, and may who are younger (Tanner, 1992). The clinical symptoms of PD include resting tremor, rigidity, bradykinesia and postural instability. The neuropathological features include neuronal loss in substantia nigra and presence of Lewy bodies. The cause of this selective neuronal loss in PD not known.

It has been suggested that PD may result from a combination of inherited differences in metabolism and environmental exposure to neurotoxins (reviewed by Tanner, 1992). Debrisoquine 4-hydroxylase is an enzyme involved in the metabolism of over 25 drugs, and is encoded by CYP2D6. Abnormalities in CYP2D6, which is a member of the cytochrome P450 gene family, could potentially generate neurotoxins. Inactivating mutations in this gene (predominantly alleles A, B and D) result in the poor metabolizer phenotype. Barbeau et al (1985) originally reported an association between the poor metabolizer phenotype and PD, based on pharmacokinetic studies. Armstrong et al (1992) and Smith et al (1992) noted that the frequency of the inactivating mutations, particularly the B allele, was higher in PD patients than in controls. Bordet et al (1996a) reported that the frequency of the B allele was significantly higher in familial PD patients than non-familial PD patients. Agundez et al (1995) observed significantly increased frequency of the B allele in early-onset PD (onset<50) than those with late-onset PD (onset≥50). However, subsequent studies have not replicated this finding. One study did not detect a difference in frequency of the B allele between early-onset PD and controls (Sandy et al., 1996), and one study did not detect a difference between early-onset PD (onset<40) and late-onset PD (onset>50) (Diederich et al., 1996). A meta-analysis of 11 independent

published data sets reported that the poor metabolizer phenotype was associated with PD and resulted in a modest, but statistically significant odds ratio of 1.47 (McCann et al., 1997). The role of CYP2D6-B allele in PD is not clear as in the same meta-analysis, there was a trend of increased risk of PD due to the presence of the B allele, however, the association did not reach significance ($p=0.06$).

The aim of the present study was to examine the role of CYP2D6-B allele as a susceptibility allele for PD, and to determine whether carriers of the B allele have an earlier onset.

SUBJECTS AND METHODS

The study population consisted of 189 unrelated, Caucasian PD patients recruited from the Movement Disorder Clinic at Oregon Health Sciences University. All patients were evaluated by a neurologist and were diagnosed with idiopathic PD, based on the presence of two or more of the cardinal features of PD (tremor, rigidity, and bradykinesia), and the lack of other signs and history that could indicate alternative diagnoses or etiologies. Patients were not demented at the time of PD diagnosis. All subjects signed an informed consent, approved by the Institutional Review Board.

Age at onset was defined as the age at which the patient noticed the first symptom indicative of PD. Patients with onset before age 30 were excluded to avoid confounding by the rare early-onset forms of PD. Subjects were genotyped for the presence or absence of the CYP2D6-B allele as described previously (Smith et al., 1992). Allele frequencies were estimated by allele counting, and allele frequency differences were tested using the Z statistic. We used the *t*-test to compare mean age at onset of PD among those with the B

allele and those without. All statistical analyses were performed using SPSS Software (Version 8, 1997).

RESULTS AND DISCUSSION

The characteristics of the study population are summarized in table 1. The frequency of the B allele in this study was 0.21, which is within the range reported for PD by other published reports. We examined the frequency of the B allele in relation to family history and age at onset of PD. Familial PD patients consisted of those with a positive family history among first- or second-degree relatives. The frequency of the B allele was not significantly different among familial PD (0.17) and non-familial PD (0.23). The observed frequency of B in familial PD and non-familial PD patients in this study are not in agreement with the report of increased frequency of B in French familial PD patients by Bordet et al (1996a). This study consisted of 65 familial patients which is 5 times the number of familial patients in the study by Bordet et al (1996a). Patients with onset <50 years were categorized as early-onset PD and those with onset ≥ 50 years as late-onset PD. Comparison of the B allele frequency did not reveal a significant difference between early-onset PD (0.16) and late-onset PD (0.23). We expected to see increased frequency of B in young-onset PD based on the report of increased frequency of B in young-onset PD (onset <50) by Agundez et al (1995). However, our results are in agreement with the results of 2 subsequent studies that did not detect any difference in the frequency of B between early and late-onset PD (Diederich et al., 1996; Sandy et al., 1996).

Next, we tested the hypothesis that those with the B allele would have earlier onset of PD. The mean age at onset of PD was 57.2 ± 10.3 years among those with the B allele

and 55.4 ± 11.8 years in those without, and the difference was not significant. This trend for delayed onset of PD was in the opposite direction of our expectations. To explore the basis of the above observation, we examined the frequency of the B allele in patients grouped by age at onset into intervals of 10-years (Figure 1). We observed considerable fluctuations in the frequency of the B allele with the lowest frequency in those with onset between 30-39, and the highest in those with onset between 60-69.

In conclusion, the results suggest that CYP2D6 may be associated with PD in a subset of cases, and if so, it may help distinguish a disease subtype.

	Study population
Male / Female	120 / 87
Familial / non-familial	65 / 124
Age (mean $\pm$ SD)	67.3 $\pm$ 10.5
Age at onset of PD (mean $\pm$ SD)	56.0 $\pm$ 11.3
CYP2D6-B frequency	
B / B	11
B / -	58
- / -	120

Table 1. Characteristics of the study population.

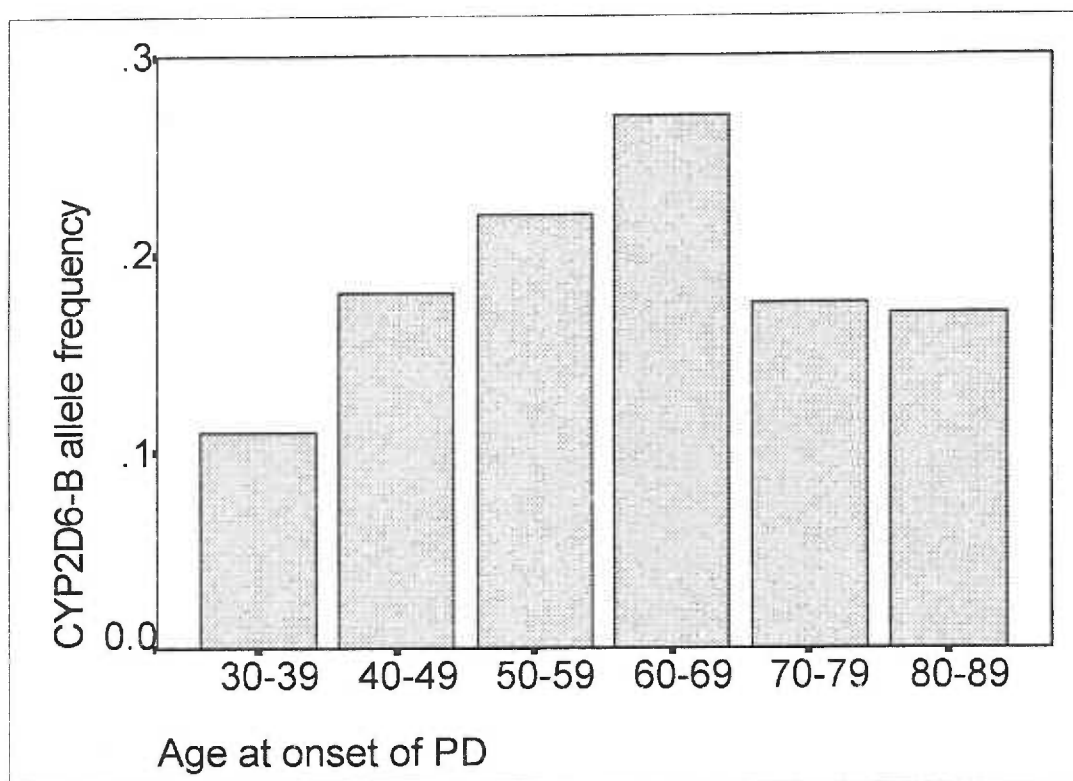


Figure 1. Frequency of the CYP2D6-B allele as a function of age at onset of PD.

CHAPTER 9

GENETIC EPIDEMIOLOGY OF PARKINSON'S DISEASE

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ABSTRACT

The cause of Parkinson's disease (PD) is unknown. The major risk factors identified to date are family history, age and elements of rural living. Nearly one third of all PD cases are familial, a small subset of which appear autosomal dominant; However, the majority exhibit no clear inheritance pattern. Autosomal dominant PD is genetically heterogeneous: two PD genes have been mapped to chromosomes 4 and 2 and there may be additional as yet unidentified gene(s). The common forms of PD – both familial and sporadic cases – appear to involve a complex interplay of genetic susceptibility and environmental exposure. The observations that rural residence and pesticide exposure increase the risk of developing PD, and that a synthetic drug, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, can cause parkinsonism, suggest at least a subset of PD may be caused by a toxin. Furthermore, modest but significant associations have been reported between PD susceptibility and genes that regulate metabolism of drugs and neurotoxins. There is also evidence for mitochondrial dysfunction in PD which was recently traced to anomalies in mitochondrial DNA. At the present time, the genetics of PD appears to be complex, involving multiple nuclear genes and possibly mitochondrial genes as well.

Idiopathic Parkinson disease (PD) is a progressive movement disorder that affects 1-2% of the population over the age of 60, and many who are younger (Tanner and Goldman, 1996). The clinical symptoms of PD can include resting tremor, rigidity, bradykinesia and postural instability. The neuropathological features include neuronal loss in substantia nigra and presence of Lewy bodies. The clinical symptoms of PD manifest when 80% or more striatal dopamine is lost (Hornykiewicz and Kish, 1986), presumably as a result of substantial neuronal loss in substantia nigra. It is not known, however, why the neurons die. Symptomatic therapy helps most of the patients, but progression of the disorder and complications of therapy cause increasing disability. Effective intervention will require an understanding of the causes of the disease, and the factors that modulate its expression.

Both genes and environmental factors seem to be involved in the pathogenesis of PD. The current task is to identify the individual culprits and decipher their mechanism of action. Scientific opinion on the relative importance of nature versus nurture in PD has changed several times over the last century, often shifting dramatically from one extreme to the other. In the 1980's, PD was thought to have little or no genetic component. A surge of family studies in the 1990's introduced new evidence in favor of the genetic hypothesis, and the localization of the first PD gene in 1996 demonstrated unequivocally that PD can be caused by genetic mutations. These recent breakthroughs have validated the existing body of evidence on familial clustering of PD and fueled the interest in genetic research.

GENETIC EPIDEMIOLOGY

PD is the second most common neurodegenerative disease, after Alzheimer disease (AD). The prevalence estimates range from 31 per 100,000 in Libya to 328 per 100,000 in the Parsi community in India (Tanner and Goldman, 1996). Community-based studies in the U.S., Europe and Canada have estimated the crude prevalence rates for Caucasians as 99 to 240 per 100,000. The prevalence of PD rises almost exponentially with age, from an estimated 3-47 per 100,000 for before the age of 50, to 2179-3096 per 100,000 for above age 80 (Schoenberg et al., 1985; Mayeux et al., 1995a; Tanner and Goldman, 1996). Prevalence of PD is higher in Europe and North America than in Africa, Japan or China (Tanner and Goldman, 1996). Most studies have found higher rates in whites than in blacks (Mayeux et al., 1995a; Tanner and Goldman, 1996). The fact that the highest prevalence is found in a genetic isolate (i.e., a closed community of Persian descendants in India) supports the notion that ethnic differences in disease frequency are at least in part due to genetic variation.

Family history

A familial component to PD was proposed by Gowers in 1888 (Gowers, 1888). In the hundred years that followed, many groups reported increased incidence of PD in relatives of patients, but the evidence for a familial component was dismissed in the 1980's because the early studies were affected by selection bias, diagnostic uncertainties and methodological limitations (Duvoisin, 1987). Also in the 1980's, twin studies failed to show a higher concordance rate in monozygotic twins than in dizygotic twins (Ward et al., 1983; Marsden, 1987; Marttila et al., 1988; Vieregge et al., 1992). The negative results of the twin studies, the lack of conclusive evidence from family studies, and the discovery

that a synthetic compound, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), can induce parkinsonism rendered the genetic hypothesis unlikely in the eyes of many scientists. Although, as was pointed out later by Johnson et al (1990), the results of twin studies may have been seriously affected by the variability in age at onset and preclinical disease in twin pairs. In fact, when positron emission tomography was used to measure ¹⁸fluorodopa uptake into basal ganglia, as the means of detecting preclinical disease, the monozygotic concordance rate increased (Burn et al., 1992). Several carefully designed family studies have been published in the 1990's, the results of which consistently and unequivocally demonstrate familial aggregation in PD (Butterfield et al., 1993; Semchuk et al., 1993; Payami et al., 1994a; Bonifati et al., 1995; Vieregge and Heberlein, 1995; De Michele et al., 1996; Marder et al., 1996; Uitti et al., 1997). All of the studies, regardless of the location of the study or the ethnic origin of the subjects, concluded that the risk to first degree relatives of patients was significantly higher than the risk to first degree relatives of controls. The estimated odds ratios varied across populations, ranging from 14.5 for an Italian population (De Michele et al., 1996), to 3.5 for US whites (Payami et al., 1994a), to 2.3 for an ethnically mixed community of whites, blacks and Hispanics in New York (Marder et al., 1996).

Clinic and population based studies estimate that 10-30% of PD are familial and the remaining 70-90% are sporadic (Butterfield et al., 1993; Payami et al., 1994a; Bonifati et al., 1995; De Michele et al., 1995; Vieregge and Heberlein, 1995; Uitti et al., 1997).

Familial PD is defined as patient having at least one affected relative; some studies include only first degree relatives, others extend to second degree relatives. Sporadic PD refers to patients who report no family history. Ideally, a sporadic case would be documented by a

large pedigree with long-lived disease-free relatives. But in reality, family histories are not always informative, yet the patient is classified as sporadic for the lack of a positive family history. Therefore, 70-90% is probably an overestimate for sporadic PD, and 10-30% is an underestimate for familial PD. Familial and sporadic PD appear similar clinically and neuropathologically (Maraganore et al., 1991; Bonifati et al., 1995; Plante-Bordeneuve et al., 1995; De Michele et al., 1996).

In some kindreds, PD follows a Mendelian autosomal dominant inheritance pattern (Golbe et al., 1993; Waters and Miller, 1994; Denson and Wszolek, 1995; Markopoulou et al., 1995; Golbe et al., 1996; Morrison et al., 1996). The majority of familial cases, however, show no clear inheritance pattern. Familial clustering of disease is usually taken as evidence for a genetic etiology, but it could also result from common exposure to environmental factors. Epidemiological studies have shown that rural living, farming, herbicide/pesticide exposure and well water drinking are associated with increased risk of PD (Tanner and Goldman, 1996). It is therefore possible that at least a subset of familial PD is due to common family exposures. For instance, in the families reported by Calne et al (1987) the pattern of disease transmission was suggestive of an autosomal dominant gene, if one assumed a genetic etiology. But the onset of symptoms tended to occur at approximately the same time, regardless of the age of the patient, which was also consistent with an environmental cause.

Several groups have favored a genetic etiology for the familial aggregation of PD and have attempted to determine the mode of inheritance. Barbeau and Roy (1984) defined two familial subtypes based on clinical features: an autosomal dominant essential-tremor related parkinsonism and an autosomal recessive familial akineto-rigid syndrome.

Maraganore et al (1991) calculated segregation ratios in 20 familial PD kindreds, and suggested an autosomal dominant transmission with incomplete penetrance. Lazzarini et al (1994) used survival analysis and calculated age adjusted segregation ratios in 80 multicase families. The life-time risk of developing PD approached 50%, and the age adjusted segregation ratio was approximately 0.5, consistent with autosomal dominant transmission. The studies that found autosomal dominant inheritance as the most likely explanation used families that were preferentially selected for having multiple affected members. To date, only one study has performed complex segregation analysis using families of patients that were selected randomly, regardless of family history. Zarepars et al (1998c) applied complex segregation analysis to 136 families of randomly selected clinic patients. They found evidence for transmission of a rare familial factor, but rejected all Mendelian inheritance models. Furthermore, their study suggested that the putative familial factor is more likely to manifest its effect in the form of earlier age at onset rather than conferring greater susceptibility to PD. The seemingly conflicting results of complex segregation analysis and the earlier studies that suggested autosomal dominant inheritance may in fact reflect differences in the patient populations that were studied. If Zarepars et al's findings are confirmed, the collective evidence would suggest that, while a subset of familial PD is inherited in a Mendelian fashion, the familial aggregation of the more common forms of PD may be more complex, and may involve factors that affect age at onset.

Age

Advancing age is a major risk factor for PD. Prevalence and incidence of PD rise almost exponentially with age in all populations (Tanner and Goldman, 1996). The reason for the

correlation with age is not known. Nor is it known if the incidence continues to rise at very high ages, eventually reaching 1.0 if one lived long enough. The variation in incidence with age may reflect a disease mechanism that is time dependent. Alternatively, there may be a "natural" and gradual neuronal loss over time that accumulates with age until the disease threshold is reached. It is believed that the onset of symptoms will occur after 80% of dopamine is lost (Hornykiewicz and Kish, 1986). The age variable may reflect individual variations in rate of progression to this threshold.

Age at onset of PD can vary by more than sixty years. It is not known why some people develop PD in their 20's and others not until they are in their 80's. Identification of factors that affect age at onset may be the first step towards developing effective strategies to delay the disease. Several lines of evidence suggest that familial factors, presumably genes, are involved in determining age at onset of PD. First, autosomal dominant PD kindreds often exhibit unusually early onset (Golbe et al., 1993; Denson and Wszolek, 1995; Markopoulou et al., 1995; Golbe et al., 1996; Morrison et al., 1996). Second, in series of consecutively ascertained patients, the average age at onset is earlier in patients that have a family history than those who do not (Bonifati et al., 1995; Payami et al., 1995). Third, results of complex segregation analysis suggest familial aggregation of PD is best explained by familial factors that affect age at onset (Zarepari et al., 1998c). Fourth, preliminary data suggest age at onset of PD may be modulated by the apolipoprotein E (APOE) genotype (Zarepari et al., 1997). Fifth, familial PD exhibits anticipation (i.e., earlier onset in successive generations).

Nine studies have examined the intergenerational difference in age at onset of familial PD and have found on average 4 to 33 years earlier onset in the offspring generation than

in the parents' generation (Bonifati et al., 1994; Bonifati et al., 1995; Markopoulou et al., 1995; Payami et al., 1995; Plante-Bordeneuve et al., 1995; Golbe et al., 1996; Maraganore et al., 1996; Morrison et al., 1996; Munoz et al., 1997). The possibility that anticipation is partly or completely an artifact of ascertainment bias can not be excluded. Genetic anticipation was first described for myotonic dystrophy (MD), but it was thought to be of no biological significance until the connection to DNA instability was discovered. In MD, and several other neurodegenerative disorders that exhibit anticipation, the gene contains a triplet repeat that can expand as it is transmitted from parent to child (Li and El-Mallakh, 1997). The triplet is often, but not always, a CAG. As a general rule, the larger the number of repeats the more severe the disease and the earlier the onset of symptoms. Using the Repeat Extension Detection Method, Carrero-Valenzuela et al (1995) found no evidence for the presence of a CAG repeat expansion in PD. However, small CAG expansions and other triplet repeats would have gone unnoticed with this method. Mitochondrial disorders can also exhibit anticipation in age at onset (Uziel et al., 1997), and as will be discussed later, there is increasing evidence for mitochondrial anomalies in PD. Whether the cause of anticipation in PD is bias or biological will not be known until a mechanism is identified.

Environmental agents

Exposure to MPTP can induce parkinsonism. MPTP was a side product and contaminant of a "designer drug" that was sold as an injectable synthetic heroin in 1970's. Some of the individuals who injected MPTP developed parkinsonism within days of exposure (Langston et al., 1983). A metabolite of MPTP, MPP⁺, is a neurotoxin that inhibits complex I of the oxidative phosphorylation system, causing energy depletion and oxidative

stress leading to neuronal death (Tipton and Singer, 1993). The striking similarities between MPTP-induced parkinsonism and idiopathic PD has triggered the idea that similar compounds may be involved in pathogenesis of PD. Genes that regulate conversion of endogenous and exogenous protoxins, like MPTP, into active toxins may underlie the genetic susceptibility to PD.

Numerous studies have linked various aspects of rural living to an increased risk of PD. In their review, Tanner and Goldman (1996) point out that although both the methods and the locations of the studies have differed, all show an association between at least one of the proposed exposures – rural residence, farming, well water drinking or herbicide/pesticide exposure – and increased risk for developing PD. Tanner and Goldman also review the literature on cigarette smoking and PD. Both retrospective and prospective studies have shown lower rates of PD among smokers. Animal studies also suggest nicotine has a protective effect against induced neuronal loss in substantia nigra (Janson et al., 1992; Prasad et al., 1994). A recent study has shown an interaction between smoking and genetic polymorphism at the monoamine oxidase B locus, suggesting the protective effect of smoking in PD is genotype specific (Checkoway et al., 1998).

GENE MAPPING IN AUTOSOMAL DOMINANT KINDREDS

Occasionally, PD exhibits a clear Mendelian transmission pattern in a family, consistent with an autosomal dominant mode of inheritance (Golbe et al., 1993; Waters and Miller, 1994; Denson and Wszolek, 1995; Markopoulou et al., 1995; Golbe et al., 1996; Morrison et al., 1996). Kindreds that clearly exhibit autosomal dominant inheritance are rare. The majority of PD families consist of only two or three affected relatives and show no

obvious inheritance pattern. Despite their rarity, autosomal dominant kindreds have been the centerpiece of genetic studies because a) they provide direct evidence for the involvement of a major disease gene, b) they are amenable to lodscore linkage analysis, the most powerful method for gene localization, and c) one large family may have sufficient power to map the gene, thus eliminating the need to pool and mix potentially heterogeneous families. The best known example of autosomal dominant PD is the Contursi kindred, an Italian-American family with 60 affected members, presenting with typical clinical and pathological features of idiopathic PD, except relatively early onset and rapid course from onset to death (Golbe et al., 1996).

Linkage to chromosome 4 and the α -synuclein gene

In 1996, Polymeropoulos et al (1996) reported linkage of PD to DNA markers on the long arm of chromosome 4 in the Contursi kindred, placing the putative gene in the 4q21-23 region. A more detailed examination of the region, revealed an association between a mutation in the α -synuclein gene and PD in the same kindred (Polymeropoulos et al., 1997). The mutation was a single base pair change from guanine (G) to adenine (A) at nucleotide position 209, resulting in an alanine to threonine substitution at amino acid position 53. All except one of the affected individuals in the Contursi kindred had A, and all of the unaffected relatives had G, suggesting a high penetrance. Thus, the G209A mutation may be a good predictor of PD, however, its utility as a pre-symptomatic diagnostic tool would be limited to the few kindreds that harbor this mutation.

In addition to the Contursi kindred, Polymeropoulos et al (1997) studied 5 Greek PD kindreds and 52 Italian sporadic PD patients. The G209A mutation was present in three of the five Greek kindreds and none of the sporadic patients. To date, over 530 PD subjects

have been screened for the G209A mutation, including 141 familial PD kindreds. None had the mutation (Munoz et al., 1997; Bennett and Nicholl, 1998; Chan et al., 1998a; Chan et al., 1998b; Farrer et al., 1998; Group, 1998; Kruger et al., 1998; Vaughan et al., 1998; Zarepari et al., 1998a).

To determine if PD is caused by other mutations in the α -synuclein gene, a number of groups have sequenced the coding regions, searching for differences between patients and controls (Chan et al., 1998a; Farrer et al., 1998; Group, 1998; Kruger et al., 1998; Vaughan et al., 1998). Kruger et al (1998), found a guanine (G) to cytosine (C) mutation at position 88 (resulting in an alanine to proline substitution in the protein) that segregated with PD in one family. No other mutation has been found in over 70 familial PD kindreds of various ethnic origins that were sequenced. These studies examined only the coding regions of the α -synuclein gene, leaving the possibility that the disease is caused by mutations in non-coding or regulatory regions. Results of linkage analyses, however, argue against that possibility. Three groups have excluded linkage to a relatively large region, including and surrounding the α -synuclein gene (Gasser et al., 1997; Scott et al., 1997; Zarepari et al., 1998a). Thus the collective data from sequence analysis and linkage studies suggest that α -synuclein mutations are rare causes of PD. Nevertheless, α -synuclein may have a central role in the pathogenesis of PD.

α -synuclein was originally discovered as a component of the amyloid plaques in the brains of patients with AD (Ueda et al., 1993). Interestingly, α -synuclein is the second most abundant component of the Alzheimer's plaques, after β -amyloid (Ueda et al., 1993). More recently, Spillantini et al (1997) demonstrated the presence of α -synuclein in Lewy bodies. They showed that Lewy bodies in substantia nigra from patients with PD stained

with antibodies raised to amino acid residues 11-34 and to residues 116-131 of human α -synuclein. α -synuclein is also present in substantia nigra of patient with dementia with Lewy bodies (Spillantini et al., 1997). Although the function of the protein is unknown, it has been postulated that α -synuclein and Lewy bodies are central to neurodegenerative process (Spillantini et al., 1997).

Linkage to chromosome 2

Recently, Gasser et al (1998) reported evidence for a PD susceptibility gene on chromosome 2p13. They were able to map this locus using linkage analysis in several familial PD kindreds that exhibited an autosomal dominant inheritance pattern. They identified a common haplotype that segregated with PD in two unrelated families, suggesting the possibility of a founder mutation. This haplotype was present in 25 family members, 10 of whom had PD, yielding a penetrance estimate of 40%. Efforts are now focused on confirming the initial linkage report and to identify and clone the disease gene. It is too early to know what proportion of familial PD kindreds may be linked to the putative locus on chromosome 2. One study has found suggestive evidence for linkage to chromosome 2 in a subset of families, while excluding it in at least one family (Zarepari et al., 1998b). The family that excluded linkage to chromosome 2 also excluded chromosome 4. This finding suggests that some families may be linked to another, as yet unidentified PD gene.

GENETICS OF JUVENILE PARKINSONISM

Juvenile parkinsonism (JP) clinically resembles typical PD, but pathologically it may differ from idiopathic PD in that many cases do not have Lewy bodies (Yokochi, 1997).

Age at onset of JP is arbitrarily set at before 40, but it can occur as early as in childhood. The absence of Lewy bodies may be an indication that JP is etiologically distinct from PD. Alternatively, Lewy bodies may not develop in young patients. In fact, an autopsied brain of a JP patient revealed cytoplasmic changes that could represent immature Lewy bodies (Hayashida et al., 1993). Identification of genes for PD and JP should help determine if they are the same or different at the molecular level.

Yokochi (1997) reports that in Japan, where JP is studied most extensively, nearly half of JP cases are familial and half are sporadic. The familial subset may present as autosomal recessive or as autosomal dominant. In 1997, Matsumine et al (1997) reported evidence for linkage of JP to chromosome 6q25.2-27 in 13 Japanese families that presented with the autosomal recessive form of the disease. This finding has been confirmed by two independent groups in families of diverse ethnic origin, including Japanese, Middle Eastern, European, American, and African (Jones et al., 1998; Tassin et al., 1998).

Kitada et al (1998) showed recently that deletions in a novel gene, parkin, segregated with JP in four Japanese families with autosomal recessive inheritance. parkin, which is on the short arm of chromosome 6 within the candidate region identified by linkage, is a large gene with twelve exons. Its protein product, Parkin, is expressed in many tissues and is abundant in the brain including substantia nigra. The function of Parkin is currently unknown, but its structure is similar to the ubiquitin family of proteins, which are a component of Lewy bodies in PD, and of paired helical filaments in AD (Kitada et al., 1998).

Genetic susceptibility to common forms of PD

We now shift the focus from the rare hereditary cases to the genetic basis of more common forms of PD. As discussed in the previous section, genetic-epidemiology studies have produced consistent results pointing to a familial, presumably genetic component in PD (Butterfield et al., 1993; Semchuk et al., 1993; Payami et al., 1994a; Bonifati et al., 1995; Vieregge and Heberlein, 1995; De Michele et al., 1996; Marder et al., 1996; Uitti et al., 1997). Deciphering the genetics of common PD will undoubtedly be more challenging than the clear cut Mendelian forms, because the syndrome that we call idiopathic PD and treat as one entity is probably a complex of several disorders that are clinically indistinguishable. The uncertainty of clinical diagnosis and the need for pathological confirmation (Hughes et al., 1992), which is not available for most studies, adds to the complication. The genetic component of PD may be a composite of modest effects of multiple loci, too subtle and too complex to be detected in every study. Despite these difficulties, considerable progress has been made in genetics of common PD. The findings have not always been consistent, not surprisingly, given the suspected heterogeneity, diagnostic difficulties and methodological limitations. The most commonly used methods for identifying susceptibility genes in complex disorders are linkage analysis (using affected relatives such as sibpairs) and allelic association studies (using unrelated patients and controls). Affected sib pair and relative pair studies of PD are underway in several laboratories. Allelic association studies have been used extensively to test for involvement of candidate genes with potential functional significance in PD pathogenesis. The candidate genes for PD may be classified in four categories: genes that regulate metabolism of xenobiotics and may therefore determine ones susceptibility to toxins, genes

involved in dopamine metabolism and transport as a possible source of dopamine deficiency in PD, genes that are associated with neurodegeneration in other disorders, and mitochondrial mutations as the possible source of the complex I deficiency in PD.

Genes associated with drug metabolism

The most extensively studied candidate gene for PD is CYP2D6, which is a member of the cytochrome P450 gene family. The enzyme encoded by CYP2D6, debrisoquine 4-hydroxylase, is involved in the metabolism of over 25 drugs, and abnormalities in this gene could potentially generate neurotoxins. The CYP2D6 locus is on chromosome 22q13. Inactivating mutations in this gene (predominantly alleles A, B and D) result in the poor metabolizer phenotype. Barbeau et al (1985) originally reported an association between the poor metabolizer phenotype and PD, based on pharmacokinetic studies in 1985. In 1992, Armstrong et al (1992) and Smith et al (1992) noted that the frequency of the inactivating mutations, particularly the B allele, was higher in PD patients than in controls. There have been numerous studies since, with inconsistent results (Kurth and Kurth, 1993; Mazzetti et al., 1994; Plante-Bordeneuve et al., 1994; Rempfer et al., 1994; Agundez et al., 1995; Akhmedova et al., 1995; Saitoh et al., 1995; Bordet et al., 1996a; Bordet et al., 1996b; Diederich et al., 1996; Gasser et al., 1996a; Lucotte et al., 1996; Sandy et al., 1996; Bandmann et al., 1997). McCann et al (1997) have performed a meta-analysis utilizing 11 independent published data sets. In the pooled data, the poor metabolizer phenotype was present in 7.4% of patients as compared to 5.4% of controls, yielding a modest, but statistically significant odds ratio of 1.47.

Monoamine oxidase (MAO) loci A and B are two homologous genes located adjacent to each other on the X chromosome (Xp11.3). They are candidate genes for PD because

they encode enzymes that metabolize dopamine (Oreland, 1991) and MPTP (Tipton and Singer, 1993). A number of groups have examined possible association of MAO polymorphisms with PD, but the results have been inconsistent. With respect to MAO-B, four studies found evidence for association with PD (Kurth et al., 1993; Hotamisligil et al., 1994; Nanko et al., 1996; Costa et al., 1997), two did not (Ho et al., 1995; Plante-Bordeneuve et al., 1997). With respect to MAO-A, two studies found an association (Hotamisligil et al., 1994; Nanko et al., 1996), two did not (Kurth et al., 1993; Plante-Bordeneuve et al., 1997). Unfortunately, it is not possible to pool the MAO studies because they used different polymorphisms.

There are additional reports of candidate gene studies in the literature, which have generated conflicting results. They include cytochrome P450IA1, which is involved in activation of mutagens in cigarette smoke (Takakubo et al., 1996), COMT, which is involved in inactivation of neurotransmitters, toxins and xenobiotics containing a catechol moiety (Hoda et al., 1996; Kunugi et al., 1997; Yoritaka et al., 1997), and N-acetyltransferase 2, which is involved in detoxification of aromatic amines (Bandmann et al., 1997).

Dopamine receptor and transporter genes

Few studies have examined the genes that govern dopamine synthesis and transport in relation to PD susceptibility. Plante-Bordeneuve et al (1997) have reported a significant distortion in the dopamine receptor D2 allele frequencies in both sporadic and familial PD, as compared to controls. Le Couteur et al (1997) has found an allelic association, and a possible effect on age at onset, with the dopamine transporter gene in Caucasian PD patients. But two other studies, one in Caucasian patients and one in Chinese patients, did

not find any association with dopamine transporter gene (Leighton et al., 1997; Plante-Bordeneuve et al., 1997).

Exploring a genetic link with other neurodegenerative disorders

There is considerable phenotypic overlap between idiopathic PD and several other neurodegenerative disorders, such as AD, essential tremor, frontotemporal dementia with parkinsonism, and dopa-responsive dystonia. The etiologies of these disorders are probably distinct, each being caused by disease-specific genes or environmental insults that damage specific populations of neurons. The mechanisms of response to neuronal damage, however, are probably genetically determined and may not be disease specific. If so, the efficacy of the response mechanism – e.g., the neuronal repair system and the inflammatory response – may affect one's general susceptibility to neurodegeneration, progression of disease and age at onset of symptoms. Hence, genes that control the response mechanisms may be associated with more than one neurodegenerative disease, and may be responsible for some of the overlap in their phenotypes.

Approximately 30% of PD patients develop dementia (Hughes, 1997), and 30% of AD patients develop parkinsonian signs (Merello et al., 1994). More over, brains from PD patients often display co-incidental AD pathology (Hughes et al., 1993), and similarly, brains from AD patients frequently contain Lewy bodies (Hulette et al., 1995). Soon after the discovery of the association of APOE with AD, several investigators questioned if APOE was a link between the two disorders. APOE, a gene on chromosome 19, has been long recognized for its role in lipid metabolism and its association with heart disease (Siest et al., 1995). APOE is also involved in repair of neuronal damage (Poirier, 1994). APOE has three common alleles, $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$. There are two common observations regarding

APOE and AD: (1) the frequency of $\epsilon 4$ is significantly elevated in AD patients while the frequency of $\epsilon 2$ is reduced, and (2) age at onset of dementia is earliest in $\epsilon 4$ -carriers, intermediate in $\epsilon 3$ homozygotes and latest in $\epsilon 2$ -carriers (Schellenberg, 1995). Numerous studies have examined $\epsilon 4$ frequency in PD; few have tested an association with age at onset. The frequency of $\epsilon 4$ was not elevated in PD, or in PD-dementia in most studies (Arai et al., 1994; Benjamin et al., 1994; Han et al., 1994; Hardy et al., 1994; Harrington et al., 1994; Marder et al., 1994; Rubinsztein et al., 1994; Koller et al., 1995; Martinoli et al., 1995; Poduslo et al., 1995; Whitehead et al., 1996; Group, 1997; Zarepari et al., 1997; Inzelberg et al., 1998). Thus, $\epsilon 4$ does not seem to be associated with susceptibility to PD or to PD-dementia. But APOE may have an effect on age at onset. Whitehead et al (1996) examined age at onset of PD in 189 subjects, all with onset at or before age 55. While there was no $\epsilon 4$ effect on onset age, they reported $\epsilon 2$ -carriers as having significantly delayed onset. Zarepari et al (1997) studied 137 PD patients whose ages of onset ranged from 34 to 80. They found age at onset was earliest in $\epsilon 4$ carriers, intermediate in $\epsilon 3$ homozygotes and latest in $\epsilon 2$ -carriers, which is the same pattern seen in AD.

Yamamoto et al (1997) studied α_1 -antichymotrypsin (ACT) polymorphism, a possible risk factor for AD (Kamboh et al., 1995), in Japanese PD patients. They found a significant allelic association with PD and a possible effect of ACT genotype on age at onset of PD. Furthermore, their data suggested a possible interaction between ACT and APOE genotypes in association with PD.

Frontotemporal dementia with parkinsonism is a collection of several neurodegenerative disorders that all map to the same locus on chromosome 17 (Foster et

al., 1997). The gene was recently identified as Tau and several mutations have been described in different kindreds (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998). Two groups have examined linkage of familial PD to this locus, and both obtained significant evidence for exclusion (Gasser et al., 1996b; Zarepari et al., 1998d).

Mitochondrial dysfunction and PD

Mitochondria are the power house of the cell and their dysfunction can result in cell death. Some of the protein components of the oxidative pathway (complexes I-V) are encoded by nuclear genes and some by mtDNA. Inhibition of this pathway, particularly at complex I or III, results in increased production of free radicals. As summarized in the review by Schapira (1997), the results of many studies suggest that there is a specific ~35% complex I deficiency in PD nigra. This defect is restricted to substantia nigra and does not affect any other parts of PD brain. Several laboratories have detected a modest complex I deficiency in platelets from PD patients as well. Recently, Swerdlow et al (1996) demonstrated that the complex I deficiency in PD is linked to mtDNA. By fusing mtDNA from PD patients into rho⁰ cells, which have a nucleus but no mtDNA, they showed that complex I defect follows the transfer of mtDNA.

The attempts to identify mtDNA mutations in PD have not been successful so far. There have been several reports of slight frequency increases of various point mutations, deletions and insertions in mtDNA in PD versus controls; none of which have been replicated (Schapira, 1997). mtDNA is transmitted through maternal lineage exclusively, thus if PD involved inherited mtDNA mutation, one would expect an excess maternal transmission in familial PD. Most studies, however, do not find that (Maraganore et al., 1991; Zweig et al., 1992; Lazzarini et al., 1994; Bonifati et al., 1995; De Michele et al.,

1995; Plante-Bordeneuve et al., 1995; Wooten et al., 1997). It is possible that mtDNA mutations in PD are acquired through exposure to exogenous toxins, rather than inherited from the mother. Given the suspected heterogeneity in PD, identification of mtDNA mutations may require carefully selected patients with demonstrated complex I deficiency or large kindreds with a clear maternal transmission pattern compatible with mitochondrial inheritance.

SUMMARY

Considering that only a few years ago PD was thought to be non-genetic, the recent progress in genetic research has been phenomenal. The genes responsible for the autosomal dominant forms of PD should be all identified within the next several years, paving the road to understanding the fundamental mechanisms of disease pathogenesis. The genetic basis of common forms of PD will be more difficult to resolve, but the work is underway. The task is difficult because there is no clue as to the degree of the underlying complexity in this disease. To maximize the chances of success, many large institutions have formed collaborative consortiums, bringing together expertise of neurologists, molecular biologists and genetic epidemiologists, and pooling resources to create large study populations with power to detect modest effects of individual genes in an etiologically heterogeneous syndrome. Success of genetic studies must not overshadow the importance of epidemiological research, rather, it should help intensify the search for environmental triggers.

CHAPTER 10

SUMMARY

The overall objective of this thesis project was to identify genetic risk factors for AD and PD, to attempt to dissect the genetic complexity of both disorders by identifying specific subtypes associated with specific genetic risk factors, and to identify genetic risk factors that may be associated with both AD and PD and may explain the observed clinical and neuropathological overlap between the two disorders.

This thesis project has made the following contributions to the field:

- We showed an interaction between gene and gender in the risk of AD
- We discovered that HLA-A2, a gene known for its involvement in the immune system, or a closely linked gene is associated with age at onset of AD
- We showed that APOE, a gene known to affect age at onset of AD, also affects age at onset of PD
- We showed that α -synuclein, a gene that is linked to rare autosomal dominant PD, is not the cause of common forms of PD
- Using formal segregation analysis, we found that contrary to the common belief, the factor(s) responsible for familial clustering of PD is not a Mendelian gene, and affects age at onset not susceptibility to PD
- We excluded tau, a gene responsible for frontotemporal dementia with parkinsonism, as the cause of familial PD in Oregon kindreds.

AD

At the time I started my thesis, four genes were known for AD. The genes for the rare early-onset autosomal dominant forms of AD are APP, PS1 and PS2. They have little if

any involvement in the common late-onset AD. The only genetic risk factor known for late-onset AD was APOE. APOE does not cause AD rather it modulates its age at onset. There was evidence for the existence of additional as yet unidentified genes for late-onset AD.

Following up on the possible association of HLA-A2 with AD (reviewed by Payami et al., 1991), we found that HLA-A2 was associated with the age at onset of AD independent of APOE, adding to our understanding of the genetic complexity of AD. Our original finding of earlier onset of AD in HLA-A2 carriers has been confirmed by two other groups (Combarros et al., 1998; Ballerini et al., 1999). Furthermore, two other studies have reported evidence for linkage to the HLA region on chromosome 6 (Collins et al., 1997; Pericak-Vance et al., 1998). The association between AD and HLA-A2 may reflect the presence of an immune/inflammatory response mechanism for AD. This is supported by previous studies that have reported the presence of immune-associated proteins and cells such as activated microglia (Aisen and Davis, 1994; McGeer et al., 1994), and delayed onset of AD in patients taking nonsteroidal anti-inflammatory drugs (Breitner et al., 1995). As a first step in exploring this association, future research is needed to distinguish whether age at onset of AD is influenced by HLA-A2 itself or a closely linked gene. We and others are currently trying to investigate this question by examining other HLA loci and nearby genes for a possible effect on age at onset of AD and studying the strength of the possible effect. If in fact, it is found that HLA-A2 or another class I HLA molecule is involved in AD, then functional studies are needed to determine the role of the immune system in the pathogenesis of AD. HLA-A2 is the most common of HLA-class I molecules and is involved in the regulation of the immune

response by presenting antigens as short peptide fragments derived from intracellular proteins to antigen-specific CD8⁺ T-cells (Browning and Krausa, 1996). The results of a recent study have implied a novel role for HLA-class I molecules (including HLA-A) in normal neuronal functioning and have suggested that the level of expression of HLA-class I molecules are reflective of the level of neuronal activity with increased expression due to increased activity and decreased expression due to decreased activity (Corriveau et al., 1998). They suggest that the decreased expression by damaged neurons may have an important adaptive value by protecting them from immune destruction and thereby preventing further damage, while the normal expression makes neurons targets for disruption by cells of the immune system in situations where the blood-brain barrier is compromised (Corriveau et al., 1998).

It has long been recognized that there are more women with AD than men. However, it was not clear whether the increased prevalence of AD in women was reflective of a gender difference in disease mechanism or an artifact due to longer survival by women. Our study showed that the APOE-associated risk of AD is different between men and women, providing evidence for an interaction between gene and gender. Our original findings have now been confirmed by numerous studies worldwide (Farrer et al., 1997; Palumbo et al., 1997; Martinez et al., 1998).

Our finding of APOE-gender interaction in AD has stimulated research into the mechanism for the increased risk of AD in women. A subsequent study has found an interaction between APOE and gender on the degree of entorhinal cortex atrophy in AD, where $\epsilon 4$ positive women were found to have the most prominent volume loss (Juottonen et al., 1998), a pattern that is consistent with our observations. One study has reported

that estrogen use is associated with a significant delay in the onset of AD and reduced risk of AD in $\epsilon 4$ heterozygous women (Tang et al., 1996). Estrogen promotes the growth of cholinergic neurons, synapse formation and may interact with APOE (Toran-Allerand et al., 1992; Honjo et al., 1995). One study has observed a gender difference on the influence of APOE on the distribution of lipids (Reilly et al., 1994). This gene-gender interaction was also found to influence treatment of AD, as one study reported that the effect of treatment with tacrine, a cholinesterase inhibitor, depended on the gender-APOE combination of the patients (Farlow et al., 1998). It is possible that the gender difference in the APOE-associated risk of AD results from reduced efficacy of neuronal repair in women, influenced by the differential effects of APOE genotypes compounded by estrogen loss, resulting in a faster deterioration and earlier onset in women with $\epsilon 4$. The role of gender differences in development and progression of AD warrants further research.

PD

The relative importance of genetic versus environmental factors in the pathogenesis of PD has been the subject of controversy for over a century, with scientific opinion shifting from one extreme to the other several times. At the time of this thesis, several carefully designed family studies consistently and unequivocally demonstrated familial aggregation in PD (Butterfield et al., 1993; Semchuk et al., 1993; Payami et al., 1994a; Bonifati et al., 1995; Vieregge and Heberlein, 1995; De Michele et al., 1996; Marder et al., 1996; Uitti et al., 1997). Approximately 10-30% of all PD patients have a positive family history (Butterfield et al., 1993; Payami et al., 1994a; Bonifati et al., 1995; De Michele et al.,

1995; Vieregge and Heberlein, 1995; Uitti et al., 1997), and in a rare subset, the mode of inheritance is autosomal dominant (Maraganore et al., 1991; Lazzarini et al., 1994; Waters and Miller, 1994; Denson and Wszolek, 1995; Markopoulou et al., 1995; Golbe et al., 1996). But the majority of the families show no discernible inheritance pattern. There have been several hypotheses regarding the mode of inheritance of PD based on the presence of genes that influence susceptibility to PD.

We performed a formal segregation analysis using randomly selected clinic patients, and found that the familial clustering of PD was best explained by non-Mendelian transmission of a rare familial factor that influences age at onset. The segregation analysis of PD presented me with a more complex picture of PD than I had previously thought. The observed non-Mendelian transmission may be reflective of genetic heterogeneity, gene-environment interaction, or a mitochondrial component to PD. The finding that the factor(s) exerts its effect by influencing age at onset rather than susceptibility to PD was very intriguing. Our results suggested that everyone would develop PD if they lived long enough, but the estimated mean onset (120 years) was beyond the human life span. However in those who carried the familial factor(s), the age at onset of PD was much earlier and was within the range of human life span (62 years). This effect on the onset of the disease rather than susceptibility urges us to consider the onset of disease as an essential feature in future studies and to not just focus on the presence or absence of the disease in a given study population. If confirmed, our results have immediate implications in gene-mapping studies because they assume that the gene affects disease susceptibility (not age at onset) and is inherited in a Mendelian fashion. The results of our study have also long-term implications in understanding the pathogenesis of PD, its treatment and

prevention, and shall encourage the search for genetic and environmental factors that influence the age at onset of PD.

A major advance in the genetics of PD was the report of linkage between PD and a region on chromosome 4q (Polymeropoulos et al., 1996), and the subsequent identification of a missense mutation in the α -synuclein gene in several rare autosomal dominant kindreds (Polymeropoulos et al., 1997). Our study along with other studies have demonstrated that α -synuclein is not the cause of common forms of PD (Munoz et al., 1997; Bennett and Nicholl, 1998; Chan et al., 1998a; Chan et al., 1998b; Farrer et al., 1998; Group, 1998; Kruger et al., 1998; Vaughan et al., 1998; Zarepari et al., 1998a). In addition, these studies collectively have shown that PD is genetically heterogeneous, which is contrary to the popular belief held by some groups (Golbe et al., 1990; Duvoisin and Golbe, 1995).

α -synuclein was originally discovered as a component of the amyloid plaques in the brains of patients with AD; interestingly it is the second most abundant component after A β (Ueda et al., 1993). The function of α -synuclein is still unknown, and a recent study has demonstrated the presence of α -synuclein in Lewy bodies (Spillantini et al., 1997). They showed that Lewy bodies in substantia nigra from patients with PD stained with antibodies to regions of α -synuclein. They also showed that α -synuclein was present in substantia nigra of patient with dementia with Lewy bodies (Spillantini et al., 1997). Further research is needed to elucidate the mechanism by which α -synuclein mutations cause PD, and the role of α -synuclein in formation of Lewy bodies and pathogenesis of PD in patients without α -synuclein mutations.

AD, PD and Related Disorders

There is considerable clinical and neuropathological overlap between PD and several other neurodegenerative diseases such as AD and frontotemporal dementia with parkinsonism. The clinical and neuropathological overlap between PD and other neurodegenerative diseases may reflect the existence of a common neurodegenerative pathway and common susceptibility genes. In order to identify the common genetic risk factors associated with several neurodegenerative diseases, we examined the role of known genetic risk factors for AD and related disorders with PD.

PD patients frequently develop dementia (Marder et al., 1995; Tison et al., 1995; Hughes, 1997), and AD patients often exhibit parkinsonian signs (Funkenstein et al., 1993; Merello et al., 1994). Neuropathological studies have shown the frequent occurrence of co-incidental AD pathology in brains of PD patients (Hughes et al., 1993), and an increased frequency of Lewy bodies in brains of AD patients (Hulette et al., 1995). AD is associated with APOE, and a common association of APOE with AD and PD may explain the overlap between AD and PD. Our finding that APOE genotypes modulate age at onset of PD in a manner similar to their effect on onset of AD suggest that APOE may play a general role in neurodegeneration, perhaps through its involvement in neuronal repair (reviewed by Beffert et al., 1998). The etiologies of AD and PD are most likely distinct, each being caused by disease-specific genes or environmental insults that damage specific populations of neurons. The mechanisms of response to neuronal damage, however, are probably genetically determined and may not be disease specific. If so, the efficacy of the response mechanism - e.g., the neuronal repair system and the inflammatory response - may affect one's general susceptibility to neurodegeneration, progression of

disease and age at onset of symptoms. Hence, genes that control the response mechanisms may be associated with more than one neurodegenerative disease, and may be responsible for some of the overlap between PD and AD. Identification of these genes will lead to a better understanding of the neurodegenerative pathway.

Mutations in *tau* cause frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17). The spectrum of clinical manifestations and pathological findings in FTDP-17 overlap with clinical and pathological features of PD and AD. In fact, an OHSU patient with clinical diagnosis of AD was found to have a mutation in *tau*. Thus, we questioned if mutations in *tau* can cause PD. We excluded *tau* as the cause of familial PD in Oregon kindreds. Similar studies are underway at other centers.

The underlying causes of common AD and PD are still unknown. Age at onset of AD ranges from before age 60 to after age 90. The presence of both HLA-A2 and two APOE- ϵ 4 alleles can reduce the onset of AD by about 10 years. The remaining variation in age at onset of AD among individuals may be explained by the existence of additional gene(s) that also influence age at onset of AD which await future identification. Age at onset of PD varies by more than 50 years. Our observation that within each APOE genotype, patients with a family history of PD still had an earlier onset than those without a family history suggests that age at onset of PD maybe influenced by additional genes, which await future identification. As these genes are identified by genetic epidemiological studies, additional studies are needed to decipher their biological mechanism of action.

Having summarized and discussed the implications of my studies, I would like to expand on the three important lessons that I have learned. The first lesson originates from the study on the role of gender in the APOE-associated risk of AD. Our study and subsequent studies which confirmed our finding showed that having one $\epsilon 4$ allele was associated with increased risk of AD in women, but not in men. This finding had a major impact on me as it points out that the biological difference between men and women can lead to a difference in disease mechanism which should be taken into account by studies examining the role of APOE in the pathogenesis of AD. The biological difference between men and women can affect not only development of disease, but also, progression of disease and response to medication.

The second lesson that I have learned is that PD is even more complex than I had previously thought. I gained this appreciation for the genetic complexity of PD from the results of the α - synuclein linkage and mutation analysis, segregation analysis, and the study on the role of CYP2D6 in PD. Although α - synuclein mutations are responsible for PD in several autosomal dominant PD kindreds, exclusion of α - synuclein by linkage analysis in at least 4 of our familial PD kindreds with autosomal dominant inheritance suggest the existence of other PD genes. Genetic heterogeneity in PD became more apparent as linkage to a susceptibility locus on chromosome 2 in several other autosomal dominant PD kindreds was reported. Linkage analysis of the chromosome 2 candidate region in our PD kindreds revealed evidence consistent with linkage in a few families, and evidence against linkage in two families. Therefore, familial PD which itself is a rare disease subtype, can be caused not only by α - synuclein and the susceptibility locus on chromosome 2, but also by at least one other as yet unidentified gene. Our segregation

analysis of PD highlighted additional complexity of PD due to the observed non-Mendelian transmission of a familial factor in our data set, which can result from any of the following possibilities. One, the genetic component of PD may include the involvement of several major genes with different modes of inheritance in clinically indistinguishable patients and families. Two, PD may have a polygenic inheritance involving several interacting loci each with a modest effect. Three, the complexity in PD may reflect gene-environment interaction which are difficult to assess. Four, the genetic component of PD may include the involvement of mitochondria, whose heteroplasmic inheritance complicates the correlation of phenotype with the presence or absence of mtDNA mutations.

The most important lesson that I have learned from the HLA study on the onset of AD, the APOE study on the onset of AD, and the segregation analysis of PD is the novel concept that genes can affect age at onset of a disease without affecting necessarily susceptibility to the disease. We found that although the frequency of HLA-A2 allele was not increased in AD patients compared to controls, age at onset of AD was shifted to an earlier onset by the presence of the A2 allele. Similarly, we found that although the frequency of APOE alleles were not altered in PD patients, those with $\epsilon 4$ had an earlier onset, those with $\epsilon 3$ had an intermediate onset, and those with $\epsilon 2$ had a later onset. Our segregation analysis of PD suggested that everyone would develop PD if they lived long enough, but the estimated mean onset (120 years) was beyond the human life span. However in those who carried the familial factor(s), the age at onset of PD was much earlier and was within the range of human life span (62 years). I was greatly surprised that the results consistently indicated that it was more likely that the familial factor(s)

influences age at onset of PD rather than susceptibility to PD. However looking at all of our results, it is clear to me that genes may explain why some people develop PD in their 30's while others develop PD in their 80's. I believe that this novel concept that genes can influence age at onset without influencing susceptibility is an important one to keep in mind, and genetic epidemiologists should develop new methodology for analyzing and dissecting the effects of genes on the onset of a disease and not rely solely on traditional linkage and association methodology. If we had relied on linkage and association methodology we would have missed both the effect of HLA-A2 on the onset of AD and the effect of APOE on the onset of PD. In addition, by identifying the genes that influence age at onset of AD or PD and understanding their role in development of disease, we may be able to develop potential strategies to delay the onset of these disorders. This line of research would be extremely beneficial to our society, given the growing number of elderly in our population.

This thesis project has provided me with an incredible learning opportunity. Having been involved in multiple facets of disease from gender to genotype gave me the opportunity to continuously synthesize my results in the big picture of disease. I am sure that I will apply the lessons that I have learned during this thesis project to the studies of other complex disorders in my future work.

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