REVIEW ARTICLE

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Alzheimer's Disease and Parkinson's Disease

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THE INCIDENCE OF MANY COMMON DISEASES IS INCREASED AMONG the relatives of affected patients, but the pattern of inheritance rarely follows Mendel's laws. Instead, such common diseases are thought to result from a complex interaction among multiple predisposing genes and other factors, including environmental contributions and chance occurrences. Identifying the genetic contribution to such complex diseases is a major challenge for genomic medicine. However, as so clearly foreseen nearly 350 years ago by the English physiologist William Harvey,[1] finding the genetic basis for rarer, mendelian forms of a disease may illuminate the etiologic process and pathogenesis of the more common, complex forms. This is illustrated in the progress made in understanding Alzheimer's disease and Parkinson's disease through the investigation of the rare, clearly inherited forms of these diseases. The molecular basis of neurodegenerative disorders was reviewed in the *Journal* in 1999.[2]

ALZHEIMER'S DISEASE

The most common neurodegenerative disease, Alzheimer's disease constitutes about two thirds of cases of dementia overall (ranging in various studies from 42 to 81 percent of all dementias), with vascular causes and other neurodegenerative diseases such as Pick's disease and diffuse Lewy-body dementia making up the majority of the remaining cases.[3,4]

Alzheimer's disease is a progressive neurologic disease that results in the irreversible loss of neurons, particularly in the cortex and hippocampus.[5] The clinical hallmarks are progressive impairment in memory, judgment, decision making, orientation to physical surroundings, and language. Diagnosis is based on neurologic examination and the exclusion of other causes of dementia; a definitive diagnosis can be made only at autopsy. The pathological hallmarks are neuronal loss, extracellular senile plaques containing the peptide β amyloid, and neurofibrillary tangles; the latter are composed of a hyperphosphorylated form of the microtubular protein tau.[6] Amyloid in senile plaques is the product of cleavage of a much larger protein, the β-amyloid precursor protein, by a series of proteases, the α-, β-, and γ-secretases.[7] The γ-secretase, in particular, appears to be responsible for generating one particular β-amyloid peptide — $A\beta_{42}$ — that is 42 amino acids in length and has pathogenetic importance, because it can form insoluble toxic fibrils and accumulates in the senile plaques isolated from the brains of patients with Alzheimer's disease.[8,9]

Measures of the prevalence of Alzheimer's disease differ depending on the diagnostic criteria used, the age of the population surveyed, and other factors, including geography and ethnicity.[10,11] Excluding persons with clinically questionable dementia, Alzheimer's disease has a prevalence of approximately 1 percent among those 65 to 69 years of age and increases with age to 40 to 50 percent among persons 95 years of age and over[10] (Fig. 1). Although the mean age at the onset of dementia is approximately 80 years,[3] early-onset disease, defined arbitrarily and variously as the illness occurring before the age of 60 to 65 years, can occur but is rare. In one community-based study in France, the prevalence of early-onset disease (defined by an onset before the age of 61 years) was 41 per 100,000; thus, early-onset cases make up about 6 to 7 percent of all cases of Alzheimer's disease.[12] About 7 percent of early-onset cases are familial, with an autosomal dominant pattern of inheritance and high penetrance.[12] Thus, familial forms of early-onset Alzheimer's disease, inherited in an autosomal dominant manner, are rare; however, their importance extends far beyond their frequency, because they have allowed researchers to identify some of the critical pathogenetic pathways of the disease.

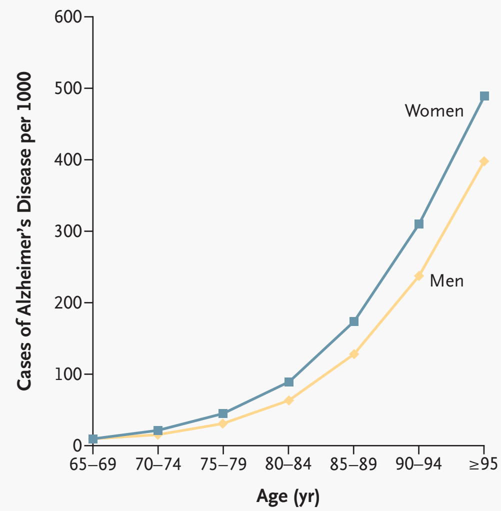

Figure 1. Prevalence of Alzheimer's Disease as a Function of Age in Men and Women.

Missense mutations that alter a single amino acid and therefore gene function have been identified in three genes in families with early-onset autosomal dominant Alzheimer's disease. Family linkage studies and DNA sequencing identified mutations responsible for early-onset autosomal dominant forms of the disease in the gene encoding β-amyloid precursor protein itself on chromosome 21 (Fig. 2), as well as in two genes with similarity to each other, presenilin 1 (*PSEN1*) on chromosome 14 and presenilin 2 (*PSEN2*) on chromosome 1. *PSEN1* mutations are more common than *PSEN2* mutations. In a study of French families, for example, half of patients with familial, early-onset Alzheimer's disease that was inherited as an autosomal dominant trait had mutations in *PSEN1*, whereas approximately 16 percent of families had mutations in the β-amyloid precursor protein (β*APP*) gene itself.[12] *PSEN2* mutations were not found, and the genes responsible for the remaining 30 percent or so of cases were unknown.

The presenilin and β*APP* mutations found in patients with familial early-onset Alzheimer's disease appear to result in the increased production of $A\beta_{42}$, which is probably the primary neurotoxic species involved in the pathogenesis of the disease[7,13] (Fig. 3). In these forms of Alzheimer's disease, mutations in β*APP* itself or in the presenilins can shift the cleavage site to favor the γ-secretase site[14] and, in particular, to favor increased production of the toxic $A\beta_{42}$ peptide over the shorter, less toxic $A\beta_{40}$ peptide. Presenilin 1 may in fact be the γ-secretase itself or a necessary cofactor in γ-secretase activity.[15] The toxic peptide is increased in the serum of patients with various β*APP*, *PSEN1*, and *PSEN2* mutations causing early-onset Alzheimer's disease.[16] Cultured cells transfected in order to express the normal β-amyloid precursor protein generally process approximately 10 percent of the protein into the toxic $A\beta_{42}$ peptide. Expression of various mutant β*APP* or *PSEN1* genes associated with early-onset familial Alzheimer's disease can result in an increase in the production of $A\beta_{42}$ by a factor of up to 10.[17-19] The identification of mutations in β*APP* and the presenilins in early-onset familial Alzheimer's disease not only suggests a common mechanism through which mutations in these genes may exert their deleterious effects (i.e., increased production or decreased clearance of $A\beta_{42}$ and formation of a protein aggregate, the amyloid plaque) but also provides evidence of a direct role of the $A\beta_{42}$ peptide and presenilins in the pathogenesis of the disease.[20] In contrast, mutations in the tau gene, which encodes a protein contained within another neuropathologic structure in Alzheimer's disease, the neurofibrillary tangle, have not been identified in families with hereditary Alzheimer's disease, although they are seen in another, rarer neurodegenerative disorder, frontotemporal degeneration with parkinsonism[21,22] (Fig. 3).

As important as the rare familial early-onset

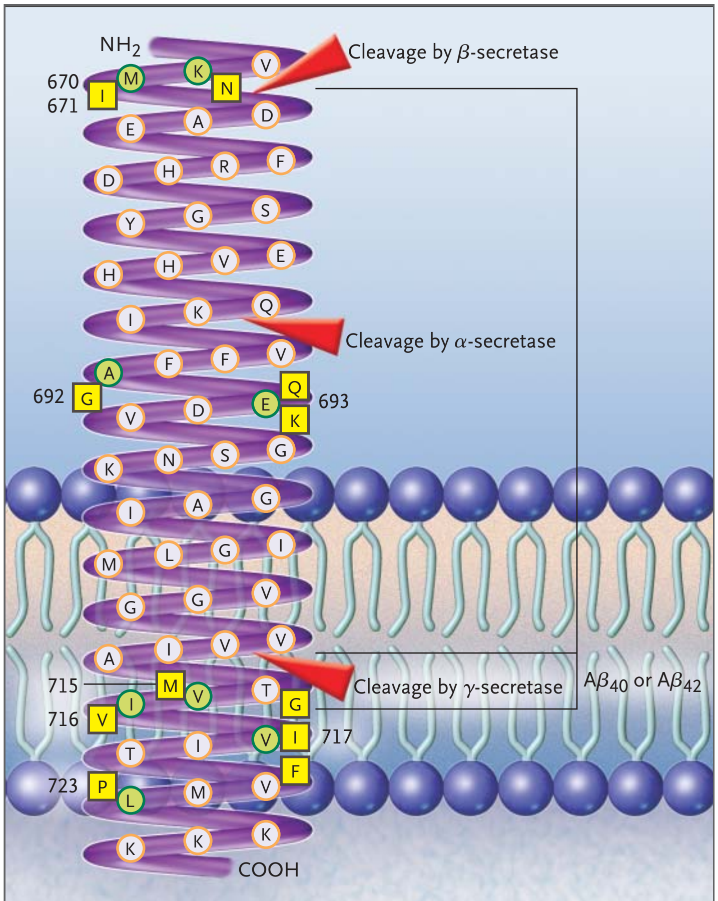

Figure 2. Altered Amino Acid Residues in a Segment of the β-Amyloid Precursor Protein Adjacent to Its Transmembrane Domain Resulting from Missense Mutations and Causing Early-Onset Familial Alzheimer's Disease.

Letters are the single-letter code for amino acids in β-amyloid precursor protein, and numbers show the position of the affected amino acid. The altered amino acid residues are near the sites of β-, α-, and γ-secretase cleavage (red triangles). Normal residues involved in missense mutations are shown as green circles, whereas the amino acid residues representing various missense mutations are shown as yellow boxes. The mutations lead to the accumulation of toxic peptide $A\beta_{42}$ rather than the wild-type $A\beta_{40}$ peptide.

forms of Alzheimer's disease have been for understanding the pathogenesis of the disease, the majority of patients of any age have sporadic (nonfamilial) disease in which no mutation in the *βAPP* or presenilin genes has been identified. However, another genetic risk factor, variants of *APOE*, the gene that encodes apolipoprotein E, a constituent of the low-density lipoprotein particle, has been associated with Alzheimer's disease.[23] Three variants of the gene and the protein are found in human populations and result from changes in single amino acids in apolipoprotein E (referred to as the *APOE* ε2, ε3, and ε4 alleles). Carrying one *APOE* ε4 allele nearly doubles the lifetime risk of Alzheimer's disease (from 15 percent to 29 percent), whereas not carrying an *APOE* ε4 allele cuts the risk by 40 percent.[24] Initially, survival curves analyzing the effect of the *APOE* ε4 allele on the occurrence of Alzheimer's disease suggested that 70 to 90 percent of persons without this allele were disease-free at the age of 80 years, whereas 30 to 60 percent of those with one *APOE* ε4 allele and only 10 percent of homozygous persons surviving to the age of 80 were disease-free.[23] A more recent study also provided evidence that *APOE* ε4 has a role in Alzheimer's disease, but the effect was less marked, with the rate of disease-free survival as high as 70 percent in homozygous persons.[25]

Although the magnitude of the effect of the *APOE* ε4 allele differs among studies, there appears to be a dose effect, in that disease-free survival was lower in homozygous persons than in heterozygous persons. This observation has led to the conclusion that the primary effect of the *APOE* ε4 allele is to shift the age at onset an average of approximately 5 to 10 years earlier in the presence of one allele and 10 to 20 years earlier in the presence of two alleles in persons with an underlying susceptibility to Alzheimer's disease.[26] The molecular mechanisms by which the various *APOE* alleles alter the age at onset and, therefore, the lifetime risk of Alzheimer's disease are unknown. A number of associations of the disease with variants of genes other than *APOE* have also been reported but remain to be confirmed and are the subject of ongoing research.[27]

Because of the relative rarity of *βAPP*, *PSEN1*, and *PSEN2* mutations in patients with late-onset Alzheimer's disease, we believe that molecular testing for mutations in these genes should be restricted to those with an elevated probability of having such mutations — that is, persons with early-onset disease or a family history of the disease. At-risk, symptomatic relatives of persons with documented mutations in *βAPP* or one of the presenilins may also request testing for the purposes of family, financial, or personal planning. Testing of a presymptomatic person should be undertaken with extreme care and only after extensive pretest counseling, so that the person requesting the test is aware of the potential for severe psychological complications of testing positive for an incurable, devastating illness. There may also be serious ramifications in the area of employment and in obtaining life, long-term care, dis-

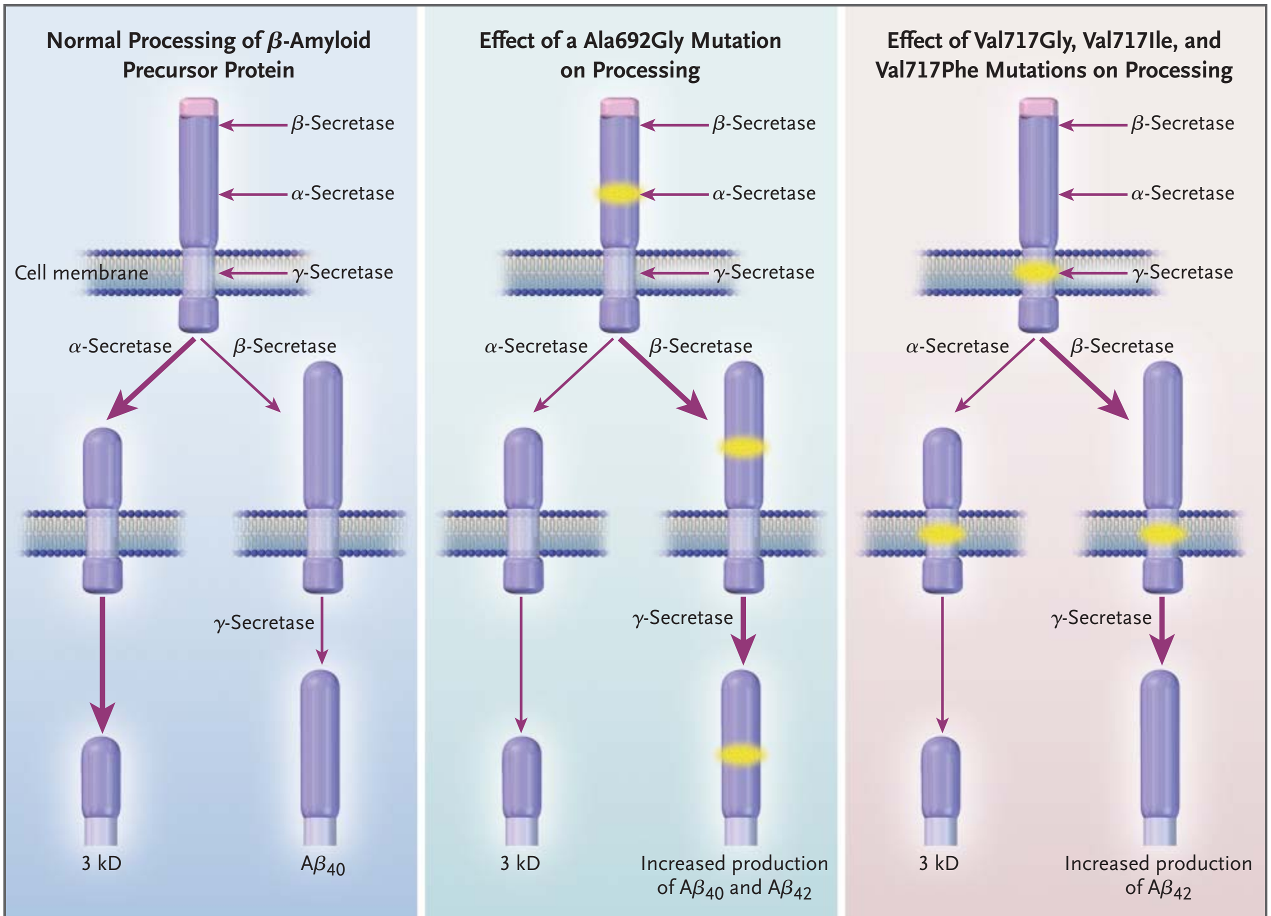

Figure 3. The Normal Processing of β-Amyloid Precursor Protein as Well as the Effect on Processing of Alterations in the Protein Resulting from Missense Mutations Associated with Early-Onset Familial Alzheimer's Disease.

These mutations (indicated by the yellow burst symbols) either interfere with α-secretase or enhance β- or γ-secretase cleavage, resulting in an increase in the production of the toxic $A\beta_{42}$ peptide rather than the wild-type $A\beta_{40}$ peptide. Drugs with selectivity for certain secretases might reduce or eliminate the processing of β-amyloid precursor protein to the toxic $A\beta_{42}$ peptide and therefore help prevent Alzheimer's disease or slow its progression. The thickness of the arrows represents the amount of each peptide being made relative to the other peptides.

ability, or health insurance. Also important is that a positive test may indicate that other family members, who may not have participated in any counseling or consented to testing, will be identified as being at a substantially increased risk for early-onset Alzheimer's disease by virtue of their relationship to the person who tests positive.

The usefulness of testing for the *APOE* ϵ4 allele is also limited. Finding one or two *APOE* ϵ4 alleles in a symptomatic person with dementia certainly increases the likelihood that one is dealing with Alzheimer's disease and might be used as an adjunct to clinical diagnosis.[28] On the other hand, since 50 percent of patients with autopsy-proved Alzheimer's disease did not carry an *APOE* ϵ4 allele, its negative predictive value in a symptomatic person is very limited.[24] *APOE* ϵ4 testing in asymptomatic persons has very poor positive and negative predictive values and should not be used.[24]

Insights derived from the identification of mutations in rare families with early-onset Alzheimer's disease are proving useful for identifying therapeutic targets and creating animal models for evaluating therapies.[29] For example, β-secretase inhibitors have been developed and may prove useful in treating Alzheimer's disease by reducing $A\beta_{42}$ production.[30] Transgenic mice expressing mutant β-amyloid precursor protein have an age-dependent increase in the amount of Aβ_{42} formation, increased plaque formation, and spatial memory deficits; they have, however, only a minimal loss of neurons.[31] In addition, mice transgenic for both a *βAPP* and a *PSEN1* mutation show accelerated deposition of Aβ_{42}, as compared with mice expressing either transgene alone.[32] In transgenic mice with a mutant β-amyloid precursor protein, immunization with Aβ_{42} resulted in a decrease in plaque formation and an amelioration of memory loss.[32-34] However, phase 2 clinical trials investigating immunization therapy with Aβ_{42}[35] had to be suspended because of an increased risk of aseptic meningoencephalitis.[35-37] In addition, other drugs such as statins, clioquinol, and certain nonsteroidal antiinflammatory drugs[38] are being evaluated in mouse models of these rare, heritable forms of Alzheimer's disease.

PARKINSON'S DISEASE

Parkinson's disease is the second most common neurodegenerative disorder, after Alzheimer's disease. It is characterized clinically by parkinsonism (resting tremor, bradykinesia, rigidity, and postural instability)[39] and pathologically by the loss of neurons in the substantia nigra and elsewhere in association with the presence of ubiquinated protein deposits in the cytoplasm of neurons (Lewy bodies)[40,41] and thread-like proteinaceous inclusions within neurites (Lewy neurites). Parkinson's disease has a prevalence of approximately 0.5 to 1 percent among persons 65 to 69 years of age, rising to 1 to 3 percent among persons 80 years of age and older.[42] The diagnosis is made clinically, although other disorders with prominent symptoms and signs of parkinsonism, such as postencephalitic, drug-induced, and arteriosclerotic parkinsonism, may be confused with Parkinson's disease until the diagnosis is confirmed at autopsy.[43]

A genetic component in Parkinson's disease was long thought to be unlikely, because most patients had sporadic disease and initial studies of twins showed equally low rates of concordance in monozygotic and dizygotic twins.[44] The view that genetics was involved in some forms of Parkinson's disease was strengthened, however, by the observation that monozygotic twins with an onset of disease before the age of 50 years do have a very high rate of concordance — much higher than that of dizygotic twins with early-onset disease.[44,45] Furthermore, regardless of the age at onset, the apparent rate of concordance among monozygotic twins can be significantly increased if abnormal striatial dopaminergic uptake in the asymptomatic twin of a discordant pair, as revealed by positron-emission tomography with fluorodopa F18, is used as a sign of presymptomatic Parkinson's disease.[46,47] An increased risk of Parkinson's disease was also seen among the first-degree relatives of patients,[48,49] particularly when the results of positron-emission tomography of asymptomatic relatives were taken into account,[50] providing further evidence of the existence of a genetic component to the disease.

However, the real advance occurred when a small number of families with early-onset, Lewy-body–positive autosomal dominant Parkinson's disease were identified.[51] Investigation of these families, of Mediterranean and German origin, led to the identification of two missense mutations (Ala53Thr and Ala30Pro) in the gene encoding α-synuclein, a small presynaptic protein of unknown function.[52,53] Although mutations in α-synuclein have proved to be extremely rare in patients with Parkinson's disease, they did provide the first clue that this protein could be involved in the molecular chain of events leading to the disease. The importance of α-synuclein was greatly enhanced by the discovery that the Lewy bodies and Lewy neurites found in Parkinson's disease in general contain aggregates of α-synuclein[54,55] (Fig. 4). Molecules of α-synuclein protein are prone to form into oligomers in vitro; proteins carrying the missense mutations Ala53Thr and Ala30Pro seem to be even more prone to do so.[56]

Although the study of families with early-onset Parkinson's disease proves that abnormal α-synuclein can cause the disease, it is still unclear whether fibrils of aggregated α-synuclein, as seen in Lewy bodies and Lewy neurites, have a critical causative role in the more common forms of Parkinson's disease or are simply a marker for the underlying pathogenetic process. Lewy bodies positive for α-synuclein are found not only in various subnuclei of the substantia nigra, the locus ceruleus, and other brain-stem and thalamic nuclei of patients with Parkinson's disease, but also in a more diffuse distribution, including the cortex in some patients with Parkinson's disease as well as in patients with dementia of the diffuse Lewy-body type.[57,58] Aggregated α-synuclein in glia is also a feature of multiple-system atrophy,[59] leading to the coining of a new nosologic term, "synucleinopathy," to refer to the class of neurodegenerative diseases associated with aggregated α-synuclein.[60]

Autosomal recessive juvenile parkinsonism is

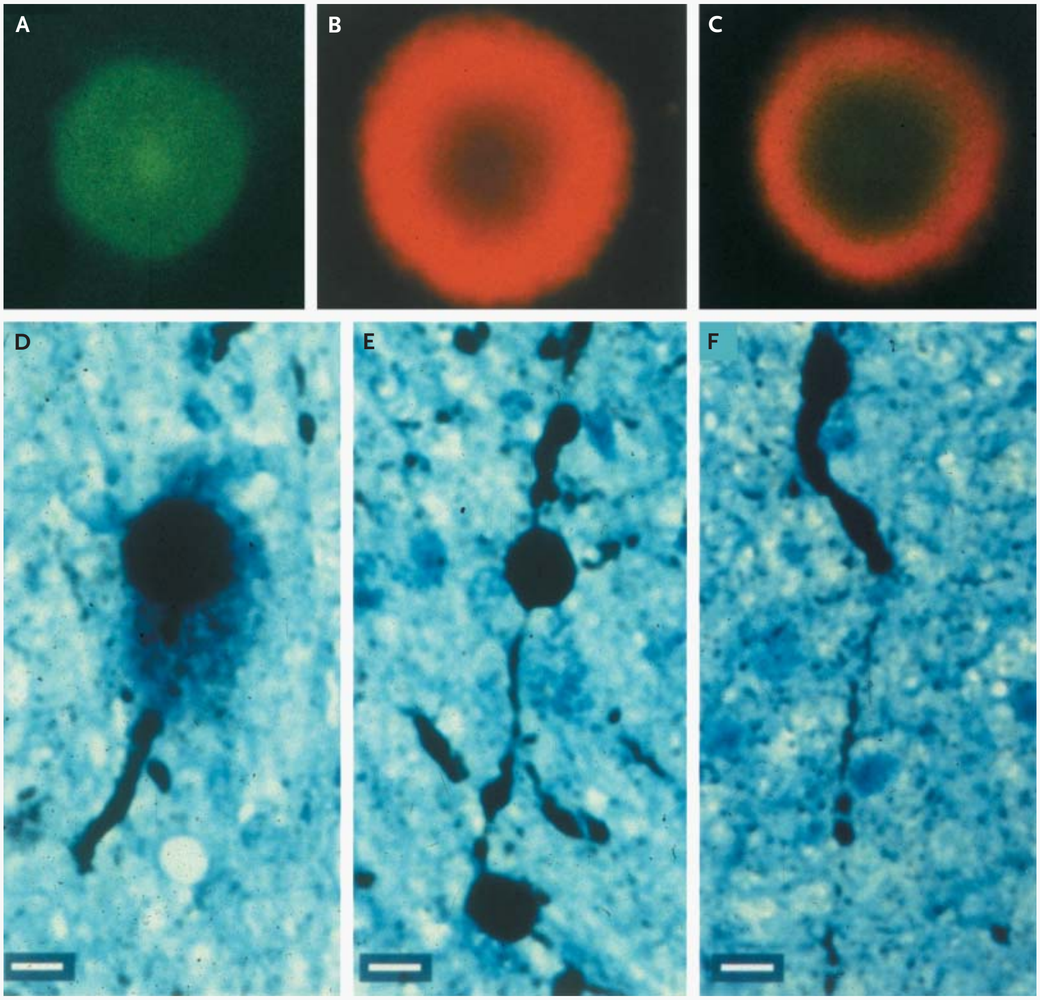

Figure 4. Immunohistochemical Analysis of Sections from the Substantia Nigra of a Patient with Sporadic Parkinson's Disease, Indicating the Involvement of α-Synuclein in the Formation of Lewy Bodies and Lewy Neurites.

Panel A shows a Lewy body stained with antibody against ubiquitin (green) (×3000). Panel B shows the same Lewy body stained with antibody against α-synuclein (red) (×3000). Panel C, which merges the images shown in Panels A and B, shows that Lewy bodies contain a central core of ubiquinated proteins and α-synuclein surrounded by a rim of α-synuclein–positive fibrillar material (×3000). Panels D, E, and F show neuronal processes from the substantia nigra of a patient with sporadic Parkinson's disease in which neurites are ballooned and dilated and stain for α-synuclein (black stain). Scale bars in Panels D, E, and F indicate 10 μm. (Adapted from Mezey et al.[55])

another genetic neurologic syndrome that has provided important insights into Parkinson's disease. Autosomal recessive juvenile parkinsonism is a relatively rare syndrome that shares many of the features of parkinsonism, including responsiveness to levodopa and loss of nigrostriatal and locus ceruleus neurons, but it has a very early onset (before the age of 40 years), a slow clinical course extending over many decades, and no Lewy bodies or Lewy neurites at autopsy.[61] Genetic mapping of the syndrome to 6q25–27 led to the identification of mutations responsible for autosomal recessive juvenile parkinsonism in a gene encoding a protein termed parkin.[62] Parkin is expressed primarily in the nervous system and is one member of a family of proteins known as E3 ubiquitin ligases, which attach short ubiquitin peptide chains to proteins, a process termed ubiquination, thereby tagging them

Table 1. Mutations in Single Genes That Lead to Parkinson's Disease.

Locus	Gene	Location	Mode of Inheritance	Where Found
PARK1	α-Synuclein	4q21	Autosomal dominant	Greece, Italy, and Germany
PARK2	Parkin	6q25–27	Autosomal recessive; may also be autosomal dominant	Ubiquitous
PARK3	Unknown	2p13	Autosomal dominant	Germany
PARK4	Unknown	4p15	Autosomal dominant	United States
PARK5	Ubiquitin C-terminal hydrolase	4p14	May be autosomal dominant	Germany
PARK6	Unknown	1p35	Autosomal recessive	Italy
PARK7	DJ-1	1p36	Autosomal recessive	Netherlands
PARK8	Unknown	12p11.2–q13.1	Autosomal dominant	Japan

for degradation through the proteosomal degradation pathway.

Autosomal recessive juvenile parkinsonism results from a loss of function of both copies of the parkin gene,[63-65] leading to autosomal recessive inheritance, as opposed to the missense mutations that alter α-synuclein and cause a dominantly inherited disorder. More recently, however, the spectrum of disease known to be caused by parkin mutations has broadened, with apparently sporadic Parkinson's disease occurring in adulthood, as late as in the fifth and sixth decades of life, in association with parkin gene mutations.[66] There have even been a few patients with apparently classic sporadic Parkinson's disease with an onset in adulthood who appear to have only one mutant parkin allele, although an exhaustive demonstration that the other allele is truly normal and not harboring an unusual mutation outside the coding sequence and its immediate environs is still lacking. Precisely what role parkin mutations have in the majority of cases of Parkinson's disease and whether the heterozygous state (which is far more common in the population than is homozygosity for two mutant alleles) represents an important risk factor remain to be established.

Recent evidence suggests that ubiquination by parkin may be important in the normal turnover of α-synuclein.[67] Also of interest is the finding in one family of a few members with Parkinson's disease who had a deleterious missense mutation in the gene encoding a neuron-specific C-terminal ubiquitin hydrolase, another gene involved in ubiquitin metabolism.[68] The obvious inference from these disparate pieces of data is that aggregation of abnormal proteins, dysfunctional ubiquitin-mediated degradation machinery, or both may be important steps in the pathogenesis of Parkinson's disease.

In addition to the α-synuclein, parkin, and ubiquitin C-hydrolase genes, at least five other loci have been proposed for autosomal dominant[69-71] and autosomal recessive[72-74] Parkinson's disease (Table 1). Genetic analysis of the more common, sporadic forms of Parkinson's disease suggests that there is a component of heritability in the forms that are not clearly inherited as autosomal dominant or recessive traits.[75-78] For example, certain alleles at a complex DNA-repeat polymorphic locus approximately 10 kilobase pairs upstream of the α-synuclein gene have been shown to be associated with sporadic Parkinson's disease in some populations, but not in others.[79-82] Positive identification of the genes at these loci is likely to provide additional genes and proteins that can be studied for their roles in the pathogenesis of the disease.

Because of the extreme rarity of α-synuclein mutations, genetic testing for these mutations should be performed only on a research basis when a strong family history of autosomal dominant Parkinson's disease is present. Homozygous parkin mutations are found in the nearly half of patients presenting with apparent Parkinson's disease in childhood and adolescence and perhaps 5 percent of young adults with Parkinson's disease.[64] There is little evidence supporting a role for mutations in the parkin gene in typical late-onset Parkinson's disease, and neither α-synuclein nor parkin gene testing is currently available as a routine clinical service.

CONCLUSIONS

The common neurodegenerative diseases are predominantly idiopathic disorders of unknown pathogenesis. As the examples of Alzheimer's disease and Parkinson's disease demonstrate, however, the genetic mapping and gene-isolation tools created by the Human Genome Project over the past decade have greatly accelerated the rate of identification of genes involved in the rare inherited forms of these diseases and are now being used to determine the genetic contributions to the more common, multifactorial forms of these diseases. The emergence of a consensus hypothesis — aggregates of $A\beta_{42}$ and α-synuclein are neurotoxic in Alzheimer's disease and Parkinson's disease, respectively — may explain the pathogenesis not only of the inherited forms of these diseases but also of the idiopathic variety. Such insights into causation and pathogenesis are helping to identify new treatment targets for these debilitating disorders.

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REFERENCES

1. Garrod A. The lessons of rare maladies. Lancet 1928;1:1055-60.
2. Martin JB. Molecular basis of the neurodegenerative disorders. N Engl J Med 1999; 340:1970-80. [Erratum, N Engl J Med 1999; 341:1407.]
3. Helmer C, Joly P, Letenneur L, Commenges D, Dartigues JF. Mortality with dementia: results from a French prospective community-based cohort. Am J Epidemiol 2001;154:642-8.
4. Aronson MK, Ooi WL, Geva DL, Masur D, Blau A, Frishman W. Dementia: age-dependent incidence, prevalence, and mortality in the old old. Arch Intern Med 1991; 151:989-92.
5. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939-44.
6. Clark CM, Ewbank D, Lee VM-Y, Trojanowski JQ. Molecular pathology of Alzheimer's disease: neuronal cytoskeletal abnormalities. In: Growdon JH, Rossor MN, eds. The dementias. Vol. 19 of Blue books of practical neurology. Boston: Butterworth–Heinemann, 1998:285-304.
7. Hutton M, Perez-Tur J, Hardy J. Genetics of Alzheimer's disease. Essays Biochem 1998;33:117-31.
8. Iwatsubo T, Odaka A, Suzuki N, Mizusawa H, Nukina N, Ihara Y. Visualization of A beta 42(43) and A beta 40 in senile plaques with end-specific A beta monoclonals: evidence that an initially deposited species is A beta 42(43). Neuron 1994;13:45-53.
9. Esler WP, Wolfe MS. A portrait of Alzheimer secretases — new features and familiar faces. Science 2001;293:1449-54.
10. Hy LX, Keller DM. Prevalence of AD among whites: a summary by levels of severity. Neurology 2000;55:198-204.
11. Hendrie HC, Ogunniyi A, Hall KS, et al. Incidence of dementia and Alzheimer disease in 2 communities: Yoruba residing in Ibadan, Nigeria, and African Americans residing in Indianapolis, Indiana. JAMA 2001;285:739-47.
12. Campion D, Dumanchin C, Hannequin D, et al. Early-onset autosomal dominant Alzheimer disease: prevalence, genetic heterogeneity, and mutation spectrum. Am J Hum Genet 1999;65:664-70.
13. Steiner H, Haass C. Intramembrane proteolysis by presenilins. Nat Rev Mol Cell Biol 2000;1:217-24.
14. Borchelt DR, Thinakaran G, Eckman CB, et al. Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta 1-42/1-40 ratio in vitro and in vivo. Neuron 1996;17: 1005-13.
15. Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ. Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature 1999;398:513-7.
16. Scheuner D, Eckman C, Jensen M, et al. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 1996;2: 864-70.
17. Murayama O, Tomita T, Nihonmatsu N, et al. Enhancement of amyloid beta 42 secretion by 28 different presenilin 1 mutations of familial Alzheimer's disease. Neurosci Lett 1999;265:61-3.
18. Mehta ND, Refolo LM, Eckman C, et al. Increased Abeta42(43) from cell lines expressing presenilin 1 mutations. Ann Neurol 1998; 43:256-8.
19. Eckman CB, Mehta ND, Crook R, et al. A new pathogenic mutation in the APP gene (I716V) increases the relative proportion of A beta 42(43). Hum Mol Genet 1997;6: 2087-9.
20. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002;297:353-6. [Erratum, Science 2002; 297:2209.]
21. Lynch T, Sano M, Marder KS, et al. Clinical characteristics of a family with chromosome 17-linked disinhibition-dementia-parkinsonism-amyotrophy complex. Neurology 1994;44:1878-84.
22. Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998;393:702-5.
23. Strittmatter WJ, Roses AD. Apolipoprotein E and Alzheimer's disease. Annu Rev Neurosci 1996;19:53-77.
24. Seshadri S, Drachman DA, Lippa CF. Apolipoprotein E epsilon 4 allele and the lifetime risk of Alzheimer's disease: what physicians know, and what they should know. Arch Neurol 1995;52:1074-9.
25. Meyer MR, Tschanz JT, Norton MC, et al. APOE genotype predicts when — not whether — one is predisposed to develop Alzheimer disease. Nat Genet 1998;19:321-2.
26. Farrer LA, Cupples LA, Haines JL, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease: a meta-analysis. JAMA 1997;278:1349-56.
27. Myers AJ, Goate AM. The genetics of late-onset Alzheimer's disease. Curr Opin Neurol 2001;14:433-40.
28. Saunders AM, Hulette O, Welsh-Bohmer KA, et al. Specificity, sensitivity, and predictive value of apolipoprotein-E genotyping for sporadic Alzheimer's disease. Lancet 1996;348:90-3.
29. Chapman PF, Falinska AM, Knevett SG, Ramsay MF. Genes, models and Alzheimer's disease. Trends Genet 2001;17:254-61.
30. Citron M. Beta-secretase as a target for the treatment of Alzheimer's disease. J Neurosci Res 2002;70:373-9.
31. Moechars D, Lorent K, De Strooper B, Dewachter I, Van Leuven F. Expression in brain of amyloid precursor protein mutated in the α-secretase site causes disturbed behavior, neuronal degeneration and premature death in transgenic mice. EMBO J 1996;15: 1265-74.
32. Holcomb L, Gordon MN, McGowan E, et al. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nat Med 1998;4:97-100.
33. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 1999;400:173-7.
34. Morgan D, Diamond DM, Gottschall PE, et al. A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. Nature 2000;408:982-5. [Erratum, Nature 2001;412:660.]
35. Helmuth L. Alzheimer's congress: further progress on a beta-amyloid vaccine. Science 2000;289:375.
36. Check E. Nerve inflammation halts trial for Alzheimer's drug. Nature 2002;415:462.
37. Birmingham K, Frantz S. Set back to Alzheimer vaccine studies. Nat Med 2002;8: 199-200.
38. De Strooper B, Konig G. An inflammatory drug prospect. Nature 2001;414:159-60.
39. Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967;17:427-42.
40. Pollanen MS, Dickson DW, Bergeron C. Pathology and biology of the Lewy body. J Neuropathol Exp Neurol 1993;52:183-91.
41. Kuzuhara S, Mori H, Izumiyama N, Yoshimura M, Ihara Y. Lewy bodies are ubiquitinated: a light and electron microscopic immunocytochemical study. Acta Neuropathol (Berl) 1988;75:345-53.

42. Tanner CM, Goldman SM. Epidemiology of Parkinson's disease. Neurol Clin 1996;14:317-35.

43. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992;55:181-4.

44. Tanner CM, Ottman R, Goldman SM, et al. Parkinson disease in twins: an etiologic study. JAMA 1999;281:341-6.

45. Duvoisin RC, Johnson WG. Hereditary Lewy-body parkinsonism and evidence for a genetic etiology of Parkinson's disease. Brain Pathol 1992;2:309-20.

46. Burn DJ, Mark MH, Playford ED, et al. Parkinson's disease in twins studied with 18F-dopa and positron emission tomography. Neurology 1992;42:1894-900.

47. Morrish PK, Rakshi JS, Bailey DL, Sawle GV, Brooks DJ. Measuring the rate of progression and estimating the preclinical period of Parkinson's disease with [18F]dopa PET. J Neurol Neurosurg Psychiatry 1998; 64:314-9.

48. Marder K, Tang M-X, Mejia H, et al. Risk of Parkinson's disease among first-degree relatives: a community-based study. Neurology 1996;47:155-60.

49. Payami H, Larsen K, Bernard S, Nutt J. Increased risk of Parkinson's disease in parents and siblings of patients. Ann Neurol 1994;36:659-61.

50. Piccini P, Morrish PK, Turjanski N, et al. Dopaminergic function in familial Parkinson's disease: a clinical and 18F-dopa positron emission tomography study. Ann Neurol 1997;41:222-9.

51. Duvoisin RC. Recent advances in the genetics of Parkinson's disease. Adv Neurol 1996;69:33-40.

52. Polymeropoulos MH, Lavedan C, Leroy E, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997;276:2045-7.

53. Kruger R, Kuhn W, Muller T, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 1998;18:106-8.

54. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. α-Synuclein in Lewy bodies. Nature 1997;388: 839-40.

55. Mezey E, Dehejia AM, Harta G, et al. Alpha synuclein is present in Lewy bodies in sporadic Parkinson's disease. Mol Psychiatry 1998;3:493-9. [Erratum, Mol Psychiatry 1999;4:197.]

56. Conway KA, Lee SJ, Rochet JC, Ding TT, Williamson RE, Lansbury PT Jr. Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. Proc Natl Acad Sci U S A 2000;97: 571-6.

57. Louis ED, Fahn S. Pathologically diagnosed diffuse Lewy body disease and Parkinson's disease: do the parkinsonian features differ? Adv Neurol 1996;69:311-4.

58. Kosaka K, Iseki E. Dementia with Lewy bodies. Curr Opin Neurol 1996;9:271-5.

59. Spillantini MG, Crowther RA, Jakes R, Cairns NJ, Lantos PL, Goedert M. Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. Neurosci Lett 1998;251:205-8.

60. Galvin JE, Lee VM, Trojanowski JQ. Synucleinopathies: clinical and pathological implications. Arch Neurol 2001;58:186-90.

61. Matsumine H, Saito M, Shimoda-Matsubayashi S, et al. Localization of a gene for an autosomal recessive form of juvenile parkinsonism to chromosome 6q25.2-27. Am J Hum Genet 1997;60:588-96.

62. Kitada T, Asakawa S, Hattori N, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 1998;392:605-8.

63. Abbas N, Lucking CB, Ricard S, et al. A wide variety of mutations in the parkin gene are responsible for autosomal recessive parkinsonism in Europe. Hum Mol Genet 1999;8:567-74.

64. Lucking CB, Abbas N, Durr A, et al. Homozygous deletions in parkin gene in European and North African families with autosomal recessive juvenile parkinsonism. Lancet 1998;352:1355-6.

65. Lücking CB, Dürr A, Bonifati V, et al. Association between early-onset Parkinson's disease and mutations in the *parkin* gene. N Engl J Med 2000;342:1560-7.

66. Farrer M, Chan P, Chen R, et al. Lewy bodies and parkinsonism in families with parkin mutations. Ann Neurol 2001;50: 293-300.

67. Shimura H, Schlossmacher MG, Hattori N, et al. Ubiquitination of a new form of alpha-synuclein by parkin from human brain: implications for Parkinson's disease. Science 2001;293:263-9.

68. Leroy E, Boyer R, Auburger G, et al. The ubiquitin pathway in Parkinson's disease. Nature 1998;395:451-2.

69. Farrer M, Gwinn-Hardy K, Muenter M, et al. A chromosome 4p haplotype segregating with Parkinson's disease and postural tremor. Hum Mol Genet 1999;8:81-5.

70. Gasser T, Muller-Myhsok B, Wszolek ZK, et al. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nat Genet 1998;18:262-5.

71. Funayama M, Hasegawa K, Kowa H, Saito M, Tsuji S, Obata F. A new locus for Parkinson's disease (PARK8) maps to chromosome 12p11.2-q13.1. Ann Neurol 2002;51: 296-301.

72. Valente EM, Bentivoglio AR, Dixon PH, et al. Localization of a novel locus for autosomal recessive early-onset parkinsonism, PARK6, on human chromosome 1p35-p36. Am J Hum Genet 2001;68:895-900.

73. van Duijn CM, Dekker MC, Bonifati V, et al. Park7, a novel locus for autosomal recessive early-onset parkinsonism, on chromosome 1p36. Am J Hum Genet 2001;69:629-34.

74. Bonifati V, Rizzu P, van Baren MJ, et al. Mutations in DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 2003;299:256-9.

75. Scott WK, Nance MA, Watts RL, et al. Complete genomic screen in Parkinson disease: evidence for multiple genes. JAMA 2001;286:2239-44.

76. Pankratz N, Nichols WC, Uniacke SK, et al. Genome screen to identify susceptibility genes for Parkinson disease in a sample without *parkin* mutations. Am J Hum Genet 2002;71:124-35.

77. Hicks AA, Petursson H, Jonsson T, et al. A susceptibility gene for late-onset idiopathic Parkinson's disease. Ann Neurol 2001;52:549-55.

78. Sveinbjörnsdóttir S, Hicks AA, Jónsson T, et al. Familial aggregation of Parkinson's disease in Iceland. N Engl J Med 2000;343: 1765-70.

79. Tan EK, Matsuura T, Nagamitsu S, Khajavi M, Jankovic J, Ashizawa T. Polymorphism of NACP-Rep1 in Parkinson's disease: an etiologic link with essential tremor? Neurology 2000;54:1195-8.

80. Kruger R, Vieira-Saecker AM, Kuhn W, et al. Increased susceptibility to sporadic Parkinson's disease by a certain combined alpha-synuclein/apolipoprotein E genotype. Ann Neurol 1999;45:611-7.

81. Farrer M, Maraganore DM, Lockhart P, et al. Alpha-synuclein gene haplotypes are associated with Parkinson's disease. Hum Mol Genet 2001;10:1847-51.

82. Izumi Y, Morino H, Oda M, et al. Genetic studies in Parkinson's disease with an alpha-synuclein/NACP gene polymorphism in Japan. Neurosci Lett 2001;300:125-7.