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Review

Pharmacogenomics of neurodegenerative diseases

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Abstract

Current knowledge of sporadic degenerative disorders suggests that, despite their multifactorial etiopathogenesis, genetics plays a primary role in orchestrating the pathological events, and even dramatically changes the disease phenotype from patient to patient. Genes may act as susceptibility factors, increasing the risk of disease development, or may operate as regulatory factors, modulating the magnitude and severity of pathogenic processes or the response to drug treatment. The goal of pharmacogenomics is the application of this knowledge to elaborate more specific and effective treatments and to tailor therapies to individual patients according to their genetic profile. Here, we outline the leading theories on the etiopathogenesis of neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer disease, and we review the potential role of genetic variations, such as gene mutations and polymorphisms, in each context. We also suggest potential targets for new therapeutic approaches and variability factors for current treatments based on genotype features. Finally, we propose a few options of preventive therapeutic interventions in patients with a high genetic risk of disease.

Keywords: Amyotrophic lateral sclerosis; Parkinson disease; Alzheimer disease; Gene polymorphism; Gene mutation; Pharmacogenomics

1. Introduction

The deciphering of the human genome means the virtual end of an age of human genomics aimed at the localization of genes on the chromosomal material. Already heralded by a few pioneering studies, a new era of post-genomics genotype–phenotype correlations has started. In the future, researchers will attempt to correlate genes with functions, assess polymorphic variations of these activities in the various populations and determine their impact on physiological and pathological conditions. If the number of genes is in the 80.000–100.000 range, the investigation of the role played by each of these genes in human diseases, and the role of reciprocal interactions, is likely to require the work of several generations of researchers.

A particular aspect of this variability is the goal of "*Pharmacogenomics*", i.e. a field of research devoted to understanding how genetic factors can direct the choice of selective therapies and influence the response to established treatments for human diseases. Treatments normally entail the interaction between an exogenously administered stimulus (protein, radiation, etc.) and a number of endogenous proteins involved in the pathogenesis of the disease. A typical interaction occurs between the receptor for a neurotransmitter and an exogenous molecule able to block this interaction. By definition, all the genes coding for these proteins are polymorphic: Mendelian mutations or allelic variations in their sequence could account for the phenotypic variability in the response to present or future therapies. In theory, any gene can affect any given clinical feature of a disease, and the majority of the genes code for still unidentified proteins. As such, it is impossible to predict which gene will be pivotal in determining the response to a given therapy. The only way to sort out these interactions is to assess them under specifically designed experimental conditions.

When applied to neurodegenerative diseases, pharmacogenomics is still in its infancy. In fact, the etiopathogenetic mechanisms underlying most of these illnesses are still poorly understood, which makes the search for the genes whose variability may account for the differential

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response to drugs observed in different individuals particularly difficult. For this reason, in this review we will attempt to summarize current knowledge of the pathogenetic mechanisms of the major neurodegenerative diseases (amyotrophic lateral sclerosis, Parkinson disease and Alzheimer disease), pointing to the most relevant molecules we believe are likely to play a crucial role in the occurrence of the disease. We will review molecules involved in the mechanisms of action of drugs commonly used for disease management along with molecules we predict to be relevant for future therapeutic approaches. We hope our effort will stimulate future attempts to perform the necessary genetic analyses.

2. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is a progressive and fatal disease, where neurodegeneration affects primarily, although not exclusively, motor neurons of the cerebral cortex, brain stem, and spinal cord. The causes of amyotrophic lateral sclerosis are still unknown, but clinical and laboratory data collected to date allow different pathogenic mechanisms for the neuronal damage to be hypothesized, and thus also provide therapeutic targets for drugs.

2.1. Selective vulnerability of motor neurons

Anatomical and molecular findings accumulated so far suggest that motor neurons may be more vulnerable than other neurons to neurodegenerative processes. The large cell body and long axonal process of spinal motor neurons predict that these cells have high energy demands and a high metabolic rate, requiring a high level of mitochondrial activity. The remarkable length of motor neuron fibers needs a robust skeleton made of neurofilaments, and the content of neurofilament proteins is increased in motor neurons compared to other neurons. More recently, two other molecular features have been reported as being typical of motor neurons and further corroborate the concept of their increased susceptibility to excitotoxic damage. The first is the subunit composition of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtype of glutamate receptors in motor neurons. These receptors are responsible for fast excitatory transmission in the central nervous system and are made of four subunits, GluR1–GluR4. The presence of the GluR2 subunit makes the AMPA receptor impermeable to Ca^{2+}, preventing a cascade of potentially toxic events triggered by the intracellular influx of Ca^{2+} ions through glutamate receptor ion channels (Day et al., 1995). In contrast with most neurons in the human central nervous system, motor neurons display low levels of mRNA and protein synthesis for the GluR2 AMPA receptor subunit (Williams et al., 1997). This evidence may imply that motor neurons are more permeable to Ca^{2+} upon AMPA receptor activation, rendering them more vulnerable to glutamate excitotoxicity. The second feature is the lack of the Ca^{2+}-binding proteins calbindin D28k and parvalbumin in those motor neurons that are mostly affected by amyotrophic lateral sclerosis (Alexianu et al., 1994). These proteins buffer intracellular calcium and amplify Ca^{2+} homeostasis, contributing to the protection of neurons against calcium-mediated damage (Lledo et al., 1992).

2.2. Oxidative stress

Ten percent of amyotrophic lateral sclerosis cases are of familial origin and 15–20% of such families show mutations of the Cu^{2+}/Zn^{2+} superoxide dismutase gene (Brown, 1997). Such mutations are dominant and the gene is located on chromosome 21q22. Mutations of the superoxide dismutase gene are also found in rare cases of sporadic amyotrophic lateral sclerosis, accounting for 2% of cases (Brown, 1997). These findings point to a deficit in oxygen radical scavenging as being responsible for motor neuron injury due to excessive oxidative stress. However, the use of transgenic mouse models, expressing different human superoxide dismutase mutated proteins, and superoxide dismutase gene knockout mice shows that a loss of function is not the case (Borchelt et al., 1994; Reaume et al., 1996). On the contrary, several lines of evidence support the hypothesis of a toxic gain of function acquired by the mutant superoxide dismutase. Mutations may induce structural changes of the superoxide dismutase, causing polypeptide unfolding around the active site with abnormal entry of reactive species (i.e., $^{C}OONO$) and subsequent nitration of tyrosine residues (Crow et al., 1997). Alternatively, increased accessibility to the Cu^{2+} active site of the mutant superoxide dismutase may lead to the use of additional substrates such as hydrogen peroxide and peroxynitrite with the production of highly toxic hydroxyl radicals (Wiedau-Pazos et al., 1996). In both cases, excessive reactive radical species may damage functionally important proteins, such as neurofilaments and neurotrophic factors, and membrane lipids, with the release of mitochondrial factors and activation of proapoptotic mechanisms (Li et al., 1997a). The oxidative stress hypothesis may warrant the search for superoxide dismutase gene mutations in patients with amyotrophic lateral sclerosis (regardless of whether it is the sporadic or familial form) before treatment is started. If abnormal superoxide dismutase activity is demonstrated, potential therapeutic approaches may include administration of free radical scavenging drugs or Cu^{2+} chelators (Table 1). Inhibition of the Cu^{2+} chaperone for superoxide dismutase, a specific protein involved in Cu^{2+} acquisition by superoxide dismutase, could also prevent Cu^{2+}-mediated toxicity. Experiments are currently underway to verify this option. Gene therapy represents a potential future perspective for super-

Table 1
Perspectives of pharmacogenomics in amyotrophic lateral sclerosis

Gene	Therapeutic
Genotype variations and corresponding therapies	
Superoxide dismutase 1	Free radical scavengers, Cu^{2+} chelators
Interleukins or interleukin receptors	Anti-inflammatory drugs, interleukin-1 receptor antagonist
Neurotrophic factors and neurotrophics factors receptors	Exogenous or transfected neurotrophic factors (CNTF, BDNF, IGF-1)
Apoptosis-related molecules	Antiapoptotic drugs, caspase inhibitors
Genotype variations and potentially affected therapies	
Glutamate receptors	Riluzole
Glutamate transporters	Riluzole

oxide dismutase-related amyotrophic lateral sclerosis forms, although the simple transfection of the wild-type superoxide dismutase gene may not be sufficient to cure the disease, as inactivation of mutated superoxide dismutase might be needed.

2.3. Altered neurofilament

Accumulation of neurofilaments in the cell body and proximal axons of motor neurons is a common finding in amyotrophic lateral sclerosis (Hirano, 1991). Such neurofilaments are often phosphorylated and may also be found within intracellular inclusions in motor neurons of patients with superoxide dismutase-related familial amyotrophic lateral sclerosis (Manetto et al., 1988; Murayama et al., 1989). Neurofilaments may also represent a favorite target for the mutated superoxide dismutase, as their light subunits are more susceptible to superoxide dismutase-catalyzed nitration than are other proteins of the central nervous system (Crow et al., 1997). Irrespective to the preceding events, the build-up of neurofilament deposits in motor neurons will eventually block axonal transport, leading to axonal degeneration. However, in a minority of amyotrophic lateral sclerosis patients, abnormal neurofilament biochemistry may represent the primary cause of motor neuron injury. Mutations of the neurofilament heavy chain gene are found in rare cases of sporadic amyotrophic lateral sclerosis (Figlewicz et al., 1994; Al-Chalabi et al., 1999). Moreover, transgenic mice expressing increased quantities of neurofilament light or heavy subunits develop motor neuron pathology (Cote et al., 1993; Xu et al., 1993). These findings suggest a role for neurofilaments in motor neuron pathology. Coding sequences of neurofilament subunits also show polymorphisms, but so far none have been demonstrated to be significantly associated with familial or sporadic amyotrophic lateral sclerosis. Thus, the search for new treatments for amyotrophic lateral sclerosis should consider neurofilament biochemistry as a potential primary site of intervention: gene therapy for mutated neurofilament subunit genes and prevention of neurofilament accumulation by inhibiting their synthesis or excessive phosphorylation and oxidation are likely candidates for this approach.

2.4. Excitotoxicity

There is a large body of evidence suggesting that glutamatergic toxicity plays a fundamental role in motor neuron injury in amyotrophic lateral sclerosis. It is a solid concept that motor neurons are highly vulnerable to increased concentrations of glutamate in vitro and in vivo (Rothstein et al., 1993). Human spinal motor neurons show a high density of glutamatergic receptors, which are functionally involved in neurotransmission from cortical motor neurons and inhibitory interneurons. Post-synaptic glutamatergic receptors are of two major classes: ionotropic, which are ligand-gated ion channels, and metabotropic, which are coupled via G proteins to second messenger pathways. The former are subdivided into three groups, according to their selective agonists: NMDA receptors, AMPA receptors, and kainate receptors. The molecular features of different glutamatergic receptors are determined by the specific assembly of receptor subunits, alternative splicing of the receptor subunit gene, and post-transcriptional mRNA editing (Leigh and Meldrum, 1996). Physiological glutamatergic transmission also involves the active re-uptake of glutamate from the synaptic cleft by the glutamate transporter proteins located on glial and neuronal cells. Five human transporters have already been cloned: the glial transporters EAAT-1 and EAAT-2, and the neuronal transporters EAAT-3, EAAT-4, and EAAT-5 (the latter two also function as glutamate-gated chloride channels) (Arriza et al., 1994, 1997; Fairman et al., 1995). Regardless of the mechanism involved, excessive stimulation of glutamatergic receptors leads to an abnormal intracellular influx of ions, including Ca^{2+}, and generates a series of biochemical reactions which may result in cell death (Leigh and Meldrum, 1996). However, glutamate may also be neurotoxic at physiological concentrations when neuronal metabolism is already compromised (Novelli et al., 1988). A decrease in intracellular energy levels weakens the voltage-dependent Mg^{2+} block of the NMDA receptor channel, leading to over-activation of

NMDA receptors even by normal levels of glutamate. Moreover, motor neurons in amyotrophic lateral sclerosis patients may be more susceptible to glutamate excitoxicity than the corresponding neurons in healthy individuals. The Glu2R subunit of the AMPA receptor, which regulates Ca^{2+} entry within the cell, is underexpressed in the ventral gray matter of amyotrophic lateral sclerosis spinal cord due to deficient mRNA editing (Takuma et al., 1999). Enhanced Ca^{2+} influx would result in an increased neuronal vulnerability to glutamate.

The transport of glutamate is impaired in the motor cortex and spinal cord from amyotrophic lateral sclerosis patients. This defect originates from a decreased expression of the glial glutamate transporter EAAT-2 (Rothstein et al., 1995). The loss of EAAT-2 is not related to genomic mutations, and selective EAAT 2 gene polymorphisms are not associated with an increased risk for amyotrophic lateral sclerosis (Aoki et al., 1998; Jackson et al., 1999). Yet, abnormal EAAT 2 mRNA species have been detected in 65% of patients with sporadic amyotrophic lateral sclerosis and these mRNAs show rapid degradation, with a net reduction in the synthesis of functional protein (Lin et al., 1998). Alternatively, EAAT-2 deficiency could derive from defective translational or post-translational mechanisms, secondary to oxidative damage induced by peroxynitrite, superoxide anions, and hydroxyl radicals (Trotti et al., 1996).

At this time, it is not clear whether glutamate excitotoxicity is the primary cause of motor neuron loss in sporadic amyotrophic lateral sclerosis, but it is very likely that glutamate excitotoxicity represents a common pathway to motor neuron damage for most, if not all, pathogenic mechanisms involved in this disease. This concept is supported by the therapeutic effects of riluzole in patients with sporadic amyotrophic lateral sclerosis (Bensimon et al., 1994; Lacomblez et al., 1996). Riluzole acts by inhibiting glutamate release and blocking Ca^{2+} entry within the cell upon NMDA receptor activation (Martin et al., 1993; Hubert et al., 1994). However, the overall efficacy of riluzole is mild, and treatment benefits are more evident in amyotrophic lateral sclerosis patients with bulbar onset of disease. The reason for this discrepancy is not clear, but clinical and therapeutic heterogeneity among amyotrophic lateral sclerosis patients may also be accounted for by minor genetic diversity. If this is the case, then genotype studies of glutamatergic receptors or glutamate transporters may prove more helpful in predicting the clinical phenotype of amyotrophic lateral sclerosis or the response to riluzole than in providing clues to the pathogenesis of the disease (Table 1).

In the next few years, research will probably discover new and more efficacious glutamate receptor antagonists or release inhibitors, and we might witness the deployment of gene therapy approaches to reduce motor neuron vulnerability to glutamatergic excitoxicity in amyotrophic lateral sclerosis patients. The GluR2 subunit of the AMPA glutamate receptor and/or the calcium-binding proteins calbindin D28k and parvalbumin seem to be reasonable targets: increasing the expression of such genes may improve the survival of residual motor neurons in amyotrophic lateral sclerosis.

2.5. Altered Ca^{2+} homeostasis and dysimmunity

Recent studies indicate that calcium levels are increased in the motor nerve terminals of amyotrophic lateral sclerosis patients (Siklos et al., 1996). Although a rise in intracellular Ca^{2+} may follow different types of neuronal injury, a leading cause of increased Ca^{2+} levels seems to be the presence of circulating autoantibodies. These immunoglobulins bind to voltage-gated Ca^{2+} channels and promote Ca^{2+} influx into motor neurons in experimental animals (Engelhardt et al., 1995). The latter effect may induce dysfunction of neurotransmitter release at the level of motor nerve terminals and, in some cases, axonal degeneration and denervation (Uchitel et al., 1992; O'Shaughnessy et al., 1998). In addition, abnormal Ca^{2+} entry into motor neurons may be aggravated by their constitutive lack of Ca^{2+}-binding proteins (i.e., calbinding-D28k and parvalbumin). Increased intracellular Ca^{2+} may trigger a cascade of events, leading to free radical synthesis, glutamate release, and, ultimately, to further Ca^{2+} influx (Dykens, 1994; Carriedo et al., 1998). The detection of potentially pathogenic autoantibodies represents strong evidence supporting the autoimmune hypothesis for amyotrophic lateral sclerosis. However, despite a series of reports showing that immune infiltrates and IgG are present in amyotrophic lateral sclerosis spinal cord (Engelhardt and Appel, 1990; Engelhardt et al., 1993), all attempts to slow the course of the disease by immunosuppressive treatments have failed (Brown et al., 1986; Drachman et al., 1994; Appel et al., 1988). At this time, immunomodulatory drugs cannot be recommended for monotherapy in amyotrophic lateral sclerosis, but they deserve to be tested further in the context of polytherapy for amyotrophic lateral sclerosis patients with autoantibodies to Ca^{2+} channels. New therapeutic options in this field may also originate from the study of cytokines and their polymorphisms at a genomic level. As already shown for other neurodegenerative diseases (Grimaldi et al., 2000), the search for an increased frequency of gene allele polymorphisms for specific cytokines or cytokine-receptors could provide more information on the role that immune responses play in amyotrophic lateral sclerosis and, perhaps, prompt the scrutiny of more selective anti-inflammatory treatments (Table 1).

2.6. Neurotrophic factors

Neurotrophic factors such as brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), and insulin-like growth factor-1 (IGF-1) enhance motor neuron

survival in vitro and can also exert beneficial effects in mouse models of amyotrophic lateral sclerosis (reviewed in Yuen and Mobley, 1996). Despite the lack of evidence for a deficient production of neurotrophic factors in amyotrophic lateral sclerosis, there could still be a rationale for using them as therapeutics. In fact, reduced neurotrophic support can be the result of the existence of inactive forms of the factors or of a relative unresponsiveness of their receptors. Based on these premises, a few clinical trails have been conducted to test the therapeutic value of neurotrophic factors in amyotrophic lateral sclerosis patients, but so far the results have been unrewarding (Lai et al., 1997; Borasio et al., 1997). The apparent therapeutic failure of neurotrophic factors in amyotrophic lateral sclerosis may also be secondary to malfunction of receptors or signal-transduction mechanisms or to inadequate delivery of the drugs to the central nervous system. New trials are underway in which the intrathecal route is used to deliver adequate amounts of neurotrophic factors beyond the blood–brain barrier.

The study of gene allele polymorphisms for neurotrophic factors is still in an early stage. A mutation of the CNTF gene occurs in 2–3% of the human population, leading to a defective CTNF protein without apparent neurological dysfunctions (Giess et al., 1998). However, the mutation of the CNTF gene may be deleterious when associated with mutations of other relevant genes, such as leukemia inhibitory factor (LIF) (Sendtner et al., 1996). LIF is another neurotrophic factor for motor neurons and a mutation of the LIF gene has been detected in a small minority of amyotrophic lateral sclerosis patients (Giess et al., 2000). Future studies will have to elucidate whether gene mutations for one or more neurotrophic factors in combination with other genetic or epistatic alterations are of primary importance in the pathogenesis of amyotrophic lateral sclerosis and require substitutive therapy (Table 1).

2.7. Apoptosis

Regardless of the initial pathogenic mechanisms, a number of data obtained with spinal cord samples from amyotrophic lateral sclerosis patients suggest that the final mechanism leading to motor neuron death is apoptosis. These findings include evidence of DNA fragmentation, a biochemical marker of apoptosis, the altered expression of mRNA for Bcl-2 and Bax (an anti-apoptotic and a proapoptotic gene, respectively), and the demonstration of caspase-1 and caspase-3 activation, which are specific intracellular proteases responsible for the execution of the cell death program (Yoshiyama et al., 1994; Martin, 1999; Li et al., 2000). The pivotal role of apoptosis in motor neuron death is also supported by data collected from experimental amyotrophic lateral sclerosis models. In spinal cord of human mutant superoxide dismutase transgenic mice, the expression of Bax and Bad (proapoptotic) genes is increased, whereas that of Bcl-2 and Bcl-xL (anti-apoptotic) is decreased (Vukosavic et al., 1999). In double transgenic mice expressing human mutant superoxide dismutase and human Bcl-2, the overexpression of Bcl-2 is associated with a significant delay in disease onset (Kostic et al., 1997). Furthermore, the administration of the caspase inhibitor *z*-Val-Ala-Asp-fluoromethylketone (zVAD-fmk) to mutant superoxide dismutase transgenic mice reduces disease progression and increases survival (Li et al., 2000). Hence, the use of caspase inhibitors may represent a potential treatment for amyotrophic lateral sclerosis and other neurodegenerative disorders in which apoptosis is thought to be the mechanism of neuronal death. The search for gene allele polymorphisms for caspases in amyotrophic lateral sclerosis patients could also provide important information and may help focus treatment on a specific member of the caspase family. Interestingly, caspase-1 (formerly called interleukin-1β converting enzyme) is responsible for the cleavage of mature interleukin-1β from its precursor and interleukin-1β levels are increased in amyotrophic lateral sclerosis spinal cord (Li et al., 2000). Therefore, gene polymorphisms of interleukin-1β-related molecules deserve careful study in amyotrophic lateral sclerosis since a new therapeutic scenario may become evident, as has already occurred in Alzheimer's disease (Table 1).

3. Parkinson's disease

Parkinson's disease is a common neurodegenerative disease whose clinical picture includes slowness of movements, rigidity, tremor, and loss of postural reflexes. The neuropathological hallmarks are the degeneration of neuromelanin-containing neurons located in the brain stem, particularly those in the pars compacta of the substantia nigra and the presence within the surviving neurons of eosinophilic inclusions known as Lewy bodies (Hornykiewicz and Kish, 1987). These inclusions appear concentric with a dense core surrounded by a filamentous halo and contain neurofilament proteins such as tubulin and ubiquitin (Forno, 1996). Neurons of the substantia nigra use dopamine as neurotransmitter and send their projections to the striatum, a nuclear group of the basal ganglia (Wooten, 1997). The clinical symptoms usually do not become evident before a loss of at least 80% of striatal dopamine has occurred (Jankovic and Marsden, 1988). The cause of sporadic Parkinson's disease is unknown, but epidemiological, neuropathological, and laboratory studies point to a mutifactorial etiology, involving a genetic predisposition, environmental toxins, and aging. However, unlike other neurodegenerative diseases, Parkinson's disease is characterized by the unique feature of having an effective pharmacological treatment. Therefore, the pharmacogenomics of this disorder must focus on the influence of the genetic background not only on the pathogenic

mechanisms but also on the clinical response to drug therapy.

3.1. Genetic hypothesis

In the last few years, epidemiological data on sporadic Parkinson's disease have demonstrated a significant familial aggregation, especially for cases with an early onset of disease (Elbaz et al., 1999). Studies of multiplex families have shown an increased risk of Parkinson's disease in siblings compared to controls (Lazzarini et al., 1994; Payami et al., 1994). Further support for a critical role of genetic factors in sporadic Parkinson's disease derives from positron emission tomography (PET) studies of monozygotic and dizygotic twins. A reduction of fluoro-DOPA uptake at the level of the striatum occurs more frequently in asymptomatic monozygotic twins of Parkinson's disease patients than in asymptomatic dizygotic twins (Piccini et al., 1999).

The genetics of Parkinson's disease was radically changed by the recognition of true hereditary forms of the disease. The first gene identified as being responsible for an autosomic dominant form of Parkinson's disease is that encoding for α-synuclein, located on chromosome 4q21 (Polymeropoulos et al., 1997). α-Synuclein is a monomeric protein of 140 amino acids, normally soluble and unfolded, which preferentially locates within the synaptic endings of central nervous system neurons. Its function is largely unknown, but it may be involved in neuronal plasticity (Clayton and George, 1999). Recent studies, though, established a primary role for α-synuclein in the formation of Lewy bodies, and its aggregation in insoluble amyloid fibrils seems to precede the accumulation of ubiquitin and neurofilaments (Goedert et al., 1998). Mutant forms of α-synuclein, such as those linked to autosomal dominant forms of Parkinson's disease, exhibit accelerated self-aggregation into fibrils, probably fostering Lewy body development (Conway et al., 1998). However, it is not known what triggers α-synuclein aggregation in sporadic Parkinson's disease.

Studies focused on the α-synuclein gene in sporadic Parkinson's disease have revealed that allele polymorphism of the promoter sequence (NAC-Rep1) is significantly associated with an increased risk of disease development, especially in combination with the apolipoprotein ε (Apoε) allele 4 (Kruger et al., 1999). These findings suggest that future drugs able to slow down α-synuclein aggregation may find an elective use in selected Parkinson's disease populations.

A second gene responsible for an autosomal recessive form of Parkinson's disease is located on chromosome 6q25.2-27. This gene is made of 12 exons and codes for a 465-amino acid protein, termed Parkin, whose function is still unknown (Kitada et al., 1998). Structural alterations of the Parkin gene result in exon deletions, early truncation of transcripts, or amino acid substitutions, with severe functional impairment of the protein (Abbas et al., 1999). Parkinsonisms due to Parkin gene mutations are associated with degeneration of the substantia nigra, but not with Lewy bodies, perhaps implying a pathogenic process different from that of sporadic Parkinson's disease (Mori et al., 1998). The role of Parkin in sporadic Parkinson's disease remains to be discovered, but allele polymorphisms in different exons of the Parkin gene may confer either an increased or a decreased risk of developing the disease (Satoh and Kuroda, 1999; Wang et al., 1999). Thus, Parkin isoforms could contribute to individual susceptibility to sporadic Parkinson's disease.

Other genes have been identified as being responsible for other autosomal dominant forms of Parkinson's disease. In a small German pedigree, a point mutation in exon 4 of the ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1) gene has been detected on chromosome 4, indicating that abnormal proteolytic mechanisms may favor aggregation and ubiquitination of proteins like α-synuclein (Leroy et al., 1998). In contrast, a common polymorphic variant of the same gene has been associated with a lower risk of sporadic Parkinson's disease (Maraganore et al., 1999). Two other loci located on chromosome 2p13 and 4p are linked with autosomal dominant Parkinson's disease, but the corresponding genes have not been sequenced yet (Riess et al., 2000). In both types of Parkinson's disease, patients have Lewy bodies and show a good response to L-DOPA.

In summary, the hereditary forms identified so far account only for a small minority of the entire Parkinson's disease population, but their study has been quite valuable to the understanding of the pathogenic mechanisms of the disease.

3.2. Oxidative stress

Dopamine molecules can generate free radicals and hydrogen peroxidase by auto-oxidation and through normal enzymatic processing by monoamino oxidase-B. Free radical-mediated cellular damage is commonly prevented by the scavenging activity of superoxide dismutase (which converts superoxide ions to hydrogen peroxide) and glutathione peroxidase (which converts hydrogen peroxide to water and oxidated glutathione disulfide). In addition, the presence of transition metals (e.g., iron, copper) may contribute to the generation of superoxide ions ($\bullet O_2^-$) and hydroxyl radicals (•OH) by oxidative reaction with O_2. Autopsy studies indicate that dopaminergic neurons in Parkinson's disease may be more susceptible to oxidative stress due to their reduced glutathione (GSH) levels and excessive free iron content (Sian et al., 1994; Olanow and Youdim, 1996).

Allele polymorphisms at the level of introns 2 and 13 of the monoamino oxidase-B gene have been identified, but studies performed to detect an association with Parkinson's

disease in small cohorts of Caucasian patients have not provided unequivocal results (Costa et al., 1997; Plante-Bordeneuve et al., 1997; Checkoway et al., 1998; Mellick et al., 1999). A recent meta-analysis, based on the available data on monoamino oxidase-B gene polymorphisms in Parkinson's disease, found a barely significant association only for the (GT)n dinucleotide repeat polymorphism on intron 2 (Tan et al., 2000). Larger population studies are needed to define whether specific monoamino oxidase-B gene polymorphisms increases the risk of Parkinson's disease development and, therefore, support an intervention with monoamino oxidase-B inhibitors (e.g., selegiline, lazabemide, rasagiline) even in preclinical stages (Table 2).

3.3. Mitochondrial dysfunction

The notion of a possible mitochondrial dysfunction in Parkinson's disease initially came from the study of the mechanism of action of methylphenyltetrahydropyridine (MTPT). MTPT is a contaminant of street drugs, which is responsible for a parkinsonian syndrome after accidental intoxication (Langston, 1987). MTPT is converted by monoamino oxidase-B into the methyl phenylpyridinium ion (MPP^+), which is actively taken up by dopaminergic neurons and concentrated in the cytoplasm, synaptosomal vesicles, or mitochondria. MPP^+ inhibits mitochondrial complex I reductase, resulting in a decrease in ATP synthesis and damage of dopaminergic neurons due to energy failure (Przedborski and Jackson-Lewis, 1998). Analysis of the respiratory chain in the brains of patients with idiopathic Parkinson's disease revealed a deficit of complex I activity confined to the substantia nigra (Shapira, 1994). Similar findings were also obtained for platelets and striated muscle tissue, but a deficiency of complex I activity appears specific for Parkinson's disease and not for other parkinsonian syndromes.

The cause of mitochondrial dysfunction and its role in the pathogenesis of Parkinson's disease remain elusive, but a deficient activity of complex I may simply be a secondary event, due to the toxicity of accumulated exogenous or endogenous compounds. Nonetheless, it has been hypothesized that mutations of mitochondrial DNA may contribute to the development of Parkinson's disease, and in some familial cases a matrilinear transmission of complex I dysfunction could be documented, as occurs in true hereditary mitochondrial diseases (Swerdlow et al., 1998). In addition, hybrid cell lines made to express mitochondrial DNA from Parkinson's disease patients are deficient in complex I activity, implying that the enzymatic defect may be secondary to a mutated mitochondrial genome (Swerdlow et al., 1996).

Accordingly, mitochondrial DNA has been repeatedly analyzed in parkinsonian patients, but results are mostly inconsistent. No clear variation in the mitochondrial genome was found to be associated with Parkinson's disease, and the majority of DNA deletions, mutations or polymorphisms detected in these patients were also common in aging controls (Reichmann and Janetzky, 2000). A meta-analysis reviewing the studies of mitochondrial DNA polymorphisms claimed there to be a significant association between A4336G at the $tRNA^{Glu}$ gene and Parkinson's disease (Tan et al., 2000), but a recent study on this topic argues against such evidence (Simon et al., 2000). Overall, it is unlikely that sequence changes in mitochondrial DNA are the primary cause of sporadic Parkinson's disease. However, heteroplasmic mutations of mitochondrial DNA may occur at low mutational burdens (i.e., less than 60%) and may be missed by sequencing analysis. Hence, multiple, randomly positioned mutations may not reach the necessary mutational burden to be detected but could still impair mitochondrial function by a cumulative effect. Such mutations could also be acquired rather than inherited (e.g., by oxidative damage to mitochondrial DNA), and

Table 2
Perspectives of pharmacogenomics in Parkinson's disease

Gene	Therapeutic
Genotype variations and corresponding therapies	
Moamino oxydase-B	Monoamino oxydase inhibitors (selegiline, rasagiline, lazabemide)
Glutamate receptors or transporters	Remacemide
Neurotrophic factors or neurotrophic factor receptors	Exogenous or transfected neurotrophic factors (GDNF)
Interleukins or interleukin receptors	Anti-inflammatory drugs, interleukin-1 receptor antagonist
Apoptosis-related molecules	Antiapoptotic drugs, caspase inhibitors
N-acetyltransferase	Exogenous neurotoxic avoidance
Glutathione transferase	Pollutant avoidance, exogenous glutathione
Genotype variations and potentially affected therapies	
Dopamine receptor D2	L-DOPA (dyskinesias)
Dopamine receptors	L-DOPA, dopamine receptor agonists

their detrimental activity on mitochondrial physiology may develop over time, as the cumulative mutational burden reaches the pathological threshold. Finally, more sensitive techniques, which enable the detection of DNA mutations at a low mutational burden, are required to determine the importance of mitochondrial genome mutations in Parkinson's disease.

3.4. Excitotoxic damage

The glutamatergic excitatory inputs going from the neocortex and subthalamic nucleus to the substantia nigra are hyperfunctional in Parkinson's disease (Bladini et al., 1996). Abnormal glutamatergic transmission is likely to contribute to the parkinsonian syndrome, as suggested by the favorable effects of glutamate antagonists (Chase et al., 2000). Although direct evidence of excitotoxicity is not available yet, excessive glutamate release may still be responsible for neuronal damage in Parkinson's disease. A mild elevation of glutamate levels would increase ion pump activity and, therefore, ATP expenditure in dopaminergic cells with a mitochondrial dysfunction due to a defective complex I. The concomitant rise in intracellular Ca^{2+} levels would activate nitric oxide synthase with the subsequent generation of toxic free radicals, such as peroxynitrites. This potential mechanism of damage to nigral neurons has already prompted a trial with remacemide, a glutamate-receptor antagonist, in Parkinson's disease (Parkinson Study Group, 2000). If this therapeutic approach proves to be beneficial, study of the glutamatergic receptor and glutamate transporter genes may provide useful information as to whether such therapy should be routinely used in Parkinson's disease or reserved for selected groups of patients (Table 2).

3.5. Altered dopamine metabolism

Dopaminergic transmission works as a complex set of sequential events, including the synthesis and the release of neurotransmitter, its binding to specific receptors, its reuptake by presynaptic terminals, and its breakdown by specific enzymes. The possibility that a dysfunction of some of these steps may predispose to Parkinson's disease prompted a series of investigations on the genes involved in the metabolism of dopamine, in a search for DNA variations able to affect dopaminergic transmission. The genes under scrutiny included those for tyrosine hydroxylase, dopamine transporter (DAT), dopamine receptor D2, D3, D4, and D5, monoamino oxidases A and B, and catechol-*O*-methyltransferase. A significant association between Parkinson's disease and specific polymorphisms of DAT, dopamine receptor D2 and D4, monoamino oxidase-A, monoamino oxidase-B, and catechol-*O*-methyltransferase genes was demonstrated in some studies, but not in others (see Tan et al., 2000 for review). However, interpretation of the available data in this field is very difficult because studies focusing on the same gene differ in methodological and technical aspects (e.g., examining different polymorphisms for the same candidate gene, sampling populations of different ethnicity, choosing different polymerase chain reaction primers, etc.). A critical meta-analysis of the literature on this topic revealed that the (GT)n dinucleotide repeat polymorphism of the monoamino oxidase-B gene (i.e., alleles greater than or equal to 188 bp) was the only gene polymorphism involved in the metabolism of dopamine to be significantly associated with sporadic Parkinson's disease (Tan et al., 2000). Interestingly, a study has recently reported a reduced risk of developing levodopa-induced dyskinesias in patients with Parkinson's disease carrying the 13 or the 14 short tandem repeat polymorphisms of the dopamine receptor D2 gene (Oliveri et al., 1999). If confirmed, this finding can have therapeutic implications (e.g., a reason to delay starting levodopa treatment in patients with Parkinson's disease who do not carry the protective polymorphisms against levodopa-induced dyskinesias). Although definitive evidence is still lacking at this time, association studies are continuously being published in this area and future data may help to define whether variations of the genes related to dopaminergic transmission can affect the risk of developing Parkinson's disease or the response to L-DOPA or dopaminergic receptor agonist therapy (Table 2).

3.6. Altered detoxification of metabolites

The occurrence of parkinsonian syndromes upon exposure to external toxin (e.g., MTPT, manganese, etc.) and the association of sporadic Parkinson's disease with environmental factors, such as agricultural pesticides or industrial pollutants (Spencer and Butterfield, 1995), led to the hypothesis that toxic injuries can contribute to the pathogenesis of Parkinson's disease. Under normal circumstances, the number of nigral neurons progressively declines with age, but exposure to neurotoxic agents could result in a rapid loss of dopaminergic nerve cells, with a premature onset of Parkinson's disease symptoms. In addition, genetic variations in the enzymes able to inactivate environmental toxins may lead to an impaired metabolism of these agents, with a subsequently increased susceptibility to Parkinson's disease.

The genotype of several enzymatic systems involved in the detoxification of exogenous metabolites has been repeatedly analyzed in patients with Parkinson's disease (see Table 3), in an attempt to identify DNA polymorphisms associated with an increased risk of disease. The genes of cytochrome *P*450 (CYP) enzymes and, particularly, its CYP2D6 polymorphism are the most extensively investigated. A few studies showed a significant association of the poor metabolizer genotype of CYP2D6 with an increased risk of Parkinson's disease, but opposite results were also reported and, therefore, the issue remains controversial (McCann et al. 1997; Christensen et al., 1998;

Table 3
Genes involved in detoxification of metabolites studied in Parkinson's disease (Tan et al., 2000)

1. Cytochrome *P*-450 enzymes
2. *N*-acetyltransferase 2
3. Glutathione transferase
4. Human heme oxygenase
5. Paroxonase
6. Alpha-ketoglutarate dehydrogenase complex
7. Manganese superoxide dismutase

Riedl et al., 1998; Sabbagh et al., 1999). In a series of investigations, polymorphisms of the *N*-acetyltransferase gene resulting in a slow acetylator phenotype were found to be significantly associated with Parkinson's disease. This evidence was also confirmed by a comprehensive meta-analysis (Tan et al., 2000), but the relevance of these findings is still elusive as in some studies slow acetylator status was more frequent only in patients with familial or early-onset Parkinson's disease (Bandmann et al., 1997; Agundez et al., 1998).

Glutathione transferase is involved in the detoxification of exogenous toxins and the frequency of deletions of its GSTT1 locus is higher in Parkinson's disease patients. (Tan et al., 2000). In one study, the genotype distribution of GSTP1 (another locus of glutathione transferase) significantly differed between patients and controls that had been exposed to pesticides (Menegon et al., 1998). A few other enzymes involved in the metabolism of xenobiotics were examined for their genotype in Parkinson's disease (see Table 2); however, the populations examined were too small to draw conclusions. Nevertheless, study of the genotype–phenotype interactions of detoxifying enzymes may prove helpful in the future not only to the understanding of the pathogenesis of Parkinson's disease, but also to prevent the development of the disease in selected cohorts living in areas with an elevated environmental risk of pollutants (Table 2).

3.7. Neurotrophic factors

A number of neurotrophic factors show protective effects on dopaminergic neurons in vivo, including glial cell line-derived neurotrophic factor (GDNF), basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor, neurotrophin 3, neurotrophin 4/5, ciliary neurotrophic factor, and transforming growth factor (TGF)-β (Dunnett and Björklund, 1999). Among these, the most promising is the GDNF family of proteins, and clinical trials with intraventricular administration of GDNF are currently underway. A polymorphism in the coding region of the GDNF gene was recently detected, but its possible association with Parkinson's disease was not demonstrated in a small cohort of patients (Wartiovaara et al., 1998). However, the analysis of DNA variations at the level of growth factor or growth factor receptor genes might be potentially rewarding, and further studies on this topic are warranted (Table 2).

3.8. Inflammatory mechanisms

In recent years, data have accumulated indicating that mild inflammation may take place in the brains of patients with Parkinson's disease. Activated microglia and astrocytosis as well as increased amounts of inflammatory cytokines, such as interleukin-1β, interferon-γ, and tumor necrosis factor-α, are detected in the parkinsonian substantia nigra (Marsden and Olanow, 1998; Hirsch, 2000). Furthermore, interleukin-2, interleukin-4, and interleukin-6 levels are elevated in the ventricular cerebrospinal fluid and in the caudate nucleus and putamen of patients with Parkinson's disease (Hirsch, 2000). Some of these cytokines can promote apoptosis and induce the synthesis of nitric oxide, which may exert toxic effects on nerve cells of the substantia nigra through the formation of peroxynitrates or the release of free iron (Hirsch, 2000). Circulating autoimmune antibodies with lethal activity on dopaminergic neurons are also found in patients with Parkinson's disease (Chen et al., 1998). Although inflammation is likely to be a secondary event in Parkinson's disease, it may contribute to the degeneration of dopaminergic neurons, accelerating the progression of Parkinson's disease. To date, there are no studies available on polymorphisms of the cytokine or cytokine receptor genes in Parkinson's disease. This field, however, deserves further attention because findings of a positive association may encourage the use of anti-inflammatory drugs as neuroprotective therapy in selected cases of Parkinson's disease (Table 2).

3.9. Apoptosis

Neuropathological findings support the concept that the death of nigral dopaminergic neurons in Parkinson's disease may also occur through the apoptotic pathway (Mochizuki et al., 1996; Hirsch et al., 1999). The apoptosis-effector molecule, caspase-3, and the anti-apoptotic molecule, Bcl-2, are overexpressed in the basal ganglia of patients with Parkinson's disease, confirming that apoptosis is a relevant mechanism of neural death in this paradigm (Marshall et al., 1997; Hartmann et al., 2000).

What triggers apoptosis in Parkinson's disease is still obscure, but any of the pathogenic mechanisms outlined in the previous paragraphs could start the cascade of events leading to programmed cell death. However, the nuclear translocation of transcription nuclear factor (NF)-kB in the dopaminergic neurons of patients with Parkinson's disease suggests that apoptosis could be induced by oxidative stress (Hunot et al., 1997). Gene polymorphisms of apoptosis-related factors (e.g., Bax and Bcl-2) or apoptosis-effector molecules (e.g., caspase enzymes) have still to be investigated in Parkinson's disease, although they may play a role in modulating the susceptibility of nigral

neurons to set off apoptosis in response to a variety of noxious stimuli. Accordingly, future drugs blocking the release of pro-apoptotic factors or inhibiting the activity of caspase enzymes may turn out to be beneficial in patients with Parkinson's disease and a genetic predisposition to an amplified apoptotic response (Table 2).

4. Alzheimer's disease

Alzheimer's disease is the most common type of dementia in the elderly. The neuropathological hallmarks of Alzheimer disease are large neuron loss along with neuritic plaques and neurofibrillary tangles, preferentially located in limbic and cortical areas of the brain (Yankner, 1996; Selkoe, 1999). Extensive loss of synapses occurs in the same regions of the brain affected by Alzheimer disease, with concomitant alterations of neurotransmitter systems (DeKosky and Scheff, 1990; DeKosky et al., 1992; Francis et al., 1994). Neuritic plaques are spherical, multicellular lesions containing extracellular deposits of β-amyloid protein, which is mostly in a fibrillar form (Selkoe, 1999). Neuritic plaques are surrounded by degenerating axons and dendrites, activated microglia, and reactive astrocytes (Selkoe, 1999). Neurofibrillary tangles are intraneuronal, cytoplasmic bundles of paired, helical filaments. They are composed of hyperphosphorylated, insoluble forms of microtubule-associated protein, *tau*, often conjugated with ubiquitin (Selkoe, 1999).

4.1. Role of the β-amyloid protein

It is a widely accepted concept that deposition of β-amyloid protein is the key event in the pathogenesis of Alzheimer disease. β-Amyloid protein derives from a precursor, named amyloid precursor protein, which in neurons consists of 695-amino acid residues. The amyloid precursor protein is a transmembrane molecule with a long extracellular portion, whose principal function in vivo is unknown. Under physiological conditions, a small amount of amyloid precursor protein undergoes secretory cleavage of a long extracellular portion of the molecule by the intervention of an endoprotease called α-secretase. The released fragment is soluble and shows trophic effects on neurons in culture (Selkoe, 1994). In Alzheimer disease, the processing of amyloid precursor protein is significantly altered. Increased amounts of amyloid precursor protein are cleaved by another endoprotease, named β-secretase, which acts more distally along the extracellular portion than α-secretase. Another cleavage by a third endoprotease, termed γ-secretase, at the level of the putative intramembranous portion leads to the generation of β-amyloid protein molecules of 40 or 42 amino acid residues. β-Amyloid protein$_{42}$ is highly amyloidogeneic and its deposition triggers a cascade of events provoking neuronal death and the formation of neuritic plaques.

4.2. Amyloid genetics

Proof of the importance of genetic factors in the development of Alzheimer disease comes from epidemiological data. A familial history of Alzheimer disease can be found in about 30% of patients, as indicated by the presence of at least one first-degree relative affected by the disease. Siblings of patients have twice the risk of controls of developing Alzheimer disease during their lifetime (Mayeux et al., 1991) and concordance for Alzheimer disease in monozygotic twins is higher than in dyzygotic twins (Breitner et al., 1995). In less than 10% of cases Alzheimer disease is transmitted as a pure autosomal dominant trait with elevated age-dependent penetrance (St George-Hyslop, 2000). About half of the autosomal dominant inherited forms of Alzheimer disease feature an early onset of disease and are accounted for by mutations of the amyloid precursor protein, presenilin-1 (PS-1), or presenilin-2 gene (PS-2) (Rosenberg, 2000).

The gene of amyloid precursor protein maps on chromosome 21q21.2, and at least 7 different mutations are known to be the cause of early-onset dominantly inherited Alzheimer disease. They are all missense mutations located at the level of α-, β-, or γ-secretase cleavage sites, altering the normal proteolysis of the amyloid precursor protein.

The PS-1 gene locates on chromosome 14q24.3 and mutations in this gene are responsible for the majority of cases of autosomal dominantly inherited Alzheimer disease. PS-1 codes for a 467-amino acid protein that is an integral membrane protein with eight transmembrane domains. PS-1 function is not entirely known, but it may be involved in protein and membrane trafficking and in the regulation of intercellular signal transduction (St George-Hyslop, 2000). More than 60 mutations of the PS-1 gene have been associated with early-onset familial Alzheimer disease and almost all of them are missense mutations (Rosenberg, 2000).

The PS-2 gene maps on chromosome 1q31-q42 and codes for a 448-amino acid protein sharing 67% sequence homology with PS-1 protein. Two missense mutations of the PS-2 gene have been identified to date and are associated with rare cases of early-onset familial Alzheimer disease (Rosenberg, 2000). The high degree of sequence homology between PS-1 and PS-2 protein implies similar functions and indirect evidence suggests that they may act as or cooperate with γ-secretase in amyloid precursor protein processing (Selkoe, 1999). There is a wide consensus on the hypothesis that missense mutations of amyloid precursor protein, PS-1, and PS-2 genes may share a common pathogenetic mechanism, finally leading to the accumulation of β-amyloid protein as a byproduct of abnormal amyloid precursor protein metabolism (Selkoe, 1999).

The fundamental role played by presenilin genes in the pathogenesis of early-onset familial Alzheimer disease

prompted investigations on their potential involvement in sporadic Alzheimer disease. Homozygotic polymorphism at the level of intron 8 of the PS-1 gene was first reported to double the risk of late-onset Alzheimer disease (Wragg et al., 1996). A few studies confirmed this finding, but others did not and a meta-analysis concluded that such a PS-1 gene polymorphism is only slightly associated with Alzheimer disease (Yasuda et al., 1999). Other polymorphisms in the 5′ regulatory region of the PS-1 gene have also been detected and are associated with a higher risk of developing early-onset Alzheimer disease, probably mediated by an altered expression of the PS-1 protein (van Duijn et al., 1999). To date, polymorphisms of the PS-2 gene have not been associated with Alzheimer disease.

If further investigations identify specific polymorphisms of presenilin genes that are associated with an increased risk of Alzheimer disease, preventive therapy may become a major issue in asymptomatic individuals carrying these gene variations. Experimental data for transgenic mice which overexpress mutant human amyloid precursor protein and develop Alzheimer-like pathology demonstrated that immunization with β-amyloid protein may prevent neuritic plaque formation and that peripheral administration of anti-β-amyloid protein antibodies reduces neuritic plaque burden (Schenk et al., 1999; Bard et al., 2000). Similar trials in humans are currently underway and, if safe, immunization with β-amyloid protein may become the first-line treatment for asymptomatic subjects with a high risk for Alzheimer disease (Table 4). Combined or alternative treatments against dysfunctioning presenilins may also involve the use of selective γ-secretase inhibitors (Esler et al., 2000) (Table 4). These molecules are under active investigation because they may provide the most direct pathogenetic treatment for patients with Alzheimer disease carrying mutations of the amyloid precursor protein, PS1, or PS2 gene.

4.3. Other genetic hypotheses

At present, the most important genetic information in patients with late-onset Alzheimer disease comes from the analysis of allele polymorphism of the apolipoprotein E (ApoE) gene. The ApoE gene maps on chromosome 19q12-q13 and contains three common coding sequence polymorphisms named $\varepsilon 2$, $\varepsilon 3$, and $\varepsilon 4$ allele. The $\varepsilon 4$ allele has been associated with a higher risk of Alzheimer disease (threefold for heterozygous and eightfold for homozygous individuals) and an earlier onset of disease, whereas the $\varepsilon 2$ allele is associated with a later onset (Rosenberg, 2000). ApoE $\varepsilon 4$ is neither necessary nor sufficient to cause Alzheimer disease. However, the increased risk of developing Alzheimer disease provided by ApoE $\varepsilon 4$ may be due to its higher affinity for β-amyloid protein compared to other alleles and to its propensity to enhance the aggregation or reduce the clearance of β-amyloid protein (Selkoe, 1999). Another single nucleotide polymorphism at position −491 in the 5′-promoter region of ApoE has been reported to be associated with an increased risk of Alzheimer disease (Bullido et al., 1998). The effect of the −491 polymorphism appears to be independent of that of ApoE $\varepsilon 4$ and is associated with a rise in ApoE plasma levels (Laws et al., 1999). Nonetheless, these data need further verification because they have not been confirmed by other investigators (Casadei et al., 1999; St George-Hyslop, 2000).

Several other gene polymorphisms have been associated with an increased risk of Alzheimer disease. Some of them code for proteins, which may participate in β-amyloid

Table 4
Perspectives of pharmacogenomics in Alzheimer's disease

Gene	Therapeutic
Genotype variations and corresponding therapies	
Amyloid protein precursor	Immunization with β-amyloid protein, Anti-β-amyloid protein antibodies β- and γ-secretase inhibitors α-secretase stimulators
Presenilin-1	Immunization with β-amyloid protein, Anti-amyloid β-protein antibodies γ-secretase inhibitors
Interleukin-1α/β	Anti-inflammatory drugs (e.g., ibuprofen, cyclooxygenase-2 inhibitors), interleukin-1 receptor antagonist
α-1-Antichymotrypsin	Anti-inflammatory drugs (e.g., ibuprofen, cyclooxygenase-2 inhibitors), interleukin-1 receptor antagonist
Lipoprotein receptor related protein	Statins
Angiotensin converting enzyme-1	ACE inhibitors
Estrogen receptor alpha	Estrogens
Neurotrophic factors or neurotrophics factor receptors	Exogenous or transfected neurotrophic factors (NGF, BNDF, IGF-1, Neotrofin, AIT-082)
Apoptosis-related genes	Antiapoptotic drugs, caspase inhibitors
Genotype variations and potentially affected therapies	
Muscarinic receptors	Acetylcholinesterase inhibitors
Apolipoprotein E	Acetylcholinesterase inhibitors

protein processing or aggregation in neuritic plaques. α-1-Antichymotrypsin is a protease inhibitor and an acute-phase protein also found in amyloid deposits in Alzheimer disease brains (Abraham et al., 1988). A polymorphism in the region coding for the signal peptide of the α-1-antichymotrypsin gene was originally reported to confer a higher risk of Alzheimer disease (Kamboh et al., 1995). These findings were confirmed in some studies, but not in others. Recent data suggest that specific polymorphism of the α-1-antichymotrypsin gene may rather increase the risk for early-onset Alzheimer disease and that this effect is enhanced by a concomitant polymorphism of the interleukin1β gene. (Licastro et al., 1999, 2000b). α-1-Antichymotrypsin release in the brains of Alzheimer disease patients may be secondary to local inflammatory reactions (Licastro et al., 2000a) and contribute to enhance β-amyloid protein aggregation (Ma et al., 1994) or hamper its degradation (Yamin et al., 1999). Therefore, polymorphism analysis of the α-1-antichymotrypsin gene, especially in conjunction with that of the interleukin-1 gene, may provide further indications for the use of anti-inflammatory drugs or interleukin-1 receptor antagonist in Alzheimer disease (Table 4).

α-2-Macroglobulin, another proteinase inhibitor, is detected in amyloid plaques and interacts with the lipoprotein receptor related protein (LRP), as do a number of other ligands, including β-amyloid protein, amyloid precursor protein, ApoE, and cholesterol (Rosenberg, 2000). α-2-Macroglobulin also binds to β-amyloid protein and such complexes may be cleared through binding to LRP or deposition in amyloid plaques. A selective polymorphism near the 5′ end of the α-2-macroglobulin gene has been associated with an increased risk of Alzheimer disease (Blacker et al., 1998) and this may reflect a genetically-determined defective removal of α-2-macroglobulin/β-amyloid protein complexes.

LRP is a member of the low-density lipoprotein receptor superfamily and is believed to contribute to the clearance of ApoE/β-amyloid protein and α-2-macroglobulin/β-amyloid protein complexes (Hyman et al., 2000). As potential candidate gene in Alzheimer disease, the LRP gene was examined for DNA variations and a tetranucleotide repeat polymorphism in the 5′ region was found to be associated with an increased risk of late-onset Alzheimer disease (Kang et al., 1997). If confirmed, this finding may have therapeutic implications. LRP, in fact, is also a receptor for cholesterol and in vitro studies showed that a reduction in cholesterol levels by lovastatin and methyl-β-cyclodextrine inhibits the production of β-amyloid protein by cultured hippocampal neurons (Simons et al., 1998). Therefore, statins may be considered a potential treatment for patients with Alzheimer disease carrying certain LRP genotypes (Table 4).

Another member of the low-density lipoprotein receptor superfamily, the very low density lipoprotein (VLDL) receptor, functions as a receptor for ApoE-containing lipoproteins and for this reason it has been hypothesized to be a potential risk factor for Alzheimer disease. Genetic investigations demonstrated the association of a triplet repeat polymorphism of the VLDL receptor gene and Alzheimer disease, first in Japanese patients and subsequently also in Caucasians (Okuizumi et al., 1995; Helbecque et al., 1998). These data provide further indirect evidence that abnormal functioning of lipoprotein receptors may worsen the course of the pathological events leading to Alzheimer disease.

Other gene polymorphisms have been reported to add to the risk of developing Alzheimer disease, including cathepsin D (Papassotiropoulos et al., 1999a), angiotensin-converting enzyme (Kehoe et al., 1999), *tau* protein (Lilius et al., 1999), and bleomycin hydrolase (Montoya et al., 1998). However, the results of these association studies are conflicting and further analyses on wider population samples are required before definitive conclusions can be drawn. Nonetheless, mutations or polymorphisms of some of these genes may suggest interesting and unexpected therapeutic approaches for genetically selected candidates (Table 4).

4.4. Neurotrophic factors

The trophic activity of nerve growth factor (NGF) on cholinergic basal forebrain neurons (Scott and Crutcher, 1994), the main population of nerve cells that degenerate in Alzheimer disease, indicated that neurotrophic factors could play a primary role in the pathogenesis of the disease. NGF levels are increased in cortical areas of brains from patients with established Alzheimer disease, whereas the number of high-affinity NGF receptors is decreased in the basal forebrain (Hock et al., 2000). A reduction in the number of high-affinity trkA receptors might mediate the loss of NGF trophic activity through impaired retrograde axonal transport (Mufson et al., 1995). Furthermore, indirect, but compelling, evidence in favor of NGF involvement in the pathogenesis of Alzheimer disease comes from a recent study where transgenic mice expressing a neutralizing anti-NGF recombinant antibody developed an Alzheimer-like pathology, including amyloid plaques and neurofibrillary tangles (Capsoni et al., 2000). Because of the inability of NGF to cross the blood–brain barrier, NGF-mimetic drugs (e.g., Neotrofin or AIT-082) have been engineered and are currently being tested in clinical trials (Emilien et al., 2000) (Table 4).

Other neurotrophic factors are thought to contribute to the pathology of Alzheimer disease. Brain-derived neurotrophic factor levels are decreased in Alzheimer disease hippocampi (Ferrer et al., 1999) and insulin-like growth factor-1 shows protective effects against β-amyloid protein neurotoxicity (Dore et al., 1999). Transforming growth factor-β1 exhibits opposing activities because it protects neurons against β-amyloid protein toxicity (Prehn et al., 1996), but enhances β-amyloid protein deposition in amyloid precursor protein transgenic mice (Wyss-Coray et al.,

1997). So far, DNA variations of neurotrophic factor or neurotrophic factor receptor genes have been only marginally investigated in patients with Alzheimer disease, but more knowledge of such variations may have a tremendous impact on future therapeutic strategies (Table 4). In addition to classic neurotrophic factors, estrogen also displays protective effects on neurons in vitro (Mattson et al., 1997) and reduces β-amyloid protein production (Xu et al., 1998). Its use in post-menopausal women has been associated with a reduced risk of Alzheimer disease (Tang et al., 1996). Moreover, a polymorphism of the estrogen receptor alpha gene is associated with a higher risk of developing late-onset sporadic Alzheimer disease (Brandi et al., 1999). These findings underscore the modulatory role of estrogen in the development of Alzheimer disease and indicate that its post-menopausal use may be of utmost importance in women with a genetic susceptibility to Alzheimer disease (Table 4).

4.5. Inflammatory mechanisms

The presence of activated microglia and the detection of inflammatory cytokines in the context of Alzheimer disease lesions are clues suggesting an important role for inflammation in the pathogenesis of the disease (Huell et al., 1995; Sheng et al., 1998). This notion is further supported by epidemiological data showing a reduced use of anti-inflammatory drugs in patients with Alzheimer disease compared to controls (Stewart et al., 1997). β-Amyloid protein likely triggers an inflammatory reaction in the brain in Alzheimer disease since it induces microglia activation and the production of several inflammatory cytokines and chemokines, including interleukin-1β, with subsequent stimulation of inducible nitric oxide synthase and further oxidative damage to neurons (Berger and Harmon, 1997; Yates et al., 2000; Akama and Van Eldik, 2000). Interestingly, interleukin-1 may contribute to the formation of Alzheimer disease plaques by upregulating the secretion of amyloid precursor protein (Rogers et al., 1999). Therefore, a self-amplifying circuit may occur between β-amyloid protein and cytokines, ultimately fostering the sustained formation of plaques and extending damage to cortical neurons.

Recently, studies on DNA polymorphisms of cytokine genes substantiated the role of these molecules as modulatory factors in the development of Alzheimer disease. A single nucleotide polymorphism in the promoter region of the interleukin-1β gene coupled with an increased production of the cytokine confers a higher risk of Alzheimer disease and favors an earlier onset of clinical symptoms (Nicoll et al., 2000; Grimaldi et al., 2000; Du et al., 2000). In contrast, the C allele of a variable number of tandem repeat polymorphism in the 3' flanking region of the interleukin-6 gene is associated with a delayed onset and a lower risk of Alzheimer disease (Papassotiropoulos et al., 1999b). These data unequivocally demonstrate that inflammation has a great influence on the course of Alzheimer disease. Therapeutic trials involving the use of anti-inflammatory drugs or interleukin-1 antagonists (e.g., interleukin-1 receptor antagonist, inhibitors of interleukin-1 converting enzyme) are now being planned: results are eagerly awaited as they may lead to substantial changes in treatment protocols for Alzheimer disease (Table 4). Furthermore, preventive therapies of this kind may be envisaged in subjects having a high risk of Alzheimer disease (i.e., siblings of Alzheimer disease patients, ApoE $\varepsilon 4$ carriers) and bearing specific polymorphisms of cytokine genes.

4.6. Neurotransmitter and neuropeptide abnormalities

Some neurotransmitter and neuropetide systems are affected in Alzheimer disease. The topographical pattern of these pathological changes generally reflects that of neuronal death and synapse loss. The cholinergic basal forebrain system is severely impaired in Alzheimer disease (Geula, 1998), and the loss of cholinergic enzymes correlates with neuritic plaque burden and cognitive dysfunction (DeKosky et al., 1992). The beneficial effects of acetylcholinesterase inhibitors also provide further clues about the role of defective cholinergic transmission in the development of cognitive and behavioral symptoms in Alzheimer disease (Emilien et al., 2000).

Other neurotransmitter systems involved in Alzheimer disease include serotoninergic neurons of the dorsal raphe nuclei (Yamamoto and Hirano, 1985), noradrenergic neurons of the locus coeruleus (Marcyniuk et al., 1986), and glutamatergic neurons of the cortex (Proctor et al., 1988). Neuropeptides such as corticotrophin-releasing factor and somatostatin are also lost in Alzheimer disease, probably reflecting damage to cortical interneurons (Nemeroff et al., 1991). Despite such a large number of neurochemical abnormalities, there is almost no information available as to whether neurotransmitter-related genes modify the susceptibility to Alzheimer disease and whether neurotransmitter replacement therapy is influenced by genetic factors. To date, only a low transcriptional activity allele of the serotonin transporter gene has been found to confer a higher risk of Alzheimer disease (Li et al., 1997a,b), whereas the ApoE genotype seems to affect the response to acetylcholinesterase inhibitors (Richard et al., 1997). Genotypes of acetylcholine-related enzymes, receptors, and transporters are currently been analyzed to clarify whether acetylcholinesterase inhibitors have a better therapeutic effect in selected cohorts of patients than in the whole Alzheimer disease population (Table 4).

4.7. Apoptosis

There is accumulating evidence that apoptosis contributes to neuronal death in Alzheimer disease (Stadelmann et al., 1999). The pathogenic events triggering apop-

tosis in Alzheimer disease are still obscure, but several data suggest that chronic deposition of β-amyloid protein may be crucial. β-Amyloid protein up-regulates pro-apoptotic molecules, such as Bax, down-regulates anti-apoptotic molecules, such as Bcl-2, and induces caspase enzymes (Paradis et al., 1996; Harada and Sugimoto, 1999; Troy et al., 2000). In turn, caspases may support β-amyloid protein synthesis by altering the normal proteolytic pathway of amyloid precursor protein (Wellington and Hayden, 2000), contributing to the establishment of a self-sustaining cycle which leads to the accumulation and, ultimately, to the aggregation of β-amyloid protein into fibrils. In addition, mutated PS-1 may promote apoptosis independently of β-amyloid protein intervention, by down-regulating neuronal survival factors (Weihl et al., 1999). Therefore, anti-apoptotic therapy based on caspase enzyme inhibitors represents one of the most promising treatments for Alzheimer disease in the near future (Robertson et al., 2000) (Table 4). If well tolerated, caspase inhibitors could also be used as preventive therapy in individuals at high genetic risk of Alzheimer disease.

5. Conclusions

The astonishing progress made by molecular biology and molecular genetics in the last decade has already led to new hypotheses on the etiopathogenesis of neurodegenerative diseases. A few therapeutic trials have already been carried out and many are currently ongoing and are based on current knowledge of the pathological mechanisms underlying neurological disorders such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer disease. In this scenario, the importance of genetics in shaping individual susceptibility and disease phenotype is growing. As result, pharmacogenomics is expected to become more important because of its potential for focusing treatments according to individual genotypes and for selecting candidates who are genetically predictable responders to specific drugs.

In addition, pharmacogenomics will certainly change the current concepts on preventive therapy. Depending on a patient's genetic profile, a personalized therapeutic intervention will probably be offered to individuals with a high risk of disease while they are presymptomatic. We are just at the beginning of a new era of pharmacology where standard therapies will be replaced by individual tailored treatments, which will hopefully halt or substantially slow the progression of devastating illnesses such as most neurodegenerative diseases.

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