CHAPTER 22.

Neurodegenerative diseases

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Summary

Degenerative diseases of the nervous system impose substantial medical and public health burdens on populations throughout the world. Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) are three of the major neurodegenerative diseases. The prevalence and incidence of these diseases rise dramatically with age; thus the number of cases is expected to increase for the foreseeable future as life spans in many countries continue to increase. Causal contributions from genetic and environmental factors are, with some exceptions, poorly understood. Nonetheless, molecular epidemiology approaches have proven valuable for improving disease diagnoses, characterizing disease prognostic factors, identifying high-risk genes for familial neurodegenerative diseases, investigating common genetic variants that may predict susceptibility for the non-familial forms of these diseases, and for quantifying environmental exposures. Incorporation of molecular techniques, including genomics, proteomics, and measurements of environmental toxicant body burdens into epidemiologic research, offer considerable promise for enhancing progress on characterizing pathogenesis mechanisms and identifying specific risk factors, especially for the non-familial forms of these diseases. In this chapter, brief overviews are provided of the epidemiologic features of PD, AD, and ALS, as well as illustrative examples in which molecular epidemiologic approaches have advanced knowledge on underlying disease mechanisms and risk factors that might lead to improved medical management and ultimately disease prevention. The chapter concludes with some recommendations for future molecular epidemiology research.

Introduction

Increasingly, epidemiologic research on neurodegenerative diseases has applied molecular techniques to identify host susceptibility factors to elucidate more clearly

pathogenesis mechanisms, and to characterize exposures to potential environmental risk factors. Advances in molecular genetics and exposure measurement have facilitated expanded use of these techniques. Largely due to the ease and availability of genotyping assays, studies of candidate gene variants have been the most common applications. In this chapter, illustrations of the contributions of molecular epidemiology related primarily to elucidating disease pathogenesis processes and identifying etiologic factors will be presented. The focus will be on Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), as they share some common clinical, pathological and epidemiologic features. Other chronic neurological disorders, such as multiple sclerosis and Huntington's disease, are also significant public health concerns, but will not be discussed in the interest of brevity.

As background, brief descriptions of the clinical and pathological features of the three disorders will be provided, as well as summaries of epidemiologic aspects, including the relative contributions of genetics and the environment. No attempt to provide comprehensive reviews of these topics will be made, as they would be far beyond the scope of this chapter. Examples of various types of molecular epidemiologic approaches applied to investigations of AD, PD and ALS will be presented in the second section of this chapter.

Context and public health significance

Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disease. It also represents the most frequent cause of dementia, accounting for roughly half of all cases. The prevalence of AD is roughly 30% among people 85 years and older. Incidence rates climb steeply from 0.5% per year for ages 65 to 75 to 6–8% per year for ages 85 and up. AD onset is rare before the age of 50, except in cases of familial AD, which comprise roughly 5–10% of cases (1).

The primary clinical manifestation of AD is dementia, which is an accelerated loss of cognitive function beyond that due to normal aging. Alterations in mood and behaviour often accompany the onset of dementia, followed by memory loss, disorientation and aphasia. The hippocampus and cerebral cortex are preferentially and severely affected in AD. Pathologically, senile or neuritic plaques and neurofibrillary tangles (NFTs) are the two characteristic lesions in affected tissues (2). Neuritic plaques in blood vessels and neurons of the hippocampus are primarily composed of amyloid β (Aβ) peptide aggregates. The second pathological hallmark, NFTs, are filamentous bundles comprised of abnormal (hyper-phosphorylated) tau proteins that accumulate in the cytoplasm of affected neurons. Tau protein is normally involved in nutrient transport along neuronal axons. Various lines of evidence indicate that AD develops primarily as a result of an “amyloid cascade” (i.e. an imbalance in the production and clearance of Aβ is the central mechanism) (3). Aggregation of hyperphosphorylated tau proteins leading to tangles may also contribute to this cascade mechanism. Other potentially relevant disease mechanisms include: microvascular damage, leading to diminished blood flow and nutrient deficiency to brain cells; oxidative stress; inflammation; and mitochondrial dysfunction (2).

Family studies have established that genetic factors play a substantial role in AD, especially in younger-onset cases (<65 years). Familial AD has an autosomal dominant inheritance pattern. Three mutations in genes encoding proteins involved in amyloid plaque formation, the amyloid precursor protein (APP), presenilin-1, and presenilin-2 genes, have been identified as causal genes for early-onset AD (4–6). Non-familial AD, typically defined as having an onset at age 65 years or older, accounts for most of cases. Non-familial AD has been associated most consistently with the ε4 allele of the *apolipoprotein* gene (*ApoE-ε4*), which is a very low-density lipoprotein carrier that is required for Aβ deposition (7). Carriers of the *ApoE-ε4* allele have reduced AD ages at onset, with 3-fold and 15-fold risk excesses observed in heterozygotes and homozygotes, respectively (8,9). Numerous other candidate genes have been investigated as AD susceptibility factors, such as sortilin-related receptor-1 gene (10), but no strong or consistent findings have emerged.

Increasing age is a clear risk factor for non-familial AD, and rates are generally higher in women than in men (6,10). Other factors that have been investigated in relation to AD risk include: cardiovascular diseases (largely motivated by the link of lipid metabolism with *ApoE-ε4*) (11), head trauma (12), smoking (13), dietary antioxidants and fats (14), alcohol (15), occupational exposures to solvents (16,17), electromagnetic fields (18), educational status (19), and occupational exposures to pesticides (20,21). Epidemiologic evidence has been mixed thus far, as exemplified by contradictory findings for cigarette smoking (22). It is possible, yet remains to be established conclusively,

whether genetic factors account for the majority of the population attributable risk for AD.

Parkinson's disease

Parkinson's disease (PD), the second most common neurodegenerative disease, is a movement disorder whose cardinal clinical features are rest tremor, rigidity, bradykinesia and postural instability (23). PD is relatively rare before age 50, after which incidence and prevalence rise sharply through the eighth decade of life. Epidemiologic surveys, mainly in western countries, indicate a small (20–30%) excess risk in men. Annual incidence rates of 10–15 per 100 000 have been noted in most surveys worldwide. Prevalence may reach 2% in persons aged 65 years and older (24).

The underlying cause of PD is a loss of dopamine-producing neurons of the mid-brain substantia nigra (SN). PD pathogenesis involves complex interactions among several mechanisms, including abnormal protein aggregation and deficient clearance of aggregates, altered dopamine metabolism, impaired mitochondrial function, oxidative stress, inflammation, necrosis and accelerated apoptosis (25). Intracellular deposits of aggregated α synuclein, ubiquitin, and other proteins (known as Lewy bodies) found in many surviving neuronal populations are considered to be the pathologic characteristic of PD (26,27). Whether Lewy bodies are themselves neurotoxic, or represent the end product of cellular defence mechanisms to sequester toxic abnormal proteins, remains to be determined.

Similar to Alzheimer's disease, epidemiologic differences in early-onset (< 50 years) and late-onset PD have been described. Genetic factors, especially specific causal mutations, appear to be more prominent in early-onset PD, although the distinctions are by no means absolute. Kindred studies of heavily affected families have identified at least five genetic loci for PD (7,28). The initial discoveries were mutations of the gene encoding the α-synuclein protein that have been related to autosomal dominant early-onset PD, typified by rapid disease onset and progression (29). The functional consequences of mutations in these genes are incompletely understood, although abnormal brain protein aggregation and clearance appears to be a common feature. Mutations in the leucine-rich repeat kinase 2 (*LRRK2* or *PARK8* gene), first identified from kindred studies in Japan (30) and subsequently confirmed in Europe (31) and North America (32), have also been associated with typical late-onset PD, and thus may also contribute to risk for non-familial PD (28). Identified mutations in other genes include: parkin (*PARK2*), PTEN-induced putative kinase I (*PINK1* or *PARK6*) and DJ-1 (*DJ-1* or *PARK7*), all of which follow a recessive inheritance mode (7).

Candidate gene studies for late-onset non-familial PD have explored associations with the same genes related to familial PD. In general, the rare causal mutations observed for familial PD have not been associated consistently with non-familial disease. Extensive efforts have also been undertaken to identify common variants of biologically-based candidate genes that may confer PD susceptibility, either independently or in combination with host or environmental factors. These include variants of genes related to the metabolism of dopamine and toxic environmental chemicals, and to presumed PD pathogenesis mechanisms (e.g. oxidative stress). Perhaps not surprisingly, numerous associations have been observed, yet attempts at replication have been largely disappointing. An illustration is the inconsistent pattern of results for the gene encoding the enzyme monoamine oxidase B (MAO-B) that catabolizes dopamine (33–35).

Apart from older age, the most consistent epidemiologic observation has been an inverse relation between cigarette smoking and PD, with smokers having approximately half the rate as never smokers, and strong evidence for an inverse dose–response ("protective") effect with duration and pack-years smoked (36–39). The reduced risk among smokers does not appear to be due to selective survival bias. A biochemical basis may be the lowering of MAO-B enzyme activity in the brain, and consequent reduced dopamine catabolism (40,41). Alternatively, aversion to novelty-seeking behaviour, such as smoking, by persons who ultimately develop PD may explain the relation with smoking. Inverse PD risk associations have also been reported for caffeine (37,42) and non-steroidal anti-inflammatory medications (43,44), although the evidence is less consistent than for smoking. Additionally, family history of PD (45) and history of severe head trauma (46,47) have been related to elevated PD risks.

The discovery in the early 1980s of PD among intravenous drug users who had injected a synthetic heroin contaminated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), prompted great interest in the possibility that there are important etiologic roles of environmental toxicants (48). Induction of PD by MPTP in experimental animals and recognition of the chemical structural similarity of MPTP provided strong impetus for a focus on pesticides (49). Occupational exposures to

pesticides have been associated with elevated risk in some studies (50–54), although consistent associations with specific pesticides have not been identified. Metals, especially manganese, have been implicated as risk factors in several epidemiologic studies (55,56). Epidemiologic findings for PD risk among welders, whose jobs entail chronic exposures to various metal mixtures including manganese, have been inconsistent (57–60). There is only limited evidence supporting associations with solvents and other environmental chemicals (61–63).

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a disease of the motor neurons of the anterior horns of the spinal cord and motor neurons in the cerebral cortex. Similar to AD and PD, there are both familial and non-familial forms of ALS, with the familial ALS accounting for about 10% of cases. The incidence of non-familial ALS is approximately 1–2 cases per 100 000 per year, and appears to be slightly more common in men (64). ALS onset usually occurs in the middle to later years of life, and the incidence rises with increasing age. ALS is generally a rapidly fatal condition within two to three years of onset (65).

Excitotoxicity mediated by glutamate and elevated calcium ion (Ca^{2+}) is considered to be a major pathogenesis mechanism in the neuronal death that occurs in ALS (66). As a consequence of neuronal cell death, neuronal muscle atrophy occurs, resulting in diminished muscle strength and bulk, fasciculations, and hyperreflexia. Effects on respiratory muscles can lead to pulmonary infection, and eventually amyotrophy leads to paralysis and death. The histopathology of ALS is characterized by intracytoplasmic inclusion bodies composed of neurofilaments and spheroids containing ubiquitinated copper and zinc superoxide dismutase (CuZnSOD) or SOD1, an enzyme that catalyses the conversion of the superoxide free radical to hydrogen peroxide.

Mutations in the *SOD1* gene are present in roughly 20% of familial cases and perhaps as much as 10% of non-familial ALS (7,67). *SOD1* mutations may cause a reduced capacity to counteract oxidative stress. Additionally, mutations may result in mis-folded *SOD1* proteins that aggregate and form toxic inclusion bodies, reminiscent of the presumed mechanisms involved in AD and PD pathogenesis (68). Mutations in a second gene associated with familial ALS, *alsin* (*ALS2*), has been identified in juvenile-onset recessive PD (69). Investigations of other genetic variants in non-familial ALS have not yielded consistent findings, although several potentially promising candidate gene loci have been identified by genetic linkage studies (70).

Potentially important etiologic roles of environmental factors are indicated, at least by default, by the absence of convincing support for ALS being a predominantly genetically-determined disease. Various environmental risk factors have been investigated, including smoking (71); pesticides and other agricultural chemicals (72–73); heavy metals, especially lead (74); electric shock; and electromagnetic fields (75). Reasonably consistent yet modest excess risks have been observed among cigarette smokers (71,76). In addition, reports of apparent case clusters of ALS among United States military personnel deployed in the first Gulf War prompted epidemiologic studies that are suggestive of associations. Exposures to pesticides, petroleum combustion products and mycotoxins have been speculated as the causative agents among Gulf War veterans, but none have been established (77).

Examples

Application of molecular epidemiology methods will be illustrated with some selected examples from the literature on neurodegenerative diseases. These examples span a range of applications, including molecular methods for biomonitoring of environmental neurotoxicants, candidate susceptibility investigation, gene–gene and gene–environment interaction analyses, and the more recently developed method of proteomics profiling to identify early disease markers.

Example 1: Occupational lead exposure and risk of ALS

An etiologic association between occupational lead exposure and ALS has been suggested primarily from case–control studies in which exposures have been based on the classification of self-reported jobs. To investigate whether lead is causally related to ALS with more precise exposure characterization, a population-based case-control study was conducted in the New England region of the USA (78). Study subjects were 109 ALS cases and age/sex/region-matched controls identified by random digit dialling. Exposures to lead were assessed as lifetime number of days worked in lead-exposed occupations, blood lead levels, and bone lead concentrations determined by X-ray fluorescence. Blood and bone lead measurements

were obtained for 107 cases, but only for 41 controls. Blood lead levels represent recent exposure (within three months, or the lifespan of red blood cells that store the majority of blood lead). Lead concentrations were measured in patella and tibia bones, representing the shorter (3–5 years) and longer-term (10–15 years) body storage compartments.

The most striking finding from this study was a monotonically increasing exposure–response relation with cumulative lifetime lead-exposure work days, with a 2.3 relative risk estimate found for the highest exposure category (≥ 2000 days) compared to 0 days. Comparisons of cases' and controls' blood and bone lead levels (Table 22.1) yielded slightly larger lead body burdens among cases. Overall, the study findings offer some support to the hypothesis that lead is a risk factor for ALS.

Several features of this example warrant comment. Measurement of exposure biomarkers, as opposed to reliance strictly on questionnaire response data, has a theoretical advantage of improved precision. However, it should also be realized that biological measurements may not necessarily be more valid than questionnaire data, even in situations where the precision of measurement techniques is well established, such as for blood and bone lead. Biological monitoring of exposure does have the advantage of taking into account multiple sources of exposures (i.e. occupational and non-occupational), which is both a strength and a limitation. The strength is that it provides a more complete picture of exposure levels than does, e.g., an occupational history. The limitation is that it can be difficult to identify specific exposure sources from biomonitoring if the goal of the epidemiologic study is intervention to minimize or eliminate exposure. Additionally, the low participation rate among controls in this study was perhaps not surprising, given that presumably healthy controls would have less motivation to undergo biological sampling, albeit relatively non-invasive.

Table 22.1. Blood and bone lead levels in ALS cases and controls

Lead measurement (units)	Cases	Controls
Blood (μg/dl)	5.2 ± 0.4[†]	3.4 ± 0.4
Patella (μg/g)	20.5 ± 2.1	16.7 + 2.0
Tibia (μg/g)	14.9 ± 1.6	11.1 ± 1.6

[†] Mean (± standard error). Adapted from (78).

Example 2: Alpha-synuclein (SNCA) promoter region variants in PD

As mentioned earlier, mutations in the alpha synuclein (*SNCA*) gene have been associated with increased risks of PD in familial, and to a lesser extent non-familial PD. Epidemiologic studies of an apparently functionally important dinucleotide repeat in the *SNCA* promoter region (Rep1) have provided mixed evidence for an association with PD risk (79,80). A pooled analysis of Rep1 variability was performed that combined data for 2692 PD cases and 2652 unrelated controls from 11 study centres (in six western European countries, the USA and Australia) (81). Common genotyping protocols and quality control assessments, including selective re-genotyping, were incorporated into the study to minimize laboratory bias.

Analyses were performed for the three most common Rep1 base pair repeat lengths: 259, 261 and 263. The results of analysis comparing the Rep1 263 base pair genotype versus all others are summarized in Table 22.2. Overall, there was a modest yet statistically significant association (OR = 1.43; 95% CI = 1.22–1.69).

Notably, PD risk was elevated among carriers of 263 base pair length in each of the 11 studies. Moreover, the findings showed positive associations for both dominant (OR = 1.44; 95% CI = 1.21–1.70) and recessive (OR = 2.46; 95% CI

Table 22.2. Associations of Parkinson's disease with the Rep1 263 base pair repeat of the alpha synuclein gene promoter region

Group	No. cases/controls	OR (95% CI)[†] P-value
All subjects	2686/2454	1.43 (1.22–1.69) <0.001
Negative family history	2241/676	1.33 (1.03–1.72) 0.03
Positive family history	413/38	1.67 (0.51–5.50) 0.40
Age ≤68	1361/1317	1.47 (1.17–1.84) 0.001
Age >68	1325/1137	1.31 (1.03–1.66) 0.03
Women	1083/1205	1.33 (1.06–1.67) 0.01
Men	1603/1249	1.54 (1.22–1.95) <0.001

[†] Odds ratio (95% confidence interval) for 263 versus other base pair lengths

= 0.95–6.37) inheritance models, and varied little with respect to age, gender or family history of PD. There was a slight, although less consistently noted, reduced risk (OR = 0.86; 95% CI = 0.79–0.94) related to the 259 base pair repeat length. The 261 base pair repeat length was unrelated to PD risk.

This study exemplifies the approach of focusing on a single candidate gene that has a plausible relation to the phenotype of interest. By combining data from multiple studies and following standardized laboratory protocols, the investigators were able to achieve greater statistical precision than was possible in any previous study while maintaining a high level of validity. As with all studies of single gene associations with complex diseases, this study could not address the interactions among genes or with environmental factors. In fact, the investigators estimated that variability of the *SNCA* Rep1 promoter region may account for a population attributable risk of only 3%, but might be a component cause of a constellation of genetic and environmental factors that confer substantially larger population effects.

Example 3: Interaction of estrogen receptor and ApoE genetic polymorphisms in Alzheimer's disease

A study was conducted in Italy of the single and combined associations of two candidate genes, *ApoE-ε4* and the estrogen receptor-α (ER-α) gene, in a case–control study of 131 non-familial AD cases and 109 age-matched controls, comprised mostly of cases' spouses (82). The rationale for selecting these candidate genes was provided by previous studies demonstrating strong risks related to *ApoE-ε4*, and suggestions from the literature, albeit controversial, that estrogen may protect against dementia (82,83). Estrogenic activity is known to be mediated by α and β estrogen receptors; reduced AD risks among users of estrogen replacement therapy has been reported previously (83). Also, previous research indicated variable associations between two *ER*-α intronic single nucleotide polymorphisms (SNPs) in intron 1, rs2234693 [-397 T→C] and rs9340799 [-351 A→G], and AD (84,85).

Consistent with previous literature, *ApoE-ε4* carrier status was strongly associated with AD in both women and men, as indicated by observed relative risk estimates OR = 6.48 (95% CI = 2.99–14.0) and 4.67 (95% CI = 1.98–11.0), respectively. No associations with AD were detected for either of the ER-α SNPs individually or in combination. In contrast, analysis of the joint effects of *ApoE-ε4* and the *ER-α* intronic alleles revealed evidence for interactive effects, as shown in Table 22.3. The strongest associations were observed in women for the combinations of *ApoE-ε4*/-397 T allele (OR = 7.24; 95% CI = 2.22–23.6) and *ApoE-ε4*/-351 A allele (OR = 8.33; 95% CI = 1.73–40.1). Evidence of combined gene effects was considerably weaker in men. Notwithstanding the relatively small sample size of the study, this pattern of results could be interpreted as a gender-specific interaction between an established high risk allele, *ApoE-ε4*, and *ER-α* gene intronic variants, where the presence of the latter enhances the effects of *ApoE-ε4*. Moreover, the results from this study are strengthened insofar as they replicate findings from a previous study (85).

As this example illustrates, investigation of associations with combinations of gene variants, rather than a focus on a single gene, can provide further etiologic insight.

Table 22.3. Alzheimer's disease risk in relation to combinations of ApoE-ε4 and estrogen receptor α (ER-α) intronic alleles

	Women	**Men**
ApoE allele	**OR (95%CI)[†] P-value**	**OR (95%CI) P-value**
ε4+	6.48 (2.99–14.0) <0.001	4.67 (1.98–11.0) <0.001
ε4-	Reference	Reference
ApoE/*ER*-α intron 1 −397[‡]		
ε4+/TT or TC	7.24 (2.22–23.6) 0.001	3.47 (0.71–16.9) 0.125
ε4+/CC	2.00 (0.43–9.26) 0.375	0.80 (0.10–6.35) 0.833
ε4-/TT or TC	0.76 (0.27–2.12) 0.603	0.51 (0.12–2.07) 0.343
ε4-/CC	Reference	Reference
ApoE/*ER*-α intron 1 −351[‡]		
ε4+/AA or AG	8.33 (1.73–40.0) 0.008	2.31 (0.44–12.1) 0.320
ε4+/GG	1.25 (0.18–8.44) 0.819	0.60 (0.05–6.80) 0.680
ε4-/AA or AG	0.73 (0.18–2.96) 0.656	0.37 (0.08–1.69) 0.199
ε4-/GG	Reference	Reference

[†] Odds ratio (95% confidence interval)
[‡] *ER*-α intron 1 −397 T/C (rs2234693); *ER*-α intron 1 −351 A/G allele (rs9340799)
Adapted from (82).

This approach can be especially informative when one of the genes under study bears a predictable relation to disease risk, as is the case for *ApoE* and AD.

Example 4: Interaction of pesticides and CYP2D6 genetic polymorphism in Parkinson's disease

Potential interactions between environmental and genetic risk factors for neurodegenerative diseases have become an increasingly prominent research focus, with the growing recognition that some persons may be especially susceptible to environmental toxicants, as illustrated by the following example.

The interaction between genetic polymorphisms of the cytochrome P4502D6 (*CYP2D6*) gene and pesticide exposure were investigated in a case–control study of PD in France (86). Pesticides have been regarded as plausible causes of non-familial PD, as reviewed earlier in this chapter. The *CYP2D6* enzyme is known to metabolize MPTP and various toxic environmental chemicals, including some pesticides (87,88). The *CYP2D6* gene is polymorphic, with carriers of the *4 (minor) allele, which contains a SNP at an intron/exon junction, having diminished metabolic capacity proportional to the number of variant alleles. Pesticide exposures, determined by exposure assessment experts, and *CYP2D6* genotypes were compared between 190 PD cases identified from the French health insurance organization for workers in agricultural occupations (Mutualite Sociale Agricole), and 419 age/gender/regionally-matched controls who were also members of this insurance organization (86). A qualitative exposure gradient was defined as "no use," "gardening use," and "professional use," assuming that the last category would represent the heaviest exposures. Analyses were adjusted for cigarette smoking, in addition to the matching variables.

For the entire study population, there was a modest gradient of PD associated with the *CYP2D6*4* genotypes: OR = 1.02; 95% CI = 0.69–1.51 and OR = 1.56; 95% CI = 0.67–3.65 for carriers of one and two *4 alleles, respectively. The joint effects of pesticides and *CYP2D6*4* (Table 22.4) suggest synergism, whereby the most pronounced exposure-response trend was found among carriers of two *4 alleles, who would be classified as 'poor metabolizers.'

The notable strengths of the study were the selection of a study population with a relatively high prevalence of the environmental exposure of interest, pesticides, and the choice of a candidate gene variant whose functional consequences are well understood and plausibly related to pesticide metabolism. As with most case–control studies, this study was prone to exposure assessment uncertainties, particularly insofar as quantification of exposure levels to specific pesticides was not possible.

Example 5: Protein analysis of cerebrospinal fluid in Alzheimer's disease

Protein biomarker profiles in biological tissues have promise as early markers of disease onset and progression that may ultimately have diagnostic and medical management benefits. In addition, protein measurements may reveal characteristic patterns of response to toxic endogenous or exogenous agents that predict disease occurrence. Thus, from a neuro-epidemiologic standpoint, protein profiles offer several potential advantages by serving as early or surrogate disease markers, improving diagnostic accuracy, and suggesting host susceptibility factors. Because of its intimate anatomical and biochemical relations to the brain, cerebrospinal fluid (CSF) is the most relevant biological

Table 22.4. Joint effects on Parkinson's disease risk of CYP2D6*4 genotype and pesticide exposure

		Pesticide exposure	
CYP2D6*4	**None**	**Gardening use**	**Professional use**
Alleles	OR (95%CI)‡	OR (95%CI)	OR (95%CI)
0	1.00 [reference] --	1.73 (0.86–3.48) 0.12	1.85 (0.96–3.55) 0.06
1	1.39 (0.70–2.76) 0.35	1.17 (0.49–2.77) 0.72	1.83 (0.84–3.95) 0.13
2	0.41 (0.04–3.99) 0.44	2.75 (0.55–13.7) 0.22	4.74 (1.29–17.5) 0.02

‡ Odds ratio (95% confidence interval). Adapted from (86).

medium that can be accessed ante-mortem for epidemiologic studies of neurodegenerative disorders. In contrast, brain tissue can only be examined directly post-mortem, and blood or urine protein levels may reflect biological processes that are not-specific to the brain.

CSF levels of Aβ-amyloid$_{1-42}$ (Aβ$_{1-42}$) have been consistently associated with AD. Specifically, Aβ$_{1-42}$ levels are lower, whereas tau protein levels are higher in AD cases compared to controls (89). This pattern probably reflects impaired brain clearance (via CSF) of Aβ$_{1-42}$ and overexpression of tau protein in AD. A study was conducted in Sweden to determine whether these proteins were predictive of conversion to AD among persons with mild cognitive impairment (MCI) (90). These groups were compared at baseline: 93 AD cases, 52 MCI cases, and 10 healthy controls. AD and MCI were diagnosed according to established criteria. During a follow-up period of 3–15 months, 29 MCI cases were determined to have converted to probable AD. As summarized in Table 22.5, Aβ$_{1-42}$ levels decreased consistently from lowest to highest among AD cases, MCI converters, MCI non-converters and healthy controls. For tau protein, a similar pattern in the opposite direction was observed. Relative to reference values established in an earlier study of 231 healthy Swedish subjects (91), abnormally low Aβ$_{1-42}$ levels were associated with an MCI conversion sensitivity of 59% and specificity of 100%. Sensitivity and specificity for MCI conversion to AD associated with abnormally high tau protein levels were 83% and 90%, respectively.

This study provides a vivid illustration of the utility of measuring well-established biomarkers of a defined clinical outcome, AD, to assess clinical progression from earlier symptomatic states. Serial measurements in addition to baseline assessments of Aβ$_{1-42}$ and tau among the MCI and control groups would have been valuable, although the requirement of multiple lumbar punctures would certainly have posed a logistical hurdle. The relatively small sample size, especially of healthy controls, is another limitation, partly offset by the availability of normative data obtained previously in a larger sample.

Strengths, limitations and lessons learned

There are some formidable and characteristic challenges that epidemiologists confront when investigating the causes and prognostic factors for AD, PD and ALS. Each is a complex disorder with varying phenotypes that may in fact represent different clinical entities. Subdivisions of disease phenotypes into familial and non-familial forms, or with respect to age at onset, is a convenient approach, although may be fraught with considerable uncertainty. For example, age 50 is often cited as the demarcation of early- versus late-onset PD, largely based on the onset ages of familial cases, yet the age distinction is arbitrary. From an epidemiologic perspective, the relative homogeneity or heterogeneity of any disease rubric is especially important for identifying risk and prognostic factors. The generally slow rate of disease progression among the majority of cases (non-familial) of the neurodegenerative diseases complicates establishing precise disease onset times. The net result is often inclusion of prevalent rather than incident disease cases in epidemiologic studies, and attendant biases due to differential survival associated with risk factors of interest. Other challenges, which are not unique to research on neurodegenerative disorders, include uncertainties of diagnoses that are based solely on clinical examination; reliance on questionnaire responses, sometimes by proxies, such as with AD, to determine exposure status; availability of very few population-based neurologic disease registries (in contrast to cancer registries, for example); and typically low

Table 22.5. Cerebrospinal fluid levels of β-amyloid and tau protein in relation to conversion from mild cognitive impairment (MCI) to Alzheimer's disease

CSF protein (ng/l)	Group[†]			
	AD (n=93)	MCI converters (n=29)	MCI non-converters (n=23)	Healthy controls (n=10)
Aβ-amyloid$_{1-42}$	545 (± 230)	577 (± 197)	805 (± 368)	962 (± 182)
Tau	725 (± 266)	640 (± 162)	576 (± 275)	341 (± 118)

[†] Mean (± standard deviation). Table compiled from (90).

response rates among controls when biological sampling is included in a study.

Due to the rarity of AD, PD and ALS, population-based case–control studies have been the predominant study design. There have been some cohort studies in which neurodegenerative and other diseases have been investigated. Large cohort sizes and thorough exposure assessments are needed. The Nurses' Health Study cohort in the USA is a good example of a valuable study population for investigating associations with common exposures, such as smoking (37). Cohorts with well-characterized environmental exposures can also be investigated for associations with specific agents, such as a study of neurodegenerative disease mortality among US workers exposed to polychlorinated biphenyls (92). Occupational cohorts, however, generally are limited by relatively small numbers of cases and reliance on death certificates for case identification. Investigations of disease incidence or clinical indicators of neurologic disease, such as symptoms determined from standardized clinical exams, may be desirable where there are clear *a priori* hypotheses regarding risk in relation to specific exposures. Studies of PD-related signs and symptoms among cohorts of career orchardists exposed to pesticides (93) and welders exposed to manganese and other metals (58) typify this approach.

With respect to study size, large samples are generally required to detect low to modest risk associations, such as those usually observed in case–control studies of candidate genes. Collaborative pooled studies following similar protocols, as illustrated in the example of *SNCA* and PD (81), are thus highly desirable in that they offer the opportunity to examine consistency of associations among various populations, with attendant increased statistical power.

The issue of sample size is especially relevant for genome-wide association studies (GWAS), which have become increasingly prominent. Typically, GWAS include extremely large sample sizes (thousands of cases and controls) assembled across multiple collaborating studies to achieve adequate statistical power to detect modest associations. A particular advantage of this method is the opportunity to replicate findings in heterogeneous study populations worldwide with high levels of statistical power. This is illustrated by two large independent GWAS of PD among persons of European ancestry (94) and of Japanese ancestry (95), both of which identified *SNCA* and *LRRK2* as important disease-related genetic loci. Similarly, several GWAS for AD have consistently replicated findings for *ApoE*, but differences were noted for other loci (96–98). For a comprehensive review of GWAS results, the reader is referred to the National Human Genome Research Institute's GWAS catalogue, at http://www.genome.gov/26525384.

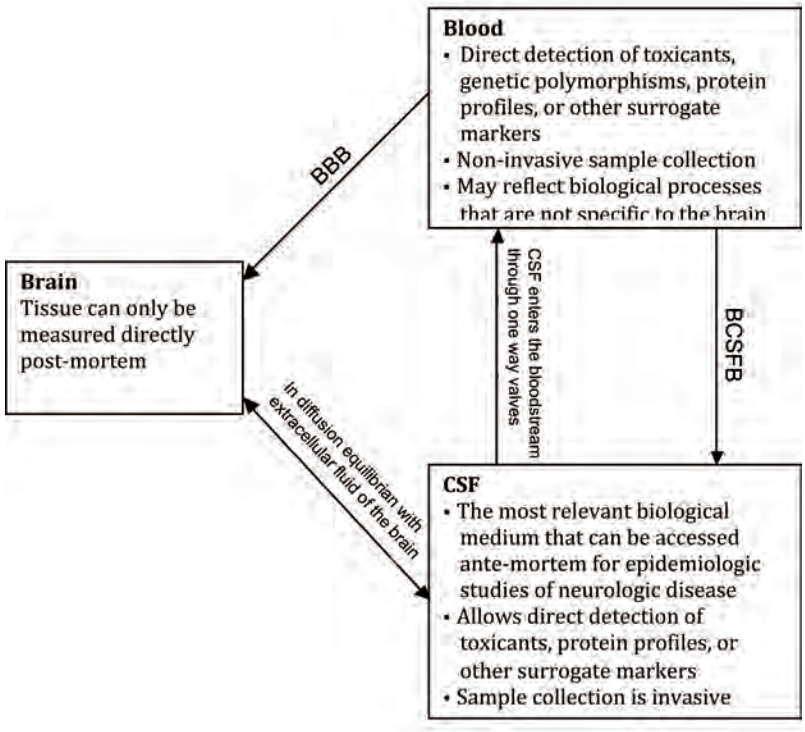
Future directions and challenges

Molecular epidemiology cannot eliminate or mitigate all of the previously mentioned research shortcomings. Nevertheless, there are some distinct advantages to incorporating molecular methods into neuroepidemiologic studies. As demonstrated with the examples presented in this chapter, the range of potential benefits include: improved diagnoses and phenotypic characterization, identification of genetic susceptibility factors, and, at least in theory, more precise exposure assessments in some instances.

A particular challenge that arises in molecular epidemiologic studies of neuro-degenerative disorders is that the target tissues of the central nervous system are not directly accessible except in post-mortem studies, which typically have epidemiologic shortcomings (e.g. convenience sampling). Consequently, surrogate measurements of toxicants, metabolites and other biomarkers are necessitated. The exception is DNA that can be assayed for genotyped validity from multiple tissue sources. Figure 22.1 summarizes the inter-relations between tissue sources for molecular biomarker assessment.

Molecular methods to date have mainly been applied to address relatively narrowly defined hypotheses, such as associations with a small number of genetic polymorphisms or exposure biomarkers. Nevertheless, as molecular technology becomes increasingly affordable and flexible, epidemiologists will be able to capitalize on technological advances to broaden the scope of research. GWAS and extensive linkage studies of PD (99) and ALS (100), and broad-based proteomic assessments of CSF in AD (101), indicate that this trend is underway. GWAS approaches are well suited to identifying common variants associated with disease, but other types of gene variation, namely rare variants and/or copy number variation, are not amenable to the current genotyping platforms. Hence, newer, more advanced approaches (whose development is in progress) will be necessary to query the genome fully, and in some cases this may necessitate even larger sample sizes. Molecular methods should also be particularly

Figure 22.1. Inter-relations between brain, cerebrospinal fluid and blood for molecular biomarker assessment



CSF, cerebrospinal fluid; CNS, central nervous system; BBB, blood-brain barrier; BCSFB, blood-cerebrospinal fluid barrier.

advantageous for investigating risk and prognostic factors for pre-clinical neurodegenerative disease outcomes, such as neuroimaging abnormalities or proteomic profiles, for which there are demonstrated high predictive values for late-stage disease.

Ultimately, consistent findings from epidemiologic studies, focused on narrow hypotheses that are corroborated by results from broader-based molecular epidemiology investigations, will be important for the prevention and management of the neurodegenerative diseases.

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