Pesticide Exposure, Host Susceptibility Factors and Risk of Parkinson's Disease: An Introduction to a Work in Progress

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Background

Parkinson's disease (PD) is a common neurological disorder (second only to Alzheimer's disease) and affects close to 1 million Americans. It occurs in all ethnic groups with an average prevalence of about 150/100,000 in North America and Western European countries. Both genetic and environmental factors are thought to influence the course of PD.[1] Prevalence of PD in the United States is highest in five midwestern states—Iowa, Minnesota, Nebraska, North and South Dakota—where agrochemicals are used intensively, suggesting that pesticide exposure may contribute to disease etiology.[2] Progressive disability results from tremor, muscular rigidity, slowing of movements (bradykinesia), and impairment of gait and balance.[3]

The clinical features of PD are the result of neuronal loss in the dopamine-producing region of the upper brainstem, the substantia nigra.[4] The presentation of PD can be broadly classified as "early onset" with symptoms typically occurring before the age of 40, or "idiopathic," also referred to as "sporadic," with symptoms appearing typically after the age of 50.

The etiology of PD has been enigmatic to clinicians, researchers, and epidemiologists. Despite considerable research, the precise causal factors remain elusive. Results of epidemiology and animal studies suggest that exposure to environmental agents (pesticides, heavy metals and solvents) and aging likely play roles in the disease process, especially in idiopathic disease.[5-10] Recently, investigators have identified genetic polymorphisms relevant to "early onset" and "idiopathic" PD etiology.[11-13] However, few studies have merged genetics with environmental epidemiology to examine the interaction between genes and the environment to determine if individuals harboring gene mutations may be more susceptible to effects of environmental exposures.

Marshfield Medical Research Foundation is conducting a population-based, case-control study to examine host susceptibility factors (age, genes) and environmental exposures on the risk for "idiopathic" PD in rural areas of central and northern Wisconsin. The study is being funded by the Centers for Disease Control and Prevention and the National Institute of Occupational Safety and Health. The long-term goal of this study is to link exposure information with biomarker data with the intention of providing a clearer understanding of the mechanisms by which agrochemical exposures may influence the occurrence of PD. The findings may help reduce incidence by improving strategies for disease prevention.

People diagnosed with and without PD are being interviewed to collect data on risk factors such as family and personal medical histories, alcohol consumption, tobacco use, residential exposures (pest control, gardening and hobbies), and lifetime occupational exposures. Blood samples from study participants will be analyzed for contaminant levels and for mutations in genes coding enzymes involved in pesticide metabolism. It is anticipated that 270 people (135 cases and 135 controls) will participate in this 5-year study.

Study Population

Study participants will be males and females, ≥40 years of age, residing in the Marshfield Epidemiologic Study Area (MESA), a 24 ZIP code area in northern and central Wisconsin (Figure 1). MESA includes approximately 77,000 people and

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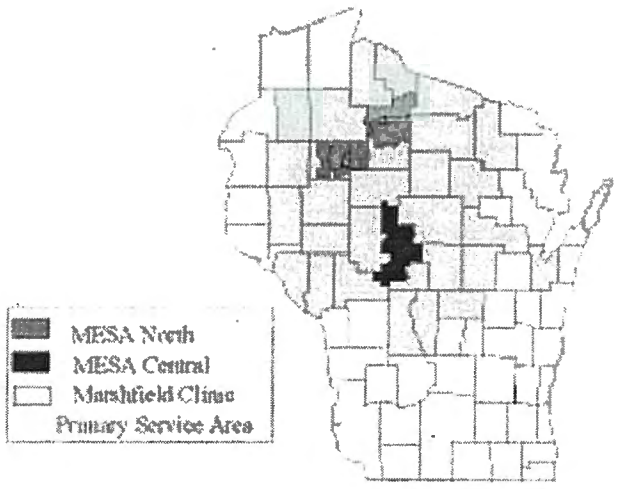
Figure 1. Marshfield Epidemiological Study Area 24 ZIP code regions in northern and central Wisconsin.



encompasses a region in which generations of central and northern Wisconsin residents have relied heavily on farming for their economy and lifestyle.[14] Other study subjects in MESA will represent families who either work or reside in rural areas, near farming activities. Cases will be Caucasian and have a neurologist confirmed diagnosis of PD based on the United Kingdom Parkinson's Society Brain Bank and will display two or more of the four cardinal signs of PD: resting tremor, muscle rigidity, bradykinesia, and disturbances of gait/equilibrium. Excluded from the study will be cases whose parkinsonian symptoms are drug-induced or could be attributed to essential tremor, Parkinson-like syndromes (progressive supranuclear palsy, normal pressure hydrocephalus, multi-infarct dementia, etc.), encephalitis, Alzheimer's disease, head trauma, tumor, or chemical intoxication.

Recruitment Strategy and Questionnaire

This project is a population-based, case-control study and is restricted to residents of MESA. Letters explaining the study are being sent to prospective participants and a follow-up phone call is being used to answer questions about the study, to determine interest and to set up an appointment with the study neurologist. Once determined eligible for the study, the participants are measured for weight and height, provide a blood sample and work with a study coordinator to answer questions about job and hobby exposures, lifestyle choices, and medical history. Serum from the blood sample is being stored for analysis of contaminant levels and for determining concentration of pesticide metabolizing enzymes. DNA will be isolated from peripheral blood cells and used to identify mutations in the genes coding for pesticide metabolizing enzymes.

Data Analysis

Associations between exposure variables such as gender, farming status, and genetic markers will be analyzed. Estimated odds ratios and 95% confidence limits will be placed in 2x2 tables to assess the associations between various dichotomous genetic markers and exposure variables. Because exposed and unexposed cases would likely differ on factors other than age, logistic regression modeling will allow for adjustment of these factors.

Study Team

A multi-disciplinary research team assembled for this project consists of a pesticide researcher, neurologist, molecular biologists, epidemiologists, biostatistician, and extramural advisors from the National Institute of Occupational Safety and Health (Table 1).

Risk Factors

Table 2 shows a summary of risk factors that may modify an individual's risk of developing PD.

Age, Sex, Race

PD incidence and prevalence rise sharply beginning at age 50 and continue to increase throughout life.[15-19] PD is rarely diagnosed before the age of 40. A small percentage of juvenile forms are thought inherited.[20] A male excess of 20% to 30% has been estimated in a few population-based studies.[16,18] Lanska[21] and Mayeux et al[16] report slightly higher PD prevalence and mortality among Caucasians than African Americans.

Family History

Family history of PD among first-degree relatives (parents and siblings) has been reported in 7% to 16% of PD cases. Affected siblings account for 6% to 7% of PD cases.[22,23]

Matsumine et al[20] localized a gene for autosomal recessive juvenile parkinsonism and Kitada et al[24] subsequently identified a novel gene (parkin) coding for a ubiquitin-like protein in this candidate region. Ubiquitin is a mo-

Table 1. Parkinson's Disease Risk Factor Study Team

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Table 2. Risk Factors for Early Onset and Idiopathic Parkinson's Disease

Risk Factor	Early Onset	Idiopathic	References
Age of onset	<40 yr	>50 yr	15-19
Family history	X*	X	11,20,22-26
Residential pesticide use		X	29
Occupational exposures			
Pesticide application		X	30-34
Solvent exposure		X	10
Heavy metals (copper, manganese, lead-copper, lead-iron, iron-copper)		X	9
Genetic polymorphisms			
Parkin	X	X	11,20,22-26
PON1		X	38
CYP 2D6 29B+		X	36
GSTP1		X	40

*Evidence exists in the scientific literature to suggest the factor may modify a person's risk of developing PD. The absence of an X either means few or no reports have been published on the topic or a relationship has not been established between the particular exposure/genotype and risk of disease.

lecular tag identifying proteins destined for destruction within the cytoplasm. Lucking et al[25] reported evidence suggesting that various mutations (exon rearrangements, point mutations) in the parkin gene are a major cause of early-onset PD and isolated juvenile-onset PD (≥20 years). Findings of Abbas et al[26] and Scott et al[11] suggest various mutations in the parkin gene may be associated with 21% of affected family members with early onset PD and 2% of affected family members with idiopathic PD.

Rural Residence

Barbeau et al[27] published the first epidemiological study suggesting that pesticide exposures in rural environments may be associated with increased risk of PD. In this study, the prevalence of PD in nine rural regions of Quebec was found unevenly distributed about the province and varied according to drinking water source. Regions with higher PD prevalence were found to be predominantly agricultural with intensive market gardening and pesticide use. In a separate study, an association was noted between prevalence of parkinsonism and residence in those areas where carbamates and organophosphates were detected in water samples.[28]

Residential Pesticide Use

People exposed to pesticides in the home or garden may have a significantly higher risk of PD. Stephenson[29] reviewed a study by Nelson et al that involved 496 patients diagnosed with PD in northern California during the years 1994-1995 and 541 age- and sex-matched controls from the same population. In-person interviews were used to collect information about the patients' lifetime history of exposure to home pesticides prior to diagnosis. After controlling for known risk factors (e.g., family history of PD, occupational exposure to pesticides, smoking), it was determined that individuals with a high level of exposure to herbicides (average of 160 exposure days) were at significantly higher risk of the disease when compared with non-users. Handling and applying insecticides in or around the home also resulted in elevated rates of PD when compared to non-users.

Farming

Case-control studies have demonstrated an increased risk for PD in farming occupations[30-32] and with occupational exposure to pesticides.[33] Semchuck et al[34] found a dose-response relationship between years worked in field crop farming or grain farming occupations and an increased risk of PD.

Enzyme Polymorphisms

In general, pesticide metabolism can be divided into three phases.[35] Phase I utilizes oxidizing and hydrolyzing enzymes such as cytochrome (CYP) P450 and paraoxonase (PON). Phase II pathways include conjugating enzymes such as glutathione S-transferase (GST). Phase III describes mechanisms for the transport of conjugated xenobiotics from the cells.

Hubble et al[36] examined environmental and genetic variables in tandem to look for interactions and the risk of PD with dementia. Subjects who had pesticide exposure and at least one copy of the CYP 2D6 29B+ allele had an 83% predicted probability of PD with dementia, supporting the possibility that a gene-toxin interaction may play an etiological role in PD with dementia.

Paraoxonase-1 (PON1) is an enzyme that functions in both normal lipid metabolism and the detoxification of several organophosphorous insecticides and nerve agents through the CYP P450/PON1 pathway.[37] Acute organophosphate toxicity is influenced by polymorphic variation in the PON1 gene that alters both the level enzyme synthesized and the enzymatic activity of the protein produced.[38] Two factors determine PON1 status of an individual, the amino acid (GLN or Arg) present at position 192 and the amount of PON1 protein in the serum.

In studies comparing normal with PON1 knockout mice, an increased sensitivity to specific insecticides processed through the CYP 450/PON1 pathway was noted in knockout mice exposed to the organophosphate chlorpyrifos.[39] Injection of purified PON1 protected mice from cholinesterase inhibition induced by chlorpyrifos. These findings suggest that variations in PON1 status may modify toxic sequellae due to organophosphorous pesticide exposures.

GSTs are a ubiquitous group of detoxification enzymes involved in the metabolism of pesticides and other toxins. Menegon et al[40] investigated the role of GST polymorphisms in the pathogenesis of idiopathic PD. They genotyped polymorphisms in four GST classes (GSTM1, GSTT1, GSTP1, and GSTZ1) in 95 PD patients and 95 controls, and collected information about pesticide exposure. Their findings suggest that the distribution of GSTP1 genotypes differed significantly between PD patients and controls that had been exposed to pesticides. No other association was found with any of the other GST polymorphisms. The significant differences in GSTP1 genotype might explain variations in susceptibility to PD after pesticide exposure.

Significance

Linking exposures with biomarker data may provide a clearer understanding of the mechanisms by which agrochemical exposures influence the occurrence of PD and may assist with formulating strategies for reducing adverse health consequences for agricultural and other workers by policy design, education, and research. It may well be that the Parkinson phenotype is not the result of a single gene defect or of exposure to a single environmental toxin, but rather multiple potential combinations of genetic and environmental interactions. Ultimately the hope is that knowledge gained in studies such as this will allow clinicians to screen individuals for susceptibility genes, and advise them on specific environmental factors that need to be avoided in order to prevent the subsequent development of PD years later.

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