Pesticides and Parkinson's disease: is it in your genes?

“A crucial question when considering the etiology of Parkinson's disease is 'what initiates this process?'”

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Extraordinary progress has been made in our understanding of the pathophysiology of Parkinson's disease (PD) over the past 30 years but the etiology of the majority of cases remains elusive. Clinicians, pathologists, biochemists, geneticists and epidemiologists have all made significant contributions to our current state of knowledge, and it is useful to consider all perspectives when discussing the relative roles of genetics and the environment in the pathogenesis of PD.

The diagnosis of PD continues to be a clinical judgment based on core motor features that are the result of dopamine depletion from neuronal loss in the substantia nigra. For this reason, PD was considered primarily a 'dopamine disease' until recently. We now know that PD is much more of a systemic disorder involving neurons in the gut, brainstem, mid-brain and cerebral cortex. Braak *et al.* have demonstrated that in the majority of cases, disease pathology appears to progress in an orderly fashion [1]. PD pathology is apparent in enteric neurons and olfactory bulb even before the classic symptoms of PD are recognized [1,2]. Clinical correlates to

this early pathology include constipation and disturbances in olfaction up to 5 years prior to the development of motor symptoms. More recently, several investigators have proposed that PD pathology spreads trans-neuronally via a prion-like process, which is consistent with this orderly progression. Support for this hypothesis has come from a variety of studies in cultured cells, animal models and PD patients having received fetal cell transplants [3]. A crucial question when considering the etiology of PD is 'what initiates this process'?

PD was generally considered to be a sporadic disorder until the discovery of a mutation in the α -synuclein (α -syn) gene in 1997 that caused PD in a small number of families. Since then, mutations in several genes have been found to cause PD [4]. Despite the fact that all of these Mendelian-inherited mutations only account for a small portion of all PD cases, identification of their gene products have provided important clues into the pathophysiology of sporadic disease. For example, the identification of α -syn as the major component of PD pathology came directly from genetic studies. Unfortunately, despite over

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15 years of genetic studies, only a small component of the risk of developing PD has been found, leaving the etiology of most cases still unknown. The 16 genetic loci that cause familial PD and the 11 PD loci identified in several GWAS studies can only account for 10–20% of all cases of PD, suggesting that the environment is likely playing an important role [4,5].

Interest in potential environmental causes of PD was stimulated by the identification of MPTP as the cause of a Parkinsonian syndrome in drug abusers. Since MPTP was found to inhibit complex I in mitochondria, it was suggested that mitochondrial dysfunction might underlie the development of PD, whether the dysfunction was due to genetic and/or environmental origins. Indeed, both have been since been implicated. Complex I alterations have been measured in mitochondria from PD brains and peripheral blood, although these have not been universal findings [6]. From a genetic perspective, mutations in mitochondria-associated genes (e.g., PINK1 and Parkin) lead to clinically defined PD. Systemic administration of rotenone, a complex I inhibitor, results in Parkinson-like pathology in rats [7] and exposure to complex I inhibitors increases the risk of developing PD in humans [8].

The interest in pesticides as a potential risk factor for PD grew from the structural similarities of MPTP and the herbicide paraquat. Approximately 1 billion pounds of pesticides are applied annually in the USA and are comprised of hundreds of compounds that differ both structurally and mechanistically. A large body of epidemiologic literature exists concerning pesticides and PD, including case reports, descriptive studies and cohort studies, although most studies have used a case–control design [9]. The majority of these studies reported an association between pesticide exposure and PD, although these findings were met with some skepticism due to methodological concerns. These concerns include potential recall bias, and inability to identify exposure to specific pesticides. Recent studies have addressed these potential problems. Ascherio and colleagues performed a prospective analysis of subjects enrolled in the Cancer Prevention Study II Nutrition Cohort to avoid potential recall bias. They analyzed surveys from 143,325 individuals who did not exhibit symptoms of PD at baseline and found that those exposed to pesticides had a 70% increased risk of developing PD [10]. Another prospective

study of pesticide applicators found that subjects exposed to pesticides that inhibit complex I and increased oxidative stress were at an increased risk of developing PD [8].

Our group has taken a different approach to address the issues of recall bias and identification of specific toxins. We have taken advantage of the California Pesticide Use Registry (PUR) and geographic information system (GIS)-based methods to estimate 40-year pesticide exposure in subjects (PD and controls) living and working in the Central Valley of California. Using these methods, we can determine if exposure alters risk of developing PD without the potential of recall bias and the specificity of the PUR allows for identification of individual pesticides or combinations of pesticides. We studied two groups of pesticides; pesticides that had previously been implicated in PD and a second group that we found to alter a biological pathway believed to be involved in the pathogenesis of PD (e.g., ubiquitin proteasome system [UPS]). Using this approach, we confirmed the association of paraquat with PD (especially if co-exposed to maneb or ziram) and identified previously unrecognized pesticides that increase the risk of PD [11–15]. For example, ziram, a dithiocarbamate fungicide, was found to inhibit E1 ligase of the UPS and selectively injure dopaminergic neurons in primary cultures and in mice [16]. Exposure to ziram in our PEG study subjects led to a 300% increase (odds ratio: 3) in risk of developing PD [12]. As a reference for effect size, the cumulative genetic risk for those with all the bad polymorphisms identified in multiple GWAS adds up to an odds ratio of only 2.51 [5]. We also found that inhibition of aldehyde dehydrogenase (ALDH) is toxic to dopaminergic neurons in cultures and zebrafish, and exposure to pesticides that inhibit ALDH is associated with an increased risk of PD [13,14].

As previously mentioned, gene mutations and polymorphisms can account for only a minority of cases of PD and the same can be said for known environmental toxins. We believe it is likely that the majority of PD is the result of gene–environment (GxE) interactions. This is a particularly difficult hypothesis to study given the enormous number of possible interactions, but there are examples that support this theory. Elbaz *et al.* were one of the first to demonstrate a GxE interaction involved in PD. They found that pesticide exposure only had a modest effect on PD risk in subjects who were good metabolizers

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(CYP2D6), but risk was markedly increased in poor metabolizers [17]. We have since found a number of other examples of GxE interactions that alter risk of developing PD. For instance, functional paraoxonase 1 variants modify the risk of PD due to organophosphate exposure and variation in UPS genes modify the risk of PD in subjects exposed to UPS-inhibiting pesticides [15,18]. In addition, *ALDH2* gene variations alone had little effect on PD risk but they significantly altered risk in subjects exposed to ALDH-inhibiting pesticides [14]. Examples of GxE interactions are not limited to pesticides. Individuals with an expanded REP1 allele in the α -syn promoter are at a significantly higher risk of developing PD with head trauma compared with those with the shorter allele [19].

Based on our current understanding of the genetics and epidemiology of PD, it is likely that GxE interactions are involved in essentially all cases, but the relative contribution of each will vary. We believe that the genetics of an individual determines how susceptible one is to specific environmental insults. Once α -syn pathology is initiated, it can progress in a prion-like manner throughout the nervous system. Since the pathology of PD appears to begin in the gut and olfactory bulb, main routes of environmental exposure, it would be logical to implicate environmental toxins in the initiation

of this process. Pan-Montojo *et al.* tested this hypothesis in an eloquent series of experiments that demonstrated the ability of small amounts of rotenone delivered to the enteric nerves to initiate α -syn pathology in mice. Strikingly, resection of the autonomic nerves prevented its spread to the CNS [20].

Both rare gene mutations and pesticide exposure provide excellent proof of principle that both genes and the environment can alter risk of developing PD; however, even these well-studied examples account for only a small percentage of cases. What the field needs now are larger studies designed to identify the complicated environmental and genetic factors that can, in a combinatorial manner, determine a more complete picture of the etiology of PD.

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