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CYP450, genetics and Parkinson's disease: gene × environment interactions hold the key

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Summary. The ecogenetic theory contends that most cases of Parkinson's disease (PD) result from the actions of environmental factors in genetically susceptible individuals on a background of normal ageing. This notion is supported by epidemiologic data; family history of PD and exposures to environmental toxins such as pesticides increase risk, while cigarette smoking reduces risk. As a result, polymorphic genes that code for metabolic enzymes have been considered as candidates for conferring differential risk for PD. Given their prominence in xenobiotic metabolism, the cytochrome P450 (CYP) genes have come under great scrutiny. The activity of CYP2D6 is largely determined by genetic variability and common sequence variants exist in human populations that lead to poor metaboliser (PM) phenotypes. These have been extensively studied as genetic risk factors for PD with inconsistent results. However, these studies have disregarded interactive effects (e.g. gene × environment interactions) despite the assertions of the ecogenetic theory. Data from our group and others suggest that the CYP2D6 PM genotype interacts with certain environmental factors such as pesticide exposure and cigarette smoking to confer differential risk for PD. Previous failure to consider such interactions might, in part, explain the inconsistencies observed in the CYP2D6 genetic risk-factor literature. Our data illustrate, using CYP2D6 as an exemplar, that it is crucial to consider both genetic and environmental factors, and their interactions, in any examination of risk factors for PD.

Introduction

Parkinson's disease is a late onset neurodegenerative disease affecting 1.6% of the population over 60 years of age and with an average age at onset in the late 7^{th} or early 8^{th} decade. The triggers for the disease remain unknown although a complex aetiology is universally accepted. This symposium discusses the cytochrome P450 (CYP) family of metabolic enzymes, their role in the CNS and the implications for PD. This paper will provide background to this discussion. Why might modulators of xenobiotic metabolism be important in PD? What role(s) do the CYPs play? How do we interpret the existing literature? What important considerations must be addressed in the study of risk factors in human populations? These issues will be addressed in this discourse using real data from the study of the CYP2D6 gene in PD as an exemplar.

Ecogenetic theory

The ecogenetic theory of PD (see Fig. 1), adapted from the cancer literature and first

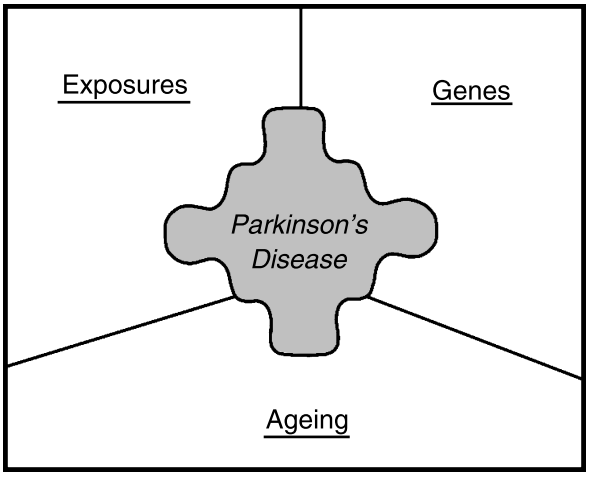

Fig. 1. The ecogenetic theory of PD

outlined by Barbeau and colleagues in their 1985 Lancet paper suggests that:

"... Parkinson's disease is the result of environmental factors acting on genetically susceptible individuals against a background of normal ageing" (Barbeau, 1985).

This statement, written 20 years ago remains totally consistent with all available current research evidence. Let's now briefly look at each aspect of this theory.

Environmental factors and PD

Intense interest in environmental exposures as risk factors for PD developed in the 1980s after reports confirmed that intoxication with the synthetic meperidine derivative, MPTP, could reproduce the features of PD in humans and animals. This stimulated a search for exogenous or endogenous molecules with similar effect. This also prompted a multitude of epidemiological surveys to assess potential environmental risk factors for Parkinson's disease. It is now clear that a number of classes of molecules have an ability to induce parkinsonism in animals and humans. Examples include the mitochondrial toxin rotenone, proteasomal pathway inhibitors and certain heavy metals. In addition, epidemiological surveys, mostly using a case-control experimental design, have identified environmental exposures that may modify risk for the disease. Exposures to pesticides, neurotoxic metals, solvents, well water and rural residency reportedly increase risk; cigarette smoking and coffee consumption appear to be associated with a reduced risk. Despite considerable noise, three consistent findings emerge from such studies: (1) Exposure to pesticides is associated with increased risk; (2) Exposure to cigarette smoke is associated with a decreased risk; (3) A family history of PD is associated with increased risk.

These findings are consistent with the ecogenetic theory. Readers interested in further information about these risk factors are directed to meta-analyses that review the literature for these risk factors in more detail. Pesticide exposure yields a summary odds ratio (OR) of 1.94 (95%CI = 1.49–2.53) (Le Couteur et al., 1999). Smoking's effect is likewise modest (OR = 0.59, 95%CI = 0.54–0.63) (Hernan et al., 2002). These effect-sizes, which constitute a less than two-fold change in risk, require large sample sizes to detect.

With the caveat that family members tend to share environments and occupations, the family history story points to inherited factors (this will be discussed below).

Genetic factors and PD

That genes are important in the aetiology of PD is unequivocal. Rare monogenic forms of parkinsonism, resulting from genetic abnormalities in the so-called *PARK* genes are now well known. These are helping to define the important biological pathways leading to the neurodegeneration seen in PD. Twin studies, family studies and more complex genetic segregation analyses also point to a genetic component for typical idiopathic PD. It is, however, pertinent to remind the reader that the genetic component of PD is relatively modest. Moreover, the vast majority of individuals with PD have no apparent family history. Thus it is logical that any examination

of genetic risk factors for PD should also consider their environmental context.

Ageing and PD

The final protagonist in the ecogenetic theory of PD is normal ageing. Ageing results in a multitude of physiological changes that could contribute to increased risk for neurodegenerative disease. Well characterised changes in the central nervous system (CNS), cardiovascular system (CVS), metabolic pathways and gene-expression profiles, many of which are highly inter-related, all contribute to this increased risk. In terms of PD, the effect of ageing appears to shift the aetiological balance more towards environmental risk factors. In other words, the older an individual gets, the greater the environmental component to PD risk. This idea is illustrated in the results of twin studies and family studies.

This extremely rudimentary summary of the major concepts of the ecogenetic theory of PD seeks only to provide a flavour for the complex and multifactorial nature of the aetiology of PD. A more comprehensive summary of age-environment and gene-environment interactions in the pathogenesis of PD can be found in a recent review article on the topic (Le Couteur et al., 2002).

Metabolic pathways link all three aspects of the ecogenetic theory

One link between all three main components of the ecogenetic theory for PD involves the body's ability to deal with endogenous or exogenous molecules that may be directly or indirectly neurotoxic; this ability is manifest in metabolic or detoxification pathways. Such pathways are responsible for the removal of environmental exposures including certain pesticides and components of cigarette smoke. They are made up of enzymes such as the CYP enzymes, the activity of which are subject to the influences of commonly occurring genetic polymorphism. Furthermore, the influence of normal ageing can result in significant physiological and gene-expression alterations, leading to important perturbations in these metabolic pathways. These have important potential implications for the risk of diseases such as PD.

Metabolic pathways

Metabolic pathways can be simply classified into three different phases. Phase I metabolism involves oxidations, phase II enzymes conjugate these oxidised substrates into more polar ones, while phase III processes actively transport these conjugated molecules across membranes and out of cells. Examples of phase I enzymes include the flavin monooxygenases (FMO), paraoxonases and the cytochrome P450 family which is the focus of this symposium. The glutathione transferases (GSTs), N-acetyl-transferases (NATs) and glucuronidases are all phase II enzymes, while the multi-drug resistance associated proteins (MRPs) are in the phase III category.

For reasons outlined above, it is possible that enzymes in any or all of these categories could be regarded as potential candidates to influence the risk for PD. Indeed, genetic polymorphism in many of them has been assessed for association with disease. In historical terms, the CYP2D6 gene has arguably received the most attention; the remainder of this paper will concentrate on these studies. However, the various issues raised in relation to the study of CYP2D6 are equally applicable to the study of other metabolic enzymes in association to differential risks for PD.

The Cytochrome P450s

Recent genomic analyses highlight the natural diversity of this family of enzymes. Approximately 4000 different cytochrome P450 genetic isoforms exist in nature, being represented in all forms of life from simple bacteria through to complex eukaryotes. There are at least 50 different human CYPs isoforms, divided into 10 different subfamilies. These enzymes are generally defined as

mix-function mono-oxygenases that act together with the cofactor NADPH-P450. In the course of this symposium you will hear of several of these human isoforms including, CYP1A1, CYP2C6, CYP2C13, CYP2D6, CYP2D7 and CYP2E1. The genes coding for these isoforms exhibit commonly occurring genetic variability in human populations that contribute to individual variability in enzyme activity. A review by A. K. Daly summarises the currently known and functionally relevant ones (Daly, 2003).

Influencers of metabolic activity

We have already mentioned that normal human ageing and common genetic sequence variation are two important influencers of metabolic enzyme activity. However, it is also pertinent to highlight that there is a complex web of interacting determinants which can modulate the gene-expression and protein function of metabolic enzymes. In particular, an individual's exposure to various external factors can have massive effects. Many of the CYP genes exhibit inducibility, suppression and competitive influences. For example, CYP1A1 expression in the lung is induced by components or cigarette smoke and CYP2E1 in liver is alcohol inducible. Conversely, the ingestion of certain dietary components (such as grapefruit juice) or drugs can result in considerable reductions in the CYP3A4 metabolism of other substrates. These effects may also be influenced by genetic background. Even for CYP2D6, a so-called non-inducible isoform, certain medications including anti-parkinsonian agents have been shown to be associated with reduced activity. These points highlight the importance of non-genetic factors resulting in phenotypic variability.

CYP2D6 phenotypic variability

CYP2D6 has attracted great attention to PD researchers because it participates in the metabolism of the parkinsonism-inducing toxin MPTP, herbicides (like atrazine and paraquat) and organophosphate pesticides. Moreover, it has been long known that there is considerable phenotypic variability in the enzyme activity of CYP2D6. CYP2D6 activity (or phenotype) was traditionally measured pharmacokinetically in terms of a metabolic ratio (MR), defined as the ratio of parent substrate (usually spartine or debrisoquin) to hydroxyl-metabolite in the urine of patients. Population frequencies of CYP2D6 phenotype can be grouped into three categories, namely extensive metaboliser (EMs, ''normals''), intermediate metabolisers (IMs) and poor metabolisers (PMs). There are also a very small number of individuals who have an extremely rapid enzyme activity (ultra-rapid metabolisers). The phenotypic activity of CYP2D6 is primarily (but not exclusively) controlled by the different versions (or alleles) of the CYP2D6 gene that exist in human populations. Thus genetic analyses using DNA from patients can be used to define the specific nucleotide sequences at the CYP2D6 locus (genotypes) and thus infer the phenotypic category of an individual (phenotype). There are several different CYP2D6 alleles that result in a PM phenotype, however three of these make up the vast majority, namely: (1) CYP2D6*3 (2549A>del frameshift); (2) CYP2D6*4 (the most common PM variant resulting from a 1846 G>A spice site variation); (3) CYP2D6*5 (a full deletion of the gene). Individuals carrying one PM allele usually act as IMs while two copies of a variant allele lead to the PM phenotype. Traditional PCR-RFLP methods have been used to genotype these alleles, but these methods are fast being replaced by newer methods such as the affymetrix CYP-chip which can simultaneously screen for 18 CYP PM variants (including 10 in CYP2D6). In this age of the $1000 genome there is an explosion of available genetic sequence information from CYP genes. It will be a tremendous challenge to determine the most appropriate ways to use this information to learn more about disease risk.

CYP2D6 PMs and PD

There have been dozens of studies that have tested the question of whether CYP2D6 PMs are over-represented in PD cases. The first such analysis, published in the Lancet in 1985, was conducted by Barbeau and colleagues who showed that IM and PM phenotypes were over-represented in their PD group (Barbeau, 1985). A series of replicate studies based on phenotyping methods yielded inconsistent results. Smith and colleagues were the first to use a purely genetic characterisation of metaboliser status (Smith et al., 1992). They showed that PMs were over-represented in their PD group with an OR = 2.54 (95%CI 1.51–4.28).

Subsequent studies again yielded equivocal findings. Five meta-analyses have now summarised the published data (McCann et al., 1997; Christensen et al., 1998; Rostami-Hodjegan et al., 1998; Joost et al., 1999; Persad et al., 2003). Interestingly, all Caucasian analyses show a slightly increased frequency of CYP2D6 PMs in PD cases compared to controls. However, the summary data appears highly influenced by the original genetic study of Smith and colleagues (Smith et al., 1992) and, taken together, the general consensus of PD researchers is reflected in the statement of Riedl and colleagues:

"As yet there is no conclusive evidence to suggest that CYP2D6 polymorphisms confer susceptibility to PD" (Riedl et al., 1998).

While it is clear from the published literature that PM status does not confer a particularly strong "main effect" on PD risk, three important facts should ensure that CYP2D6 remains on the research radar of PD researchers: (1) Current thinking suggests that there are many determinants of PD risk, most with modest effect sizes similar to the apparent PM effect (consider pesticides, cigarette smoking and family history as "main effects" of similar magnitude); (2) None of the previous literature reports seriously considered adjustment of analyses for important potential confounders such as age, gender, ethnic background or environmental factors (the most critical being smoking which may be related to CYP genotype); (3) These studies may not be asking the right question with respect to CYP2D6. Clearly, if a polymorphism at the CYP2D6 locus influences susceptibility to PD by altering the metabolism of an environmental neurotoxin, then studies will only show an association if they examine those subjects that have had such neurotoxin exposure. Thus studies need to examine interactive, as opposed to main effects if we are to better understand the impact of CYP genotype on PD risk.

Do interactions between CYP2D6 PM genotype and environmental exposures influence the risk for PD?

To explore this question we examined 400 neurologist diagnosed PD cases and 400 unrelated, unaffected aged individuals for whom we had well characterised epidemiological information (including cigarette smoking and pesticide exposure data) and DNA for CYP2D6 genotype analysis. The results are summarised in Table 1.

PD cases were more likely to have claimed regular pesticide exposures as defined as weekly exposure for a period of six months or more (adjusted OR = 3.57, 95%CI = 1.15–11.07). However, a particularly interesting result is observed when this data was stratified for CYP2D6 PM genotype. We considered unexposed, EMs as the reference. For individuals who have never been exposed to pesticides, PM genotype appears protective, suggesting that perhaps CYP2D6 might actually be involved in the metabolism of as yet unidentified substrates with a protective effect. EMs, who are exposed to pesticides, don't appear to be at substantially increased risk. However as exposure dose is increased, and the ability to metabolise CYP2D6 substrates (such as pesticides) decreases the risk for PD increases;

Table 1. Studying CYP2D6 PM gene × environment interactions in 400 Australian PD cases and 400 unaffected aged controls

	Exposure to Pesticides (regular = weekly > 6 months)	
	Never	Regular
Cases	151 (38%)	61 (16%)
Controls	208 (54%)	21 (5%)
	OR* (95%CI) = 3.57 (1.15–11.07)	

	Exposure to Pesticides		
Group	Never OR	Occasional OR	Regular OR
	(95%CI)	(95%CI)	(95%CI)
EMs	1.00	1.24 (0.82–1.86)	1.3 (0.63–2.83)
IMs	0.95 (0.59–1.54)	0.89 (0.54–1.47)	3.27 (1.21–8.80) P > 0.02
PMs	0.29 (0.11–0.80) P > 0.02	2.09 (0.80–5.43)	8.47 (1.01–69.76), P > 0.05

All OR calculated using logistic regression adjusted for: age, sex, family history of PD & smoking. The respective freq. of the *3 & *4 alleles were 1.15% & 22.3% in cases and 1.29% & 2.3% in controls. 3 cases and 1 control were homozygous *5. All genotypes were in HWE

PMs who claim regular exposure exhibit an OR of 8.47 (95%CI = 1.01–69.76). In isolation this result was considered interesting but probably simply a chance finding. Therefore, it was with much interest to discover that almost an identical finding was reported by the group of Alexis Elbez and colleagues, who were studying a cohort of French farmers, also with known pesticide exposures (Elbaz et al., 2004). Their data also clearly show a reduced OR for unexposed PMs and an increase in risk with increasing pesticide exposure and reduced metabolic capacity. Clearly these interesting findings warrant further examination.

Given that CYP2D6 participates in the metabolism of nicotine and possibly other components of cigarette smoke, we thought that we should also consider the possibility for interactions between PM genotype and cigarette smoking influencing risk for PD. While these data are in an extremely preliminary stage, we have made two interesting observations: (1) PM genotype may be related to smoking status in healthy normal subjects (we examined 540 unaffected aged Australians and showed that PMs are less likely to smoke and smoke less if they happen to be smokers); this highlights the possibility that cigarette smoking could be a crucial confounding variable in any examination of PM genotype and PD and (2) An analysis of 434 PD cases who claimed no pesticide exposure, revealed that PMs who smoke exhibit a later age-at-onset of disease (69 ± 5ys) compared to non-smokers and EMs who smoke (61 ± 11ys) $P = 0.02$; this relationship is dependent on dose. One explanation for this tantalizing result is that PMs, who have a reduced ability to eliminate nicotine or other potential neuroprotective components of cigarette are somewhat protected from the development of PD. It is important to note, however, that we did not observe any direct effect of PM status on the OR for smoking in this analysis. Both of these results are speculative and require further serious examination in replicate cohorts. None-the-less this provides further evidence for the existence of relevant gene-environment interactions influencing an individual's risk for PD.

Conclusions

This paper summarises the ecogenetic theory for the aetiology of PD. It provides arguments for why genetic variables in enzymes involved in the metabolism of endogenous and exogenous substrates (particularly the CYP genes) are valid candidates for conferring differential risk to the development of PD. Using CYP2D6 PM genotypes as an exemplar, data are presented to highlight the importance of considering the environmental context when examining genetic variability in metabolic pathways. Failure to consider crucial gene × environment interactions in such analyses may result in the inappropriate dismissal of important risk-altering factors. The aetiology of PD is, by its nature, complex and single main effects are unlikely to account for major proportions of the attributable risk. Single gene × environment interactions are also likely to be poor oversimplifications of the reality. However, with the concerted efforts of collaborative researchers, examining well-defined study cohorts of adequate sample size, with standardised and validated instruments for assessing environmental exposures, and an appreciation that neither genetic nor environmental risk factors for PD can be examined with mutual exclusivity, there is much to be gained from the continued development of the ecogenetic theory for PD.

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