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NFE2L2, *PPARGC1a*, and pesticides and Parkinson's disease risk and progression

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Abstract

Objective—To investigate three expression-altering *NFE2L2* SNPs and four *PPARGC1a* previously implicated SNPs and pesticides on Parkinson's disease (PD) risk and symptom progression.

Methods—In 472 PD patients and 532 population-based controls, we examined variants and their interactions with maneb and paraquat (MB/PQ) pesticide exposure on PD onset (logistic regression) and progression of motor symptoms and cognitive decline (n=192; linear repeated measures).

Results—*NFE2L2* rs6721961 T allele was associated with a reduced risk of PD (OR=0.70, 95% CI=0.53, 0.94) and slower cognitive decline (β=0.095; p=0.0004). None of the *PPARGC1a* SNPs were marginally associated with PD risk. We estimate statistical interactions between MB/PQ and *PPARGC1a* rs6821591 (interaction p=0.009) and rs8192678 (interaction p=0.05), such that those with high exposure and the variant allele were at an increased risk of PD (OR≥1.30, p≤0.05). *PPARGC1a* rs6821591 was also associated with faster motor symptom progression as measured with the UPDRS-III (β=0.234; p=0.001).

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Conflict of Interest: None

Conclusion—Our study provides support for the involvement of both *NFE2L2* and *PPARGC1α* in PD susceptibility and progression, marginally and through pathways involving MB/PQ exposure.

Keywords

NFE2L2; PPARGC1α; paraquat; Parkinson's disease; cognition

Introduction

Parkinson's disease (PD), the second most common neurodegenerative disease, is characterized by the progressive loss of dopaminergic neurons in substantia nigra region of the brain. Several major molecular pathways are implicated in cellular dysfunction and neuronal death in PD. Many, including impaired ubiquitin-proteasome system (UPS), mitochondrial dysfunction, and neuroinflammation, involve oxidative stress as an underlying mechanism[1–3]. Although it is not well understood how oxidative stress contributes to motor or non-motor symptom progression, a long history of post-mortem studies indicate increases in oxidative stress at the end stage of the illness when neuronal loss was marked, reporting excess free radicals, increased iron levels, and decreased glutathione (GSH) among other findings[1]. Furthermore, the influence of oxidative stress may accelerate as cellular dysfunction (e.g. mitochondrial dysfunction, etc.) accelerates and disease progresses. Similarly, a genetically determined inability to cope with oxidative stress may contribute to the underlying processes and enhance the effects of exposure to toxicants that increase oxidative stress, such as pesticides.

Cells have many antioxidant mechanisms to counteract reactive oxygen species (ROS)/ oxidative stress, including a battery of endogenous antioxidant enzymes[4]. Nuclear factor-erythroid 2 related factor 2 (Nrf2), encoded by the *NFE2L2* gene, and peroxisome proliferator activator receptor γ coactivator 1α (PGC-1α), encoded by the *PPARGC1α*, are transcription factors involved in the activation of many antioxidant enzymes in response to oxidative stress and targets for neurodegenerative disease therapy[4].

Once activated by oxidative stress, Nrf2 binds to specific promoter regions of multiple cytoprotective genes to upregulate the transcription of antioxidant enzymes. These include GSH, one of the factors which may determine vulnerability of dopaminergic (DA) neurons to oxidative stress, and NAD(P)H quinone oxidoreductase-1 (NQO1), which exerts a protective effect against toxic DA metabolites implicated in PD pathogenesis[3]. In a non-diseased population, three *NFE2L2* promoter polymorphisms have been shown to have functional significance, influencing transcriptional activity through action on Nrf2 antioxidant response element (ARE)-like and MZF1 promoter binding sites. One SNP, rs6721961, also significantly affected basal Nrf2 expression, resulting in attenuation of ARE-mediated gene transcription[5]. This 3-SNP *NFE2L2* promoter haplotype has been linked with both decreased risk of developing PD and older age of PD onset in a European case-control study[6,7]. Additionally, animal models provide support for *NFE2L2* involvement in PD; Nrf2 deficient mice and neuronal cultures showed increased MPTP toxicity, the toxic metabolite known to acutely induce Parkinsonism in humans[8,9]. On the other hand, over

expression of Nrf2 protected against locomotor activity loss in Drosophila modeled Parkinsonism[10].

PGC-1α is believed to mediate the protective responses of many antioxidant enzymes located primarily in the mitochondria, of great interest for PD given that mitochondrial dysfunction has been strongly implicated in PD etiology[4]. Further, PGC-1α, a multifunctional protein, is also a critical regulator of metabolism, including the adipogenesis and gluconeogenesis pathways[4]. This again is compelling for PDas some research associated type 2-diabetes and glucose sensitivity with PD risk and symptom severity[11]. In a population based genome-wide expression meta-analysis of PD, *PPARGC1A* was robustly associated with PD across all 3 stages of analysis and replication. Finding 425 PGC-1α– responsive genes under expressed in PD patients[12]. A recent study suggested the *PPARGC1α* SNPs rs6821591 and rs2970848 are associated with age at PD onset while rs8192678 and rs6821591 were linked to longevity in PD patients[13]. Interestingly, *PPARGC1α* knockout mice experienced over 5 times the loss of DA neurons in response to MPTP exposure than wild-type mice[14].

Pesticide exposures have long been implicated in idiopathic PD. The herbicide paraquat (PQ), in particular, is of interest because it is structurally similar to the toxic MPTP metabolite. In fact, PQ is used to induce Parkinsonism in some animal models[15]. The cellular toxicity of PQ is due primarily to redox cycling, in which PQ is reduced to form a PQ mono-cation free radical. The PQ mono-cation free radical is then rapidly re-oxidized in the presence of oxygen generating the superoxide radical (O_2^-), which sets off a well-known cascade of reactions leading to the generation of other ROS[15]. Animal models have also shown that combination exposures to PQ and the fungicide maneb (MB) result in even greater PD related pathology[16]. Maneb and paraquat (MB/PQ) are often used on the same crops in California. In our population, we have shown that exposures to agricultural application of MB/PQ near participants residence greatly increased the risk of developing PD[17]. We hypothesize that genetic variations in *NFE2L2* and *PPARGC1α* may modify the endogenous antioxidant response, and thus the risk associated with MB/PQ exposure in our population.

Here we aim investigate the influence of variation in an expression-altering region of *NFE2L2* and candidate *PPARGC1α* SNPs on PD risk and symptom progression in our population-based study of PD patients and community controls. And further, we explore how genetic variation modifies the risk of PD associated with exposure to oxidative stress inducing pesticides, MB/PQ.

Methods

All procedures described were approved by the University of California at Los Angeles (UCLA) Human Subjects Committee and informed consent was obtained from all participants.

Study Population

To investigate PD onset, we used 472 PD patients and 532 controls of European ancestry from the Parkinson's Environment and Gene (PEG) population-based case-control study living in three agricultural Central California counties (Kern, Fresno, and Tulare), enrolled between 2001–2015. Participants were considered eligible if they were 35 or older, had lived in California for at least 5 years, and were residing in one of the three counties at the time of enrollment. All patients were seen by study movement disorder specialists (JB, YB) at least once at baseline, many on multiple occasions, and confirmed as having probable idiopathic PD according to published criteria[18].

Patients were recruited initially (from 2001–2007) through large medical groups, neurologists, and public service announcements, and then from 2010–2015 through the state-mandated pilot California Parkinson's Disease Registry (CAPDR), see figure 1. During the first round (2001–2007) of patient recruitment, we identified 1,167 potential PD patients; 604 were not eligible (397 not diagnosed with PD within 3 years of recruitment, 134 did not live in the three county study area, and 73 did not have PD). Of 563 potential cases, our movement disorder specialists were able to examine 473 patients. Of these examined patients, 94 did not meet criteria for idiopathic PD, 13 were reclassified as not having idiopathic PD during follow-up, and 6 subjects withdrew so that 360 incident (diagnosed within 3 years) PD patients were enrolled. For the second round of case recruitment (2010–2015), there were 4,672 registry recorded potential PD patients with an address in the three county study area; we were able to contact and assess case reporting accuracy for 2,363. Overall 1,648 were found not to be eligible for our study (158 were diagnosed with PD more than 3 years before recruitment, 327 did not have PD, 935 were deceased, 156 were too ill, institutionalized, or unable to communicate/contact, and 92 lived outside the three county area), and 247 potential patients refused to participate. Out of 581 potential cases, 472 were seen by our movement disorder specialists at the time of this analysis; 69 participants did not have idiopathic PD, and for 10 a PD diagnosis could not be established reliably, 13 were too ill, and 1 withdrew. Thus during the second round, we enrolled 376 confirmed PD patients. However, genotyping (2014) took place before the enrollment of 114 PD patients from this strategy, thus they are not included in analysis. Additionally, in order to avoid potential confounding in the genetic analysis by population stratification, we excluded 150 patients of non-European ancestry, leaving 472 PD patients of European ancestry for analysis.

A total of 879 controls were enrolled from 2001–2011 in the same three county area and included in genotyping. Initially, we identified potential eligible controls through Medicare enrollee lists (2001) but switched to publicly available residential tax-collector records after the Health Insurance Portability and Accountability Act (HIPAA) was implemented. We employed two sampling strategies: 1) random selection of residential parcels and mail or phone enrollment and 2) clustered random selection of five households we visited in person[19]. Using the first sampling strategy, we contacted 755 eligible control participants. Of these eligible control participants, 409 declined participation and 346 population controls were enrolled. Additionally, from an early mailing with an unknown number of eligible subjects who declined, we enrolled 62 controls. We identified 1,241 eligible population controls from the second sampling strategy, 634 declined participation and 607 individuals

were enrolled, although 183 only completed an abbreviated interview and 77 participants were not genotyped. Of the 755 controls with the necessary data, we excluded 221 participants of non-European ancestry, leaving 534 controls for analysis.

For our PD progression analyses, we relied on a prospective, longitudinal patient only cohort, which attempted to follow patients from the first round of recruitment (n=360). These patients were invited to participate (for more detail see Ritz etal 2012[20]). Briefly, at first attempted re-contact, 108 patients (29%) could not be re-examined (64 were deceased, 6 too ill, 17 withdrew, and 21 could not be contacted). Of the remaining 252 patients, 11 did not provide the data necessary for the progression analyses, and we excluded 49 participants of non-European ancestry. Of the 192 patients included in the longitudinal analyses, 37 participated in two exams and 155 in three, for a mean follow-up of 5.3 years (SD=2.1) and a mean of 7.3 years of PD duration (SD=2.8). Figure 1 details the flow of recruitment for both the PD onset and progression studies.

Data Collection

Trained interviewers recorded information on demographic and risk factors for all patients and controls; physical exams for patients were performed by movement disorder specialists (JB, YB) at baseline and at each follow-up, confirming PD diagnosis and assessing disease progression. Motor symptoms were assessed by the physicians with the Unified Parkinson's Disease Rating Scale (UPDRS) part III. A higher score indicates worse motor symptoms. If possible, patients were examined off PD medications (82% of the baseline exams and 80% of follow-up exams). For patients we could only examine on medication, we estimated an off-score value by adding the difference of the whole study population's mean off- and mean on- scores at the time of exam to the patient's on-score[20]. Cognitive function was assessed at each exam with the Mini-Mental State Exam (MMSE), a common 30-point test, including tests of orientation, attention, memory, language, and visual-spatial skills. A lower score indicates worse cognitive performance. A 26-point telephone version of the MMSE exam, validated to estimate the in-person MMSE, was administered in lieu of an in-person exam for 3 patients at baseline exams and 6 at the first follow-up; for these participants, we applied validated weights to create scores comparable with the 30-point in-person interview[21].

Maneb/Paraquat Pesticide Exposure

We estimated ambient exposure to pesticides, primarily from commercial agricultural application, using a geographic information system (GIS) based computer model. More information on this method has been published[22], which we briefly summarize here. The model links California pesticide use reports (CA-PUR), which are state mandated for all commercial pesticide application since 1974 and contain information on date, location, type and amount of pesticide applied[23], with land use surveys providing the location of specific crops[24], and with geocoded lifetime address histories for each of our participants (both residential and occupational addresses). For both maneb (MB) and paraquat (PQ), we summed the pounds of each pesticide applied per year and per acre within a 500-m buffer of each geocoded address to create a study-period (1974- diagnosis or baseline interview (controls)) average exposure by summing the pounds applied each year and dividing by the

number of years in the time period. We dichotomized exposure to both MB and PQ according to the pesticide-specific median average exposure in the exposed control population. We considered participants exposed to MB/PQ if they were exposed above the median to the one or both of the chemicals based on the dichotomized measure.

SNP Selection and Genotyping

We selected three SNPs from the *NFE2L2* gene, rs35652124 (base pair (BP) position -653), rs6706649 (BP -651), rs6721961 (BP -617), that make up a haplotype in the promoter region of the gene, which has been associated with transcriptional activity[5,6]. Rs35652124 and rs6706649 are only two base pairs apart from each other, and are in linkage disequilibrium (LD) as measured with D′ (D′=1), meaning there is no evidence of recombination between the SNPs. This is seen in both our population controls and the 1000 Genomes CEU population. However, we estimated R-square between the SNPs to be 0.43. Thus, those with the minor allele at rs6706649 (minor allele frequency (MAF)=0.12) will have the minor allele at rs35652124 (MAF=0.30), but the inverse is not necessarily the case. Also, for rs6721961 (MAF=0.13) the LD with the other SNPs is of moderate size (R-square=0.43). Thus, we analyzed SNPs individually, however, in secondary analysis, we inferred haplotype frequencies from the three SNPs (ordered rs35652124, rs6706649, rs6721961 based on genome location) using the PHASE haplotype software, to compare with previous reports. PHASE uses a Bayesian approach to provide *a-posteriori* haplotype estimates assuming *a priori* haplotype distributions based on a coalescent genealogy[25,26].

We further selected four *PPARGC1α* SNPs, three tag SNPs based on previous marginal genetic associations, rs6821591, rs2970848, and rs4235308, and one missense polymorphism, rs8192678 (Gly482Ser), where variants alter the protein sequence and structure for PGC-1α[13]. The *PPARGC1α* SNPs were in low to moderate LD among controls (R-square between 0.01 and 0.49). DNA was extracted from blood or saliva samples at the UCLA Biologic Specimen Core Facility. Genotyping was done using the Fluidigm BioMark system (Fluidigm Corporation, South San Francisco, CA).

Statistical Methods

We limited our study population to non-Hispanic participants of European ancestry due to concerns about population stratification. We examined Hardy-Weinberg equilibrium in control participants for all polymorphisms using a chi-square test. To evaluate differences between patients and controls and those followed versus those lost to follow-up in the cohort we used either chi-square (categorical variables) or student's two-tailed t-tests (continuous variables). For genetic analyses related to PD onset and progression, we relied on an allelic genetic model to examine the influence of alleles; we used the same model for haplotype analyses. As each individual has two alleles, one on each paired chromosome, the sample size is twice the number of individuals (2n). For the *NFE2L2* haplotype we set the reference level to the "TCT" haplotype, in order to assess the influence of the "TCG" haplotype, the most frequent in our population, which was previously reported as being related to PD risk[6].

For PD susceptibility analyses, we used unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs). For PD progression, we used repeated-

measures linear regression analyses (Proc MIXED; SAS 9.4, SAS Institute, Cary, NC) to investigate associations between alleles and symptom scores (MMSE, UPDRS) over time. In primary, hypothesis-based testing, we assessed statistical interactions with MB/PQ exposure for SNPs associated with PD onset or progression by introducing a multiplicative interaction term into the logistic models and additive in linear repeated measures models. For linear models, we report the interaction term between SNPs and age (in lieu of follow-up time due to collinearity), which allows us toes timate the difference in annual change in score for the outcome measures by alleles; age refers to the age at each exam, centered at the mean age at time of baseline exam (68.9 years). In secondary, exploratory analysis we assess interactions with other SNPs.

In all models, we adjusted for age (at interview for controls and diagnosis for patients), sex, smoking status (ever/never), and education (<12 years, 12 years, >12 years). All analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Demographic characteristics of the PD onset and progression study population can be found in table 1. The PD patients were slightly older (69.4 vs 67.7 years), had less years of education, a lower proportion of smokers, and a higher proportion of males and MB/PQ pesticide exposure relative to the control population. In the PD progression study, the patients followed were younger, had more years of education, and scored better on baseline PD symptom scores (UPDRS and MMSE) compared to the patients lost to follow-up; see Ritz et al, 2012 for a more in depth discussion on the progression cohort and attrition due to death and withdrawal. There were no statistically significant differences in the allele frequencies between patients included in the PD progression study and those patients lost to follow up (supplemental table 1).

All SNPs investigated were in HWE in the control population. The individual *NFE2L2* SNP allele frequencies and PD associations can be found in table 2. *NFE2L2* rs6721961 was marginally associated with PD onset (Tvs G (ref): OR=0.70, 95% CI=0.53, 0.94, p=0.02). SNP associations with progression are found in table 3. The same *NFE2L2* rs6721961 “T” allele was also associated with significantly slower annual cognitive decline as measured with the MMSE (higher MMSE, better cognition) in the PD symptom progression cohort (β=0.095, SE=0.03, p=0.0004) relative to the wildtype “G” allele. This association remains significant even after a strict Bonferroni multiple testing correction (14 marginal genetic analysis tests, significance level 0.05/14=0.0036). The *NFE2L2* rs6706649 “T” allele was associated with the slower motor symptom progression as measured by the UPDRS (lower UPDRS, better movement) (β=−0.257, SE=0.12, p=0.03) relative to the “C” allele.

In sensitivity analyses we analyzed the *NFE2L2* haplotype for comparison with prior investigations. The *NFE2L2* “CCG” haplotype was associated with a significant increase in risk of developing PD (OR=1.39, 95% CI=1.01, 1.91), and the “TCG” haplotype with an even larger risk increase (OR=1.50, 95% CI=1.11, 2.03) relative to the “TCT” haplotype in our population (supplemental table 2). The same two haplotypes, “TCG” and “CCG”, were also associated with faster cognitive decline (“TCG”: β=−0.101, SE=0.03, p=0.0003;

"CCG":β= −0.099, SE=0.02, p=0.0006) relative to the "TCT" genotype (supplemental table 3). Again, these progression associations remain significant after Bonferroni multiple testing correction (20 marginal haplotype analysis tests, significance level 0.05/20=0.0025); none of the *NFE2L2* haplotypes were associated with annual change in the UPDRS score (supplemental table 3). After running haplotype analyses, we found in our population there was not a meaningful gain in information using the haplotypes over rs6706649 alone (models are nested and compared using a likelihood ratio test, p=0.37).

None of the *PPARGC1α* SNPs were marginally associated with PD onset (table 2). *PPARGC1α* rs6821591 was strongly associated with faster motor symptom progression (Tvs C (ref): β=0.234, SE=0.07, p=0.0013; table 3). None of the other *PPARGC1α* SNPs were associated with PD onset or symptom progression.

Assessing the effects of the SNPs associated with PD onset or progression (*NFE2L2* rs6721961, rs6706649, and *PPARGC1α* rs6821591) in combination with MB/PQ exposure, our results suggest an interaction between *PPARGC1α* rs6821591 and exposure. We found no differences in risk by the T allele or high exposure alone, but those with joint exposure and T allele were at an increased risk of PD (OR=1.30, 95% CI=1.01, 1.69; table 4). When introducing the interaction into the linear repeated measures progression models, we still find the *PPARGC1α* rs6821591 T is strongly associated with faster motor symptom progression, but did not detect a significant interaction with MB/PQ exposure (table 5).

We did not detect statistical interactions between either *NFE2L2* SNP and MB/PQ exposure on PD onset (table 4). We did estimate an interaction between exposure and the rs6721961 and motor symptom progression (table 5). We found that while MB/PQ exposure was associated with higher UPDRS scores (worse symptoms; (β=1.552, SE=0.76, p=0.041; table 5) and exposure in conjunction with the rs6721961 protective T allele was associated with ~4.6 points less on the UPDRS relative to the G allele (β=−4.567, SE=2.27, p=0.044; table 5).

In secondary, exploratory analysis, in which we continued to use an allelic model, we also found a statistical interaction between *PPARGC1α* rs8192678 and MB/PQ and PD onset (p=0.05), again finding those with both high exposure and the variant allele were at the highest risk of PD (OR=1.47, 95% CI=1.12, 1.93; supplemental table 4); and near significant statistical interaction between the same rs8192678 and cognitive symptom progression (p=0.085), such that those with exposure and the variant allele showed faster cognitive decline (supplemental table 5). Additionally, we estimated a near significant interaction between *PPARGC1α* rs2970848, MB/PQ, and PD onset (p=0.09, supplemental table 4).

Since several movement disorder specialists performed the UPDRS-III assessment in our study, we also conducted sensitivity analysis for the UPDRS-III based progression models that included the clinician examiner as a covariate. Results only changed minimally and none of the SNPs lost statistical significance (supplemental table 6).

Discussion

Oxidative stress and pathways associated with cellular oxidative stress like mitochondrial dysfunction are generally thought to contribute to PD etiology. Nrf2 and PGC-1α play complementary and overlapping roles in regulating the endogenous cellular antioxidant defense system[4]. Here, we provide support for the involvement of an expression-altering *NFE2L2* rs6721961 promotor SNP with PD onset based on our case/control data and importantly also found it contributes to faster cognitive decline in patients followed longitudinally. We also identified exonic and intronic SNPs in *PPARGC1α* as modifiers of MB/PQ pesticide exposure risk in PD onset, and our progression data from patients suggested a possible involvement in motor symptom progression as well for variants in this gene.

The three *NFE2L2* promoter SNPs have been shown together to influence functional activity, with rs6721961 affecting basal *NFE2L2* expression[5]. This same SNP, rs6721961, was the only SNP marginally associated with PD risk in our population, and further showed a strong association with cognitive symptom progression. Nrf2 is a ubiquitous transcription factor, activating a host of antioxidant response element (ARE) related genes. Rs6721961 specifically has been found to significantly influence luciferase activity of promoter constructs containing the SNP and binding affinity[5]. Epidemiologic evidence for a role of *NFE2L2* in PD is limited and rs6721961 has not been associated with PD in any GWAS, including PD Gene. This association in our population might be observable due to ubiquitous oxidative stress inducing pesticide exposures. More than 60% of our population has lived or worked in close proximity to commercial applications of paraquat, primarily for agriculture[27].

Our haplotype findings (supplemental table 2 and table 3) contradict those from a previous Polish study; while we associate the “TCG” haplotype with an increased risk of PD, they estimated a protective influence of the same haplotype against PD risk (OR=0.6, 95% CI=0.4, 0.9)[6]. However, this European association lost magnitude and significance when combined with other European populations in a meta-analysis (OR=0.92, 95% CI=0.81, 1.04)[7]. Study population heterogeneity or disease misclassification may explain between-study inconsistencies[28]. But it is also plausible that the differences in findings may have resulted from unaccounted for environmental factors which modify the genetic response, given that Nrf2 is regulated and activated through oxidative stress related pathways. Our population was recruited from highly agricultural communities, and we have found a number of specific pesticides to be positively associated with PD risk in our studies, many which may induce oxidative stress[19,29,30]. This is supported in our analysis, as we estimated a statistical interaction between rs6721961 and MB/PQ pesticide exposure in modeling motor symptom progression (table 5). MB/PQ pesticides have been previously associated with PD risk in our population and experimentally with reactive oxygen species (ROS) production[16,17].

In our PD progression cohort, we detected a strong association between the same *NFE2L2* rs6721961 protective allele (T), and slower cognitive symptom progression. Nrf2 is not only a therapy target for PD, but for several neurodegenerative diseases, including Alzheimer’s

disease (AD)[31]. Cognitive decline and dementia in PD and AD are hypothesized to have overlapping etiologies, supported by observations of PD patients with dementia having a higher cortical amyloid-β(Aβ) plaques burden (a feature of AD) than PD patients without dementia and many AD patients are found to have Lewy bodies (a feature of PD)[32,33]. Furthermore, *NFE2L2* variation has been associated with faster progression of AD[34], supporting the involvement of *NFE2L2* in cognition decline.

We did not detect any marginal associations between the *PPARGC1α* SNPs and PD risk. However, *PPARGC1α* rs6821591 (T) was strongly associated with faster motor symptom progression. PGC-1α, like Nrf2, binds to promoter regions of AREs, genes coding for antioxidant enzymes, to upregulate their transcription[4]. PGC-1α regulated antioxidant enzymes, however, are believed to exert their influence primarily on mitochondria[4]. Our results, therefore, seem to implicate mitochondrial related oxidative stress as relevant for PD progression. Interestingly, the same *PPARGC1α* SNP rs6821591, as well as rs8192678, seem to modify the risk of MB/PQ pesticide exposure in our case/control population. We have previously reported in our population that ambient agricultural MB/PQ exposure increases the risk of PD[17]. Paraquat has been widely used to create animal models of PD, and its cytotoxicity is heavily related to ROS production[16]. Combination exposures (maneb and paraquat) have been shown to result in even greater PD related pathology in the animal models[16]. Of particular note, MB/PQ pesticides have also been related to mitochondrial dysfunction[35]. Again the involvement of *PPARGC1α* in PD risk related to MB/PQ exposure further implicates mitochondrial dysfunction and related ROS generation as an important mechanism for neurodegeneration in the dopaminergic system. This supports a growing body of evidence that *PARGC1α* contributes to neurodegenerative disorders, including PD. As mentioned earlier, both of these SNPs (rs6821591 and rs8192678) have previously been associated with age of PD onset and the others with longevity; *PPARGC1α* is also associated with AD, Huntington's disease, and amyotrophic lateral sclerosis (ALS)[13,36–38].

The PEG case/control study population provides many advantages that allow us to pose and investigate mechanistic hypotheses. Most epidemiologic studies rely of self-reported information for pesticide exposure assessment, a method prone to differential recall error, and which generally does not allow for investigation of specific pesticides. We assessed ambient MB/PQ exposure with a GIS approach utilizing state mandated pesticide use reports. Thus, we do not rely on participant recall for pesticide use, and are able to investigate specific chemicals. However, biomonitoring over decades to document chronic pesticide exposure is not feasible in human populations and our pesticide exposure measurement method does not account for factors such as wind patterns at the time of application, geographic features that may influence pesticide drift, and assumes that participants were at the recorded location during relevant time periods during or after applications occurred. Thus, there is a possibility for exposure misclassification. However, since this would be the same in cases and controls, the resulting bias would most likely move our estimates of effect towards not finding any association. Additionally, while a prior study has shown that the three *NFE2L2* SNPs affect transcriptional activity, we do not have any measures of transcription activity in our population to corroborate this.

PD is a commonly misdiagnosed disease[39,40]; our PD patients were all seen and well characterized by UCLA movement disorder specialists at least once and many multiple times as part of our PD progression cohort, minimizing bias from disease misclassification. Additionally, the population controls were drawn from the same region as the cases, likely providing adequate representativeness of the source population. Further, our prospectively followed PD progression study cohort is one of less than a handful of population-based PD patient cohorts worldwide, and the first to our knowledge to investigate these genes. We were able to follow patients on average more than seven years into disease. Although, as expected in a cohort of elderly patients, we were not able to follow-up all patients enrolled at baseline, mostly because the patient was too ill or deceased (n=70). Those lost to follow-up were older and scored worse on baseline health indicators UPDRS and MMSE (table 1); consequently, selection bias is possible, although there were no differences in allele frequencies by follow-up status (supplemental table 1).

Additionally, given that we do not have follow-up data on a non-PD population, we cannot tell whether the longitudinal findings are specific to cognitive decline in PD or whether the same type of decline would be observed among a matched control population. Though, our study provides an independent population of adequate sample size (≥80% power to detect previously reported marginal effect sizes), and we were able to restrict to Caucasian participants of European ancestry to limit confounding by population stratification.

Although our findings need to be re-examined and replicated in future studies with larger sample sizes and longer follow-up, our study provides support for the involvement of *NFE2L2*, specifically the expression-altering SNP rs6721961, and *PPARGC1α* in both PD susceptibility and symptom progression, and modifying PD risk in MB/PQ exposed subjects, consistent with the importance of oxidative stress-inducing mechanisms in PD onset and progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Nuclear factor-erythroid 2 related factor 2 (Nrf2) and peroxisome proliferator activator receptor (PPAR) γ coactivator 1α (PGC-1α) are important transcription factors that activate antioxidant defense mechanisms
- In 472 PD patients and 532 population-based controls, we examined three expression-altering *NFE2L2* SNPs and four previously implicated *PPARGC1α* SNPs and their interactions with maneb and paraquat (MB/PQ) pesticide exposure on Parkinson's disease (PD) risk and symptom progression
- *NFE2L2* rs6721961 T allele was associated with a reduced risk of PD and slower cognitive decline
- Statistical interactions were estimated between MB/PQ and two *PPARGC1α* SNPs, such that those with high exposure and the variant allele were at an increased risk of PD
- *PPARGC1α* rs6821591 was associated with faster motor symptom progression as measured with the UPDRS-III
- Our study provides support for the involvement of both *NFE2L2* and *PPARGC1α* in PD susceptibility and progression, marginally and through pathways involving MB/PQ exposure

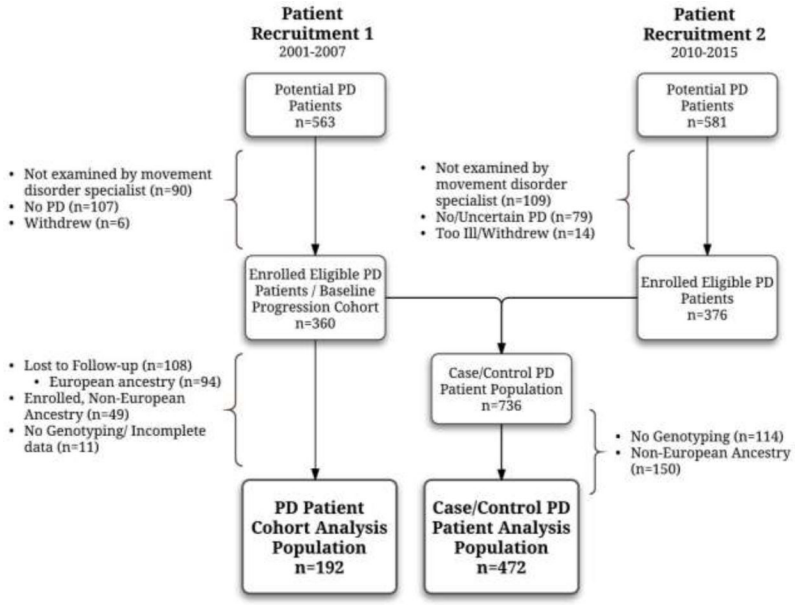

Figure 1.

PD patient recruitment flow diagram for both the PD onset and progression studies

Table 1

Population exposure and characteristics of PEG onset and progression study populations with genotyping and European ancestry only.

	PD Onset Study			PD Progression Cohort		
Variable (Mean ± SD/n (%))	**PD Patients n=472**	**Controls n=532**	**P value**	**PD Patients w/follow-up n=192**	**PD Patients lost to follow-up n=94**	**P value**
Age (y)	69.40 ± 10.0	67.7 ± 11.7	0.01	67.5 ± 9.9	72.4 ± 10.6	0.0002
Male	290 (61)	265 (50)	0.0002	107 (56)	54 (57)	0.78
Ever Smoker	213 (45)	285 (54)	0.008	81 (42)	48 (51)	0.16
Education (years)						
<12	40 (8)	34 (6)		16 (8)	16 (17)	
12	122 (26)	107 (20)	0.03	56 (29)	28 (30)	0.07
>12	310 (66)	391 (74)		120 (63)	50 (53)	
UPDRS III[a]	21.04 ± 10.2	--	--	18.8 ± 9.0	24.1 ± 11.0	<.0001
MMSE[a]	27.77 ± 2.6	--	--	28.3 ± 2.1	26.8 ± 3.0	<.0001
High MB/PQ Exposure	252 (53)	249 (47)	0.04	102 (53)	47 (50)	0.62
Follow-up (y)	--	--	--	5.3 ±2.1	--	--

Abbreviations: MB/PQ=Maneb/Paraquat pesticide; UPDRS=Unified Parkinson's Disease Rating Scale; MMSE=Mini-Mental State Exam.

[a]Scores at baseline (case/control study) interview

Table 2

Marginal *NFE2* and *PPARGC1A* SNP associations with PD onset, assuming an allelic genetic model (2n).

SNP	Major/Minor Allele	Major Allele (ref)		Minor Allele		
		Case/Control	OR (95% CI)	Case/Control	OR (95% CI)	P-value
NFE2 rs35652124	T/C	647/738	1.00 (ref)	267/314	1.00 (0.82, 1.22)	0.97
NFE2 rs6706649	C/T	823/928	1.00 (ref)	107/130	0.93 (0.71, 1.23)	0.61
NFE2 rs6721961	G/T	844/924	1.00 (ref)	90/138	0.70 (0.53, 0.94)	0.02
PPARGC1A rs6821591	C/T	479/537	1.00 (ref)	451/511	1.00 (0.83, 1.19)	0.96
PPARGC1A rs8192678	G/A	608/703	1.00 (ref)	320/349	1.06 (0.88, 1.28)	0.55
PPARGC1A rs2970848	A/G	615/710	1.00 (ref)	317/348	1.03 (0.85, 1.25)	0.76
PPARGC1A rs4235308	T/C	562/652	1.00 (ref)	370/402	1.04 (0.87, 1.26)	0.64

Models control for age, sex, smoking status, and education

Table 3

NFE2L2 and PPARGC1A SNPs and PD progression outcomes, UPDRS (motor score) and MMSE (cognitive score), using linear repeated measures model, assuming an allelic genetic model (2n).

Loci/Allele	n (%)	UPDRS			MMSE		
		β[a]	SE	P Value	β[a]	SE	P Value
***NFE2L2* SNPs**							
rs35652124							
T	264 (0.69)	ref	--	--	ref	--	--
C	118 (0.31)	0.123	0.08	0.12	−0.015	0.02	0.34
rs6706649							
C	339 (0.88)	ref	--	--	ref	--	--
T	45 (0.12)	−0.257	0.12	0.03	0.037	0.02	0.10
rs6721961							
G	343 (0.89)	ref	--	--	ref	--	--
T	41 (0.11)	0.063	0.14	0.64	0.095	0.03	0.0004
***PPARGC1A* SNPs**							
rs6821591							
C	204 (0.53)	ref	--	--	ref	--	--
T	180 (0.47)	0.234	0.07	0.001	−0.011	0.01	0.45
rs8192678							
G	264 (0.69)	ref	--	--	ref	--	--
A	120 (0.31)	−0.106	0.08	0.20	−0.008	0.02	0.64
rs2970848							
A	244 (0.63)	ref	--	--	ref	--	--
G	140 (0.36)	0.115	0.07	0.12	−0.014	0.01	0.31
rs4235308							
T	233 (0.61)	ref	--	--	ref	--	--
C	151 (0.39)	−0.043	0.07	0.56	0.003	0.01	0.82

Models control for age, sex, smoke, education, and PD duration prior to baseline (0–3 years)

[a] β represents interaction with age (centered); e.g. annual difference in outcome score over

Table 4

Maneb/Paraquat Pesticide Exposure- SNP interactions and PD onset, assuming an allelic genetic model.

SNP	No/low MB/PQ Exposure			High MB/PQ Exposure			Interaction P Value
	Case/Control	OR (95% CI)	P Value	Case/Control	OR (95% CI)	P Value	
***NFE2L2* rs6706649**							
C	389/502	1.00 (ref)	--	436/426	1.30 (1.07, 1.58)	0.008	--
T	47/62	1.02 (0.68, 1.53)	0.92	60/68	1.09 (0.75, 1.60)	0.64	0.50
***NFE2L2* rs6721961**							
G	400/490	1.00 (ref)	--	445/434	1.21 (1.00, 1.47)	0.05	--
T	36/74	0.56 (0.37, 0.86)	0.008	55/64	1.03 (0.69, 1.53)	0.89	0.16
***PPARGC1A* rs6821591**							
C	232/263	1.00 (ref)	--	249/274	1.02 (0.80, 1.32)	0.85	--
T	200/295	0.79 (0.61, 1.02)	0.07	251/216	1.30 (1.01, 1.69)	0.05	0.009

Models control for age, sex, smoke, and education

Table 5

Maneb/Paraquat Pesticide Exposure-SNP interactions and PD susceptibility, assuming an allelic genetic model.

Model Term	MMSE		UPDRS	
	β Coefficient	P value	β Coefficient	P value
***NFE2L2* rs6706649**				
Age	−0.061	<.0001	0.221	<.0001
T allele (vs C ref)*Age	0.037	0.107	−0.252	0.031
Maneb/Paraquat	0.136	0.361	0.923	0.224
T allele*Maneb/Paraquat	−0.223	0.616	0.659	0.775
***NFE2L2* rs6721961**				
Age	−0.064	<.0001	0.191	<.0001
T allele (vs G ref)*Age	0.096	0.0004	0.054	0.693
Maneb/Paraquat	0.078	0.598	1.552	0.041
T allele*Maneb/Paraquat	0.262	0.560	−4.567	0.044
***PPARGC1A* rs6821591**				
Age	−0.061	<.0001	0.098	0.033
T allele (vs C ref)*Age	0.010	0.487	0.233	0.001
Maneb/Paraquat	0.317	0.095	0.945	0.328
T allele*Maneb/Paraquat	−0.479	0.086	−0.016	0.991

Abbreviations: MMSE = Mini-mental State Exam; UPDRS=Unified Parkinson's Disease Rating Scale

Models control for sex, smoke, education, and PD duration prior to baseline (0–3 years)

Results shown as regression coefficients (β); interaction term between allele and age represents the difference in annual score change between the alleles