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Pooled analysis of the HLA-DRB1 by smoking interaction in Parkinson's disease

Yu-Hsuan Chuang¹, Pei-Chen Lee², Tim Vlaar³, Claire Mulot⁴, Marie-Anne Lorient⁵, Johnni Hansen⁶, Christina M. Lill⁷, Beate Ritz^{1,8,9}, and Alexis Elbaz³

¹Department of Epidemiology, Fielding School of Public Health, University of California, Los Angeles (UCLA), Los Angeles, USA

²Department of Health Care Management, College of Health Technology, National Taipei University of Nursing and Health Sciences, Taipei, Taiwan

³Université Paris-Saclay, Univ. Paris-Sud, UVSQ, CESP, INSERM, Villejuif, France

⁴INSERM UMR-S 1147, CRB EPIGENETEC, Univ. Paris Descartes, Sorbonne Paris Cité, 75006 Paris, France

⁵Assistance Publique-Hopitaux de Paris, Hopital Européen Georges Pompidou, Biochimie, Pharmacogénétique et Oncologie Moléculaire, 75015 Paris, France

⁶Danish Cancer Society Research Center, Danish Cancer Society, Copenhagen, Denmark

⁷Genetic and Molecular Epidemiology Group, Institute of Neurogenetics, University of Luebeck, Luebeck, Germany

⁸Department of Neurology, School of Medicine, UCLA, Los Angeles, USA

⁹Department of Environmental Health Sciences, Fielding School of Public Health, UCLA, Los Angeles, USA

Abstract

Objective—Inflammatory response plays an important role in Parkinson's disease (PD). Previous studies have reported an association between human leukocyte antigen (HLA)-*DRB1* and the risk of PD. There has also been growing interest in investigating whether inflammation-related genes interact with environmental factors such as smoking to influence PD risk. We performed a pooled analysis of the interaction between *HLA-DRB1* and smoking in PD in three population-based case-control studies from Denmark and France.

Methods—We included 2,056 cases and 2,723 controls from three PD studies (Denmark, France) that obtained information on smoking through interviews. Genotyping of the rs660895 polymorphism in the *HLA-DRB1* region was based on saliva or blood DNA samples. To assess

Correspondence: Alexis Elbaz, Inserm U1018-CESP, Hôpital Paul Brousse, Bâtiment 15/16, 16 avenue Paul Vaillant-Couturier, 94807 Villejuif cedex, France. alexis.elbaz@inserm.fr.

Author Contributions:

AE and BR contributed to the conception and design of the study; AE, BR, CM, MAL, CL, and JH contributed to the acquisition of data; YC, PL, and TV contributed to the analysis of data; YC and AE contributed to drafting the text and preparing the tables.

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interactions, we used logistic regression with product terms between rs660895 and smoking. We performed random-effects meta-analysis of marginal associations and interactions.

Results—Both carrying rs660895-G (AG vs. AA: OR= 0.81; GG vs. AA: OR= 0.56; p-trend=0.003) and ever smoking (OR=0.56, p<0.001) were inversely associated with PD. A multiplicative interaction was observed between rs660895 and smoking using codominant, additive (interaction parameter=1.37, p=0.005), and dominant (interaction parameter=1.54, p=0.001) genetic models without any heterogeneity ($I^2=0.0\%$): the inverse association of rs660895-(AG+GG) with PD seen in never smokers (OR=0.64, p<0.001) disappeared among ever smokers (OR=1.00, p=0.99). Similar interactions were observed when we investigated light and heavy smokers separately.

Interpretation—Our study provides the first evidence that smoking modifies the previously reported inverse association of rs660895-G with PD, and suggests that smoking and *HLA-DRB1* are involved in common pathways, possibly related to neuroinflammation.

Keywords

Parkinson's Disease; inflammation; immune system; HLA-DRB1; smoking

The human leukocyte antigen (HLA) system that encodes the major histocompatibility complex (MHC) proteins is a crucial part of the immune system. Class II MHC presents extracellular antigens to T lymphocytes. HLA-DR is a MHC class II cell surface receptor encoded by the human leukocyte antigen complex on chromosome 6p21.31 and is composed of an alpha and beta chain coded by the *HLA-DRA* and *HLA-DRB* genes, respectively. While the *HLA-DRA* locus is largely monomorphic and not prominent in HLA-disease association studies, the *HLA-DRB* locus has been reported to influence both autoimmune (e.g., rheumatoid arthritis,^{1,2} multiple sclerosis^{3,4}) and neurodegenerative diseases (e.g., Alzheimer's disease,⁵ Parkinson's disease (PD)^{6–10}).

We previously examined 51 single nucleotide polymorphisms (SNPs) in the *HLA-DR* region in two French case-control datasets. We found that rs660895-G (in the *HLA-DRB1* region) showed the strongest association with PD (OR=0.70, 95% CI=0.57–0.87),⁷ and did not confirm the previously reported association with the *HLA-DRA* locus.⁶ In the same study, SNP-based imputation of HLA alleles showed an inverse association with the *HLA-DRB1*04* allele.⁷ Rs660895 was also the strongest HLA region hit in a genome-wide association study.⁸ While a British case-control study also reported a lower frequency of the *HLA-DRB1*04* allele in PD patients than in matched controls,⁹ a Chinese study found a reduced risk of PD associated with the *HLA-DRB1*0406* allele.¹⁰ Finally, based on SNP-based imputation of HLA alleles, two GWAS showed an inverse association between *HLA*DRB1*0404* and PD.¹¹

PD is likely to be caused by a combination of genetic and environmental factors, acting in concert with each other. While smoking increases the risk of many diseases, in particular through inflammatory mechanisms,^{12,13} it has consistently been shown to be inversely associated with PD.¹⁴ It remains unknown, however, whether this robust inverse association is causal or not. Some have argued that it cannot be explained by survival bias or

confounding,¹⁵ but reverse causation cannot be discarded as an alternative explanation, due to increasing evidence of a long premotor period.^{16,17}

The identification of gene-environment interactions represents a possible approach to better understand the biological mechanisms underlying the association between environmental exposures and disease and to improve causal inference. In our previous study, we showed that the rs660895-PD association was restricted to never smokers (OR=0.63, 95% CI= 0.49–0.81) while there was no significant association in ever smokers (OR=0.93, 95% CI=0.61–1.37); however, the interaction test was not significant at the traditional α -level of 0.05 ($p=0.11$),⁷ and larger studies are necessary to investigate this interaction.

The aims of this study were: (i) to investigate the interaction of rs660895 in the *HLA-DRB1* region and smoking in PD in a large population-based case-control study conducted in Denmark; (ii) to perform a pooled analysis of the smoking-rs660895 interaction based on individual data from the Danish and aforementioned French datasets.

Materials and Methods

Study population

The data used for analyses have been assembled in the Parkinson's disease in Denmark (PASIDA) study, and the French *Terre* and *Partage* studies.

The PASIDA study—The PASIDA study enrolled idiopathic PD patients treated at 10 neurological treatment centers and identified from the Danish National Hospital Register between 1996 and 2009. Population controls were selected from the Danish Central Population Registry and matched on year of birth, sex, and being alive and free of PD at the time of case identification. Detailed recruitment information was published previously.¹⁸ Briefly, from 2,762 eligible patients, 949 (34%) were excluded due to the following reasons: lack of a PD diagnosis on medical records ($n=179$), declined to participate ($n=497$), failure to confirm idiopathic PD according to accepted criteria in medical record review ($n=273$).¹⁹ From 3,626 eligible controls, 1,887 (52%) completed an interview and questionnaire. Of these, DNA samples were available for 1,575 (87%) patients and 1,607 (85%) controls. Later, 20 subjects with dementia prior to interview and 20 subjects with missing information for either rs660895, ever smoking, or covariates were excluded. Overall, 1547 PD cases and 1595 controls were retained in the analysis. Previous studies showed that the Danish population has high genetic homogeneity and that population structure is a lesser concern for large-scale gene-mapping studies in the country.²⁰

Terre and Partage studies—*Terre* and *Partage* studies two population-based case-control studies were performed within a French health insurance system for persons working in agriculture and related occupations (*Mutualité Sociale Agricole*, MSA). PD patients from 62 metropolitan districts who received treatment between 1998 and 1999 were enrolled in the *Terre* study, whereas those from five districts treated between 2006 and 2007 were enrolled in *Partage*. These patients were examined by neurologists and PD was diagnosed using standard criteria.²¹ We randomly selected eligible controls from among all MSA members who requested reimbursement for health expenses (*Terre*) or who were listed on

electronic insurance records (*Partage*). Controls were matched to cases on age, sex, and district of residency, and did not report cardinal signs of PD. Subjects with dementia or those who were bedridden were excluded. Participation rates were high and similar in cases and controls in both studies (*Terre*: cases, 83%, controls, 75%; *Partage*: cases, 82%, controls, 77%).^{22,23} We further excluded subjects lacking DNA samples, of non-European ancestry, or with missing data, leaving 202 PD and 529 controls (*Terre*) and 307 PD and 599 controls (*Partage*) for analysis.^{24,25} The majority (>90%) of the participants were of French ancestry; these populations of farmers are not very residentially mobile, with about 75% of cases and controls born in the same district as both their parents.⁷

The institutional review boards (IRB) of UCLA, the Danish Data Protection Agency, and the ethics committee of Copenhagen approved the PASIDA study, whereas the IRB of the ethnics committees of the Kremlin-Bicêtre and Pitié-Salpêtrière University Hospitals approved the *Terre* and *Partage* studies. All subjects provided written informed consent.

Data collection and smoking assessment

Participants provided information on demographics and lifestyle through telephone interviews or in writing (PASIDA) and in-person interviews (*Terre*, *Partage*). In PASIDA, we defined ever (including ex- and current) smokers as persons who had smoked at least one cigarette, pipe, or cigar/cheroot per week for at least 6 months. In the French studies, ever (including ex- and current) smokers are those who had smoked at least one cigarette per week for at least 6 months; there were few pipe or cigar/cheroot smokers and all reported smoking cigarettes too.

For all studies, we collected lifetime smoking information, i.e., the amount and duration of smoking and changes of smoking behavior, and used this information to calculate total pack-years of cigarette smoking. Only smoking behaviors that occurred prior to the date of the first motor symptom or PD diagnosis in cases and the same index date in matched controls were considered. Information on coffee drinking was available in the PASIDA and *Partage* studies only.²²

Genotyping

DNA was extracted from saliva (*PASIDA*, *Partage*) or blood (*Terre*) samples. PASIDA samples were genotyped for rs660895 using multiplex Taqman allelic discrimination assays (Applied Biosystems) according to the manufacturer's protocol. For *Terre* and *Partage* samples, rs660895 was included in a microarray that was processed by Illumina (Illumina, San Diego, CA) using Illumina technology and Infinium iSelect custom genotyping.⁷ In the *Terre* study, we previously genotyped with Taqman 3 SNPs that were also present on the microarray; the agreement between the two methods was >99% for the 3 SNPs. All three datasets passed quality checks, i.e., genotyping efficiency >95% and accuracy >99.5%; there were no difference in genotyping call rates between cases and controls.

Rs660895 is an intergenic SNP located 32.6 kb upstream of HLA-DRB1 (from the nearest transcription start site) that tags the *HLA-DRB1*04* allele. Based on chromatin immunoprecipitation sequencing and DNaseI-seq peak data (ENCODE project), this SNP was shown to be located within regulatory regions.²⁶

Statistical analysis

We assessed Hardy-Weinberg equilibrium in controls from each study using Pearson's chi-square test. Because there was no DNA for some cases and controls, for each study, we broke the matching and used unconditional logistic regression analysis adjusted for the matching factors (PASIDA: year of birth, sex, onset/index age (age at index date); *Terre*, *Partage*: index age, sex, and district of residency) to increase statistical efficiency. We first assessed the marginal associations of rs660895 and ever smoking with PD, and computed odds ratios (OR) and 95% confidence intervals (CI). We also considered family history of PD as a potential confounder. Similar to what has been previously reported in the literature, we analyzed rs660895 using co-dominant, additive, and dominant genetic models; recessive models were not considered due to the low frequency of homozygotes for the minor allele G.

We then examined the independent and joint effects of ever smoking and rs660895 in each study. In further analyses, we used pack-years of smoking defined as a three level variable (never smoker, 18, >18²⁴). The 18 pack-years cutoff was the median among ever smokers in controls in the *Terre* study, for PASIDA and *Partage*, the median pack-years among ever smokers in controls was 15 and 12, respectively; we chose the cutoff of 18 to separate light/moderate from heaviest smokers. We also conducted a sensitivity analysis using 12 pack-years as the cut-off. Multiplicative interactions between ever smoking and rs660895 were examined by introducing a product term between these variables into the regression model; the interaction parameter is the exponential of the regression coefficient associated with this product term. Interactions were tested on a multiplicative scale, as departure from multiplicativity is expected when at least one of the interacting factors is preventive.²⁷

Both for analyses of marginal associations and interactions, we pooled the estimates from the three studies using random-effects meta-analysis.²⁸ We tested for between-study heterogeneity with the chi square-based Q statistic (deemed significant for $p < 0.10$) and quantified its extent with I^2 , which ranges from 0% to 100% and represents the proportion of between-study variability ascribed to heterogeneity rather than to chance;^{29,30} I^2 values of 0%–24% suggest little heterogeneity, 25%–49% reflect moderate heterogeneity, 50%–74% reflect large heterogeneity, and >75% reflect very large heterogeneity.

Lastly, we assessed the association between rs660895 and smoking among cases and controls separately using unconditional logistic regression adjusted for onset/index age, sex, and matching factors in each study, and again pooled the three estimates using random-effects meta-analysis. Case-only studies have been shown to be statistically more powerful than case-control studies when assessing gene-environment interactions assuming that the gene-environment independence assumption holds, i.e., that rs660895 is not associated with smoking among controls.³¹

In sensitivity analyses, we checked whether there was an interaction between rs660895 and coffee drinking and whether adjusting for coffee drinking modified our results for the rs660895-smoking interaction.

All analyses were performed using SAS v9.4 (SAS Institute, Cary, NC, USA) and STATA v14.1 (Stata Corporation, College Station, TX, USA).

Power calculation

Assuming the following parameters, based on 1000 Genomes Project European ancestry samples and the literature (minor allele frequency, MAF: 28%; ever smoking: 70%; marginal genetic OR: 0.70;⁷ marginal smoking OR: 0.64;³² $n_{\text{cases}}=2,056$, $n_{\text{controls}}=2,723$), our study had a statistical power of 97.3% to detect an interaction of 1.5 (as we previously reported⁷) at a 0.05 two-sided alpha level under an additive genetic model; power was 90.0% for an interaction of 1.4. For the case-only design, our study had 99.9% statistical power to detect an interaction of 1.5 and 98.9% for an interaction OR of 1.4. Power calculations were performed in Quanto v.1.2.4 (USC, Los Angeles, CA, USA).³³

Results

The three studies included a total of 2,056 cases (PASIDA, 1,547; *Terre*, 202; *Partage*, 307) and 2,723 controls (PASIDA, 1,595; *Terre*, 529; *Partage*, 599) for analysis (Table 1). There was no deviation from Hardy-Weinberg equilibrium for rs660895 in controls in any dataset (PASIDA, $p=0.18$; *Terre*, $p=0.91$; *Partage*, $p=0.61$). The distribution of rs660895 genotypes was very similar in the controls from the two French studies. As expected, compared with control subjects, PD patients were more likely to be male, to report a positive family history of PD, and to have never smoked (Table 1). Table S1 shows the cross tabulation of smoking and rs660895 in cases and controls from each study.

In each study and in the pooled analysis, ever-smoking was inversely associated with PD (pooled OR=0.56, 95% CI: 0.49–0.64, $p<0.001$; Table 2) with a dose-effect relation (p for trend <0.001) and without any heterogeneity across studies ($I^2=0.0\%$). Similar to what we reported previously,⁷ we observed an inverse association between rs660895-G and PD in the Danish study (OR per one-minor allele=0.85, 95% CI=0.75–0.96, $p=0.010$). In the French studies, OR estimates adjusted solely for onset/index age and sex did not differ from estimates further adjusted for district of residency, thus only data from the former analysis are shown. The inverse association between rs660895-G and PD was significant in pooled analyses (OR per one-minor allele=0.79, 95% CI=0.68–0.93, $p=0.003$) with moderate heterogeneity ($I^2=31.3\%$) and no heterogeneity under a dominant model (OR=0.79, 95% CI=0.70–0.90, $p<0.001$, $I^2=0.0\%$).

Table S2 shows analyses of the interaction between smoking and rs660895 in each study and Table 3 shows results of the pooled analysis. In PASIDA, the interaction between ever smoking and rs660895 was statistically significant, no matter which genetic model was considered. Under the dominant model, the inverse association between the rs660895-(AG+GG) genotypes and PD in never smokers (OR=0.66, 95% CI=0.53–0.82) was lost in ever smokers (OR=0.46/0.46=1.00, 95% CI=0.82–1.21) corresponding to an interaction parameter of 1.52 (95% CI=1.13–2.04, $p=0.005$); similarly, the inverse association between smoking and PD seen in carriers of the rs660895-AA genotype (OR=0.46, 95% CI=0.38–0.56) became weaker in carriers of the rs660895-(AG+GG) genotypes (OR=0.46/0.66=0.70, 95% CI=0.54–0.86). Interactions were of a similar size, but not statistically significant, in the French studies (*Terre*, interaction parameter=1.74, 95% CI=0.79–3.84, $p=0.17$; *Partage*, interaction parameter=1.52, 95% CI=0.76–3.06, $p=0.24$). In pooled analyses of the three studies (Table 3), there was no heterogeneity for any of the interaction estimates. Under a

dominant model, carriers of the rs660895-(AG+GG) genotypes were at decreased PD risk among never smokers (OR=0.64, 95% CI=0.54–0.77) but not in ever smokers (OR=0.48/0.48=1.00, 95% CI=0.84–1.19), corresponding to an interaction of 1.54 (95% CI=1.19–2.00, $p=0.001$, $I^2=0.0\%$); similarly, the inverse association between smoking and PD seen in carriers of the rs660895-AA genotype (OR=0.48, 95% CI=0.41–0.57) was weaker in carriers of the rs660895-(AG+GG) genotypes (OR=0.48/0.64=0.75, 95% CI=0.60–0.90). In analyses based on pack-years, interactions were significant for both light (interaction parameter=1.64, 95% CI=1.20–2.25, $p=0.002$) and heavy (interaction parameter=1.48, 95% CI=1.02–2.13, $p=0.040$) smoking; the interaction was somewhat stronger for light than heavy smoking but the difference was not significant ($p=0.68$). Results remained consistent when the cutoff of 12 pack-years was used (light smokers: interaction parameter=1.71, 95% CI=1.22–2.41, $p=0.002$; heavy smokers: interaction parameter=1.47, 95% CI=1.06–2.05, $p=0.021$).

Table 4 presents the results of case-only and control-only analyses. Case-only analyses showed a significant and positive association between ever smoking and rs660895-G in cases from PASIDA under all genetic models considered. This pattern was confirmed in pooled analyses without any heterogeneity across studies ($I^2=0.0\%$ for all genetic models). Among controls, rs660895-G tended to be less frequent among ever smokers than never smokers, but this difference was not significant in any of the studies and in pooled analyses.

Both in the *PASIDA* and *Partage* studies, smoking and coffee drinking were positively associated in controls. There was no significant interaction between rs660895 and coffee drinking (either as a binary variable or in cups per day) in both datasets and in pooled analyses; in addition, the rs660895-smoking interaction remained significant in PASIDA and in pooled analyses after adjusting for coffee consumption (data not shown).

Discussion

In this pooled analysis of individual data from three population-based case-control studies, we replicate an inverse association between the rs660895-G allele and PD, and provide the first evidence of a statistically significant interaction between rs660895 and smoking for the risk of PD. The decreased risk of PD in carriers of the rs660895-G allele was restricted to never smokers.

We previously reported significant associations of the *HLA-DRB1*04* allele and rs660895 with PD, and a strong correlation of rs660895-G with *HLA-DRB1*04* (Spearman's $\rho=0.77$, $p<0.0001$) in the French studies included here.⁷ These results are in agreement with our findings for rs660895 in the Danish study. Additionally, a British study of 528 cases and ~3000 controls previously reported a lower *HLA-DRB1*04* allele frequency in PD patients compared with controls (17% vs. 20%, $p=0.01$).⁹ A subtype of *HLA-DRB1*04*, *HLA-DRB1*0406*, which is rare in European but common in Asian populations, was also significantly related to PD in a Han Chinese study of 567 cases and 746 controls (OR=0.12, $p=5\times 10^{-5}$).¹⁰ An inverse association between *HLA*DRB1*0404* and PD was also reported in two GWAS using SNP-based imputation.¹¹ These findings support a role of *HLA-DRB1* in the etiology of PD across ethnicities. The *HLA-DRB1* gene encodes MHC class II beta

chain proteins, which are widely expressed on antigen presenting cells such as B cells, dendritic cells, and macrophages, including brain microglia. Studies have reported microglial activation in the *substantia nigra* of PD patients and in animal models, and some suggested that modulation of microglia related oxidative stress and inflammation might be a treatment strategy for PD.^{34,35} Observational studies showing an inverse association between NSAIDs use and PD also support a role for neuroinflammation in PD.³⁶

In addition, our findings suggest that the inverse association between *HLA*DRB1* and PD is present only in never smokers, and that the inverse association between smoking and PD was less pronounced in carriers of the rs660895-G allele. In analyses based on pack-years of smoking, although interaction ORs tended to be higher for light smokers than heavy smokers, the difference was small and not statistically significant. The finding that the strength of the interaction decreased with increasing exposure to an environmental factor has previously been termed 'low exposure-gene effect' with several examples available in the cancer literature:³⁷ according to this pattern, the association between the exposure and the outcome is modified by genetic susceptibility at low exposure levels only, and not at higher levels, thus implying that genetic susceptibility is no longer relevant at high or very high exposure levels. Even larger studies would be needed to further investigate such dose dependent patterns for smoking and *HLA-DRB1* in PD.

Epidemiologic studies have consistently reported a significant inverse association between smoking and PD with a dose-effect relation.¹⁴ This has led some researchers to advocate the use of nicotine to prevent or treat PD.³⁸ There is however no consensus yet whether this association is causal or is due to bias or reverse causation, such that those who will develop PD have a diminished nicotinic response allowing them to quit more easily in the prodromal phase of PD; indeed, in PASIDA, the risk of developing PD was higher among former smokers who reported that quitting had been easy and who did not use nicotine substitutes.¹⁶ Moreover, older quitting age was strongly associated with nicotine substitute use, while age at smoking initiation was not.¹⁶ In the Terre study, ex-smokers were at decreased PD risk even if they stopped smoking 40 years before PD onset.²⁵ Gene-environment studies may represent a way to improve our understanding of the mechanisms underlying the puzzling association between smoking and PD. Our finding of a multiplicative interaction between *HLA-DRB1* and smoking suggests that they are involved in common pathways, possibly related to neuroinflammation; further research is needed to better understand the mechanism underlying the *HLA-DRB1*-PD association and the interaction with smoking. In this context, it is of interest that smoking and HLA have been previously reported to interact in other conditions, including multiple sclerosis (MS),^{4,39} rheumatoid arthritis (RA),^{2,40,41} lymphoma,⁴² and adult idiopathic inflammatory myopathies.⁴³ In MS patients, smoking influenced the proliferation of immune cells in the lung; the magnitude of the increase in alveolar macrophage concentration was associated with *HLA-DRB1* alleles.⁴ In RA sufferers, smoking and *HLA-DRB1*04* interacted to influence their risk of cardiovascular disease;⁴⁴ the hypothesized mechanism was that both *HLA-DRB1*04* and smoking enhanced T-cell activation in the lung, which may lead to inflammatory processes in the endothelial cells of the vessel walls and subsequent development of cardiovascular disease. Regarding lymphoma, a recent study showed a positive association with *HLA-DRB1* shared epitope alleles (including *HLA-DRB1*04*) as well as a positive additive interaction between

HLA-DRB1 shared epitope alleles and smoking, such that there was no association with smoking in subjects without shared epitope alleles and a significant positive association in those who carried two shared epitope alleles.⁴² Smoking induces the release of intracellular antigens and the authors hypothesized that shared epitope-containing *HLA-DRB1* variants may facilitate the presentation of these antigens to helper T cells and induce B-cell chronic activation which may increase the risk of abnormal proliferation.⁴² Interestingly, a meta-analysis showed PD patients to have a 24% reduced risk of leukemia and lymphoma.⁴⁵ This finding is in agreement with associations in opposite directions for *HLA-DRB1* with PD and lymphoma; although it is unclear how smoking and *HLA-DRB1* may interact at the biological level, the observation of interactions in opposite directions in both diseases, one characterized by cell proliferation and the other by neuronal death, deserves further investigation. Lastly, smoking and *HLA-DRB1* have also been reported to interact in adult idiopathic inflammatory myopathies.⁴³

Our study has several strengths including its large size and study design (pooled analysis of individual data from three population-based studies) that provided us with sufficient statistical power to identify an interaction of the size previously reported⁷ or even weaker. In case-only analyses, rs660895 was associated with smoking among cases and the assumption of gene-environment independence among controls was met. As studies were population-based, controls were representative of the underlying population from which the cases arose. In addition, in each study, participants were ethnically homogeneous and of European ancestry. The diagnosis of PD was clinically confirmed by neurologists in the two French studies, and by careful systematic review of complete medical records in PASIDA. Finally, the effect size of ever smoking was consistent with that reported in a previous meta-analysis of 67 studies (OR=0.64, 95% CI=0.60–0.69).³² However, there are also limitations. Although rs660895-G tags the *HLA-DRB1**04 allele, we cannot provide more detailed information about allele subtypes and haplotypes as additional SNPs were not typed. Besides, the recruitment of PD patients includes both incident and prevalent cases, but stratifying by disease duration produced the same results making survival bias unlikely (data not shown). Finally, residual confounding due to unmeasured factors cannot be ruled out; however, under gene-environment independence in the population, it has been shown that only unmeasured confounders that also interact with the gene under investigation may bias estimates of gene-environment interactions.⁴⁶ In our analyses, coffee did not interact with rs660895-G for the risk of PD, and adjusting for coffee drinking had no impact on our findings. In our previous paper, we also reported that there was no interaction between *HLA-DRB1* and professional pesticide exposure in the French dataset (p for interaction=0.88).⁷

Conclusion

The main contribution of this pooled analysis of three population-based case-control studies of PD is to show that the inverse association between the rs660895-G allele in the *HLA-DRB1* region and PD is restricted to never smokers. This finding suggests that genetic variation in the *HLA-DRB1* locus has an effect on the risk of PD in interaction with smoking, and allows to generate the hypothesis that *HLA-DRB1* and smoking may be involved in common pathways, possibly related to neuro-inflammation. In order to better understand the role of HLA and smoking in PD, further research is needed to elucidate

whether a biological basis underlies this interaction and what the mechanisms involved might be.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of participants by study

Characteristics	Denmark (PASIDA)		France (Terre)		France (Partage)	
	Cases	Controls	Cases	Controls	Cases	Controls
N	1547	1595	202	529	307	599
Mean onset/index age (SD)	61.3 (9.6)	61.6 (9.7)	63.8 (7.0)	63.3 (7.8)	66.2 (7.6)	66.3 (7.7)
Mean age at diagnosis (SD)	62.3 (9.2)	–	64.8 (7.0)	–	66.8 (7.5)	–
Mean age at study (SD)	67.7 (8.4)	68.3 (8.7)	67.3 (6.7)	66.9 (7.4)	72.7 (6.6)	72.6 (6.7)
Sex, male (%)	926 (59.9)	970 (60.8)	116 (57.4)	311 (58.8)	177 (57.7)	356 (59.4)
European ancestry (%)	1547 (100.0)	1595 (100.0)	202 (100.0)	529 (100.0)	307 (100.0)	599 (100.0)
Family history of PD (%)	210 (13.6)	88 (5.5)	19 (9.4)	24 (4.5)	36 (11.7)	24 (4.0)
Smoking						
Never Smoker	773 (50.0)	572 (35.9)	150 (74.3)	349 (66.0)	233 (75.9)	402 (67.1)
Ever smoker	774 (50.0)	1023 (64.1)	52 (25.7)	180 (34.0)	74 (24.1)	197 (32.9)
Smoking intensity (%)						
Never Smoker	773 (56.1)	572 (42.1)	150 (74.6)	349 (66.0)	233 (75.9)	402 (67.1)
Light Smoker (18 pack-years) ^{a,b}	409 (29.6)	441 (32.4)	32 (15.9)	86 (16.3)	48 (15.6)	125 (20.9)
Heavy Smoker (> 18 pack-years) ^{a,b}	197 (14.3)	347 (25.5)	19 (9.5)	94 (17.7)	26 (8.5)	72 (12.0)
rs660895 (%)						
AA	974 (63.0)	929 (58.3)	146 (72.3)	354 (66.9)	231 (75.3)	399 (66.6)
AG	510 (33.0)	589 (36.9)	52 (25.7)	157 (29.7)	74 (24.0)	182 (30.4)
GG	63 (4.0)	77 (4.8)	4 (2.0)	18 (3.4)	2 (0.7)	18 (3.0)

^aNumber of subjects with missing data for pack-years: PASIDA n=403, Terre n=1.^bMedian number of pack-years among ever smokers in controls in the Terre study (PASIDA: median 15 pack-years, Partage: median 12 pack-years).

Table 2

Associations between smoking, rs660895, and PD, in each study and overall

Characteristics	Denmark (PASIDA)			France (<i>Terre</i>)			France (<i>Partage</i>)			Pooled analysis		
	OR (95% CI)	p		OR (95% CI)	p		OR (95% CI)	p		OR (95% CI)	p	I ² % p-het
Smoking												
Never	1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)		
Ever	0.55 (0.47–0.63)	<0.001		0.62 (0.41–0.93)	0.022		0.60 (0.42–0.86)	0.005		0.56 (0.49–0.64)	<0.001	0.0 0.778
Smoking												
Never	1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)		
Light ^a	0.69 (0.57–0.82)	<0.001		0.78 (0.48–1.26)	0.306		0.62 (0.42–0.93)	0.021		0.68 (0.59–0.80)	<0.001	0.0 0.793
Heavy ^a	0.41 (0.33–0.50)	<0.001		0.44 (0.25–0.78)	0.005		0.57 (0.34–0.94)	0.029		0.43 (0.36–0.51)	<0.001	0.0 0.492
	p for trend=	<0.001		p for trend=	0.005		p for trend=	0.008		p for trend=	<0.001	
rs660895												
AA	1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)		
AG	0.83 (0.72–0.97)	0.016		0.83 (0.57–1.21)	0.331		0.71 (0.52–0.98)	0.035		0.81 (0.71–0.92)	0.001	0.0 0.675
GG	0.78 (0.55–1.10)	0.155		0.53 (0.17–1.59)	0.255		0.19 (0.04–0.84)	0.028		0.56 (0.28–1.11)	0.098	43.5 0.170
Additive model	0.85 (0.75–0.96)	0.010		0.80 (0.58–1.10)	0.162		0.65 (0.49–0.87)	0.003		0.79 (0.68–0.93)	0.003	31.3 0.233
Dominant model	0.83 (0.71–0.95)	0.009		0.80 (0.56–1.15)	0.222		0.66 (0.49–0.91)	0.010		0.79 (0.70–0.90)	<0.001	0.0 0.463

^aDefinition for smoking: non-smoker, light smoker (< 18 pack-years), and heavy smoker (>18 pack-years).

All models adjusted for index age (continuous), sex, family history of PD, and matching factor year of birth in the PASIDA study. p-het., p for heterogeneity.

Table 3

Pooled analysis of the joint effects of smoking and rs660895 for PD

		OR (95% CI)	p	I ²	p-het	Interaction parameter (95% CI)	p	I ²	p-het
Smoking rs660895 (codominant)									
Never	AA	1.00 (Ref.)							
Never	AG	0.65 (0.54–0.78)	<0.001	0.0%	0.989				
Never	GG	0.60 (0.37–0.98)	0.043	6.2%	0.344				
Ever	AA	0.48 (0.41–0.57)	<0.001	0.0%	0.767	1.00 (Ref.)			
Ever	AG	0.49 (0.41–0.60)	<0.001	0.0%	0.409	1.59 (1.22–2.07)	0.001	0.0%	0.811
Ever	GG	0.41 (0.25–0.66) ^b	<0.001	NE	NE	1.33 (0.66–2.68) ^b	0.430	NE	NE
Smoking rs660895 (additive)									
Never	AA	1.00 (Ref.)							
Never	AG	0.69 (0.60–0.81)	<0.001	0.0%	0.597				
Never	GG	0.48 (0.36–0.65)	<0.001	0.0%	0.597				
Ever	AA	0.50 (0.42–0.58)	<0.001	0.0%	0.743				
Ever	AG	0.47 (0.40–0.56)	<0.001	0.0%	0.913	1.00 (Ref.)			
Ever	GG	0.46 (0.35–0.61)	<0.001	0.0%	0.927	1.37 (1.10–1.71)	0.005	0.0%	0.992
Smoking rs660895 (dominant)									
Never	AA	1.00 (Ref.)							
Never	AG+GG	0.64 (0.54–0.77)	<0.001	0.0%	0.887				
Ever	AA	0.48 (0.41–0.57)	<0.001	0.0%	0.768	1.00 (Ref.)			
Ever	AG+GG	0.48 (0.40–0.57)	<0.001	0.0%	0.752	1.54 (1.19–2.00)	0.001	0.0%	0.952
Smoking rs660895 (dominant)									
Never	AA	1.00 (Ref.)							
Never	AG+GG	0.65 (0.54–0.77)	<0.001	0.0%	0.884				
Light ^a	AA	0.57 (0.47–0.69)	<0.001	0.0%	0.885	1.00 (Ref.)			
Light	AG+GG	0.61 (0.48–0.77)	<0.001	0.0%	0.736	1.64 (1.20–2.25)	0.002	0.0%	0.962
Heavy ^a	AA	0.38 (0.30–0.48)	<0.001	0.0%	0.560	1.00 (Ref.)			
Heavy	AG+GG	0.35 (0.27–0.47)	<0.001	0.0%	0.475	1.48 (1.02–2.13)	0.040	0.0%	0.645

^aDefinition for smoking: non-smoker, light smoker (< 18 pack-years), and heavy smoker (> 18 pack-years).

Only the Danish study provided information for estimation.
 p -het., p for heterogeneity; NE, not estimable.

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Table 4

Association of ever vs. never smoking with rs660895 among a) PD cases-only and b) non-PD controls-only, in each study and overall.

rs660895	Denmark (PASIDA)			France (Terre)			France (Partage)			Pooled analysis		
	OR (95% CI)	p		OR (95% CI)	p		OR (95% CI)	p		OR (95% CI)	p	I ² % p-het
Case-only analysis												
AA	1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)		
AG	1.37 (1.10–1.71)	0.005		0.93 (0.43–2.01)	0.857		1.71 (0.87–3.37)	0.121		1.36 (1.11–1.67)	0.003	0.0% 0.505
GG	1.20 (0.71–2.03)	0.488		NE	NE		NE	NE		1.20 (0.71–2.03) ^a	0.487	NE NE
Additive model	1.25 (1.05–1.50)	0.014		0.85 (0.41–1.76)	0.652		1.50 (0.79–2.87)	0.217		1.24 (1.05–1.47)	0.012	0.0% 0.494
Dominant model	1.35 (1.09–1.67)	0.005		0.89 (0.41–1.91)	0.761		1.63 (0.83–3.20)	0.153		1.34 (1.10–1.62)	0.004	0.0% 0.483
Control-only analysis												
AA	1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)			1.00 (Ref.)		
AG	0.92 (0.74–1.15)	0.482		0.67 (0.42–1.07)	0.092		0.83 (0.54–1.29)	0.412		0.86 (0.72–1.04)	0.114	0.0% 0.468
GG	0.82 (0.50–1.34)	0.432		0.94 (0.31–2.85)	0.918		0.65 (0.18–2.36)	0.516		0.82 (0.54–1.25)	0.353	0.0% 0.913
Additive model	0.92 (0.77–1.10)	0.336		0.78 (0.53–1.13)	0.191		0.83 (0.57–1.21)	0.320		0.88 (0.76–1.02)	0.089	0.0% 0.698
Dominant model	0.91 (0.74–1.13)	0.392		0.70 (0.45–1.09)	0.112		0.82 (0.53–1.25)	0.352		0.86 (0.72–1.02)	0.087	0.0% 0.553

ORs (95% CI) estimate the association between rs660895 and ever smoking (dependent variable) in cases and controls. All models adjusted for index age (continuous), sex, and matching factor year of birth in the PASIDA study. p -het., p for heterogeneity; NE, not estimable. Please see Table S1 for the cross-tabulation of ever smoking and rs660895 in cases and controls.

^aOnly the Danish study contributed information.