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A genetic basis for the variable effect of smoking/nicotine on Parkinson's disease

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Abstract

Prior studies have established an inverse association between cigarette-smoking and the risk of developing Parkinson's disease (PD), and currently, disease-modifying potential of the nicotine-patch is being tested in clinical trials. To identify genes that interact with the effect of smoking/nicotine, we conducted genome-wide interaction studies in humans and in *Drosophila*. We identified *SV2C* which encodes a synaptic-vesicle protein in PD-vulnerable substantia-nigra

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SUPPLEMENTARY INFORMATION is available at *The Pharmacogenomics Journal*'s website.

DATA ACCESS

NGRC genome-wide genotype, phenotype, and environmental data are available from dbGaP (http://www.ncbi.nlm.nih.gov/gap, accession number phs000196.v2.p1). Microarray data on *Drosophila* paraquat-nicotine model are available from Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/, accession number GSE35930).

CONFLICT OF INTEREST

Authors have no conflicts of interest to disclose.

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($P=1\times10^{-7}$ for gene-smoking interaction on PD risk), and *CG14691* which is predicted to encode a synaptic-vesicle protein in *Drosophila* ($P=2\times10^{-11}$ for nicotine-paraquat interaction on gene-expression). *SV2C* is biologically plausible because nicotine enhances release of dopamine through synaptic vesicles, and PD is caused by depletion of dopamine. Effect of smoking on PD varied by *SV2C* genotype from protective to neutral to harmful ($P=5\times10^{-10}$). Taken together, cross-validating evidence from humans and *Drosophila* suggest *SV2C* is involved in PD pathogenesis and it might be a useful marker for pharmacogenomics studies involving nicotine.

Keywords

SV2C; Nicotine; Parkinson's Disease

INTRODUCTION

PD is a progressive degenerative disorder of the central nervous system. Dopamine-producing neurons in the substantia nigra selectively degenerate, resulting in a drastic reduction in the brain dopamine levels. Dopamine is a neurotransmitter and can impact many functions including voluntary movement, cognition, mood, behavior and sleep, all of which are altered in PD. Current treatments are directed towards dopamine replacement. While they help with early motor difficulties, they do not slow the progression of the disease and are associated with several late complications. To date, none of the clinical trials for neuroprotective treatments of PD have succeeded. We suspect that the inability so far to account for genetic differences that affect drug response has been a hindrance to treatment trials.

Epidemiological studies have shown that caffeinated-coffee and cigarette-smoking are inversely associated with risk of developing PD [1, 2]. Although neuroprotective effects of caffeine and nicotine have been demonstrated in animal models of PD [3, 4], there is controversy as to whether the inverse associations in humans signify true protective effects or a personality trait that renders those predisposed to PD to avoid habit forming behaviors [5]. Previously, we identified *GRIN2A* as a novel gene for PD through a genome-wide interaction study with caffeine, and showed that the genetic association was specific to the risk of PD and not to the tendency for caffeine use [6]. In the present study, we sought to identify genes that influence the effect of smoking on PD.

We conducted genome-wide studies in humans, searching for genes that interact with the effect of smoking, and in *Drosophila*, searching for genes whose expression was affected by the interaction between paraquat, which we used to induce parkinsonism in the fly, and nicotine, which we used to rescue the flies from paraquat toxicity. Several genetic and toxin animal models of parkinsonism are available. We chose the paraquat model in *Drosophila* because paraquat is an environmental risk factor for PD [7-9], and *Drosophila* is amenable to powerful genetic analyses. The paraquat fly model of parkinsonism is created by feeding paraquat to flies, which results in selective and progressive loss of dopaminergic neurons, motor abnormalities and shortened lifespan [10]. Thus, in this model many of the phenotypic hallmarks of the human parkinsonism are recapitulated, and the significantly shortened life

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span provides a parkinsonism-associated phenotype that is amenable to rapid screening. The two experiments, conducted in humans and *Drosophila*, converged on a pair of homologous genes, *CG14691* and *SV2C*, which encode a synaptic vesicle protein involved in release of dopamine.

MATERIALS AND METHODS

Human Study

All research participants gave informed consent as approved by the Institutional Review Boards of the participating institutions. Subjects were from NeuroGenetics Research Consortium (NGRC) [11]. PD was diagnosed using standard criteria [12]. Controls were self-reported as not having any neurologic disease. Subjects were classified as being an ever- or never-smoker, using the common definition of having smoked 100 cigarettes in the lifetime [2]. All subjects were unrelated, Caucasian Americans of European ancestry, by self-report and confirmed by principal component (PC) analysis [11]. DNA was extracted from whole blood and unamplified. The Illumina HumanOmni1-Quad_v1-0_B array was used for genotyping, achieving a call rate of 99.92% and 99.99% reproducibility. Details of genotyping procedures and quality control have been published [11]. Every NGRC subject for whom genome-wide genotype and smoking data were available was included in the present study (1 600 persons with PD and 1 506 without PD; see Table S1).

Genotypes in the NGRC dataset had been previously cleaned for GWAS [11], but since we used only subjects who had smoking data, we rechecked all single nucleotide polymorphisms (SNPs) and included only those that had a minor allele frequency (MAF) 0.01 in the subset of data used here. This yielded 811 597 genotyped SNPs. Smoking was treated as a binary variable as ever- or never-smoked at least 100 cigarettes in the life-time. We tested SNP*smoking interaction using logistic regression and adjusting for four covariates that associate with PD risk in the NGRC dataset; [11] namely, PC1 and PC2, sex, and age at blood draw. We used PLINK software version 1.07 [13].

Haplotype blocks were constructed using Haploview [14]. Independence of SNPs was tested using step-wise conditional analysis [15, 16] performed in R version 2.14.1. Association of *SV2C* with smoking was tested in R using logistic regression and adjusting for sex. A copy number variation (CNV) exists in *SV2C*. Using the PennCNV software [17], we detected 4 cases and 4 controls with a CNV in *SV2C* (all were duplications). The detected CNV ranged from 6kb to 20kb, and were all contained in intron 2 which is downstream to and does not overlap with the region that exhibits evidence for interaction with smoking.

Drosophila study

All experiments were conducted with *D. melanogaster* w^{1118} females (Bloomington Drosophila Stock Center) kept at consistent conditions at 25°C, 55% humidity, and ambient light. Flies were collected within 24 hrs after eclosion, placed on media with or without nicotine for eight or ten days, and then placed on food with or without paraquat while continuing on nicotine at the dose they were pretreated on. Flies were transferred to new vials every other day. Nicotine ([-]–Nicotine in PESTANAL, Sigma-Aldrich) and paraquat

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(Methyl viologen dichloride hydrate, Sigma-Aldrich) were dissolved in dH_2O at stock concentrations of 50mg/ml and 1M, respectively. These solutions were prepared fresh before each batch of media preparation and were added to warmed, liquefied standard fly food (prepared with agar, cornmeal, sucrose and yeast) and mixed thoroughly prior to pouring into vials. Food was prepared fresh at least monthly.

Three sets of experiments were conducted: a pilot study, a large-scale survival experiment, and a gene expression study. For the pilot study, flies were pretreated with 0, 0.05, 0.1, 0.2, or 0.4 mg/ml of nicotine for eight days and then transferred to food containing 0, 2.5, 5, or 10 mM paraquat while continuing on nicotine at the dose they were pretreated on. For each nicotine-paraquat dose combination, we had six vials each with 30 flies for a total of 180 flies. The number of dead flies was counted daily until nearly all flies were dead (Figure S1).

For the full experiment we set up 420 flies (14 vials each containing 30 flies) for each dose combination of 0, 0.01, 0.05, 0.1, 0.2, 0.4 mg/ml for nicotine pretreatment for eight days followed with addition of 0 or 5 mM paraquat. We did not pursue paraquat at 2.5 mM or 10 mM. 2.5 mM paraquat was associated with high vial-to-vial variability in survival times. At 10 mM, nicotine did not have a notable effect on survival. Flies were followed according to the same protocol, counting dead flies daily until all were dead. Survival data were plotted using Kaplan Meier survival analysis [18], mean and median survival times were calculated, and the differences between the survival curves were tested using log rank statistics in SPSS (Figure S2).

For gene expression study, we had four dose combinations, each conducted in triplicate, at the same time and under the same conditions. 30 flies per vial were pretreated with 0 or 0.1 mg/ml nicotine for ten days and then co-treated with 0 or 5 mM of paraquat for six days. At the end of the treatment period, heads were dissected from 20 flies per vial and frozen at -80°C for up to a month. RNA was extracted from each sample using TriReagent and its provided protocol, and cleaned using Qiagen RNeasy Cleanup kit and the associated protocol. RNA was stored at -80°C for approximately two months. Affymetrix GeneChip *Drosophila* Genome 2.0 arrays were used for genome-wide quantification of transcript abundance. Expression data were analyzed using Bioconductor version 2.9 [19] packages in R version 2.14.1. Raw signal data were examined for signs of RNA degradation using AffyRNAdeg implemented in Bioconductor, and for inconsistencies in overall probe intensity by visually inspecting log(intensity) density plots. One replicate of the paraquat-only treatment was found to be an outlier and excluded from data analysis (Figure S3). Data were normalized using the GCRMA [20] algorithm including quantile normalization, pmonly correction, and median polish summarization. Statistical interaction between nicotine and paraquat on gene expression (specified as $\log_2$(signal)) was tested using the linear model implemented in limma [21]. Expression differences were tested for 18 954 transcripts. Microarray data P-values were corrected using multiple testing adjustment [22] included in the limma package.

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RESULTS

Drosophila and Human studies, independently, revealed a pair of homologous genes as the most significant signal for interaction with nicotine and smoking, respectively. We present the results of the *Drosophila* study first because the signal passed the genome-wide significance threshold for discovery. The human study was highly significant as corroborating evidence for validation.

Drosophila paraquat-nicotine model

It has previously been established that reduced lifespan is a part of the paraquat-induced parkinsonism phenotype [10]. Consistent with this notion, we found that paraquat shortened flies' lifespan by 63% ($P=9\times10^{-168}$). When co-treated with nicotine, nicotine improved survival for paraquat-treated flies in a dose-dependent manner by up to 25% ($P=2\times10^{-23}$). A beneficial effect of nicotine on survival was evident in both paraquat-treated ($P=1\times10^{-5}$) and untreated ($P=4\times10^{-3}$) flies, up to 0.2 mg/ml nicotine. However, at high dose (0.4 mg/ml), nicotine became toxic for flies that were not exposed to paraquat causing a 21% decline in median survival ($P=1\times10^{-17}$), though it continued to extend the lifespan of paraquat-treated flies up to 25% ($P=2\times10^{-23}$). These results, detailed in Table 1, Figure 1, and Figure S2, demonstrate successful construction of a nicotine-paraquat model in *Drosophila*. Furthermore, they reaffirm that nicotine can be protective against paraquat toxicity.

Gene-expression study in *Drosophila*

Test of statistical interaction between paraquat and nicotine on 18 954 transcripts gave a very strong signal for *CG14691* ($P_{Interaction}=2\times10^{-11}$, adjusted for multiple testing $P_{Interaction}=4\times10^{-7}$), followed by marginal signals for *skpB* ($P_{Interaction}=1\times10^{-6}$, adjusted $P_{Interaction}=0.01$) and *CG1885* ($P_{Interaction}=5\times10^{-6}$, adjusted $P_{Interaction}=0.03$). Figure 2 shows that compared to untreated flies, paraquat-treated flies had a modest (8%) but highly significant ($P=5\times10^{-8}$) rise in *CG14691* expression, whereas flies that were treated with nicotine+paraquat or nicotine alone were similar to untreated flies (P=1.0).

_CG14691_ is predicted to encode a synaptic vesicle membrane protein—(http://flybase.org/reports/FBgn0037829.html). Genome comparison analysis revealed that the *Drosophila CG14691* is orthologous to the SV2A/SV2B/SV2C family of synaptic vesicle proteins in humans [23]. The next step was to look for evidence of interaction between *SV2* genes and the protective effect of smoking on PD in humans.

Genome-wide gene-environment interaction in Human

As depicted in the Manhattan plot of genome-wide interaction with smoking (Figure 3), none of the SNPs achieved the genome-wide significance threshold of $P<5\times10^{-8}$. However, the highest peak was on chromosome 5 and mapped to the 5' of the synaptic vesicle protein *SV2C* gene (Figure 3, Table S2). Although the significance of *SV2C* ($P=2\times10^{-6}$ for the top SNP and $P=1\times10^{-7}$ for the joint effect of two independent SNPs) did not meet genome-wide significance criteria for discovery, it surpassed the significance threshold for validation of a candidate gene that was discovered, at genome-wide significance, in *Drosophila*.

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Eighteen SNPs spanning from ~90kb upstream in 5' prime to intron 1 of *SV2C* achieved $10^{-3}>P_{Interaction}\quad 2\times10^{-6}$ for interaction with smoking. Most of the SNPs were in moderate to strong linkage disequilibrium (LD) with each other, with correlation-coefficients (r^2) ranging from 0.66 to 0.98, and formed one large haploblock (Figure 4). The top SNP, rs30196 (minor allele frequency (MAF) =0.46, $P_{Interaction}=2\times10^{-6}$), was in this block. Another SNP, rs183766 (MAF=0.33, $P_{Interaction}=4\times10^{-4}$), showed weak LD with the SNPs in the large block (r^2=0.3-0.4). And finally, rs10214163 (MAF=0.21, $P_{Interaction}=4\times10^{-4}$), which showed virtually no correlation with other SNPs (r^2=0.01-0.06). When conditioned on rs30196*smoking interaction, the rs183766*smoking signal was abolished ($P_{Interaction}=0.51$), but rs10214163*smoking remained significant ($P_{Interaction}=0.01$). This analysis suggested that rs183766 was not an independent signal so we removed it from further consideration. There was no evidence for three-way interaction between rs30196, rs10214163 and smoking (P=0.71). Considered individually, rs30196 and rs10214163 yielded $P_{Interaction}=2\times10^{-6}$ and $P_{Interaction}=4\times10^{-4}$ for interaction with smoking (Figure 3, Table 2 top row); considered together in an additive two-SNP model (see below), the interaction test yielded $P_{Interaction}=1\times10^{-7}$.

The following analyses were performed for rs30196 and rs10214163 individually and then jointly. We used the additive model throughout where genotypes are defined by the number of minor alleles (rs30196_A, rs10214163_C). For a single-SNP, the genotypic classes are 0, 1, 2. For two-SNPs, the genotypic classes are 0, 1, 2, 3 and 4 where 0 denotes homozygous for common allele at both SNP (CC-TT), 1 denotes presence of one minor allele at one or the other locus (CA–TT or CC–CT), 2 is having two minor alleles which could be homozygous at one locus (AA-TT or CC-CC) or heterozygous at both loci (CA-CT), 3 is having three minor alleles which must be homozygous at one locus and heterozygous at the other (AA-CT or CA-CC) and 4 is homozygous for minor alleles at both loci (AA-CC).

We tested whether *SV2C* was associated with smoking *per se*. The test was conducted in cases and controls combined. We found no evidence for association of smoking with rs30196 genotype (OR=1.02, P=0.70), rs10214163 genotype (OR=1.07, P=0.27) or the joint genotype of rs30196 and rs10214163 (OR=1.03, P=0.36). Therefore, the signal for *SV2C*-smoking interaction on PD risk ($P=1\times10^{-7}$) cannot be attributed to an association between the gene and the smoking habit.

We performed stratified analysis to see whether the evidence for interaction was uniform or varied across subtypes (Table 2). The evidence for interaction was robust across all disease-related strata (familial and sporadic PD, early and late onset, males and females), the four sites of data collection (Washington, Oregon, Georgia and New York), and the ethnic and geographic origin of the ancestors (Jewish vs. non-Jewish ancestry, and European country of origin).

Overall (ignoring genotype), smoking was associated with 19% risk reduction in our dataset (OR±SE=0.81±0.06). The evidence for interaction implies that the magnitude of risk reduction conferred by smoking varies by genotype and that the overall estimate (19%) is only an average for all genotypes combined. To gain an insight to genotype-specific effects, we stratified the data by genotype, tested the association of smoking with PD risk for each

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genotype separately, and then performed formal tests of heterogeneity to determine whether the genotypic differences were statistically significant. The effect of smoking on PD risk was significantly different across genotypes ($P_{Heterogeneity}=5\times10^{-10}$, Table 3). The strongest protective effect was observed for individuals homozygous for the common alleles: OR(±SE)=0.52±0.08 for rs30196 _CC, OR=0.67±0.07 for rs10214163_TT, and OR=0.44±0.08 for being homozygous at both rs10214163_TT and rs30196 _CC. The protective effect of smoking waned with the increasing numbers of minor alleles from 56% risk *reduction* ($P=2\times10^{-6}$) for individuals homozygous for the common alleles, to 223% risk *increase* (P=0.04) for individuals homozygous for the minor alleles. The pattern was evident for rs30196 and rs10214163 individually and together (Table 3). When data were stratified by the site of data collection, Washington, Oregon and New York (but not Georgia, small N) exhibited similar effect sizes and directions indicating decreasing protection by increasing number of minor alleles (Table S3). There was no evidence for heterogeneity across the four sites (1.0 P>0.42). Our data suggest that only a fraction of the population benefits from the protective effect of nicotine, and that this group can be identified by genotyping *SV2C*.

DISCUSSION

We set out to find genes that modulate the effect of smoking on PD risk reduction, with the goal of carrying them forward as markers into upcoming clinical trials of nicotine for PD. We used an integrated approach where we conducted genome-wide studies in *Drosophila* and in humans, in parallel. The human genome-wide interaction study did not achieve the genome-wide significance threshold of 5×10^{-8}; which was not surprising considering that detecting interactions requires much larger sample sizes than the standard genome-wide association studies [24]. The *Drosophila* study, however, revealed a gene with genome-wide significance. We had planned that if the *Drosophila* study were successful in identifying a gene, we would test the association of its human homologue with PD to establish its relevance to disease. Surprisingly, the gene that emerged from the *Drosophila* study (*CG14691)* was a homologue of the gene that displayed the most significant evidence for interaction with smoking in the human study (*SV2C*). Thus, while *SV2C* did not achieve the genome-wide significance level of 5×10^{-8} required to qualify as a discovery, it did reach a highly significant level ($P=2\times10^{-6}$, or 1×10^{-7}) to qualify as a validation of the discovery made in the fly. We also noted that *SV2C* genotype was not associated with the smoking habit. Thus, our data point to an interactive effect of *SV2C* genotype and nicotine on protection against parkinsonism.

The *Drosophila* and the human experiments were set up with the same goal of identifying genes that are involved in protection by smoking, although the study designs were inherently quite different. The fly experiment was done on a uniform genetic background, parkinsonism was induced with a single neurotoxin, and rescue was with controlled doses of pure nicotine. In contrast, humans were genetically diverse, represented an unknown level of heterogeneity in the causes of PD, and were exposed to all ~600 toxins in cigarettes. Furthermore, in flies, paraquat and nicotine were the predictors and gene expression the outcome; whereas in humans, genes and smoking were the predictors and PD the outcome. That two distinct, hypothesis-free experiments conducted in two species converge on a pair

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of homologues (*CG14691* and *SV2C*) as the most significant genes argues for an important role for *SV2C* in the pathogenesis of parkinsonism and protection by nicotine.

In the brain, nicotine binds to nicotinic acetylcholine receptors with high affinity and enhances vesicular release of dopamine [25]. Dopamine depletion is a hallmark of PD. A growing body of work has implicated altered synaptic transmitter release in the pathogenesis of PD [26]. Synaptic vesicle proteins SV2A/SV2B/SV2C are integral membrane components of synaptic vesicles and have been implicated in storage and release of neurotransmitters [27, 28]. A recent study has shown that modest changes in *SV2* expression, in either direction, can have a significant impact on synaptic function [29]. *SV2C* is densely expressed in dopaminergic neurons in substantia nigra [30]. The findings of our study may therefore reflect a connection between nicotinic enhancement of vesicular dopamine release and altered neurotransmission due to changes in expression of *SV2C*.

The association of smoking with PD was genotype-specific and varied from highly protective to neutral and even harmful depending on *SV2C* genotype. This suggests that efficacy of nicotine as a neuroprotective treatment will not be uniform for all individuals and that clinical trials may benefit from pre-classification of subjects as high and low responders based on genotype. Nicotine has long been considered as a possible therapeutic agent for PD [31]. Clinical studies of the symptomatic efficacy of transdermal nicotine treatment for PD have been relatively small, and most, but not all, have shown a beneficial effect on motor and cognitive functions [32-35]. A randomized, placebo-controlled, double-blind multi-center trial was recently launched, with a larger sample size than attempted previously, to examine for the first time disease modifying potential of a nicotine patch for early PD (http://www.michaeljfox.org/research). This study provides an opportunity to evaluate utility of *SV2C* genotype for improving power and precision in assessing the efficacy of transdermal nicotine treatment. We acknowledge the distinction that our study examined risk of developing PD, whereas the trials are aimed at disease modification after onset of symptoms. It is however possible that nicotine plays a similar role in disease process before and after the onset of symptoms, a simple hypothesis that can be tested easily in a clinical trial setting.

The study had a number of limitations which need to be addressed in future studies. The interaction of *SV2C* genotype with the protective effect of smoking must be independently replicated. The molecular mechanism of the observed interactions needs to be worked out. Although the human data point to *SV2C*, the signal may be originating from another locus that is physically linked to *SV2C*. Similarly, the change in the expression of *SV2C* in the fly does not necessarily mean that this gene is directly involved in nicotine protection of neurons after toxic insult of paraquat. Experiments with*SV2C* mutant flies would be necessary to determine if the effect of nicotine is actually via *SV2C* pathway.

In summary, we have identified a novel PD-associated gene via interaction with protective effect of smoking/nicotine. Taken together, the cross-validating evidence from human and *Drosophila* studies, and the biological plausibility of the pathway that has emerged, suggest that *SV2C* plays a role in the pathogenesis of PD and that *SV2C* genotype might be a useful marker for pharmacogenomics studies of PD involving nicotine. The study provides leads

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and several testable hypotheses that have the potential to make a significant positive impact on personalized prevention and treatment strategies for PD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Hernan MA, Takkouche B, Caamano-Isorna F, Gestal-Otero JJ. A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. Ann Neurol. 2002; 52(3):276–284. [PubMed: 12205639]
2. Powers K, Kay D, Factor S, Zabetian C, Higgins D, Samii A, et al. Combined effects of smoking, coffee and NSAIDs on Parkinson's disease risk. Mov Disord. 2008; 23(1):88–95. [PubMed: 17987647]
3. Quik M, Parameswaran N, McCallum SE, Bordia T, Bao S, McCormack A, et al. Chronic oral nicotine treatment protects against striatal degeneration in MPTP-treated primates. J Neurochem. 2006; 98(6):1866–1875. [PubMed: 16882311]
4. Chen JF, Xu K, Petzer JP, Staal R, Xu YH, Beilstein M, et al. Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson's disease. J Neurosci. 2001; 21(10):RC143. [PubMed: 11319241]
5. Evans AH, Lawrence AD, Potts J, MacGregor L, Katzenschlager R, Shaw K, et al. Relationship between impulsive sensation seeking traits, smoking, alcohol and caffeine intake, and Parkinson's disease. J Neurol Neurosurg Psychiatry. 2006; 77(3):317–321. [PubMed: 16484638]
6. Hamza TH, Chen H, Hill-Burns EM, Rhodes SL, Montimurro J, Kay DM, et al. Genome-Wide Gene-Environment Study Identifies Glutamate Receptor Gene GRIN2A as a Parkinson's Disease Modifier Gene via Interaction with Coffee. PLoS Genet. 2011; 7(8):e1002237. [PubMed: 21876681]
7. Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. Am J Epidemiol. 2009; 169(8):919–926. [PubMed: 19270050]
8. Wang A, Costello S, Cockburn M, Zhang X, Bronstein J, Ritz B. Parkinson's disease risk from ambient exposure to pesticides. Eur J Epidemiol. 2011; 26(7):547–555. [PubMed: 21505849]
9. Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, et al. Rotenone, paraquat, and Parkinson's disease. Environ Health Perspect. 2011; 119(6):866–872. [PubMed: 21269927]
10. Chaudhuri A, Bowling K, Funderburk C, Lawal H, Inamdar A, Wang Z, et al. Interaction of genetic and environmental factors in a Drosophila parkinsonism model. J Neurosci. 2007; 27(10): 2457–2467. [PubMed: 17344383]

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11. Hamza TH, Zabetian CP, Tenesa A, Laederach A, Montimurro J, Yearout D, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. Nat Genet. 2010; 42(9):781–785. [PubMed: 20711177]
12. Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ. The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. Brain. 2002; 125(Pt 4):861–870. [PubMed: 11912118]
13. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81(3): 559–575. [PubMed: 17701901]
14. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–265. [PubMed: 15297300]
15. Payami H, Joe S, Farid NR, Stenszki V, Chan SH, Thomson G. Relative predispositional effects (RPE's) of marker alleles with disease: HLA-DR and autoimmune thyroid disease. Am J Hum Genet. 1989; 45:541–546. [PubMed: 2491013]
16. Hill-Burns EM, Factor SA, Zabetian CP, Thomson G, Payami H. Evidence for More than One Parkinson's Disease-Associated Variant within the HLA Region. PLoS One. 2011; 6(11):e27109. [PubMed: 22096524]
17. Wang K, Li M, Hadley D, Liu R, Glessner J, Grant SF, et al. PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data. Genome Res. 2007; 17(11):1665–1674. [PubMed: 17921354]
18. Kaplan E, Meier EL. Nonparametric estimation from incomplete observations. J Am Statist Assoc. 1958; 53:457–481.
19. Gentleman R, Carey V, Bates D, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biology. 2004; 5(10):R80. [PubMed: 15461798]
20. Wu Z, Irizarry RA. Preprocessing of oligonucleotide array data. 2004; 22(6):656–658.
21. Smyth, G. limma: Linear Models for Microarray Data.. In: Gentleman, R.; Carey, VJ.; Huber, W.; Irizarry, RA.; Dudoit, S., editors. Bioinformatics and Computational Biology Solutions Using R and Bioconductor. Springer; New York: 2005. p. 397-420.
22. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. J Roy Statist Soc Ser B. 1995; 57:289–300.
23. Hu Y, Flockhart I, Vinayagam A, Bergwitz C, Berger B, Perrimon N, et al. An integrative approach to ortholog prediction for disease-focused and other functional studies. BMC Bioinformatics. 2011; 12(1):357. [PubMed: 21880147]
24. Thomas D. Gene--environment-wide association studies: emerging approaches. Nat Rev Genet. 2010; 11(4):259–272. [PubMed: 20212493]
25. Turner TJ. Nicotine enhancement of dopamine release by a calcium-dependent increase in the size of the readily releasable pool of synaptic vesicles. J Neurosci. 2004; 24(50):11328–11336. [PubMed: 15601939]
26. Esposito G, Ana Clara F, Verstreken P. Synaptic vesicle trafficking and Parkinson's disease. Dev Neurobiol. 2012; 72(1):134–144. [PubMed: 21563316]
27. Feany MB, Lee S, Edwards RH, Buckley KM. The synaptic vesicle protein SV2 is a novel type of transmembrane transporter. Cell. 1992; 70(5):861–867. [PubMed: 1355409]
28. Dardou D, Dassesse D, Cuvelier L, Deprez T, De Ryck M, Schiffmann SN. Distribution of SV2C mRNA and protein expression in the mouse brain with a particular emphasis on the basal ganglia system. Brain Res. 2011; 1367:130–145. [PubMed: 20869353]
29. Nowack A, Malarkey EB, Yao J, Bleckert A, Hill J, Bajjalieh SM. Levetiracetam reverses synaptic deficits produced by overexpression of SV2A. PLoS One. 2011; 6(12):e29560. [PubMed: 22220214]
30. Janz R, Sudhof TC. SV2C is a synaptic vesicle protein with an unusually restricted localization: anatomy of a synaptic vesicle protein family. Neuroscience. 1999; 94(4):1279–1290. [PubMed: 10625067]
31. Quik M, O'Leary K, Tanner CM. Nicotine and Parkinson's disease: implications for therapy. Mov Disord. 2008; 23(12):1641–1652. [PubMed: 18683238]

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32. Itti E, Villafane G, Malek Z, Brugieres P, Capacchione D, Itti L, et al. Dopamine transporter imaging under high-dose transdermal nicotine therapy in Parkinson's disease: an observational study. Nucl Med Commun. 2009; 30(7):513–518. [PubMed: 19451839]
33. Villafane G, Cesaro P, Rialland A, Baloul S, Azimi S, Bourdet C, et al. Chronic high dose transdermal nicotine in Parkinson's disease: an open trial. Eur J Neurol. 2007; 14(12):1313–1316. [PubMed: 17941858]
34. Lemay S, Chouinard S, Blanchet P, Masson H, Soland V, Beuter A, et al. Lack of efficacy of a nicotine transdermal treatment on motor and cognitive deficits in Parkinson's disease. Prog Neuropsychopharmacol Biol Psychiatry. 2004; 28(1):31–39. [PubMed: 14687854]
35. Vieregge A, Sieberer M, Jacobs H, Hagenah JM, Vieregge P. Transdermal nicotine in PD: a randomized, double-blind, placebo-controlled study. Neurology. 2001; 57(6):1032–1035. [PubMed: 11571330]

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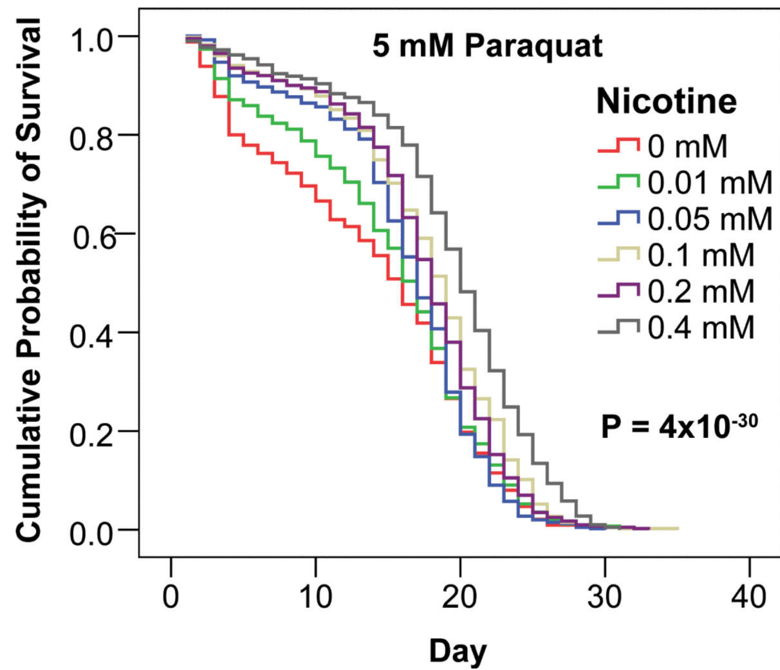

Figure 1. *Drosophila*: Effects of paraquat and nicotine on survival

Nicotine improved survival of paraquat-treated flies in a dose-dependent manner. Each treatment combination was started with 420 flies. Survival Curves were plotted using Kaplan Meier survival analysis, and differences between survival curves were calculated using log rank statistics ($P=4\times10^{-30}$).

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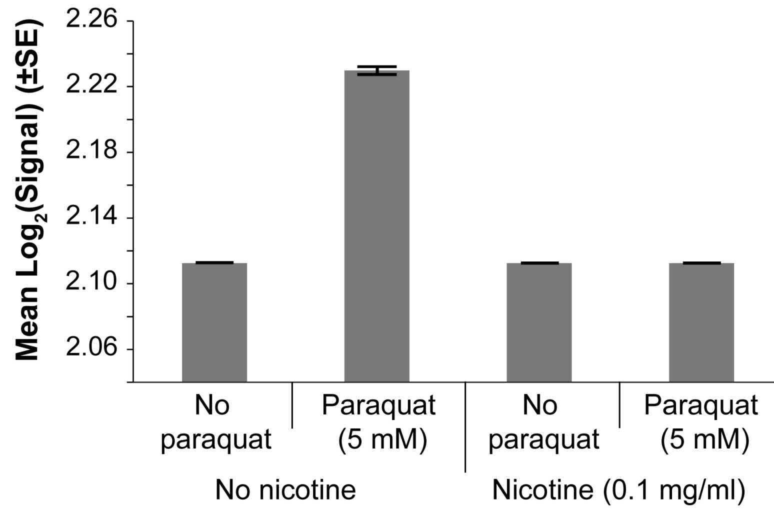

Figure 2. *Drosophila*: Effects of paraquat and nicotine on gene expression

CG14691 gene expression was increased significantly ($P=5\times10^{-8}$) in response to paraquat and restored to normal with co-treatment with nicotine (paraquat-nicotine interaction $P=2\times10^{-11}$).

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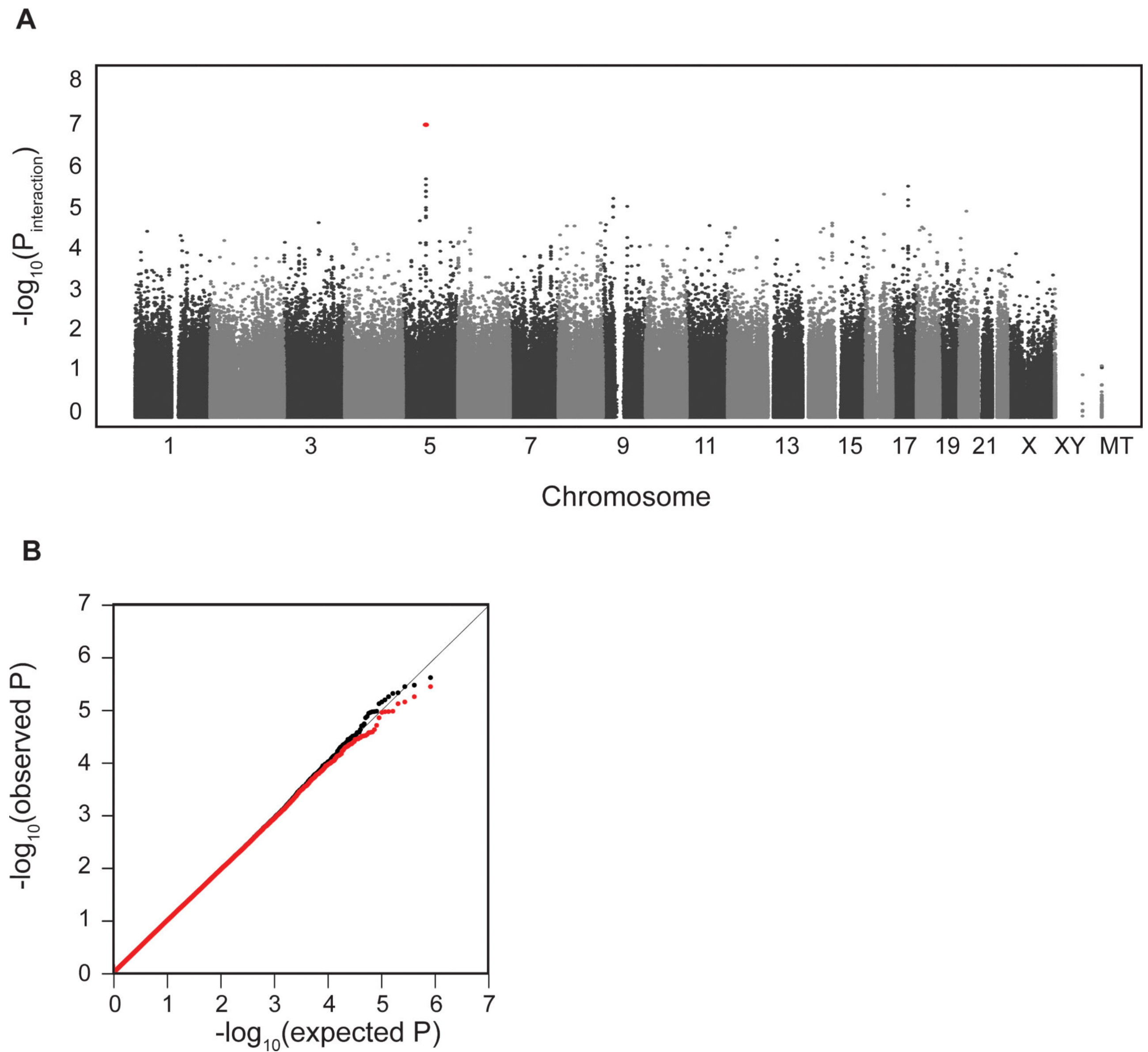

Figure 3. Human: Genome-wide SNP*smoking interaction study

(A) Manhattan plot of $-\log_{10}(P)$ values for interaction tested between 811 597 genotyped SNPs and smoking. The strongest signal came from SNPs in two closely linked haploblocks in *SV2C* on chromosome 5. The best P for any SNP was 2×10^{-6} (Black dots). The region contained two independent signals, which when considered together in an additive model, yielded $P=1\times10^{-7}$ (the red dot was added manually to the Manhattan plot to depict the two-SNP effect). (B) Quantile-quantile plot of SNP*smoking interaction P values. Black: full data. Red: excluding *SV2C* region.

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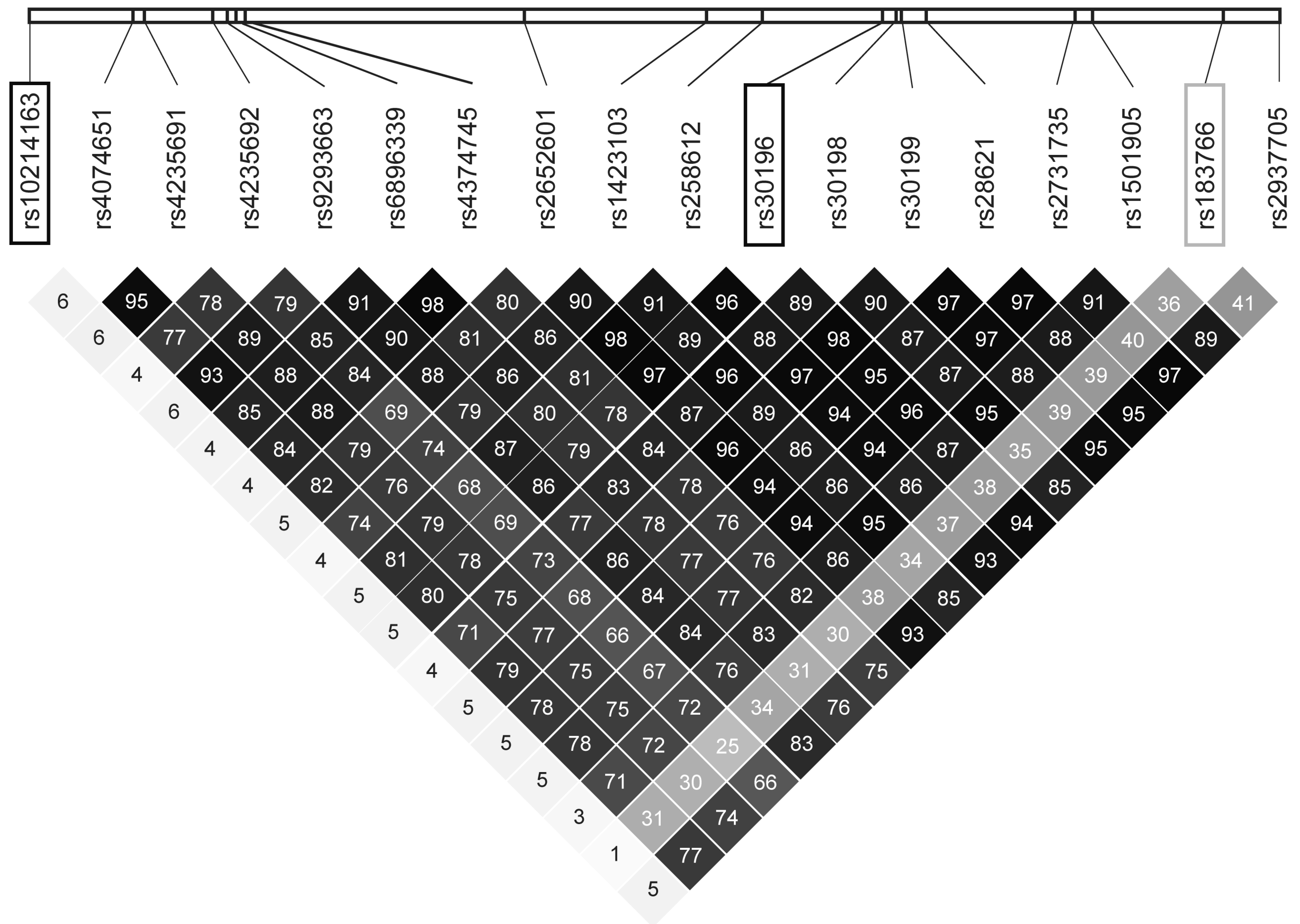

Figure 4. Human: Linkage disequilibrium in *SV2C* region

Genotyped SNPs that achieved $P_{Interaction} < 10^{-3}$ for SNP*smoking interaction in the *SV2C* region were tested for LD; the numbers in the grid represent the correlation (r^2) between each pair of SNPs. Although there appear to be three haploblocks, the SNP in the far-right block (rs183766 shown in grey box) did not have an effect independent of the other blocks. Signals from the other two haploblocks (rs30196 and rs10214163 shown in black boxes) appeared to be independent and additive, as indicated by persistent significance of one when conditioned on the more significant one. In line with this evidence for independent effects, joint consideration of genotypes at rs30196 and rs10214163 improved significance level for interaction with smoking to $P=1\times10^{-7}$.

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Table 1

Drosophila: Effects of paraquat and nicotine on survival.

Nicotine mg/ml	No paraquat			5 mM paraquat		
	Mean survival Days ± SE	**Median survival Days ± SE**	**P**	**Mean survival Days ± SE**	**Median survival Days ± SE**	**P**
0	43.3 ± 0.7	43 ± 1.0	Ref	14.0 ± 0.4	16 ± 0.5	Ref
0.01	44.4 ± 0.6	46 ± 0.8	0.48	15.2 ± 0.3	17 ± 0.4	0.15
0.05	45.1 ± 0.6	46 ± 0.8	0.56	16.1 ± 0.3	17 ± 0.3	0.14
0.1	44.4 ± 0.6	44 ± 0.6	0.96	17.6 ± 0.3	19 ± 0.3	6×10^{-9}
0.2	46.4 ± 0.7	49 ± 1.2	4×10^{-3}	17.3 ± 0.3	18 ± 0.3	1×10^{-5}
0.4	35.3 ± 0.7	34 ± 0.6	1×10^{-17}	19.4 ± 0.3	20 ± 0.3	2×10^{-23}

Paraquat reduced median survival by 63% (43±1.0 vs. 16±0.5, $P=9\times10^{-168}$). Nicotine restored survival of paraquat-treated flies by up to 25% ($P=2\times10^{-23}$).

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Table 2

Human: *SV2C**smoking interaction in strata defined by study- and disease-related variables. Two *SV2C* SNPs (rs30196 and rs10214163) which showed evidence for independent effects were tested individually and jointly. Evidence for interaction was present in all strata.

Strata	N case	N control	rs30196				rs10214163				rs30196 + rs10214163			
			OR_{Int}	SE	P_{Int}	P_{Het}	OR_{Int}	SE	P_{Int}	P_{Het}	OR_{Int}	SE	P_{Int}	P_{Het}
All	1600	1506	1.68	0.19	2×10^{-6}	-	1.61	0.22	4×10^{-4}	-	1.50	0.12	1×10^{-7}	-
PD-associated risk factors														
Familial PD	346	1506	1.90	0.34	3×10^{-4}		1.54	0.33	0.05		1.57	0.20	3×10^{-4}	
Sporadic PD	1254	1506	1.63	0.19	3×10^{-5}	NC	1.61	0.23	9×10^{-4}	NC	1.48	0.12	2×10^{-6}	NC
Male	1082	606	1.60	0.24	2×10^{-3}		1.55	0.28	0.02		1.44	0.15	5×10^{-4}	
Female	518	900	1.59	0.27	6×10^{-3}	0.99	1.74	0.35	6×10^{-3}	0.67	1.51	0.18	4×10^{-4}	0.78
Early onset (50 yrs)	416	1506	1.53	0.29	0.03		1.67	0.39	0.03		1.44	0.19	6×10^{-3}	
Late onset (> 50 yrs)	1184	1506	1.74	0.20	2×10^{-6}	NC	1.62	0.23	7×10^{-4}	NC	1.53	0.13	2×10^{-7}	NC
Coffee - heavy	510	385	1.49	0.30	0.05		1.30	0.33	0.29		1.34	0.19	0.04	
Coffee - light	940	545	2.06	0.35	2×10^{-5}	0.22	1.61	0.33	0.02	0.51	1.65	0.19	2×10^{-5}	0.26
OTC NSAIDs - ever	969	657	1.39	0.21	0.03		1.47	0.27	0.04		1.33	0.14	6×10^{-3}	
OTC NSAIDs - never	588	326	2.35	0.49	5×10^{-5}	0.04	1.65	0.42	0.05	0.71	1.82	0.27	6×10^{-5}	0.08
Rx NSAIDs - ever	512	346	1.57	0.33	0.03		1.88	0.50	0.02		1.54	0.23	4×10^{-3}	
Rx NSAIDs - never	1020	631	1.68	0.25	5×10^{-4}	0.78	1.43	0.26	0.05	0.41	1.44	0.15	4×10^{-4}	0.72
Recruitment site														
New York	376	294	1.92	0.45	0.01		1.48	0.42	0.17		1.55	0.25	8×10^{-3}	
Oregon	241	633	1.80	0.50	0.03		1.48	0.50	0.24		1.53	0.29	0.03	
Georgia	230	113	1.28	0.43	0.47		0.95	0.40	0.90		1.16	0.27	0.52	
Washington	753	466	1.70	0.30	3×10^{-3}	0.89	1.66	0.36	0.02	0.81	1.54	0.19	6×10^{-4}	0.80
Ashkenazi Jewish (genetically defined by principal components)														
Yes	73	36	4.92	3.23	0.02		1.07	0.84	0.93		2.18	0.96	0.08	
No	1527	1470	1.64	0.18	1×10^{-5}	0.10	1.61	0.22	5×10^{-4}	0.61	1.48	0.12	6×10^{-7}	0.39
Paternal and Maternal ancestry														
Great Britain	105	61	1.33	0.73	0.60		0.88	0.56	0.84		1.14	0.46	0.74	
Germany / Austria	87	44	1.72	1.08	0.39		1.22	0.83	0.77		1.30	0.53	0.53	

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Strata	N case	N control	rs30196				rs10214163				rs30196 + rs10214163			
			OR_{Int}	SE	P_{Int}	P_{Het}	OR_{Int}	SE	P_{Int}	P_{Het}	OR_{Int}	SE	P_{Int}	P_{Het}
Ireland	37	16	3×10^{8}	8×10^{11}	0.99		0.38	0.61	0.55		3.13	2.67	0.18	
Scandinavia	48	29	2.66	1.94	0.18		0.09	0.10	0.03		0.99	0.52	0.99	
Eastern Europe	31	28	1.10	1.00	0.92		0.32	0.39	0.35		0.76	0.48	0.66	
Italy	47	34	18.89	18.38	3×10^{-3}		1.94	1.80	0.48		5.68	3.90	0.01	
Russia	17	10	8.59	13.42	0.17	0.35	1.20	2.04	0.92	0.51	3.08	3.22	0.28	0.18
Paternal or Maternal ancestry														
Great Britain	445	284	1.89	0.44	6×10^{-3}		1.58	0.46	0.12		1.62	0.27	4×10^{-3}	
Germany / Austria	357	213	1.19	0.30	0.49		1.47	0.45	0.21		1.25	0.22	0.21	
Ireland	196	138	2.17	0.76	0.03		1.42	0.58	0.40		1.67	0.41	0.04	
Scandinavia	187	112	1.50	0.55	0.26		1.40	0.62	0.45		1.36	0.35	0.23	
Eastern Europe	78	66	1.98	1.01	0.18		3.25	2.22	0.08		1.93	0.68	0.06	
Italy	76	62	7.54	4.56	8×10^{-4}		2.43	1.81	0.23		4.23	1.97	2×10^{-3}	
France	68	50	3.12	2.02	0.08		0.88	0.63	0.86		1.60	0.66	0.26	
Russia	48	20	3.37	3.14	0.19	0.10	1.29	1.23	0.79	1.00	1.73	1.00	0.35	0.41

Tests were conducted for interaction of genotype*smoking. Genotype is defined as the number of minor alleles in an additive model (0, 1, 2 minor alleles for single- SNP analysis and 0, 1, 2, 3, 4 for joint analysis of two SNPs). Tests were performed in R and adjusted for age, sex (except for male and female strata), PC1, and PC2. OR_{Int}: odds ratio of interaction calculated as the odds ratio of disease when SNP and smoking are present, divided by the product of the individual odds ratios of SNP and smoking. SE= standard error of OR_{int}. P_{int}= statistical significance of interaction. P_{Het}=statistical significance of heterogeneity in evidence for interaction across strata. NC=not calculated because the two strata shared the same controls. OTC NSAIDs = over-the-counter nonsteroidal anti-inflammatory drug use. Rx NSAIDs = prescription nonsteroidal anti-inflammatory drug use.

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Table 3

Human: Variation in the effect of smoking on PD risk according to *SV2C* genotype.

No. of minor alleles	*SV2C* genotype	Genotype frequency	Smoking	N case	N control	PD risk smoker vs. non-smoker		
						OR	SE	P
Irrespective of genotype		1.00	No	866	811	0.81	0.06	7×10^{-3}
			Yes	734	695			
rs30196								
0	CC	0.30	No	303	224	0.52	0.08	9×10^{-6}
			Yes	198	211			
1	AC	0.49	No	400	380	0.86	0.10	0.18
			Yes	379	353			
2	AA	0.21	No	163	206	1.41	0.25	0.05
			Yes	157	131			
Heterogeneity across genotypes								6×10^{-7}
rs10214163								
0	TT	0.63	No	581	496	0.67	0.07	8×10^{-5}
			Yes	441	443			
1	CT	0.32	No	258	262	0.97	0.13	0.83
			Yes	254	225			
2	CC	0.05	No	27	53	2.43	0.94	0.02
			Yes	39	27			
Heterogeneity across genotypes								2×10^{-4}
rs30196 + rs10214163								
0	CC - TT	0.23	No	242	168	0.44	0.08	2×10^{-6}
			Yes	145	162			
1	CA - TT CC - CT	0.37	No	321	278	0.81	0.10	0.10
			Yes	281	264			
2	AA - TT CA - CT CC - CC	0.27	No	204	239	0.98	0.15	0.88
			Yes	199	190			

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No. of minor alleles	*SV2C* genotype	Genotype frequency	Smoking	N case	N control	PD risk smoker vs. non-smoker		
						OR	SE	P
3	AA - CT	0.11	No	87	97	1.42	0.35	0.15
	CA - CC		Yes	90	66			
4	AA - CC	0.02	No	12	28	3.23	1.89	0.04
			Yes	19	13			
Heterogeneity across genotypes								5×10^{-10}

rs30196 and rs10214163 were analyzed individually and jointly. The effect of smoking on PD risk varied significantly by genotype (P for heterogeneity across genotypes). The protective effect was strongest in individuals homozygous for the common alleles and waned with the increasing numbers of minor alleles. (See Table S3 for site-specific analysis).