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Exploration of the Association between Cathepsin B and Parkinson's Disease, and Integrative Analyses of Genome-Wide Association and Transcriptomics Data Linking Herpes Simplex Virus 1 Infection and Parkinson's Disease

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ABSTRACT

This thesis comprises two independent studies investigating two different aspects regarding Parkinson's disease (PD)–related associations.

Study 1: Exploration of the Relationship between Cathepsin B and PD

Objective: The present work sought to examine the relationship between Cathepsin B and PD, placing specific emphasis on the potential mediating function of N-acetylaspartate.

Methodology: GWAS summary data were utilized to perform a two-sample Mendelian randomization (MR) analysis assessing the relationship involving Cathepsin B (3,301 cases) and PD (4,681 cases). A sequential two-step MR analysis involving 8,148 cases was further conducted to evaluate the mediating effect of N-acetylaspartate.

Findings: The MR analysis indicated higher Cathepsin B levels were associated with a lower likelihood of developing PD. Conversely, there was limited evidence to suggest that PD influenced Cathepsin B levels. The estimated mediating effect of N-acetylaspartate was 7.52%.

Conclusion: The findings suggest that increased Cathepsin B levels may reduce the risk of developing PD, potentially through a mediating effect of N-acetylaspartate. Further investigations are warranted to clarify the underlying mechanisms of this relationship. **The main findings of this study have been published as a first-author paper in Brain Sciences (Lu et al., 2024).**

Study 2: Integrative Analyses Linking Herpes Simplex Virus 1 Infection and PD

Objective: The present work seeks to examine the possible association between infection with Herpes Simplex Virus type 1 (HSV-1) and Parkinson's disease (PD) using data derived from GWAS. In addition, transcriptomic datasets are analyzed to identify molecular features shared between these conditions to enhance understanding of their underlying mechanisms and to explore opportunities for drug repurposing.

Methods: Summary-level data obtained from GWAS were applied to perform causal inference analyses assessing the relationship involving herpes keratitis—primarily attributed to HSV-1 infection—and PD. Furthermore, transcriptomic data were examined to detect overlapping molecular signatures that may provide insight into pathogenic mechanisms and inform potential drug-repurposing strategies.

Findings: This work suggested that HSV-1 infection is associated with PD. The up-regulated shared geneset between HSV-1 infection and PD is predominantly enriched in neuroinflammation. Whereas the down-regulated shared geneset is primarily enriched in stem cell and cellular metabolism. And the drug-repurposing target for the shared molecular signature is Nalfurafine.

Conclusions: HSV-1 infection is associated with PD. These two conditions exhibit shared molecular signatures such as neuroinflammation and stem cell, which may serve as targets for drug-repurposing. **This work has been submitted to Parkinson's Disease.**

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Chapter I. General Introduction

1.1 Overview of PD

PD affects more than 10 million people globally. Its incidence rises steeply with advancing age, although roughly 4% of cases are diagnosed before the age of 50[1]. Disease onset prior to 40 years is rare and defined as young-onset PD (YOPD), while occurrence before 21 years constitutes juvenile parkinsonism. Epidemiological data show that men face approximately 1.5 times greater risk than women[2, 3].

The diagnosis of PD relies mainly upon clinical evaluation. Patients, typically older than 55, usually exhibit a slowly progressive and asymmetric motor syndrome. The hallmark motor symptoms include resting tremor, bradykinesia, and muscular rigidity.[4].

PD predominantly affects the basal ganglia, which comprise a group of nuclei at the base of the forebrain. The striatum (caudate and putamen) is the largest complex within the basal ganglia. It receives excitatory cortical input and both inhibitory and excitatory dopaminergic projections originating from the substantia nigra pars compacta (SNc). Inputs converge on spiny projection neurons of two types: those projecting directly to the internal globus pallidus (GPi), the major output nucleus, and those projecting to the external globus pallidus (GPe), forming an indirect pathway to the GPi via the subthalamic nucleus (STN)[5, 6]. The two major hallmarks are loss of pigmented dopaminergic neurons within the substantia nigra pars compacta (SNpc)[7] and Lewy bodies and Lewy neurites, predominantly composed of α-synuclein aggregates[8]. Neuronal loss is most pronounced in the ventrolateral SNpc, with approximately 60–80% dopaminergic neuron loss before motor symptoms appear. Some neurologically normal individuals have incidental Lewy bodies at autopsy, possibly representing a presymptomatic phase; prevalence increases with age. Although characteristic of PD, Lewy bodies are not entirely specific and can occur in atypical parkinsonism, Hallervorden-Spatz disease, and other disorders[9][10].

Although this topic has been widely investigated from multiple perspectives, current therapeutic strategies remain confined to the management of symptoms rather than addressing the underlying causes, with no therapies available to heal the disease[11, 12]. Levodopa continues to represent the gold-standard treatment for motor symptoms, yet prolonged use frequently causes motor fluctuations and dyskinesias[13]. The need for new understanding using new methods is urgent.

1.2 Scope of the Thesis

This thesis comprises two independent studies addressing distinct but equally important aspects of PD pathogenesis: Study 1 investigates whether Cathepsin B, a lysosomal protease, is causally associated with PD, with N-acetylaspartate evaluated as a potential mediator; Study 2 examines the possible causal relationship between HSV-1 and PD, integrating GWAS and transcriptomic data. Together, these studies contribute novel insights into the PD.

Chapter II. Study 1: Exploration of the Association between Cathepsin B and PD

2.1 Introduction

PD is the second most prevalent neurodegenerative condition, primarily impacting those over 60 years of age, with a greater incidence in males[12, 14, 15]. As the disease advances, individuals encounter escalating difficulties that existing therapeutic interventions can't mitigate[11]. Findings from neuropathological investigations indicate that Parkinson’s disease is chiefly characterized by the progressive loss of dopaminergic neurons located within the substantia nigra in the midbrain.

From a clinical standpoint, the disorder is primarily manifested through motor impairments, notably bradykinesia, muscular rigidity, tremor, and disturbances in postural control[16]. In addition to these hallmark motor features, PD is also associated with a broad spectrum of non-motor manifestations, such as olfaction dysfunction, exhaustion, and cognitive decline. Diagnosis mostly depends on neurological examination, as imaging modalities presently provide restricted diagnostic precision[17]. The care of PD remains symptomatic, as no disease-modifying drug has been identified to date.

Accumulating evidence suggests that lysosomal pathways may contribute to the underlying mechanisms of PD[18]. Impairment of lysosomal function is thought to disrupt the normal degradation of α-synuclein, a protein centrally implicated in PD pathogenesis. Within this context, Cathepsin B, a lysosomal protease, has attracted attention as both a potential biomarker and a genetic risk factor for PD[19]. Numerous cohort studies have substantiated this correlation. Chang and associates performed a GWAS using 6,476 participants from the 23andMe Parkinson's Disease cohort and 302,042 controls genotyped [20]. Their analysis identified common variants within the CTSB gene, which encodes Cathepsin B, as being significantly associated with increased PD susceptibility. Complementary evidence was provided by Milanowski et

al., who applied WES in a familial PD context and confirmed a potential role for CTSB [21]. In addition, Jones-Tabah and colleagues, through the integration of genetic datasets from several large consortia, demonstrated that rare CTSB variants are also linked to elevated PD risk [22]. Despite these converging findings, the relationship between Cathepsin B concentrations and PD onset remains inconclusive. This uncertainty is largely attributable to constraints such as limited cohort sizes, relatively short follow-up durations, and the potential influence of reverse causation.

MR has emerged as a robust analytical framework for inferring causality, particularly with the increasing availability of large-scale GWAS datasets. This approach leverages genetic variants, most commonly SNPs, as instrumental variables to evaluate whether an observed association between an exposure and an outcome reflects a causal effect [23].

This study aims to investigate the causal relationship between Cathepsin B and PD, specifically assessing N-acetylaspartate as a potential mediator, given its multiple roles in brain homeostasis, including the regulation of cerebral fluid balance and serving as a precursor for the neuronal dipeptide N-acetylaspartylglutamate. This study was formulated after a thorough examination of prior research connecting Cathepsin B and PD, with the goal of aiming to enhance existing understanding. The originality of our research resides in presenting a novel perspective by examining the mediating function of N-acetylaspartate in this relationship—a facet that has not been well explored. This method signifies a significant progress in comprehending the pathogenesis of PD.

To accomplish this objective, we utilized a rigorous two-sample Mendelian randomization framework employing extensive GWAS summary data from credible public repositories. We specifically examined the correlation between Cathepsin B (3,301 cases) and PD (4,681 cases), employing a sequential two-step Mendelian Randomization technique including 8,148 cases to investigate the mediating effect of N-acetylaspartate. The substantial sample size, coupled with a stringent methodological framework, reduces potential bias and bolsters the credibility of our results.

2.2 Materials and Methods

2.2.1 Research Design

The analysis conducted in this study utilized publically available data, therefore negating the necessity for extra approvals. We conducted a two-sample Mendelian randomization design to evaluate the bidirectional causation between Cathepsin B and PD. A two-step Mendelian randomization analysis, utilizing SNPs as instrumental variables, found possible mediators, with special emphasis on N-acetylaspartate levels. The MR procedure is illustrated in Figure 1. Figure 1 illustrates a conceptual framework for our MR analysis.

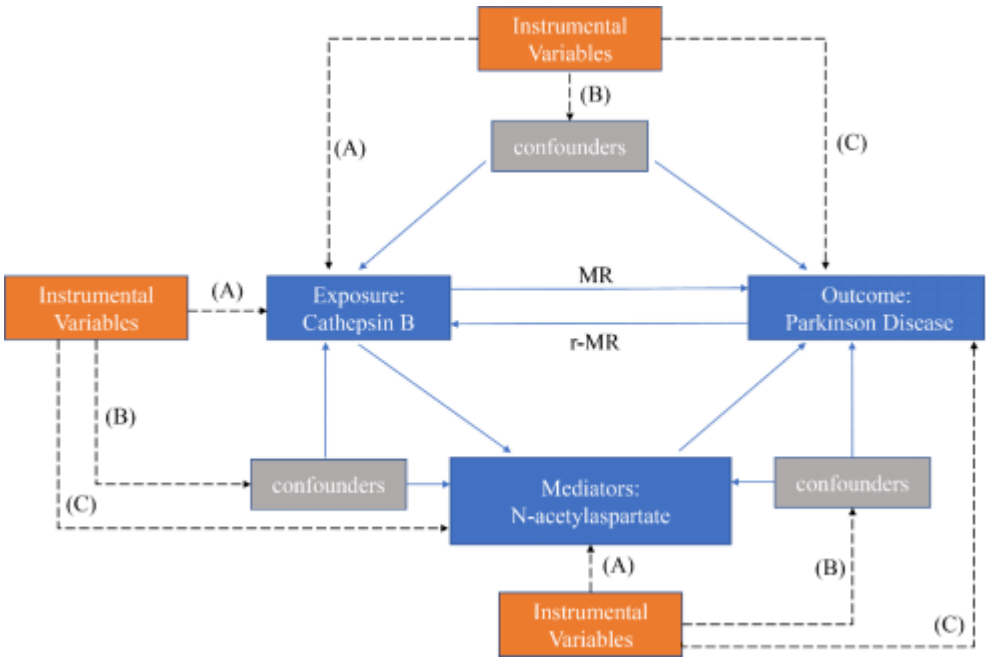


Figure 1 The flowchart presents a conceptual framework for our MR analysis.

2.2.2 Sources of Data for GWAS Summary Statistics

The GWAS data for this research were sourced from publically accessible databases, predominantly consisting of cohorts of European heritage (see Supplementary Table S1 for specifics). Genetic correlations of Cathepsin B were derived from a GWAS meta-analysis conducted by Sun et al[24], involving 3,301 European participants (Supplementary Table S2). Data on PD were acquired from the

FinnGen R10 study[25], comprising 4,681 cases and 407,500 controls (Supplementary Table S3). N-acetylaspartate level data were obtained from the GWAS summary data by Chen et al.[26], encompassing 8,148 individuals (Supplementary Table S4). The GWAS datasets were obtained from separate populations, guaranteeing no data overlap.

2.2.3 Selection of Instrumental Variables and Data Harmonization

SNPs exhibiting genome-wide significance ($P < 5 \times 10{-}5$) were designated as instrumental variables (IVs). The SNPs were grouped based on linkage disequilibrium, utilizing a 10,000 kb window and a r2 threshold of less than 0.001. F-statistics for each SNP were computed using the formula $F = R2 (N - K - 1) / (K (1 - R2))$, which assesses the strength of the instrumental variable, where R2 represents the explained genetic variance in exposure, K denotes the number of SNPs, and N indicates the sample size. SNPs exhibiting an F-statistic over 10 were selected to reduce mild instrument bias in Mendelian randomization analysis.

2.2.4 Mediation Analysis

The MR analysis was performed utilizing R (version R-4.3.0) and the Two Sample MR package (version 0.5.6). Results were presented as odds ratios (ORs) accompanied by 95% confidence intervals (CIs) per standard deviation (SD). Mediation proportions were computed using the formula $(\beta1 \times \beta2) / \beta$, where β denotes the total effect from the primary study, $\beta1$ signifies Cathepsin B's effect on the mediator, and $\beta2$ represents the mediator's influence on PD. Standard errors and confidence intervals were calculated via the delta approach.

2.2.5 Statistical Examination

The strength of the causal inferences was evaluated using many sensitivity analyses. This encompassed Cochran's Q statistics for heterogeneity in the Inverse Variance Weighting (IVW) model, MR-Egger intercept tests for horizontal pleiotropy, and leave-one-out sensitivity analyses. Cochran's Q statistics evaluated heterogeneity in IVW models[27, 28], suggesting probable heterogeneity with significant p values

($p < 0.05$). The MR-Egger intercept test was utilized to assess horizontal pleiotropy[29, 30]. The presence of a non-zero intercept in the Mendelian randomization impact estimates of instrumental variables indicates horizontal pleiotropy. Leave-one-out analysis was conducted by sequentially eliminating each SNP to evaluate the impact of individual SNPs on the overall causal estimates[31].

2.3 Results

2.3.1 Primary Analysis

Various MR techniques were utilized to investigate the causal association between Cathepsin B and PD. The IVW method functioned as the primary analytical technique, integrating the Wald ratios obtained from each SNP to assess the cumulative causal influence[32]. To augment robustness, supplementary methods—including MR-Egger regression[33], the weighted median technique, and both simple mode and weighted mode estimators—were utilized[30, 34].

2.3.2 Mediation Analysis

A two-step MR framework was implemented to determine whether N-acetylaspartate acts as a mediator in the causal pathway linking Cathepsin B to PD. This design decomposed the total causal effect into direct effects (independent of mediation) and indirect effects (mediated by N-acetylaspartate). The proportion mediated was calculated by dividing the indirect effect by the total effect, and corresponding 95% confidence intervals (CIs) were computed using the delta method.

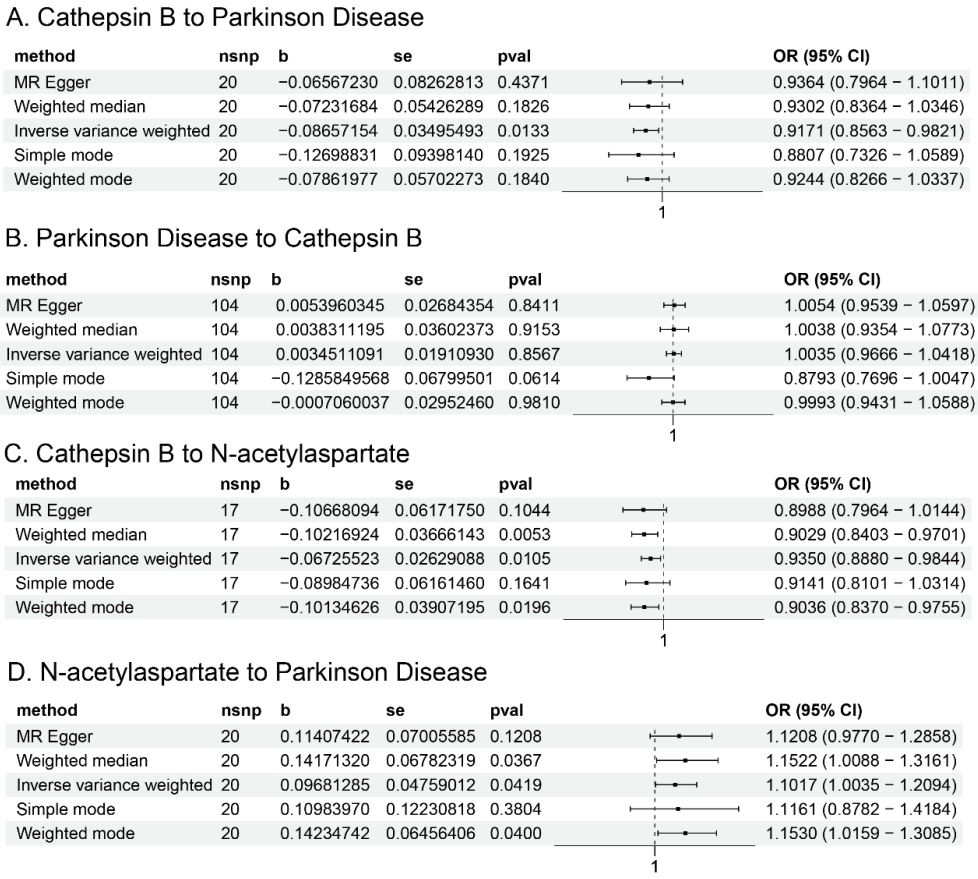


Figure 2 MR results Forest plot.

A. The potential causal effect of Cathepsin B serving as the exposure variable on the risk of Parkinson's disease as the outcome was evaluated. B. The reverse direction was also examined, assessing whether Parkinson's disease, considered as the exposure, exerts a causal influence on Cathepsin B levels as the outcome. C. Five distinct analytical approaches were applied to estimate the causal association between Cathepsin B (exposure) and N-acetylaspartate concentrations (outcome). D. Similarly, the causal impact of N-acetylaspartate levels as the exposure on Parkinson's disease as the outcome was quantified using five complementary methodological strategies.

2.3.3 Association Between Cathepsin B and PD

Univariate MR results examining the causal association between Cathepsin B and PD are shown in Figure 2A and detailed in Supplementary Table S5. A total of 20 SNPs were selected as instrumental variables (IVs). The IVW analysis revealed a significant inverse association between Cathepsin B and PD (OR = 0.9171, 95%CI: 0.8563-0.9821, p = 0.0133), indicating that higher Cathepsin B levels corresponded to

an approximately 8.3% reduction in PD risk.

The funnel plot demonstrated symmetry, suggesting no substantial bias in SNP selection (Supplementary Figure 1A). The scatter plot illustrated the causal relationship based on MR estimates (Supplementary Figure 1B). The IVW forest plot for individual SNPs (Supplementary Figure 1C) and leave-one-out analysis (Supplementary Figure 1D) confirmed the robustness of the findings. Genetic variant characteristics are summarized in Supplementary Table S7, and expression analyses indicated that CTSB was downregulated in PD (Supplementary Figure 2).

The reverse analysis—testing the causal effect of PD on Cathepsin B—employed 104 SNPs as IVs (Figure 2B). None of the MR methods yielded significant results (IVW: $p = 0.8567$), indicating a lack of evidence supporting a reverse causal association. Diagnostic plots for this direction are presented in Supplementary Figure 3.

2.3.4 Association Between Cathepsin B and N-acetylaspartate Levels

Seventeen genome-wide significant SNPs were used as IVs for Cathepsin B. The IVW, weighted median, and weighted mode analyses consistently demonstrated that genetically elevated Cathepsin B levels were negatively associated with N-acetylaspartate concentrations. These results suggest that higher Cathepsin B levels are associated with an approximate 6.5% reduction in N-acetylaspartate levels (Figure 2C; Supplementary Table S5). Funnel, scatter, and forest plots, along with leave-one-out analyses, are shown in Supplementary Figure 3, and the genetic variant details are listed in Supplementary Table S8.

2.3.5 Association Between N-acetylaspartate Levels and PD

As shown in Figure 2D and Supplementary Table S5, genetically predicted N-acetylaspartate levels exhibited a significant positive association with PD risk. Diagnostic plots supporting these results are provided in Supplementary Figure 3.

2.3.6 Proportion of the Association Mediated by N-acetylaspartate

The two-step MR mediation analysis indicated that N-acetylaspartate partially

mediates the causal pathway between Cathepsin B and PD. Specifically, Cathepsin B was inversely associated with N-acetylaspartate levels, which in turn were positively associated with PD risk. The estimated proportion mediated was 7.52%, suggesting that N-acetylaspartate levels accounted for approximately 9.87% of the total effect of Cathepsin B on PD.

2.3.7 Sensitivity Analyses

Cochran's Q tests revealed no significant heterogeneity among the selected IVs. The MR-Egger intercept analyses produced p-values > 0.05, indicating the absence of substantial horizontal pleiotropy. The MR-PRESSO test identified and removed outlier SNPs with evidence of pleiotropy; reanalysis after their exclusion yielded consistent results. The leave-one-out analysis further confirmed the stability of the findings, as no single SNP exerted a disproportionate influence on the overall causal estimates. Detailed sensitivity results are presented in Supplementary Table S6.

2.4 Discussion

PD is a prevalent neurodegenerative disorder affecting over 10 million individuals worldwide. Its incidence increases markedly with age, although a minority of cases, approximately 4%, are identified before the age of 50 [1]. When onset occurs before 40 years, the condition is classified as YOPD, whereas cases arising before 21 years are referred to as juvenile parkinsonism. Epidemiological studies consistently indicate a higher susceptibility in males, with a risk approximately 1.5-fold greater than that observed in females [2, 3]. In clinical practice, the diagnosis of PD is primarily based on neurological assessment rather than definitive laboratory testing. Most patients, commonly over the age of 55, present with a gradually progressive motor disorder that typically begins asymmetrically. The cardinal motor manifestations include resting tremor, bradykinesia, and rigidity, which together form the core diagnostic features of the disease [4]. From a neuropathological perspective, PD predominantly involves dysfunction within the basal ganglia, a collection of subcortical nuclei located at the base of the forebrain. The striatum, comprising the

caudate nucleus and putamen, represents the principal input structure of this system. It integrates excitatory signals from the cortex alongside dopaminergic inputs originating in the SNc, which exert both excitatory and inhibitory effects. These convergent signals are processed by two populations of spiny projection neurons. One group forms the direct pathway, projecting to the internal segment of the GPi, the primary output nucleus. The second group contributes to the indirect pathway, projecting first to the GPe and subsequently influencing the GPi via the STN [5, 6]. The defining pathological features of PD include a substantial loss of pigmented dopaminergic neurons in the SNpc [7], together with the presence of Lewy bodies and Lewy neurites, which are largely composed of aggregated α-synuclein [8]. Degeneration is particularly severe in the ventrolateral region of the SNpc, where it is estimated that 60–80% of dopaminergic neurons are lost before clinical motor symptoms become evident. Notably, Lewy body pathology has also been identified in neurologically asymptomatic individuals at autopsy, suggesting a potential preclinical stage whose frequency increases with advancing age. However, although these inclusions are characteristic of PD, they are not entirely disease-specific and may also be observed in conditions such as atypical parkinsonian syndromes and Hallervorden–Spatz disease [9, 10]. Despite extensive research efforts across multiple domains, current therapeutic approaches remain largely symptomatic and do not modify the underlying disease process. No treatment is currently available that halts or reverses neurodegeneration [11, 12]. Levodopa remains the most effective pharmacological option for alleviating motor symptoms; however, long-term administration is commonly associated with complications, including motor fluctuations and dyskinesias [13]. These limitations underscore the pressing need for novel mechanistic insights and the development of more effective therapeutic strategies.

This study investigated the intricate relationship between Cathepsin B and PD, with particular emphasis on the potential mediating contribution of N-acetylaspartate. To address this objective, we employed a two-sample MR framework alongside a two-step MR approach, drawing on data derived from well-characterized European

cohorts. This analytical design provides a robust basis for exploring potential causal pathways underlying the observed associations.

Existing evidence has suggested that Cathepsin B, a lysosomal protease, may function both as a biomarker and as a contributor to PD susceptibility. Multiple lines of research, including genetic [18, 35], clinical[31], and experimental investigations[32, 33]. Research across several groups has confirmed a potential genetic association between Cathepsin B and PD. For instance, a genome-wide association study conducted by Chang et al. analyzed 6,476 individuals from the 23andMe PDWBS alongside 302,042 controls genotyped using customized Illumina platforms. Their findings indicated that common variants within the CTSB gene, which encodes Cathepsin B, are associated with an increased likelihood of PD development[20].

Further support arises from familial genetic analyses. Milanowski et al. performed whole-exome sequencing in a PD-affected family and identified a rare CTSB variant (p.Gly284Val) that may underlie the observed clinical phenotype. Their work included functional characterization in patient-derived fibroblasts, thereby providing insight into how this mutation might influence gene expression and cellular processes relevant to PD pathology[21].

Complementary large-scale investigations have reinforced these observations. Jones-Tabah et al. integrated genetic datasets from multiple consortia—including BioFIND, HBS, PPMI, PDBP, iLBDGC, and STEADY-PD III. Their findings aligned with prior studies, indicating that uncommon mutations in the CTSB gene may contribute to an elevated risk of PD[22].

Sjödin and colleagues executed a pilot research integrating solid-phase extraction with parallel reaction monitoring mass spectrometry, revealing a reduction in the content of Cathepsin B[36]. McGlinchey and colleagues identified Cathepsin B as crucial for the lysosomal breakdown of α-synuclein[37, 38]. Jones-Tabah and colleagues conducted multiple tests indicating that Cathepsin B may facilitate the clearance of fibrillar alpha-synuclein, augment lysosomal functionality, and promote glucocerebrosidase activity in dopaminergic neurons[22].

The findings from our MR study indicated a protective causal connection between increased levels of genetically predicted Cathepsin B and a reduced risk of acquiring PD. This discovery is especially significant considering the traditional comprehension of PD pathogenesis. Our study expanded this paradigm by emphasizing the possible involvement of lysosomal dysfunction.

A strong correlation between PD and lysosomal dysfunction has already been identified as a critical factor in the pathogenesis models of PD[39]. Lysosomal storage disorders, marked by the dysfunction of lysosomal proteins, have a common clinical characteristic with PD[40]. In this context, the involvement of lysosomal enzymes, particularly glucocerebrosidase (encoded by GBA1), can substantially affect the etiology of PD[41-45]. Among the 90 loci identified as associated with PD susceptibility, variations in the GBA1 gene, acknowledged as the predominant genetic risk factor for PD, have garnered considerable attention in the research. Data revealed that roughly 5% of persons diagnosed with PD possessed a mutation in the GBA1 gene[46]. The existence of either heterozygous or homozygous mutations in GBA1 was associated with a 20 to 30-fold elevation in the probability of PD onset[47, 48]. Impaired glucocerebrosidase function diminished lysosomal activity, leading to decreased catabolism of α-synuclein, promoting its aggregation and the ensuing neurodegenerative cascade[49].

The mediation study indicated that N-acetylaspartate has a modest impact in the relationship between Cathepsin B and PD. N-acetylaspartate participates in various physiological functions, such as osmoregulation in neurons, providing acetate for myelin lipid synthesis, synthesizing the neuropeptide N-acetylaspartylglutamate, and facilitating energy consumption in neuronal mitochondria. Recent investigations indicate that N-acetylaspartate may play a function in protein stabilization and the inhibition of protein aggregation[50]. In this regard, Gröger and associates observed markedly reduced N-acetylaspartate levels in the substantia nigra of PD patients relative to controls, employing three-dimensional magnetic resonance spectroscopic imaging[51]. Additional research is required to thoroughly assess these findings and comprehend the complex pathophysiology of PD.

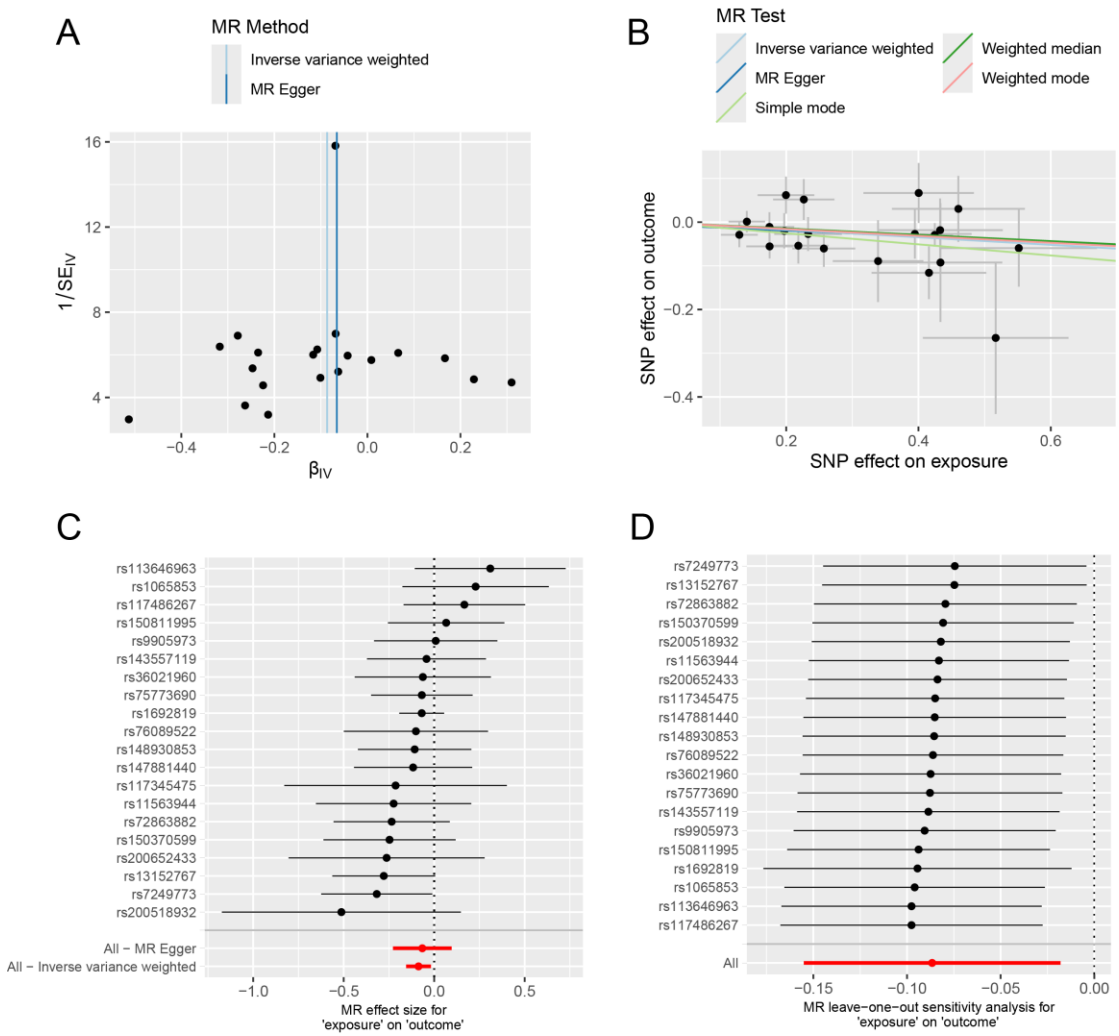
The present study possesses limitations. Our focus was on European individuals; thus, data from other patient populations are required to validate the current findings. This will be feasible once further GWAS data encompassing information on Cathepsin B and N-acetylaspartate from non-European populations becomes accessible. Moreover, the precise biological roles of several SNPs have yet to be comprehensively elucidated. Notwithstanding these constraints, our research elucidated the association between Cathepsin B and PD, establishing a foundation for future inquiries. The MR methodology is significantly less vulnerable to confounding variables and reverse causality than conventional observational and interventional research. Further inquiry and data are necessary to clarify the causal relationship between Cathepsin B and PD. A major drawback of our work is its exclusive focus on a single type of Cathepsin; thus, additional research should explore the potential relationship between PD and other Cathepsins, such as Cathepsin D and Cathepsin L[19].

2.5 Conclusions

Our research enhanced the existing comprehension of PD pathophysiology. The research validated the potential function of Cathepsin B as a biomarker and risk factor for the development of PD, while also introducing fresh insights into the metabolic pathways associated with the condition. The mediating effect of N-acetylaspartate, albeit appearing restricted, offered a novel insight for further research.

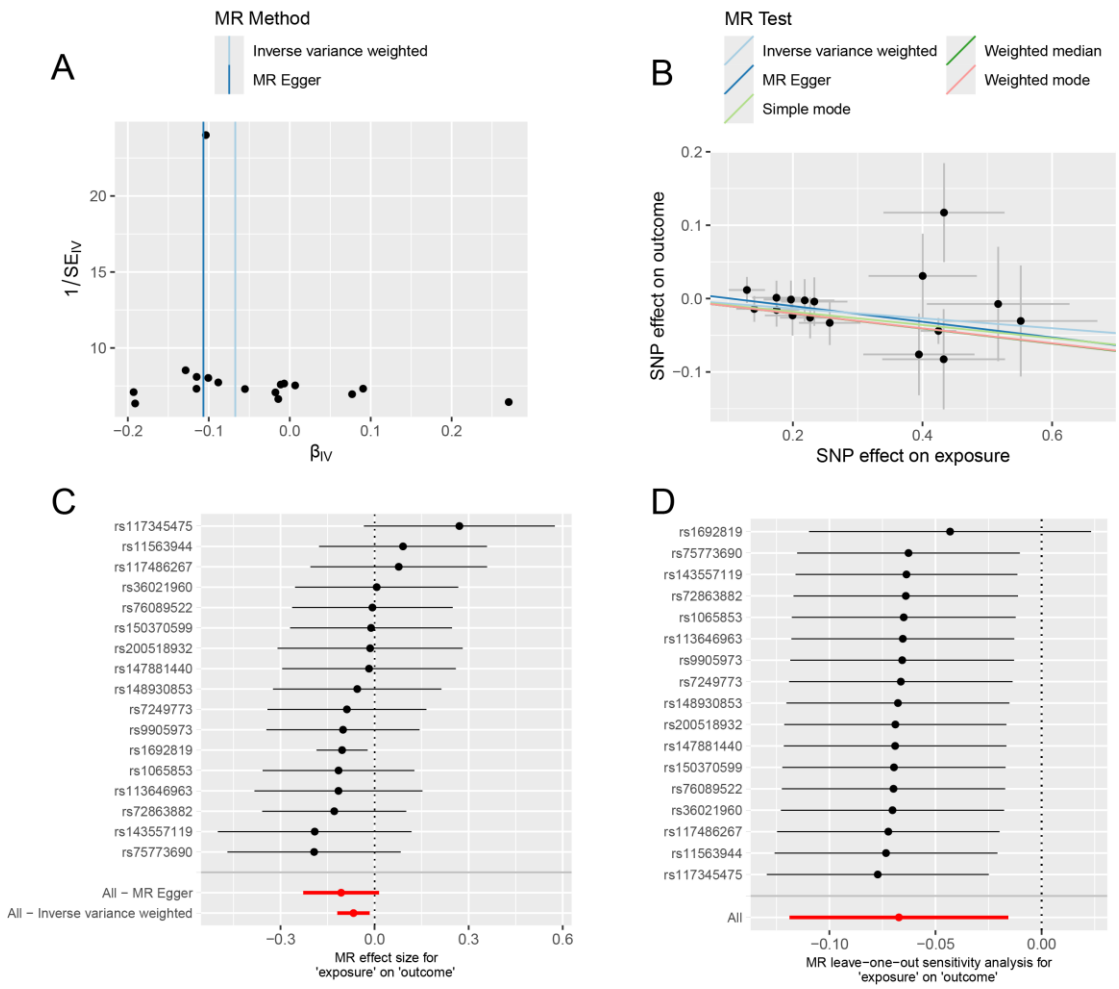
2.6 Supplementary Materials

2.6.1 Supplementary Figures



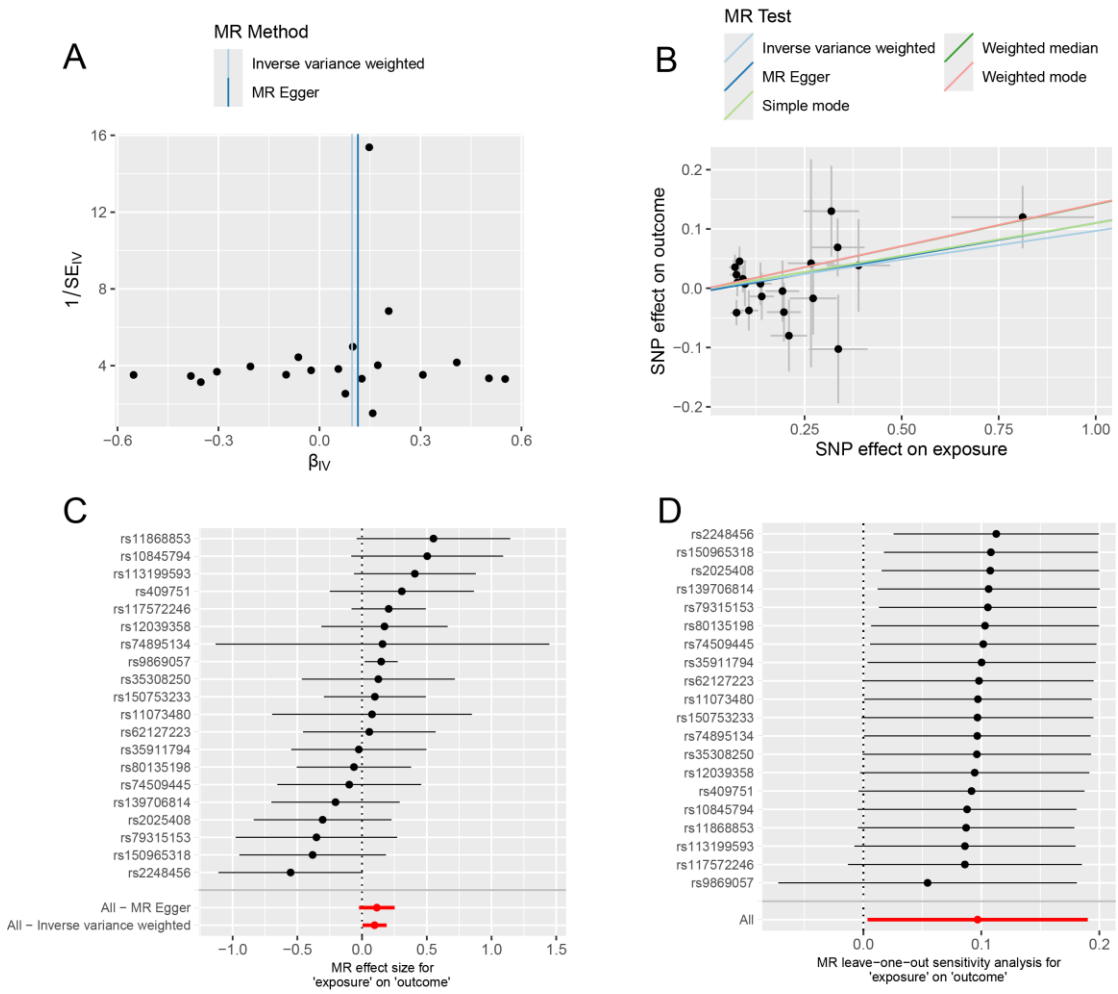
Supplementary Figure S1. Analysis Figure.

(A) Funnel plot. (B) Scatter plot. (C) Forest plot. (D)Forest plot.



Supplementary Figure S2. Analysis Figure.

(A)Funnel plot. (B)Scatter plot. (C)Forest plot. (D)Forest plot.



Supplementary Figure S3. Analysis Figure.

(A) Funnel plot. (B) Scatter plot. (C) Forest plot. (D) Forest plot.

2.6.2 Supplementary Tables

Supplementary Table S1: Information for Database.

Data sources	Phenotypes	sample description
Sun Benjamin B et al. GWAS	Cathepsin B	3,301 European ancestry individuals
FinnGen R10 study data	PD	4681 cases and 407500 controls
Chen Y et.al GWAS	N-acetylaspartate (naa) levels	8,148 European ancestry individuals

Supplementary Table S2: IV used in Cathepsin B.

rsid	effect_allele	other_allele	p_value
rs75773690	G	A	4.17E-06
rs113646963	C	T	2.95E-06
rs76089522	G	A	2.88E-06
rs200518932	C	CA	2.63E-06
rs13152767	G	A	1.62E-06
rs36021960	G	A	3.02E-06
rs11563944	A	G	3.55E-06
rs1692819	G	A	5.25E-54
rs148930853	T	C	3.02E-06
rs150811995	A	C	4.57E-06
rs72863882	G	A	6.46E-08
rs150370599	C	T	1.78E-06
rs143557119	G	A	4.79E-06
rs117345475	A	G	3.47E-06
rs117486267	T	C	1.58E-06
rs9905973	G	A	3.55E-07
rs147881440	G	A	4.79E-06
rs200652433	C	T	7.24E-07
rs7249773	G	A	6.03E-07
rs1065853	G	T	1.00E-06

Abbreviations: rsid, reference SNP identifier; effect_allele, the allele associated with the reported effect; other_allele, the alternative allele; p_value, P-value.

Supplementary Table S3: IV used in PD

rsid	effect_allele	other_allele	p_value
rs74773666	C	G	3.56E-05
rs1240761	C	T	2.75E-05
rs61814205	C	A	3.51E-05
rs35603727	A	G	1.87E-12
rs12747451	G	A	2.05E-05

rs57848722	T	C	3.53E-05
rs78479546	A	C	2.10E-05
rs12759573	G	A	2.72E-05
rs150333605	C	T	3.11E-05
rs2502428	G	A	4.80E-05
rs75218231	G	A	2.50E-06
rs72776604	A	G	2.80E-05
rs1401852	C	T	1.14E-05
rs116661870	T	C	1.50E-06
rs142753050	C	A	1.58E-05
rs62150277	T	C	4.54E-05
rs10928512	T	G	3.20E-05
rs7606168	C	T	2.12E-05
rs72484023	A	G	2.72E-05
rs71323011	C	T	3.60E-05
rs146070700	A	G	3.95E-05
rs75942641	A	G	1.50E-05
rs16854140	T	C	3.06E-06
rs80117947	T	C	2.59E-05
rs7650456	G	C	4.80E-06
rs9841498	T	C	9.16E-06
rs34311866	C	T	9.77E-07
rs13105679	C	G	2.25E-05
rs4373196	A	C	4.71E-05
rs36067287	C	T	9.00E-06
rs145343218	T	C	8.56E-06
rs72881504	C	G	9.55E-06
rs2583990	G	A	4.10E-08
rs17046072	T	C	1.52E-05
rs12331618	G	A	1.45E-05
rs67569121	C	A	4.16E-05
rs115620277	G	A	2.73E-05
rs66465003	G	A	2.64E-05
rs74558405	A	G	2.39E-05
rs3934591	G	A	5.35E-07
rs845890	C	A	4.60E-05
rs142919280	A	T	1.10E-05
rs114335056	G	A	1.36E-06
rs12662228	A	G	3.52E-05
rs118136794	G	A	2.49E-05
rs151305702	A	C	4.68E-07
rs45480197	A	G	1.83E-05
rs61756902	C	G	2.43E-05
rs151246279	G	A	1.75E-05

rs141184703	T	A	4.50E-05
rs10271158	G	A	3.18E-06
rs71260019	T	C	1.48E-05
rs145166201	C	T	2.43E-05
rs147219362	T	C	4.34E-05
rs374417259	C	T	2.30E-05
rs1346278	C	T	3.69E-05
rs62498020	C	G	1.52E-05
rs55667647	GTA	G	6.09E-07
rs148452891	G	C	1.51E-05
rs2509770	A	T	3.08E-06
rs6470633	C	T	2.73E-05
rs72728578	C	T	6.44E-06
rs71370984	ATT	A	1.06E-05
rs10981484	T	C	2.54E-05
rs78246462	C	T	3.66E-05
rs61845846	T	C	8.41E-06
rs118049046	T	C	4.60E-05
rs6585335	T	C	1.13E-05
rs66915007	G	C	9.76E-06
rs7116239	T	C	8.85E-06
rs117893454	A	G	1.48E-05
rs11226928	T	C	5.18E-06
rs4285931	C	T	7.49E-06
rs3847922	T	G	2.78E-05
rs75665194	G	C	8.67E-06
rs79024095	G	T	4.92E-05
rs74415029	G	A	2.63E-05
rs4489814	G	A	3.16E-05
rs11612666	G	T	1.94E-05
rs3748268	G	A	2.01E-05
rs1956626	A	G	8.26E-06
rs186771067	T	A	4.46E-06
rs78746245	A	C	3.25E-05
rs72708683	G	C	3.78E-05
rs36185677	T	A	4.21E-05
rs11072689	A	G	3.60E-05
rs141243902	A	T	2.06E-05
rs117503845	A	G	1.91E-08
rs11646097	C	T	6.25E-06
rs75280493	C	T	2.60E-05
rs4284662	T	G	4.25E-05
rs11075309	C	T	1.90E-05
rs371828274	A	G	5.10E-06

rs8051415	G	T	4.03E-05
rs117310434	A	G	4.35E-05
rs77704942	C	T	3.12E-05
rs190268994	T	G	1.37E-05
rs76670471	G	A	2.87E-05
rs2532362	T	G	1.68E-09
rs2696499	G	C	9.12E-11
rs73323419	C	T	3.51E-05
rs874132	T	G	1.93E-05
rs140855659	A	G	1.40E-05
rs12611282	A	G	1.03E-05
rs73536723	A	G	2.41E-06
rs1039106	G	A	2.59E-05
rs74963755	C	A	4.44E-06
rs17804573	C	A	3.09E-05
rs148983668	A	G	3.92E-05
rs140529886	C	G	2.19E-05
rs73210245	A	T	5.74E-06
rs144174710	G	A	1.64E-06
rs77628790	G	T	1.26E-07
rs45529631	C	T	2.82E-05

Abbreviations: rsid, reference SNP identifier; effect_allele, the allele associated with the reported effect; other_allele, the alternative allele; p_value, P-value.

Supplementary Table S4: IV used.

rsid	effect_allele	other_allele	p_value
rs12039358	G	T	8.49E-07
rs35308250	C	A	6.13E-06
rs139706814	G	A	6.90E-06
rs62127223	A	G	1.62E-06
rs9869057	C	T	9.51E-06
rs150965318	C	T	7.80E-06
rs544693361	T	C	4.22E-06
rs79315153	G	A	7.26E-06
rs2248456	A	G	1.49E-06
rs35911794	C	T	7.13E-06
rs10845794	T	G	8.14E-06
rs74895134	A	G	8.89E-06
rs117572246	A	G	1.29E-06
rs2025408	A	C	8.76E-06
rs150753233	G	A	1.81E-06
rs11073480	G	A	6.16E-06
rs113199593	G	A	8.90E-06

rs74509445	T	C	8.45E-06
rs11868853	G	A	2.93E-06
rs80135198	T	C	6.65E-06
rs409751	T	C	4.26E-06

Abbreviations: rsid, reference SNP identifier; effect_allele, the allele associated with the reported effect; other_allele, the alternative allele; p_value.

Supplementary Table S5: Effect estimates.

exposure to outcome	method	pval
Cathepsin B to Parkinson Disease	MR Egger	0.437088476
	Weighted median	0.182626093
	Inverse variance weighted	0.013261734
	Simple mode	0.192500568
	Weighted mode	0.183991127
Parkinson Disease to Cathepsin B	MR Egger	0.841084809
	Weighted median	0.915304751
	Inverse variance weighted	0.856682833
	Simple mode	0.061421581
	Weighted mode	0.980968758
Cathepsin B to N-acetylaspartate	MR Egger	0.104414039
	Weighted median	0.005322608
	Inverse variance weighted	0.01052398
	Simple mode	0.164131194
	Weighted mode	0.019584391
N-acetylaspartate to Parkinson Disease	MR Egger	0.120830676
	Weighted median	0.036667195
	Inverse variance weighted	0.041920776
	Simple mode	0.380395013
	Weighted mode	0.039996324

Abbreviations: pval, P-value.

Supplementary Table S6: Heterogeneity and horizontal pleiotropy analysis

Exposure	Outcome	Horizontal pleiotropy test		Heterogeneity test	
		MR-Egger regression	MR-PRESSO	IVW	MR-Egger
		P value	Global test p value	P value	P value
Cathepsin B	Parkinson Disease	0.7833	0.585	0.5591	0.4973
Parkinson	Cathepsin B	0.918	0.713	0.705	0.6808

Disease				4	
Cathepsin B	N-acetylaspartate	0.491	0.802	0.8186	0.797
N-acetylaspartate	Parkinson Disease	0.7356	0.314	0.2652	0.2212

Supplementary Table S7: Cathepsin B-related SNP's effects on PD

SNP	p	or
rs1065853	0.266973004	1.257239392
rs113646963	0.14510461	1.36327143
rs11563944	0.305837761	0.799138079
rs117345475	0.496505299	0.807867961
rs117486267	0.330305808	1.181441377
rs13152767	0.054548253	0.756820519
rs143557119	0.800228811	0.958440413
rs147881440	0.484098817	0.890005852
rs148930853	0.500321749	0.897802875
rs150370599	0.185274231	0.78125807
rs150811995	0.686911364	1.068416569
rs1692819	0.276152205	0.933479023
rs200518932	0.127985019	0.598811629
rs200652433	0.341293807	0.768811539
rs36021960	0.744109936	0.939261167
rs7249773	0.042772664	0.728082191
rs72863882	0.151462312	0.790627167
rs75773690	0.633056064	0.933973964
rs76089522	0.619177239	0.903924319
rs9905973	0.961154666	1.008500553

Supplementary Table S8: Cathepsin B-related SNP's effects on N-acetylaspartate

SNP	p	or
rs1065853	0.351819031	0.89136221
rs113646963	0.399379591	0.891174137
rs11563944	0.507082552	1.094853374
rs117345475	0.081609783	1.310590553
rs117486267	0.592079245	1.080076882
rs143557119	0.225423564	0.826120942
rs147881440	0.900310442	0.982452943
rs148930853	0.686452589	0.946180798
rs150370599	0.932427476	0.988893562
rs1692819	0.012817023	0.901523562
rs200518932	0.925009993	0.985920399
rs36021960	0.959912253	1.006694259
rs7249773	0.494726111	0.915461432

rs72863882	0.272441744	0.879279213
rs75773690	0.171275601	0.824577072
rs76089522	0.958041876	0.993152018
rs9905973	0.419261914	0.90425928

Supplementary Table S9: N-acetylaspartate-related SNP's effects on PD

SNP	p	or
rs10845794	0.092634926	1.654210192
rs11073480	0.845277647	1.079854281
rs113199593	0.089117546	1.503617903
rs117572246	0.159156063	1.228328132
rs11868853	0.068318392	1.735851521
rs12039358	0.486092226	1.1891236
rs139706814	0.417025992	0.814570416
rs150753233	0.621980468	1.10396431
rs150965318	0.186160706	0.682544086
rs2025408	0.261722042	0.737441124
rs2248456	0.052115259	0.575711372
rs35308250	0.676399475	1.133973836
rs35911794	0.925558719	0.975435791
rs409751	0.27932672	1.359517745
rs62127223	0.830258388	1.05761216
rs74509445	0.726758528	0.905688826
rs74895134	0.810063611	1.171259269
rs79315153	0.268235176	0.703001217
rs80135198	0.780776483	0.939226816
rs9869057	0.023132376	1.159173084

Chapter III. Integrative Analyses Linking Herpes Simplex Virus 1 Infection and PD

3.1 Introduction

PD is characterized by progressive loss of dopaminergic neurons located in the substantia nigra[52], This neuronal degeneration leads to a range of motor impairments as well as non-motor manifestations, both of which gradually worsen during disease progression[16, 53]. Although extensive investigations have examined PD from multiple scientific perspectives[12], current therapeutic approaches primarily focus on alleviating symptoms rather than reversing or curing the disease[11]. Consequently, there is a pressing need to obtain deeper insights into PD pathogenesis through the application of new analytical strategies.

HSV-1 is highly prevalent worldwide, with approximately 67% of individuals younger than 50 years estimated to be infected[54]. Numerous studies have attempted to clarify whether HSV-1 infection is associated with PD; however, their conclusions remain inconsistent. Some investigations reported that individuals with idiopathic PD display increased titers of HSV-1-specific antibodies and a greater frequency of HSV-1 infection[55-59]. In contrast, findings from a large population-based case-control analysis involving Medicare beneficiaries aged 66–90 years (89,790 PD cases and 118,095 controls) indicated that a history of herpes simplex infection was associated with a reduced risk of PD (OR 0.79, 95% CI 0.74–0.84)[60]. These conflicting observations may partly reflect variability in HSV-1 infection characteristics as well as inherent methodological limitations of observational studies, including confounding influences[61], selection bias[62], and the possibility of reverse causality[63].

Herpes keratitis are mainly caused by HSV-1 direct infection or its reactivation from latency to cornea, conjunctiva[64]. While numerous investigations have examined the broader association between HSV-1 infection and PD, the potential link between herpes keratitis, representing a specific clinical manifestation of HSV-1, and

PD has not yet been systematically evaluated. Examining herpes keratitis as a defined form of HSV-1 infection may offer methodological advantages compared with studies that consider HSV-1 exposure in general terms. Restricting the analysis to a specific clinical condition could reduce heterogeneity and limit confounding influences related to other HSV-1–associated manifestations. Such an approach may therefore facilitate a more precise assessment of the potential relationship between HSV-1–related ocular disease and PD.

Observational studies are often affected by methodological limitations, particularly the influence of confounding variables. Randomized controlled trials (RCTs) are typically considered the most reliable approach for overcoming these issues, as the random allocation of participants to exposure groups promotes a balanced distribution of potential confounders. Nevertheless, conducting RCTs in the context of human herpes keratitis and PD would be neither ethically acceptable nor practically achievable. As an alternative strategy, MR can be applied to investigate causal relationships. This analytical framework uses SNPs as instrumental variables (IVs) to approximate the randomization process that occurs in controlled trials. Through this approach, it becomes possible to evaluate the potential causal association between herpes keratitis and PD while minimizing ethical concerns and reducing biases commonly present in observational research. In addition to employing MR to explore causality, transcriptomic datasets were analyzed to identify molecular features shared by herpes keratitis and PD. Characterizing these overlapping molecular signatures may contribute to a deeper understanding of the biological mechanisms involved and may also highlight potential opportunities for drug repurposing.

This study hypothesized that infection with HSV-1, particularly when manifested as herpes keratitis, may represent a potential causal risk factor for PD through defined molecular pathways. To minimize confounding arising from the biological and clinical heterogeneity of HSV-1 infections, the analysis focused specifically on herpes keratitis as a representative and well-defined manifestation. MR was employed as the primary analytical framework because it enables the estimation of causal effects while

reducing bias from confounding and reverse causation, thereby providing a robust strategy for addressing the previously debated association. In addition, transcriptomic analysis was conducted to identify molecular features shared between the two conditions. This integrative approach allowed the investigation of potential biological links from a systems-level perspective and provided a basis for identifying targets that may be relevant to drug repurposing. Accordingly, the study was designed to address three key questions: whether herpes keratitis has a causal relationship with PD; which molecular signatures are common to both diseases; and how such molecular insights might contribute to identifying repurposable therapeutic agents. By systematically examining these issues, the present work clarifies the potential association between herpes keratitis and PD while also highlighting shared molecular characteristics that may improve mechanistic understanding and inform future drug-repurposing strategies aimed at enhancing therapeutic outcomes.

3.2 Materials and Methods

3.2.1 Study Design

All datasets analyzed in this study were retrieved from publicly available GWAS repositories, and therefore additional ethical approval was not required. To evaluate the potential causal relationship between herpes keratitis and PD, a bidirectional MR framework was applied. Furthermore, pathway enrichment analysis and drug repurposing exploration were conducted based on the identification of shared molecular signatures between the two conditions. The overall analytical design and workflow of the study are illustrated in Figure below.

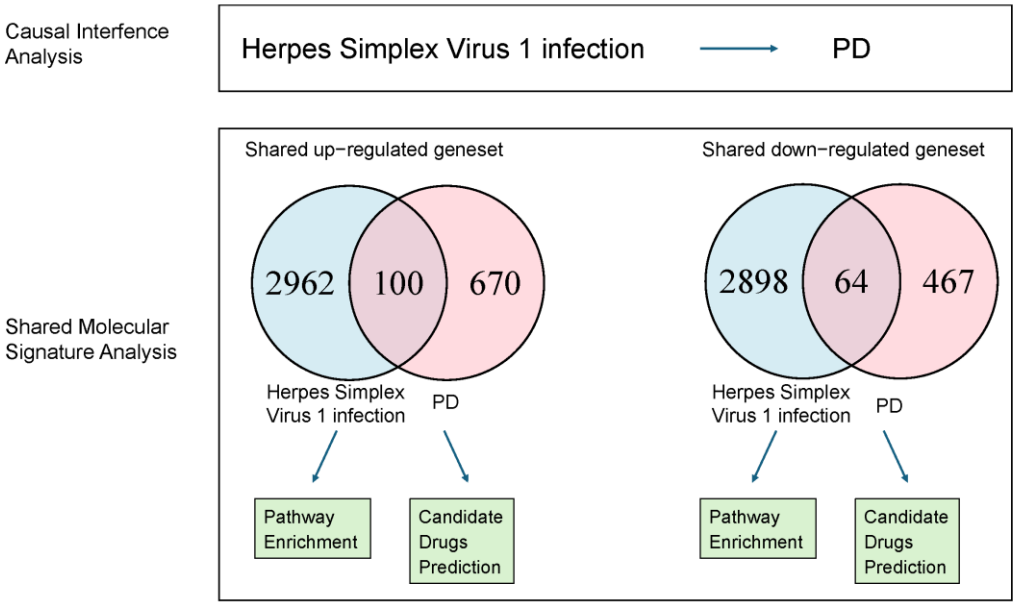


Figure 1 illustrates the overall analytical framework of the study, highlighting the integration of GWAS datasets with transcriptomic analyses to investigate the potential relationship between HSV-1 infection and PD.

3.2.2 GWAS data Source

The GWAS datasets utilized in this study were retrieved from publicly available resources, with detailed information summarized in Supplementary Table S1. Genetic association data for herpes keratitis were derived from the FinnGen R10 release[25], the work led by Mitja I Kurki et al, encompassing 1,252 cases, 390,647 controls. PD data were obtained from the work led by Mike A Nalls et al[65].

3.2.3 Source of PD transcriptomics differential expression data

Differential gene expression data comparing PD cases with controls within the Parkinson's Progression Markers Initiative (PPMI) cohort were obtained from the study conducted by Craig Scherzer and colleagues. In their analysis, factors including genetic background, age, and sex were taken into account[66]. The dataset comprised bulk RNA-sequencing profiles generated from 4,871 longitudinal whole-blood samples collected from 1,570 individuals diagnosed with Parkinson’s disease. Detailed procedures for identifying differentially expressed genes are described in the original publication by Craig and collaborators.

3.2.4 HSV-1 infected fibroblast KMB17 as a model for transcriptomics differential expression data

To model host transcriptional responses associated with HSV-1 infection, transcriptomic data derived from HSV-1–infected KMB17 fibroblast cells were analyzed. These data are available in the Gene Expression Omnibus under accession number GSE103763. Differential gene expression between infected and control samples was evaluated using the GEO2R online analysis platform[67]. For the identification of significantly altered transcripts, a threshold of $|\log_2$ fold change$|$ greater than 1 was applied together with an adjusted P value below 0.05. Genes meeting both criteria were considered to exhibit statistically significant expression changes.

3.2.5 Instrumental variables selection and data harmonization

Within the MR framework[23], instrumental variables (IVs)[68] should be SNPs that closely related to the exposure. Based on this principle, SNPs significantly linked to herpes keratitis and Parkinson's disease were initially identified. To minimize potential confounding arising from genetic correlation, linkage disequilibrium (LD) pruning was subsequently performed. This procedure applied a window size of 10,000 kb and an r^2 threshold below 0.001, allowing the removal of correlated variants as well as non-biallelic SNPs. To reduce the influence of weak instrumental variables, only SNPs with an F-statistic greater than 10 were retained for subsequent MR analyses[69]. Furthermore, to ensure the selected SNPs are consistent on effect direction and alleles across two samples, "harmonise_data" function was employed to align alleles and effect directions across both samples, while also identifying and excluding variants with inconsistent directions, palindromic SNPs, SNPs with mismatched frequencies.

3.2.6 Bidirectional two sample mendelian randomization

In the bidirectional two-sample MR framework, herpes keratitis and PD were alternately treated as exposure and outcome variables. Causal relationships were evaluated in both directions using multiple MR analytical approaches. All statistical

procedures were conducted using the TwoSampleMR package implemented in R[70, 71]. The IVW approach served as the primary MR estimator. This method synthesizes the Wald ratio estimates derived from individual SNPs through a meta-analytic framework, thereby generating an overall estimate of the causal effect associated with the genetic instruments[32, 72]. In addition, the weighted median method was employed to obtain a robust causal estimate, assigning weights to each SNP according to the inverse of the standard error of its estimated effect. The simple mode method determines the causal effect by identifying the most frequently occurring estimate across all instrumental variables. While the weighted mode method incorporates SNP-specific weights again based on the inverse standard error of their effect estimates and provide a robust causal estimate. To further enhance inference, Bayesian Weighted Median Regression (BWMR) was applied. This method integrates Bayesian modeling with the weighted median framework, allowing greater flexibility in estimating causal effects and providing probabilistic inference regarding the underlying causal relationship[73]. The strength of the causal inference was evaluated by Cochran's Q statistics, MR-Egger intercept tests, and leave-one-out sensitivity analyses[74]. Cochran’s Q statistics evaluated heterogeneity in IVW models[27]. The MR Egger regression is used to detect and adjust for pleiotropy bias of genetic instruments. It introduces an intercept to assess the average pleiotropic effects across IVs and allows for the IVs' effects to be partially independent of the exposure[30]. A statistically significant deviation of the intercept from zero suggests the presence of horizontal pleiotropy influencing the MR estimates[28].

3.2.7 Pathway enrichment analysis

Pathway enrichment analysis was conducted to investigate the biological pathways associated with the gene sets that were commonly up-regulated and down-regulated in both herpes keratitis and PD. This analysis was performed using the Metascape platform[75]. Multiple functional annotation databases were included in the analysis, such as KEGG Pathways, Gene Ontology (GO) Biological Processes, Reactome Gene Sets, and several additional curated resources. Default parameter

settings in Metascape were applied for the minimum gene overlap, statistical significance threshold (p-value cutoff), and enrichment factor. These standard criteria ensured stringent screening for pathways showing statistically meaningful enrichment. Visualization of the enrichment results was generated in the R environment using the "ggplot2" package.

3.2.8 Drug repurposing analysis using IDG Drug Target 2022

To identify potential pharmacological targets linked to the gene sets shared between herpes keratitis and PD, drug repurposing analysis was carried out using the Enrichr platform[76]. The analysis focused on both the shared up-regulated and shared down-regulated genes. In particular, the IDG (Illuminating the Druggable Genome) Drug Target 2022 library was selected as the reference dataset. This database compiles recent knowledge on druggable targets derived from contemporary genomic and pharmacological research[77]. Candidate targets were filtered according to the adjusted p-value calculated by Enrichr, and only results with an adjusted p-value less than 0.05 were considered statistically significant.

3.3 Results

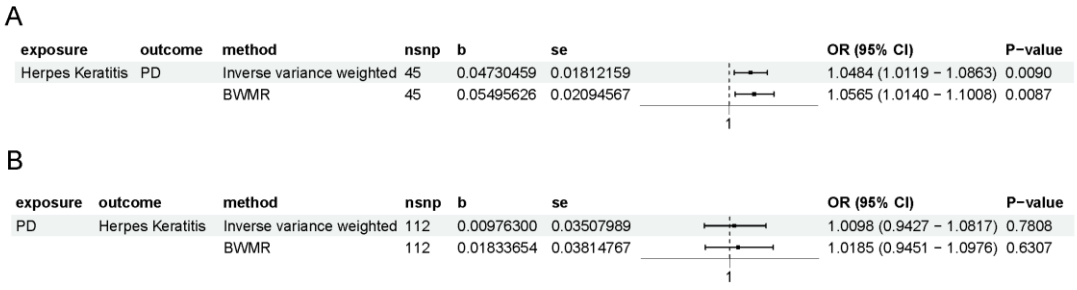


Figure 2 presents the forest plot illustrating the findings from the bidirectional Mendelian randomization analyses conducted using the IVW method and the BWMR approach.

A. The causal influence of herpes keratitis on PD was evaluated by treating herpes keratitis as the exposure and PD as the outcome. The IVW analysis yielded an odds ratio (OR) of 1.0484 with a 95% confidence interval (CI) ranging from 1.0119 to 1.0863 (p = 0.0090). Consistent results were obtained using BWMR, which produced

an OR of 1.0565 with a 95% CI of 1.0140–1.1008 (p = 0.0087). Both estimates indicate a statistically significant association. B. The reverse causal direction was subsequently examined, considering PD as the exposure and herpes keratitis as the outcome. In this analysis, the IVW method estimated an OR of 1.0098 (95% CI: 0.9427–1.0817; p = 0.7808), while the BWMR approach produced an OR of 1.0185 (95% CI: 0.9451–1.0976; p = 0.6307). Neither method provided evidence supporting a statistically significant causal effect in this direction. Statistical significance was defined as $p < 0.05$.

3.3.1 Assessment of the Unidirectional Causal Effect of Herpes Keratitis on PD

From the GWAS dataset for herpes keratitis, 45 SNPs were identified and used as instrumental variables (IVs), details including p-values, beta coefficients, standard errors and the effect allele can be seen in Supplementary Table S2. In parallel, 112 SNPs were selected as IVs from the GWAS dataset for PD, with detailed information presented in Supplementary Table S3. The strength of each instrumental variable was evaluated by calculating the corresponding F-statistic, and all values exceeded the conventional threshold of 10, indicating that weak instrument bias was unlikely. Using the IVW approach as the principal analytical method, the MR analysis indicated a causal relationship between herpes keratitis and PD. Specifically, the findings suggested that herpes keratitis is associated with an increased likelihood of developing PD, details seen in Figure 2 (A). This primary result was supported by the BWMR approach and further examined using several additional MR methods, including MR-Egger, weighted median, simple mode, and weighted mode (Supplementary Figure 2). The genetic variants associated with herpes keratitis and their corresponding estimated effects on PD are summarized in Supplementary Table S4. Conversely, characteristics of PD-associated genetic variants and their potential effects on herpes keratitis are described in Supplementary Table S5. After excluding outlier SNPs, the recalculated effect estimates for the association between herpes keratitis and PD are reported in Supplementary Table S6. To assess potential violations of MR assumptions, several sensitivity analyses were conducted. MR-Egger regression produced an intercept of 0.006230545 with a non-significant p-value (p = 0.55215), indicating no detectable horizontal pleiotropy. Similarly, the MR-PRESSO global test yielded a residual sum of squares (RSSobs) of 56.03422

with a non-significant p-value (p = 0.163), also suggesting the absence of horizontal pleiotropic effects. Heterogeneity among the instrumental variables was evaluated using both IVW and MR-Egger approaches. The IVW heterogeneity test produced a Q-statistic of 52.90191 (p = 0.16812), while the MR-Egger heterogeneity test yielded a Q-statistic of 52.46377 (p = 0.15275). In both cases, the non-significant results indicated no substantial heterogeneity across the selected SNPs. Additional graphical assessments supported the robustness of these findings. Funnel plots demonstrated symmetrical distributions, suggesting minimal bias in SNP selection (Supplementary Figure 1A). Scatter plots illustrated the estimated causal relationship between herpes keratitis and PD, where the slope of the regression lines reflected effect estimates derived from different MR methods (Supplementary Figure 1B). The SNP-specific causal estimates generated using the IVW approach are presented in Supplementary Figure 1C. Furthermore, leave-one-out sensitivity analysis was visualized using a forest plot, where each point represents the IVW estimate obtained after excluding a single SNP, thereby evaluating the influence of individual variants on the overall result (Supplementary Figure 1D). We subsequently investigated the reverse causal direction, examining whether PD might influence the risk of herpes keratitis. When the IVW method was applied as the primary analytical framework, no evidence of a causal effect of PD on herpes keratitis was observed (Figure 2B). This conclusion was further supported by BWMR and additional MR estimators, including MR-Egger regression, weighted median, simple mode, and weighted mode analyses (Supplementary Table S6). Sensitivity analyses evaluating heterogeneity and horizontal pleiotropy for the reverse-direction analysis are summarized in Supplementary Table S7. MR-Egger regression yielded an intercept estimate of −0.001123141 with a non-significant p-value (p = 0.912001), indicating no evidence of directional pleiotropy. The MR-PRESSO global test produced an RSSobs value of 102.3366 with a non-significant p-value (p = 0.735), again suggesting no detectable pleiotropic bias. Heterogeneity assessments also indicated consistent results across instrumental variables. The IVW heterogeneity test generated a Q-statistic of 99.99259 with a p-value of 0.764103, while the MR-Egger heterogeneity test produced a Q-statistic of 99.98032 with a p-value of 0.742762. Both findings indicate the absence of significant heterogeneity among the selected SNPs.

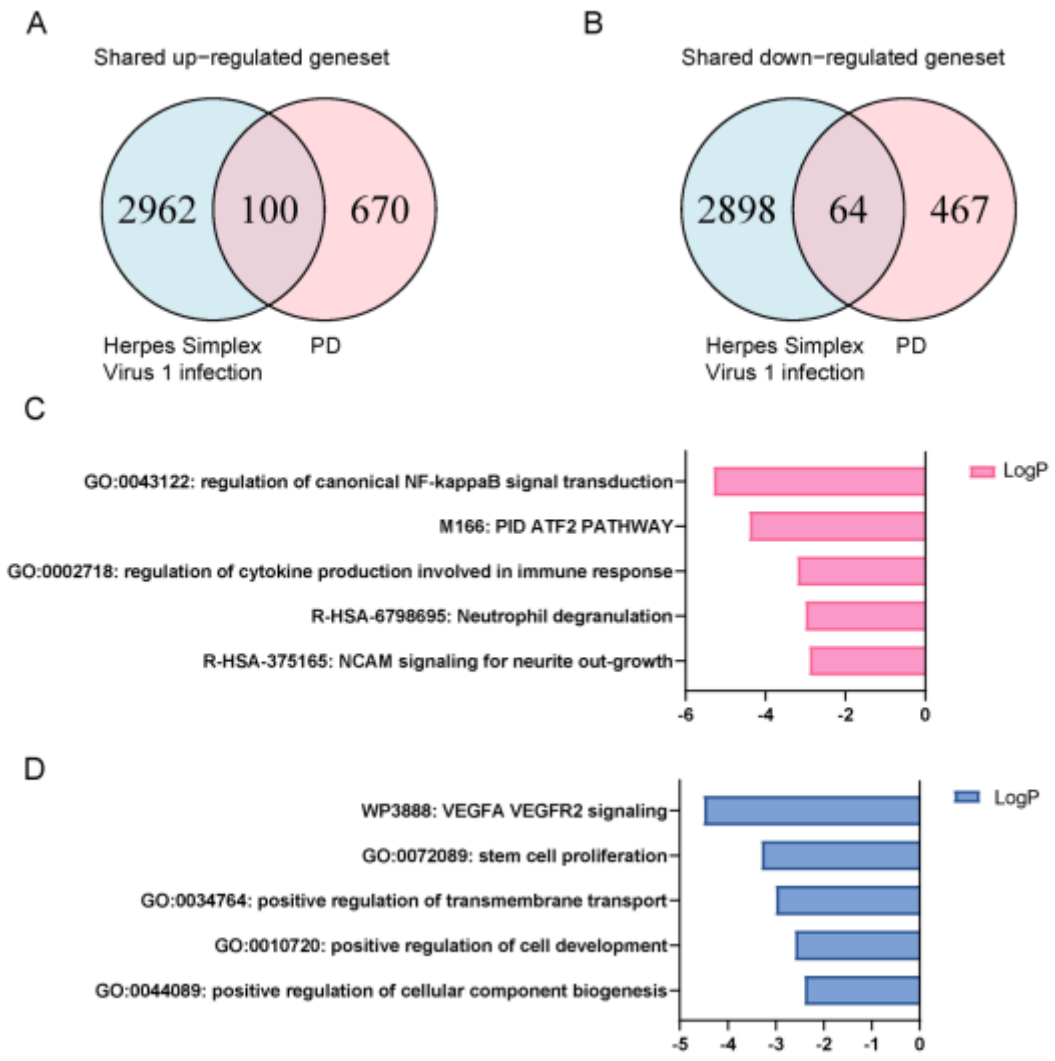


Figure 3 Identification of shared molecular signatures between herpes keratitis and PD.

A. Common up-regulated geneset. B. Common down-regulated geneset. C. Functional pathway enrichment of the shared up-regulated geneset. D. Functional pathway enrichment of the shared down-regulated geneset.

3.3.2 Identification of overlapping molecular features between herpes keratitis and PD

Differential gene expression data comparing PD cases with controls in the PPMI cohort were derived from the study conducted by Craig and colleagues. To represent herpes keratitis–related transcriptional changes, transcriptomic profiles obtained from HSV-1–infected KMB17 fibroblasts were utilized as a surrogate model. Through comparative analysis, a total of 100 genes exhibiting concordant up-regulation in both herpes keratitis and PD datasets were identified (Supplementary Table S8). Functional

enrichment of this shared up-regulated gene set indicated significant associations with several biological processes and signaling pathways, including “G0:0043122: regulation of canonical NF-kappaB signal transduction”, “M166: PID ATF2 PATHWAY”, “G0:0002718: regulation of cytokine production involved in immune response”, “R-HSA-6798695: Neutrophil degranulation”, “R-HSA-375165: NCAM signaling for neurite out-growth”, detail seen in Supplementary Table S9. 64 shared down-regulated genes between herpes keratitis and PD were found, detail seen in Supplementary Table S8. Functional enrichment of this shared down-regulated geneset was mainly enriched in “WP3888: VEGFA VEGFR2 signaling”, “GO:0072089: stem cell proliferation”, “G0:0034764: positive regulation of transmembrane transport”, “G0:0010720: positive regulation of cell development”, “G0:0044089: positive regulation of cellular component biogenesis”, detail seen in Supplementary Table S10.

3.3.3 Drug repurposing analysis based on the IDG Drug Target 2022

To identify potential therapeutic candidates targeting the overlapping molecular signatures between herpes keratitis and PD, a drug repurposing analysis was conducted using Enrichr. Both the shared up-regulated and shared down-regulated gene sets were queried against the IDG (Illuminating the Druggable Genome) Drug Target 2022 library. This database integrates updated genomic knowledge with curated information on pharmacologically relevant molecular targets. Significance of enrichment results was evaluated using adjusted p-values generated by Enrichr, and only results with an adjusted p-value below 0.05 were considered statistically meaningful. Based on this criterion, the ten compounds most strongly associated with the shared up-regulated gene set, ranked by increasing adjusted p-value, are summarized in Table 1. Similarly, Table 2 lists the top ten candidate compounds linked to the shared down-regulated gene set. Among the compounds identified, Nalfurafine emerged as the only molecule predicted to interact with targets within the shared up-regulated gene set common to herpes keratitis and PD.

Table 1 List of top 10 drugs targeting shared up-regulated geneset between herpes keratitis and PD, ranked by minimum adjusted P-value.

Term	Genes	P-value	Adjusted P-value
Nalfurafine	ACHE; CACNA1F	0.0062	0.0368

Cisplatin	ACHE; NT5E; SLC2A4	0.0066	0.0681
Tannic Acid	CACNA1F	0.0159	0.0681
Zafirlukast	SLC12A3	0.0159	0.07031
Nelfinavir	ACHE	0.0159	0.07031
Sulconazole	ACHE	0.0159	0.1032
Hexachlorophene	SLC12A3	0.0159	0.1077
Bosutinib	SLC12A3	0.0159	0.1273
Sorafenib	SLC12A3	0.0159	0.1273

Table 2 List of top 10 drugs targeting shared down-regulated geneset between herpes keratitis and PD, ranked by minimum adjusted P-value.

Term	Genes	P-value	Adjusted P-value
Nifedipine	ACHE; CACNA1F	0.0062	0.0828
Quercetin	ACHE; NT5E; SLC2A4	0.0066	0.0828
(S)-nitrendipine	CACNA1F	0.0159	0.0828
Cyclothiazide	SLC12A3	0.0159	0.0828
Alfuzosin	ACHE	0.0159	0.0828
Stearic Acid	ACHE	0.0159	0.0828
Hydrochlorothiazide	SLC12A3	0.0159	0.0828
Bendroflumethiazide	SLC12A3	0.0159	0.0828
Benzthiazide	SLC12A3	0.0159	0.0828

3.4 Discussion

PD is a common neurodegenerative condition, with an estimated global burden exceeding 10 million affected individuals. The likelihood of developing PD rises substantially with advancing age. Nevertheless, a small proportion of cases, around 4%, manifest before the age of 50 [1]. When symptoms begin prior to 40 years of age, the disorder is categorized as YOPD, while onset before 21 years is defined as juvenile parkinsonism. Epidemiological evidence consistently demonstrates a sex-related disparity, with males exhibiting approximately a 1.5-fold higher risk compared to females [2, 3]. In routine clinical settings, PD is diagnosed predominantly through neurological examination rather than through specific laboratory-based confirmation. Patients, most often older than 55 years, typically exhibit a slowly progressive motor syndrome that initially presents in an asymmetric

manner. The principal motor features—resting tremor, bradykinesia, and muscular rigidity, constitute the fundamental criteria for clinical diagnosis [4]. At the level of neuropathology, PD is characterized by functional impairment within the basal ganglia, a network of subcortical nuclei situated at the base of the forebrain. Within this system, the striatum, composed of the caudate nucleus and putamen, serves as the main input region. It receives excitatory projections from the cerebral cortex alongside dopaminergic modulation originating from the SNc, which exerts both facilitatory and inhibitory influences. These inputs converge onto two distinct populations of spiny projection neurons. One population gives rise to the direct pathway, projecting to the internal segment of the GPi, the principal output nucleus. The other population contributes to the indirect pathway, first connecting to the GPe and subsequently modulating GPi activity through the STN [5, 6]. The hallmark pathological features of PD include marked degeneration of pigmented dopaminergic neurons within the SNpc [7], accompanied by the accumulation of Lewy bodies and Lewy neurites, which are primarily composed of aggregated α-synuclein [8]. Neuronal loss is especially pronounced in the ventrolateral portion of the SNpc, where approximately 60–80% of dopaminergic neurons are depleted prior to the onset of overt motor symptoms. Importantly, Lewy body pathology has also been observed in individuals without clinical neurological manifestations during post-mortem examination, suggesting the existence of a preclinical phase that becomes more prevalent with increasing age. Although these inclusions are characteristic of PD, they lack absolute specificity and may also occur in other disorders, including atypical parkinsonian syndromes and Hallervorden–Spatz disease [9, 10]. Despite significant advances in understanding the disease, current treatment strategies primarily address symptom management rather than disease modification. At present, no therapeutic intervention has been demonstrated to arrest or reverse the neurodegenerative process [11, 12]. Levodopa remains the most effective agent for controlling motor symptoms; however, prolonged use is frequently associated with adverse effects such as motor fluctuations and dyskinesias [13]. These challenges highlight the necessity for deeper mechanistic understanding and the development of therapies capable of altering

disease progression.

Although substantial research has been devoted to PD, currently available therapeutic strategies remain largely focused on alleviating symptoms rather than modifying the underlying disease process. No treatment has yet demonstrated the ability to halt or reverse neurodegeneration. Consequently, expanding our understanding of PD pathogenesis through alternative methodological approaches and new conceptual perspectives remains essential. The possible involvement of viral infections in PD was first proposed by Von Economo, who reported that lethargic encephalitis was associated with inflammatory lesions in the midbrain tegmentum and the substantia nigra—brain regions critically implicated in PD pathology[78]. Subsequent investigations provided further support for this hypothesis. For example, highly pathogenic H5N1 influenza viruses have been shown to invade the central nervous system, triggering neuroinflammatory processes and neuronal degeneration that resemble pathological features observed in PD[79]. Among viruses investigated in relation to PD, HSV-1 has received considerable attention. Multiple studies have explored the possible relationship between HSV-1 infection and PD, although the findings remain complex. Marttila R.J. and colleagues reported that individuals with PD exhibited elevated levels of IgG antibodies directed against HSV-1 compared with control subjects[55]. Their results suggested that PD patients may mount a stronger immune response to HSV-1 infection. However, analyses of cerebrospinal fluid (CSF) did not reveal differences in HSV-1 antibody levels between PD patients and controls. This observation suggests that HSV-1 may influence PD indirectly through inflammatory mechanisms rather than by directly infecting neural tissue[57]. Additional evidence was provided by Agostini and collaborators, who found increased titers of HSV-1-specific antibodies in the serum of PD patients relative to healthy controls. Their work also identified polymorphisms in PILRA, a gene associated with HSV-1 infection susceptibility, indicating that HSV-1 may contribute to PD pathogenesis through neuroinflammatory pathways[80]. In contrast, Camacho-Soto A and collaborators reported an opposite trend. Using a population-based case-control analysis involving 2009 Medicare beneficiaries aged 66–90 years (including 89,790

PD cases and 118,095 matched controls), they observed that HSV-1 and HSV-2 infections were associated with a reduced risk of PD rather than an increased risk. Their findings suggested the possibility of an inverse association and emphasized the need for further research to determine whether such relationships reflect causal mechanisms[60]. The inconsistencies observed across studies may partly arise from the heterogeneity of HSV-1 infections and the inherent limitations of observational research designs, including confounding effects[61], selection bias[62], and reverse causation[63].

To reduce heterogeneity associated with HSV-1 infections, the present study focused on a specific clinical manifestation—herpes keratitis. To evaluate whether a causal relationship exists between herpes keratitis and PD, we employed a bidirectional MR framework. A total of 45 SNPs were extracted from the herpes keratitis GWAS dataset and used as instrumental variables (IVs). These IVs satisfied the three core assumptions required for MR analysis: relevance, independence, and exclusion restriction. First, the relevance assumption requires that IVs be strongly associated with the exposure. This condition was verified by calculating F-statistics greater than 10, thereby reducing potential weak-instrument bias. Second, the independence assumption requires that IVs be independent of confounding factors. This requirement was addressed by selecting multiple SNPs from GWAS data that showed no association with known confounders. Third, the exclusion restriction assumption requires that IVs influence the outcome only through the exposure rather than through alternative pathways. To evaluate this assumption, MR-Egger regression and leave-one-out sensitivity analyses were conducted. MR-Egger regression assesses potential horizontal pleiotropy by examining the intercept term; if the intercept deviates significantly from zero, this indicates that IVs may exert direct effects on the outcome, thereby violating the exclusion restriction assumption. The leave-one-out procedure repeatedly recalculates the causal estimate after excluding each IV individually. Large fluctuations in the estimated effect after removal of a specific IV would indicate that the exclusion restriction assumption may be violated. In MR analysis, IVs are not expected to have a direct causal relationship with the outcome;

instead, they serve as genetic proxies to evaluate the causal relationship between exposures and outcomes.

Using the 45 IVs derived from the herpes keratitis GWAS dataset, the IVW method, applied as the primary analytical approach, identified a causal effect of herpes keratitis on PD. The results suggested that herpes keratitis is associated with approximately a 4.8% increased risk of developing PD. The robustness of this finding was supported by additional MR methods, particularly the BWMR approach, which also indicated a causal relationship between herpes keratitis and PD. Furthermore, MR-Egger regression and the MR-PRESSO global test showed no evidence of horizontal pleiotropy. Heterogeneity tests conducted using both IVW and MR-Egger methods indicated no significant heterogeneity among the IVs. A reverse MR analysis was subsequently performed to determine whether PD might causally influence herpes keratitis. In this analysis, 112 SNPs obtained from PD GWAS datasets were used as IVs. The results did not support a causal effect of PD on herpes keratitis. Taken together, these findings suggest a unidirectional causal relationship in which herpes keratitis increases the risk of PD. To our knowledge, this study provides the first evidence supporting a directional causal association between herpes keratitis, primarily caused by HSV-1 infection, and PD. This discovery offers additional support for the hypothesis that viral infections may contribute to PD etiology.

To further explore potential molecular links between herpes keratitis and PD, transcriptomic analyses were performed. Differential gene expression data comparing PD patients and controls from the PPMI cohort were derived from the study conducted by Craig and collaborators. In addition, transcriptomic data obtained from HSV-1-infected fibroblast KMB17 cells were used as a model system to represent herpes keratitis-related transcriptional responses. Comparative analysis revealed 100 genes that were up-regulated in both conditions and 64 genes that were down-regulated in both datasets. These shared transcriptional alterations suggest common molecular features between herpes keratitis and PD.

Genetic background plays a significant role in determining both the age of onset and clinical progression of PD[81, 82]. Certain genetic variants are known to

influence disease trajectories differently. For instance, alleles within the GBA gene (including p.E326K, p.N370S, and p.T369M) are associated with varying rates of motor and cognitive decline. Variants in MAPT and SNCA have also been linked to differences in motor progression and disease severity. Mutations in LRRK2 produce heterogeneous clinical outcomes; carriers of the G2019S mutation generally show slower motor deterioration compared with individuals carrying the R1441C mutation, which operates through distinct molecular mechanisms. Furthermore, cumulative genetic risk scores (GRS), calculated by aggregating the additive effects of multiple SNPs, have been shown to predict disease progression. Individuals with higher GRS values tend to reach Hoehn and Yahr stage 3 more rapidly and experience earlier disease onset compared with individuals with lower genetic risk scores[82]. These observations highlight the potential for uncontrolled genetic heterogeneity to bias results in epidemiological studies. Importantly, the datasets used in the present study addressed this issue. The PD case-control differential expression dataset from PPMI, derived from the work of Craig and collaborators, incorporated considerations of genetic background and age distribution[66]. Therefore, it is unlikely that the associations observed between HSV-1 infection and PD in our analysis were confounded by genetic variability.

Sex differences in PD have been documented across epidemiological patterns, clinical manifestations, and molecular mechanisms[83]. Epidemiological studies indicate that PD occurs approximately 1.5–2 times more frequently in men than in women, with disease onset occurring about 2.2 years earlier in males. Mortality risk is also slightly higher in men[2, 84]. Clinical characteristics also vary by sex. Women are more likely to present with tremor-dominant PD and generally experience milder early motor impairment, whereas men tend to develop more severe rigidity, gait disturbances, and postural instability[85, 86]. Non-motor symptoms also show sex-specific patterns. Women with PD more frequently report anxiety, depression, fatigue, pain, and dysautonomia, while men more commonly exhibit orthostatic hypotension, urinary dysfunction, and REM sleep behavior disorder[87-89]. Cognitive outcomes also display sex differences. Male PD patients appear to have a

higher risk of cognitive decline and dementia, particularly involving deficits in executive function and verbal fluency. In contrast, female patients appear relatively protected, although they may demonstrate greater impairment in visuospatial abilities[90]. At the molecular level, estrogen is thought to enhance the resilience of dopaminergic neurons by stabilizing mitochondrial function, suppressing inflammatory signaling, and modulating α-synuclein aggregation. The reduction of estrogen levels after menopause may therefore contribute to the increase in PD incidence observed among aging women[91]. Although sex-related differences are an important aspect of PD biology, the PPMI case-control differential expression dataset used in the present study already accounted for sex as a factor in the analytical design conducted by Craig and collaborators. Consequently, the observed association between HSV-1 infection and PD is unlikely to be confounded by sex differences.

Our study of pathway analysis on the shared up-regulated geneset was mainly enriched in “G0:0043122: regulation of canonical NF-kappaB signal transduction”, “M166: PID ATF2 PATHWAY”, “G0:0002718: regulation of cytokine production involved in immune response”. These findings highlight inflammatory mechanisms as potential mediators linking herpes keratitis and PD. The NF-κB signaling pathway plays a critical role in both conditions. In HSV-infected human corneal epithelial cells, activation of NF-κB signaling leads to increased production of pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α[92]. In PD, NF-κB signaling interacts with the NLRP3 inflammasome and Notch signaling pathways in microglia, promoting the release of inflammatory cytokines that exacerbate neuroinflammation and contribute to neuronal damage[93-95]. The p38MAPK/ATF-2 pathway is also associated with neuronal survival and inflammatory regulation[96]. Cytokine production plays an essential role in the immune response in both herpes keratitis and PD. In herpes keratitis, IL-17 acting together with TNF-α or IFN-γ can stimulate the production of IL-6, IL-8, and MIP3-α, thereby promoting inflammatory progression[97]. Additionally, chemokines produced in HSV-1-infected corneal tissue facilitate infiltration of inflammatory cells into the cornea[98]. In PD, circulating cytokines such as IL-1β, IL-2, IL-10, IFN-γ, and TNF-α have been associated with both the

severity and progression of PD symptoms[98-100].

Pathway analysis of the shared down-regulated gene set revealed enrichment in “WP3888: VEGFA VEGFR2 signaling”, “GO:0072089: stem cell proliferation”, “G0:0034764: positive regulation of transmembrane transport”. These pathways are critical for cellular maintenance and tissue development. In PD, astrocyte-derived VEGFA contributes to blood–brain barrier (BBB) regulation, synaptic remodeling, and angiogenesis. Disruption of this signaling pathway may therefore contribute to BBB dysfunction and neurodegeneration[101]. The enrichment of genes associated with stem cell proliferation also reflects the degenerative nature of PD, which is characterized by progressive loss of dopaminergic neurons, formation of Lewy bodies, and reduced neural regenerative capacity[102, 103].

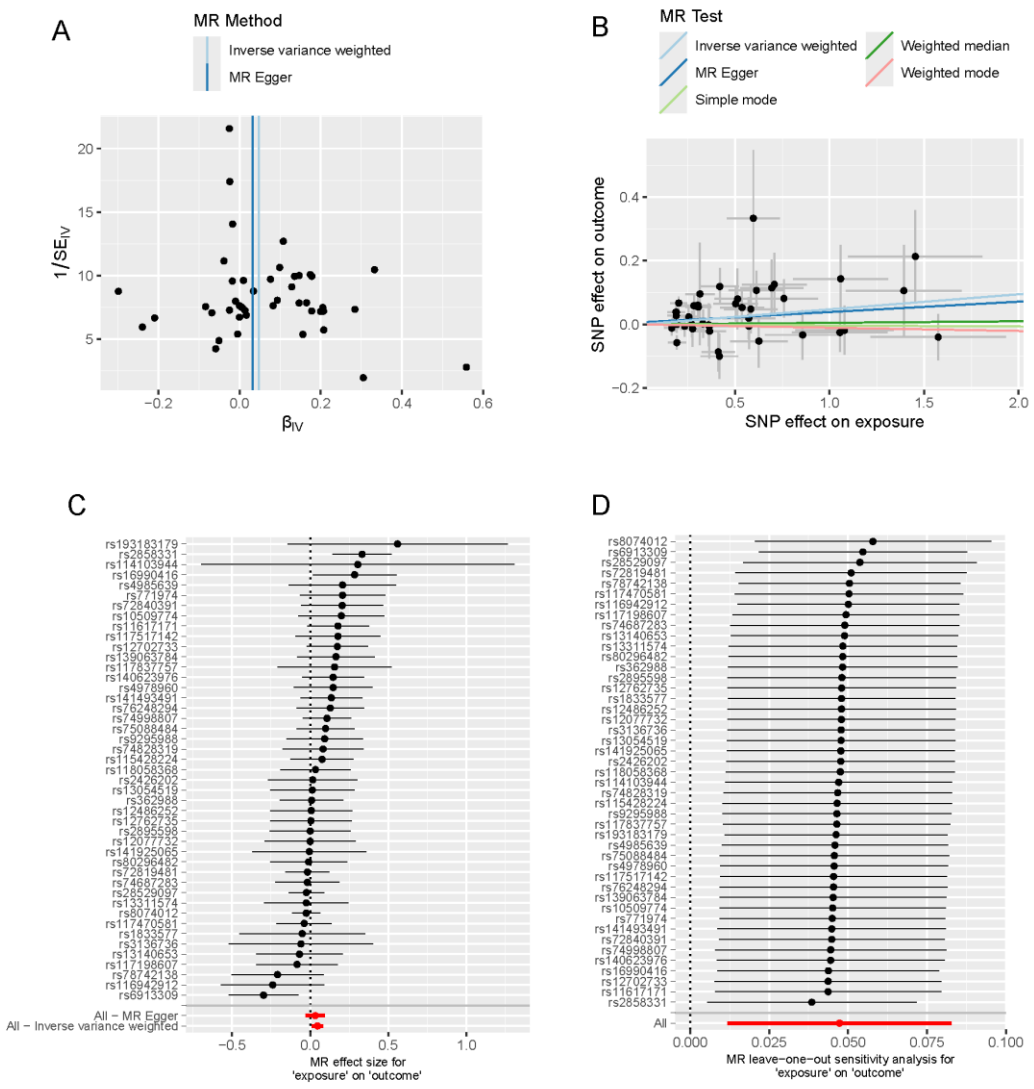
Drug repurposing analysis identified Nalfurafine as the only candidate molecule interacting with the shared up-regulated genes between herpes keratitis and PD. Nalfurafine is a selective κ-opioid receptor agonist originally synthesized for clinical use[104], It is currently used in Japan as an antipruritic agent for treating uremic pruritus in patients with chronic kidney disease undergoing hemodialysis[105]. Notably, Nalfurafine was the first selective κ-opioid receptor agonist approved for clinical application[106, 107]. Recent experimental studies have demonstrated that Nalfurafine can alleviate herpetic and post-herpetic pain in mice in a dose-dependent manner[105]. Moreover, topical application of Nalfurafine to the cornea has been shown to reduce both neovascularization and inflammatory responses[108]. Beyond these effects, Nalfurafine has also demonstrated neuroprotective properties, including the promotion of remyelination and the reduction of levodopa-induced dyskinesia [109]. In light of these findings, and in combination with the results of the present study, Nalfurafine may represent a promising candidate for therapeutic intervention. It may potentially alleviate pain and inflammation in herpes keratitis patients while also contributing to the management of PD risk.

3.5 Conclusions

This study integrates GWAS-based Mendelian randomization analysis with transcriptomic data to identify a causal relationship between herpes keratitis and Parkinson's disease. The two conditions share molecular signatures, particularly those related to neuroinflammation and stem cell-associated processes. Furthermore, the findings highlight Nalfurafine as a potential candidate for drug repurposing, suggesting that it may represent a promising therapeutic strategy for future investigation.

3.6 Supplementary Materials

3.6.1 Supplementary Figures



Supplementary Figure 1: Analysis Figure.

A. Funnel plot. B. Scatter plot. C. Forest plot. D. The causal association between herpes keratitis and PD for each individual SNP.

3.6.2 Supplementary Tables

Supplementary Table S1: Detailed information for Database.

Data sources	Phenotypes	sample description
Mitja I Kurki et al, FinnGen R10 study data	Herpes Keratitis	European, 1,252 cases, 390,647 controls
Mike A Nalls et al	PD	European, 33,674 cases, 449,056 controls

Supplementary Table S2: Instrumental variables used in MR analysis in Herpes Keratitis.

rsid	effect_allele	other_allele	p_value
rs12077732	A	T	1.40E-05
rs6600441	G	A	4.71E-05
rs114103944	C	T	4.51E-05
rs12037737	T	C	2.57E-05
rs115428224	C	T	5.11E-06
rs9309089	G	A	4.56E-05
rs148697861	C	A	3.47E-05
rs140623976	G	T	4.27E-05
rs111668950	T	TACAC	4.39E-05
rs74603152	C	G	2.76E-05
rs1368965	C	A	4.36E-05
rs62148039	T	C	2.83E-05
rs72819481	G	A	3.20E-05
rs139063784	C	T	3.25E-05
rs59257065	C	T	1.99E-05
rs619969	A	G	2.72E-05
rs371635343	C	CAATAAATAAATA	1.55E-05
rs141925065	G	A	1.86E-05
rs12486252	G	C	3.16E-05
rs13079154	C	T	3.04E-05
rs71323484	G	A	1.03E-05
rs55996057	T	G	4.38E-05
rs3914911	T	A	4.24E-05
rs141493491	C	T	2.47E-05
rs1242046	G	A	2.92E-05

rs260591	A	G	4.28E-05
rs74775034	G	A	2.79E-05
rs62301581	T	C	4.48E-05
rs13140653	C	T	4.57E-05
rs10018114	G	A	1.88E-05
rs34561493	G	A	2.20E-05
rs75088484	A	G	2.87E-06
rs1833577	C	T	2.10E-05
rs9295988	C	G	2.74E-05
rs616634	A	G	1.22E-05
rs56326741	C	T	2.80E-05
rs6913309	T	A	4.88E-05
rs113399573	C	T	1.01E-05
rs2858331	A	G	3.28E-06
rs117198607	A	G	3.30E-05
rs13311574	C	T	4.43E-05
rs12702733	G	A	2.57E-05
rs7783714	G	T	4.64E-05
rs116942912	G	A	2.85E-05
rs13230481	T	C	5.52E-06
rs55848335	A	T	6.89E-07
rs76248294	G	A	1.91E-05
rs3136736	T	A	2.02E-05
rs13264419	T	C	7.86E-06
rs117837757	G	A	2.35E-05
rs371767469	T	TTGTGTGTG	4.79E-05
rs2380826	C	G	4.11E-05
rs112179183	C	T	3.96E-05
rs12342287	G	T	1.02E-05
rs78742138	T	C	2.35E-06
rs4978960	A	T	3.50E-05
rs10759934	A	T	1.60E-05
rs2895598	A	C	3.88E-05
rs12762735	G	A	3.99E-05
rs74687283	G	A	1.66E-05
rs10509774	C	T	1.16E-05
rs118058368	T	C	4.51E-05
rs193183179	C	T	1.88E-05
rs117517142	C	G	3.85E-05
rs61890048	T	C	1.19E-05
rs12365133	A	G	4.12E-05
rs771974	G	A	2.71E-05
rs74998807	G	A	2.15E-05
rs117470581	C	T	1.45E-05

rs80296482	C	T	4.67E-05
rs11617171	G	A	7.80E-06
rs137858115	A	G	3.18E-05
rs28529097	A	G	2.75E-05
rs4985639	G	A	4.14E-05
rs8068962	T	C	3.15E-05
rs8064540	C	A	2.12E-05
rs8074012	T	C	1.09E-05
rs72840391	C	T	2.31E-05
rs74828319	A	G	3.25E-05
rs62115085	C	T	3.45E-05
rs1962457	G	A	3.32E-05
rs362988	G	A	2.93E-05
rs1535221	A	C	2.73E-05
rs2426202	G	C	4.57E-05
rs16990416	T	C	3.59E-05
rs13054519	C	T	2.10E-05
rs12628403	A	C	8.31E-06
rs131783	C	T	2.58E-05

Abbreviations: rsid, reference SNP identifier; effect_allele, the allele associated with the reported effect; other_allele, the alternative allele; p_value, P-value for the association between the SNP and the exposure trait.

Supplementary Table S3: Instrumental variables used in MR analysis in PD.

rsid	effect_allele	other_allele	p_value
rs111972941	G	A	1.37E-06
rs114546866	A	G	3.04E-05
rs181951218	G	T	4.17E-05
rs77047773	A	G	7.60E-06
rs77516059	A	G	1.92E-05
rs35749011	A	G	5.02E-30
rs114797774	T	C	9.43E-07
rs7518590	A	G	1.27E-05
rs3753539	T	C	3.01E-05
rs2153904	G	T	4.45E-10
rs61835654	C	T	1.85E-06
rs10927035	T	C	2.11E-05
rs1647265	G	A	2.34E-05
rs112413063	C	T	4.62E-06
rs4851487	T	C	3.25E-06
rs116476950	T	C	5.79E-06
rs6741007	G	T	2.09E-12
rs1474055	T	C	1.14E-12

rs6715875	C	T	1.25E-06
rs72922699	A	G	3.27E-05
rs35449298	A	G	5.54E-06
rs6718063	C	T	2.57E-05
rs73032517	G	A	1.27E-06
rs7634267	C	T	3.73E-05
rs4387990	A	G	3.52E-05
rs1872957	A	G	2.62E-05
rs9840232	T	C	6.56E-07
rs10513789	G	T	3.18E-13
rs34311866	C	T	7.97E-23
rs4389574	G	A	1.49E-13
rs78991542	T	G	3.28E-05
rs76676224	A	G	3.89E-05
rs7695720	C	A	1.53E-09
rs6857779	C	T	4.61E-05
rs356219	A	G	3.47E-41
rs17388944	G	A	3.85E-05
rs189458449	A	T	6.89E-06
rs62347195	G	C	4.54E-05
rs158699	C	T	6.90E-13
rs369776	G	A	4.80E-05
rs411648	T	A	3.32E-05
rs13177306	C	G	3.71E-05
rs6860978	A	G	1.45E-05
rs115579738	A	G	9.71E-06
rs116353880	G	A	4.41E-05
rs6918131	A	G	3.78E-05
rs35265698	G	C	3.93E-11
rs111891129	A	G	3.42E-05
rs9442714	T	C	7.73E-06
rs1028122	C	T	4.21E-05
rs41286192	G	A	2.30E-06
rs58120286	A	C	4.30E-05
rs466225	T	G	4.28E-09
rs2024032	T	C	3.02E-05
rs117797654	A	G	6.48E-06
rs2949739	G	T	2.91E-05
rs118133461	A	G	1.28E-05
rs1736065	G	A	2.15E-05
rs9644642	C	T	2.14E-09
rs12542358	T	A	1.15E-05
rs116975474	T	A	6.39E-06
rs62519800	C	T	2.86E-05

rs149865921	C	T	3.28E-05
rs1028594	G	A	9.97E-07
rs10756906	A	G	3.00E-07
rs3892039	G	T	4.81E-05
rs28442014	T	C	1.91E-05
rs143957786	C	A	3.43E-05
rs148371284	A	G	5.81E-06
rs34260311	C	T	1.56E-05
rs12570561	A	G	3.31E-05
rs2339624	T	C	4.99E-05
rs1665659	T	C	3.98E-05
rs117896735	A	G	2.83E-10
rs7940104	G	T	3.62E-05
rs77541067	C	T	4.07E-05
rs10766298	A	G	4.09E-06
rs510306	C	A	5.44E-10
rs28370649	G	A	2.20E-07
rs10459264	T	C	2.15E-05
rs75505347	T	C	6.12E-09
rs79436216	G	A	3.02E-06
rs151166702	T	C	3.23E-05
rs11060180	G	A	6.08E-07
rs12319016	A	G	2.35E-05
rs188411092	G	C	4.46E-05
rs12147950	C	T	9.46E-06
rs1951633	G	A	1.82E-05
rs10148864	T	C	1.70E-05
rs12909666	T	C	5.45E-06
rs12591188	G	C	1.46E-05
rs2414739	A	G	2.20E-07
rs2667684	G	A	1.57E-05
rs8052245	G	C	9.75E-11
rs12929797	T	C	1.32E-06
rs34679758	G	A	8.72E-07
rs76706469	G	C	1.63E-05
rs78346960	A	C	1.39E-05
rs2285582	A	G	4.60E-08
rs58879558	C	T	1.36E-21
rs74676292	T	C	1.58E-06
rs142587417	A	G	4.72E-05
rs7213778	C	T	2.98E-05
rs116916712	C	T	3.70E-05
rs11874767	G	T	2.71E-05
rs77517353	T	C	4.29E-05

rs9962882	C	T	9.78E-08
rs62105204	G	A	3.77E-05
rs12606671	G	A	1.02E-05
rs145217940	A	T	8.91E-07
rs2295547	C	A	8.77E-07
rs6088287	C	T	2.23E-05
rs6094835	G	A	1.60E-05
rs2248244	A	G	2.00E-06
rs5754947	C	T	1.41E-05

Abbreviations: rsid, reference SNP identifier; effect_allele, the allele associated with the reported effect; other_allele, the alternative allele; p_value, P-value for the association between the SNP and the exposure trait.

Supplementary Table S4: Characteristic of the Herpes Keratitis-related genetic variants and their effects on PD (45 SNPs)

SNP	SNP-PD			
	beta	se	eaf	pval
rs10509774	-0.0605	0.0423	0.9174	0.152999848
rs114103944	0.0957	0.1602	0.9669	0.550200524
rs115428224	0.1059	0.1434	0.9866	0.460300027
rs11617171	-0.0543	0.0307	0.842	0.076849315
rs116942912	0.1	0.0702	0.9672	0.154100128
rs117198607	-0.0525	0.0828	0.9772	0.525800506
rs117470581	0.033	0.0769	0.973	0.667899318
rs117517142	-0.1257	0.0982	0.0185	0.200499826
rs117837757	0.08	0.0957	0.9834	0.402999957
rs118058368	1.96E-02	0.0652	0.9625	0.764199941
rs12077732	1.00E-04	0.0403	0.0942	0.997599999
rs12486252	0.0023	0.0442	0.0866	0.958799944
rs12702733	-1.07E-01	0.0608	0.9539	0.079350561
rs12762735	-8.00E-04	0.0226	0.5091	0.970800072
rs13054519	0.0024	0.0249	0.2974	0.924600023
rs13140653	-0.0113	0.0233	0.6056	0.628100299
rs13311574	-0.0058	0.032	0.8486	0.856400082
rs139063784	0.1145	0.0883	0.9788	0.194700022
rs140623976	0.2133	0.1453	0.9867	0.142099975
rs141493491	0.1433	0.1065	0.9851	0.178400062
rs141925065	-0.0018	0.0667	0.9639	0.978700004
rs16990416	0.119	0.0571	0.9518	0.037289795
rs1833577	0.014	0.0564	0.9457	0.804599948
rs193183179	-0.3334	0.214	0.9884	0.11919993
rs2426202	0.0029	0.0258	0.2467	0.909900073

rs28529097	0.0256	0.0606	0.9547	0.672699338
rs2858331	-0.0671	0.0193	0.5767	0.000496901
rs2895598	0	0.0251	0.7266	0.999299999
rs3136736	-0.0215	0.0861	0.0237	0.803099952
rs362988	0.0016	0.0174	0.5494	0.926700079
rs4978960	0.0277	0.0241	0.3031	0.251000161
rs4985639	-0.0388	0.0327	0.1888	0.236499921
rs6913309	0.0573	0.0219	0.2817	0.008827953
rs72819481	-0.0187	0.0767	0.9775	0.807499942
rs72840391	0.0388	0.0253	0.7561	0.125800104
rs74687283	0.0065	0.038	0.9032	0.86409999
rs74828319	-0.0481	0.0765	0.9749	0.530100239
rs74998807	-0.0816	0.0597	0.9593	0.171799937
rs75088484	-0.053	0.0505	0.9467	0.294699908
rs76248294	-0.0647	0.0552	0.9548	0.241700195
rs771974	-0.0584	0.0392	0.0889	0.136300045
rs78742138	-0.0861	0.0617	0.972	0.162899968
rs80296482	-0.0058	0.0718	0.9733	0.935899898
rs8074012	0.0399	0.073	0.9737	0.585199572
rs9295988	-0.0237	0.0317	0.0945	0.454900066

Abbreviations: beta, effect size; se, standard error; eaf, effect allele frequency; pval, P-value

Supplementary Table S5: Characteristic of the PD-related genetic variants and their effects on Herpes Keratitis (112 SNPs)

SNP	SNP-HVKK			
	beta	se	eaf	pval
rs10148864	-0.0145754	0.0463744	0.25025	0.753295
rs1028122	-0.021272	0.0877619	0.0567128	0.808483
rs1028594	0.0127695	0.0430669	0.318709	0.766844
rs10459264	-0.0765061	0.0833514	0.0623341	0.358686
rs10513789	0.0304696	0.0506048	0.195657	0.547102
rs10756906	-0.0523731	0.0457349	0.260917	0.252149
rs10766298	0.00980712	0.0409293	0.406096	0.810632
rs10927035	0.0177821	0.0411809	0.610042	0.665883
rs11060180	-0.0283015	0.0407916	0.414741	0.487802
rs111891129	-0.172156	0.112714	0.0339934	0.126669
rs111972941	0.000536478	0.0851598	0.0592195	0.994974
rs112413063	-0.0799584	0.0684827	0.0954405	0.24298
rs114546866	0.0245639	0.0937749	0.0472086	0.793363
rs114797774	0.0595905	0.14796	0.0190857	0.687133
rs115579738	-0.0741328	0.0784183	0.0725246	0.34448

rs116353880	-0.0264085	0.115509	0.0309334	0.819158
rs116476950	0.0538836	0.250498	0.0064758	0.829685
rs116916712	-0.115531	0.117663	0.0305201	0.326158
rs116975474	-0.095554	0.250096	0.00687206	0.702409
rs117797654	0.0629343	0.15686	0.0174366	0.688262
rs117896735	0.000420076	0.219963	0.00858398	0.998476
rs118133461	0.044312	0.125266	0.0262259	0.723531
rs11874767	-0.0868134	0.0560385	0.151291	0.12134
rs12147950	-0.00689292	0.0410471	0.597791	0.866641
rs12319016	-0.00212548	0.0405903	0.438438	0.958238
rs12542358	0.0589477	0.0401513	0.481641	0.142066
rs12570561	0.0908022	0.0567204	0.148585	0.109404
rs12591188	0.0172399	0.0447223	0.281781	0.699876
rs12606671	0.231441	0.0906512	0.0443489	0.0106773
rs12909666	0.0250815	0.103953	0.0390135	0.809341
rs12929797	-0.0403486	0.0423696	0.340069	0.340944
rs13177306	0.00518849	0.0428978	0.325662	0.903731
rs142587417	0.0592656	0.170635	0.0142507	0.728348
rs143957786	0.168703	0.148204	0.0190054	0.254989
rs145217940	0.0855032	0.337724	0.00376572	0.800133
rs1474055	0.0473576	0.0653121	0.106124	0.468393
rs148371284	0.56716	0.200831	0.00726169	0.00474176
rs149865921	0.083167	0.160484	0.0160464	0.604302
rs151166702	-0.0596547	0.0761509	0.0749538	0.433407
rs158699	-0.0849898	0.0676851	0.902591	0.209237
rs1647265	-0.0332767	0.0406369	0.426365	0.412856
rs1665659	-0.109688	0.0948865	0.047241	0.247683
rs1736065	0.00432515	0.0512966	0.193917	0.932805
rs17388944	-0.0158792	0.0568005	0.149251	0.779814
rs181951218	0.0311319	0.0953013	0.0485192	0.74392
rs1872957	0.0292034	0.0468328	0.755229	0.532913
rs188411092	-0.168581	0.17784	0.0133024	0.343163
rs189458449	0.00846568	0.194283	0.0110196	0.965244
rs1951633	-0.0282138	0.046037	0.259629	0.539974
rs2024032	0.0147678	0.0493024	0.790306	0.764533
rs2153904	-0.0729933	0.0547942	0.8391	0.182815
rs2248244	0.0672012	0.042402	0.341821	0.112998
rs2285582	0.0578917	0.0408948	0.594798	0.156885
rs2295547	0.0253627	0.0442157	0.296418	0.566229
rs2339624	-0.0119519	0.067411	0.0988864	0.859273
rs2414739	-0.0163553	0.0441925	0.706891	0.711315
rs2667684	0.0048697	0.0403957	0.522693	0.904047
rs28370649	-0.475155	0.246316	0.00678839	0.0537254
rs28442014	0.0316546	0.0467243	0.246632	0.498103

rs2949739	0.0426601	0.0401212	0.493787	0.287655
rs34260311	-0.0564225	0.190025	0.0120888	0.766526
rs34311866	0.0448487	0.0497439	0.206586	0.367273
rs34679758	-0.00439404	0.0695737	0.0909426	0.949642
rs35265698	0.0429102	0.0563584	0.149705	0.44643
rs35449298	-0.0215614	0.0478383	0.229367	0.652194
rs356219	-0.00137753	0.0419532	0.64142	0.973806
rs35749011	-0.0983741	0.099597	0.0427756	0.323289
rs369776	-0.0250267	0.0451625	0.272625	0.579478
rs3753539	0.066719	0.0528413	0.176171	0.206723
rs3892039	-0.0410123	0.0574556	0.144357	0.475346
rs411648	0.00944725	0.0431841	0.315429	0.826832
rs41286192	0.217731	0.107457	0.035563	0.0427425
rs4387990	0.0337154	0.042599	0.346799	0.428676
rs4389574	0.0174168	0.0404525	0.558741	0.666798
rs466225	0.103999	0.0415182	0.379298	0.0122487
rs4851487	-0.0383207	0.0428783	0.324129	0.371478
rs510306	-0.0397116	0.0436054	0.692505	0.362451
rs5754947	-0.0622992	0.0895547	0.0532001	0.486645
rs58120286	-0.123705	0.105504	0.0381643	0.240989
rs58879558	-0.00110893	0.0608466	0.123854	0.985459
rs6088287	0.0110865	0.0429284	0.322049	0.796209
rs6094835	0.0186163	0.0403241	0.530744	0.644321
rs62105204	-0.10872	0.103489	0.0405551	0.293469
rs62347195	0.0394659	0.0576049	0.141498	0.493273
rs62519800	-0.116747	0.0985069	0.0436174	0.235952
rs6715875	0.321862	0.135676	0.0230988	0.0176787
rs6718063	-0.022181	0.12991	0.024438	0.864427
rs6741007	0.000152027	0.0424381	0.345706	0.997142
rs6857779	-0.0440545	0.0410971	0.396862	0.283738
rs6860978	0.117921	0.0898738	0.0517173	0.189496
rs6918131	0.0354527	0.0410493	0.601053	0.387774
rs7213778	0.00628498	0.0451937	0.274573	0.889397
rs72922699	0.0364415	0.0723727	0.0858074	0.614595
rs73032517	0.0802295	0.0964999	0.0455717	0.405751
rs74676292	-0.000659806	0.0549025	0.160257	0.990411
rs7518590	-0.0110198	0.0516241	0.187278	0.830966
rs75505347	0.292319	0.16909	0.0142956	0.0838494
rs7634267	0.279352	0.153733	0.0179624	0.0691974
rs76676224	-0.0212128	0.0872996	0.0577713	0.808014
rs76706469	0.113423	0.0874266	0.0564604	0.194511
rs7695720	-0.0147433	0.048484	0.218216	0.761062
rs77047773	0.016316	0.1945	0.0111788	0.933146
rs77516059	0.189022	0.184725	0.0121305	0.306184

rs77517353	-0.192063	0.1031	0.0402028	0.0624784
rs77541067	-0.00411045	0.14606	0.0192179	0.977549
rs78991542	0.00718026	0.105352	0.0380293	0.945663
rs7940104	-0.0458853	0.0627809	0.116164	0.464852
rs79436216	0.121089	0.10545	0.0371739	0.250844
rs8052245	0.0167753	0.0421874	0.656985	0.690898
rs9442714	0.0221002	0.04664	0.246029	0.635609
rs9644642	-0.00293291	0.0429607	0.326985	0.945571
rs9840232	-0.0477398	0.0600526	0.130151	0.426634
rs9962882	-0.024299	0.0485901	0.220543	0.617017

Abbreviations: beta, effect size; se, standard error; eaf, effect allele frequency; pval, P-value

Supplementary Table S6: Effect estimates of the associations of Herpes Keratitis and Parkinson Disease after excluding outliers SNPs.

exposure	outcome	method	pval
HVKK	PD	MR Egger	0.292741799
		Weighted median	0.857418376
		Inverse variance weighted	0.009043644
		Simple mode	0.952896534
		Weighted mode	0.748333775
		BWMR	0.008696689
PD	HVKK	MR Egger	0.807174039
		Weighted median	0.932205878
		Inverse variance weighted	0.780776241
		Simple mode	0.821600005
		Weighted mode	0.863128661

Supplementary Table S7: Evaluation of heterogeneity and horizontal pleiotropy using different method

Exposure	Outcome	Horizontal pleiotropy test		Heterogeneity test	
		MR-Egger regression	MR-PRESSO	IVW	MR-Egger
		P value	Global test p value	P value	P value
Herpes Keratitis	Parkinson Disease	0.55215007	0.163	0.16812498 3	0.152746431
Parkinson Disease	Herpes Keratitis	0.912000719	0.735	0.76410305	0.742762254

Supplementary Table S8: Shared up-regulated geneset and shared down-regulated geneset

Shared up-regulated geneset	Shared down-regulated geneset
ACSS3	ACHE
ACTN1	ADAMTS4
ALOX5AP	ADD2
ALPK1	BCL7A
ALPL	C12orf40
ANGPT1	C1orf116
ANXA3	C3
ARHGEF40	C4orf50
ASAH1	CACNA1F
BOK	CELSR3
BST1	CISD2
C1RL	COL19A1
CASS4	CUX2
CCDC153	DNAJC6
CLCN4	DPF3
COL9A3	DYRK3
CPPED1	EBF1
CPQ	EDA
CRISPLD2	ELL3
CSAD	EPB41
CYP27A1	ESM1
DAAM2	FLT1
ECE1	GREM2
ECHDC3	GYPC
EEPD1	HAGH
EPHB4	HDAC9
ERV3-1	IRF6
F2RL1	KCNH8
FAT4	KIAA1671
FBXL13	KIF14
FCGRT	KRT79
FES	LRRN3
FLOT1	MFSD2B
FLOT2	MMP14
FOS	NPIPB4
GCA	NT5E
GOLGA8K	OCLN
HLX	PCDH1
HSD17B11	PIP5KL1
INHBB	PLA2G4C
INKA2	PLEK2
JDP2	PLEKHD1
KBTBD7	RAB6B

KCNE3
KIAA0825
KIF3C
LASP1
LIN7A
LITAF
LMNB1
LRRK2
MANSC1
MAPK3
MINDY1
MX2
NHS
NLRX1
OPLAH
OPRL1
PDLIM2
PDLIM7
PEX11G
PGGHG
PLBD1
PLEKHG3
PLXDC2
PPARGC1A
PPP1R12B
PPP1R3B
PYGL
RAB11FIP1
RASSF2
RFLNB
RGS18
RGS2
RNASET2
RNF130
RNF141
SECTM1
SGK1
SKAP2
SLC40A1
SORL1
ST8SIA4
SULT1A1
TBXAS1
TEK
RAPGEF5
RGS6
RIPOR3
RYR1
SFRP2
SLC12A3
SLC2A14
SLC2A4
SLC35G2
SLC4A1
SLC7A5
SMOX
ST6GALNAC4
SYN3
TAL1
TESC
TMC5
TMEM63B
TSPAN13
UBE2O
UGT8

TMEM119
TMEM91
TNFRSF10C
TP53I11
TRIOBP
TSC22D3
TSPAN2
VSIR
ZFP36L1
ZNF117
ZNF467
ZNF608
ZNF66

Supplementary Table S9: Pathway analysis on the shared up-regulated geneset

GO	Description	LogP
GO:0043122	regulation of canonical NF-kappaB signal transduction	-5.3
hsa04910	Insulin signaling pathway	-5.2
GO:0008015	blood circulation	-5
M92	PID ANGIOPOIETIN RECEPTOR PATHWAY	-4.7
M166	PID ATF2 PATHWAY	-4.4
GO:0044057	regulation of system process	-4
GO:0031960	response to corticosteroid	-3.8
GO:0036075	replacement ossification	-3.8
GO:0001958	endochondral ossification	-3.8
GO:0051591	response to cAMP	-3.7
R-HSA-5579029	Metabolic disorders of biological oxidation enzymes	-3.7
WP481	Insulin signaling	-3.7
WP4331	Neovascularisation processes	-3.6
WP1772	Apoptosis modulation and signaling	-3.6
GO:0090066	regulation of anatomical structure size	-3.5
GO:0030097	hemopoiesis	-3.4
WP98	Prostaglandin synthesis and regulation	-3.3
R-HSA-194315	Signaling by Rho GTPases	-3.3
GO:0007507	heart development	-3.2
GO:0042594	response to starvation	-3.2
GO:0045444	fat cell differentiation	-3.1
R-HSA-9012999	RHO GTPase cycle	-3.1
GO:0060350	endochondral bone morphogenesis	-3
GO:0002040	sprouting angiogenesis	-3
GO:0051338	regulation of transferase activity	-2.9
WP2324	AGE RAGE pathway	-2.9
R-HSA-8980692	RHOA GTPase cycle	-2.8
WP3303	RAC1 PAK1 p38 MMP2 pathway	-2.8

M121	PID MTOR 4PATHWAY	-2.8
WP2880	Glucocorticoid receptor pathway	-2.8
GO:0097305	response to alcohol	-2.8
GO:0035150	regulation of tube size	-2.8
GO:0046777	protein autophosphorylation	-2.7
GO:0019725	cellular homeostasis	-2.7
R-HSA-9013106	RHOC GTPase cycle	-2.7
R-HSA-168898	Toll-like Receptor Cascades	-2.6
GO:0048545	response to steroid hormone	-2.6
M115	PID REG GR PATHWAY	-2.6
GO:0001503	ossification	-2.6
GO:0055082	intracellular chemical homeostasis	-2.5
R-HSA-6794362	Protein-protein interactions at synapses	-2.5
GO:0006873	intracellular monoatomic ion homeostasis	-2.5
R-HSA-556833	Metabolism of lipids	-2.5
GO:0008217	regulation of blood pressure	-2.5
WP2355	Corticotropin releasing hormone signaling pathway	-2.4
hsa05323	Rheumatoid arthritis	-2.4
WP2882	Nuclear receptors meta pathway	-2.4
GO:0030029	actin filament-based process	-2.4
GO:0009725	response to hormone	-2.4
GO:0060349	bone morphogenesis	-2.4
GO:0006631	fatty acid metabolic process	-2.4
hsa04510	Focal adhesion	-2.3
GO:0042445	hormone metabolic process	-2.3
GO:0051402	neuron apoptotic process	-2.3
GO:0034612	response to tumor necrosis factor	-2.3
WP4172	PI3K Akt signaling pathway	-2.3
R-HSA-5357801	Programmed Cell Death	-2.3
hsa04931	Insulin resistance	-2.3
R-HSA-2672351	Stimuli-sensing channels	-2.2
hsa04066	HIF-1 signaling pathway	-2.2
GO:0050821	protein stabilization	-2.2
GO:0043583	ear development	-2.2
R-HSA-211859	Biological oxidations	-2.2
hsa04151	PI3K-Akt signaling pathway	-2.2
GO:0000902	cell morphogenesis	-2.1
GO:0030278	regulation of ossification	-2.1
GO:0003014	renal system process	-2.1
GO:0002688	regulation of leukocyte chemotaxis	-2.1
GO:0031175	neuron projection development	-2.1
R-HSA-372790	Signaling by GPCR	-2
WP3915	Angiopoietin like protein 8 regulatory pathway	-2
GO:0016310	phosphorylation	-2

Abbreviations: LogP, logarithm of the P-value representing the significance of pathway enrichment.

Supplementary Table S10: Pathway analysis on the shared down-regulated geneset

GO	Description	LogP
WP3888	VEGFA VEGFR2 signaling	-4.5
GO:0022411	cellular component disassembly	-4
R-HSA-425407	SLC-mediated transmembrane transport	-3.7
GO:0072089	stem cell proliferation	-3.3
GO:0001525	angiogenesis	-3.1
GO:0008643	carbohydrate transport	-3.1
hsa04912	GnRH signaling pathway	-3
GO:0048871	multicellular organismal-level homeostasis	-2.8
GO:0043588	skin development	-2.5
GO:0034329	cell junction assembly	-2.5
GO:0030097	hemopoiesis	-2.5
GO:0035239	tube morphogenesis	-2.5
GO:0008283	cell population proliferation	-2.5
GO:0034762	regulation of transmembrane transport	-2.4
GO:0001501	skeletal system development	-2.4
hsa04921	Oxytocin signaling pathway	-2.4
GO:0098739	import across plasma membrane	-2.3
GO:0034330	cell junction organization	-2.3
GO:0007369	gastrulation	-2.3
GO:0098609	cell-cell adhesion	-2.2
GO:0001944	vasculature development	-2.2
WP4223	Ras signaling	-2.2
GO:0048598	embryonic morphogenesis	-2.1
GO:0015711	organic anion transport	-2

Abbreviations: LogP, logarithm of the P-value representing the significance of pathway enrichment.

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