Title

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Journal

Movement Disorders, 36(10)

ISSN

0885-3185

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Publication Date

2021-10-01

DOI

10.1002/mds.28760

Peer reviewed

Published in final edited form as:

Mov Disord. 2021 October ; 36(10): 2264–2272. doi:10.1002/mds.28760.

Increased menopausal age reduces the risk of Parkinson's disease: a Mendelian randomization approach

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Authors' Roles

Cynthia DJ Kusters: performed all parts of this project, including conception, organization, execution, statistical analysis and manuscript writing and editing

Kimberly C Paul and Aline Duarte Folle: have been extensively involved in the research project conception, organization and execution, design, review and critiquing of the statistical analysis, and review and critiques of the manuscript

Adrienne M Keener, Jeff M. Bronstein and Johnni Hansen were extensively involved in gathering of phenotypical data, reviewing and critiquing statistical analysis, and with the review and critiquing of the manuscript

Lars Bertram and Christina M. Lill were critical in analyzing the genomic data for all patients, as well as reviewing and critiquing statistical and genetic analyses, and with the review and critiquing of the manuscript

Steve Horvath, and Janet S. Sinsheimer were extensively involved in the design, execution and critiquing the statistical analysis, as well as review and critiquing of the manuscript.

Beate R. Ritz was extensively involved in overseeing the project, gathering all required information (phenotypical and genetics data), the conception, organization and execution of the original PEG and PASIDA studies and this particular project, design and critiquing of the statistical analysis and in reviewing and critiquing the manuscript.

Financial disclosure: No conflicts of interest

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Abstract

Background: Studies of PD and the association with age at menarche or menopause have reported inconsistent findings. Mendelian Randomization (MR) may address measurement errors due to difficulties accurately reporting the age these life events occur.

Objective: We employed MR to assess the association between age at menopause and age at menarche with PD risk.

Methods: We performed inverse variant-weighted (IVW) MR analysis using external GWAS summary data from the UK biobank, and the effect estimates between genetic variants and PD among two population-based studies (PASIDA, Denmark, and PEG, USA) that enrolled 1,737 female and 2,430 male subjects of European ancestry. We then replicated our findings for age at menopause using summary statistics from the PD consortium (19,773 women), followed by a meta-analysis combining all summary statistics.

Results: For each year increase in age at menopause, the risk for PD decreased (OR: 0.84, 95%CI: 0.73-0.98, P:0.03) among women in our study, while there was no association among men (OR: 0.98, 95%CI: 0.85-1.11, P:0.71). A replication using summary statistics from the PD consortium estimated an OR of 0.94 (95%CI: 0.90 – 0.99, P: 0.01), and we calculated a meta-analytic OR of 0.93 (95%CI: 0.89 – 0.98, P: 0.003). There was no indication for an association between age at menarche and PD (OR: 0.75, 95%CI: 0.44-1.29, P:0.29).

Conclusions: A later age at menopause was associated with a decreased risk of PD in women, supporting the hypothesis that sex hormones or other factors related to late menopause may be neuroprotective in PD.

Keywords

Parkinson’s disease; females; menopause; menarche; Mendelian Randomization

Introduction

It is well established that Parkinson’s disease (PD) is more prevalent among males than females.[1] Neuroprotective effects of sex hormones have long been hypothesized as a potential reason for this difference. A number of animal studies have provided support for the hypothesis that estrogen protects dopaminergic neurons against death.[2–4] However, the relationship between sex hormones and the risk of developing PD is still unclear, as results from human epidemiological studies appear equivocal.

In observational studies, the association between lifetime levels of female sex hormones and PD has extensively been explored through the proxy measures of self-reported age at menarche and age at menopause. Unfortunately, the study findings, including a meta-

analysis, have been inconsistent.[1,5–15] There are a number of possible explanations for these discrepancies. First, there may indeed be no association between menarche or menopausal age and PD. Other explanations include that the associations might be population-specific, i.e. depend on interactions with other factors that influence lifelong hormone levels and vary across populations such as the prevalence of surgical menopause, parity, and hormone supplementations. Finally, there is the possibility of biases, including residual confounding and measurement error due to inaccurate or invalid reporting. Further, assessing age at menopause by self-report is difficult. It is often defined as the age at which a woman has had amenorrhea for twelve months ending the menopausal transition. Menopausal transition is the process in which hormone levels change over a long period, and women go from early to late perimenopausal to postmenopausal stages.[16] During the perimenopausal period, the menstrual cycle duration becomes more inconsistent and women may skip periods.[16] Hence, it is quite likely that women find it increasingly hard to recall the time of their last menses accurately with length of time since menopause occurred: inconsistencies between reported menopausal age from repeated interviews supports this.[17–19] Misreporting and missing values for age at menopause or menarche are unlikely to be random events and are more likely in case-control studies of aging-related outcomes as this information is often collected many years after the menopausal transition occurred.

One way to address some of these technical issues is through the use of Mendelian randomization (MR) analyses. MR analysis is performed based on an association between a genetic variant and the exposure, as well as the association between the genetic variant and disease status, such that the genetic variant acts as an instrumental variable.[20] Here, we employ a MR approach to study associations between the age at menopause or age at menarche with PD. Summary statistics from a large external GWAS (UK Biobank) were used to establish associations between the genetic variants and exposure. Associations between these genetic variants and PD risk were then estimated in two population-based studies of PD. The MR analysis was followed by a replication and meta-analysis using summary statistics from a large PD consortium.[21] Based on the hypothesis that female hormones may protect dopamine neurons, we assessed whether an older age at menopause or younger age at menarche is associated with a decreased risk of PD among women.

Methods

We combined information of subjects from two population-based studies: The Parkinson's Environment and Gene study (PEG), and the Parkinson's disease in Denmark (PASIDA) study. Detailed recruitment procedures have previously been described, both for the PEG study[22,23] and the PASIDA study.[15,24]

Briefly, the recruitment in the PEG study, was performed in the Fresno, Tulare, and Kern counties in Central California. We recruited newly diagnosed PD cases in 2001-2007 and from 2011 through present. Case eligibility criteria were: 1) diagnosed with PD <=5 years before recruitment; 2) residence in counties and in CA for >=5yrs; 3) an evaluation by UCLA movement neurologists confirmed "probable" PD clinically according to published criteria; [25] 4) no other neurodegenerative disease diagnosis (including dementia before motor symptom onset); 5) not terminally ill or institutionalized. Starting in 2011, we

enrolled PD cases diagnosed since 2007, who were identified in the population-based California PD registry. Disease classifications were reassessed during follow-up visits with the study neurologists. Recruitment and follow-up occurred in several stages between 2001 and 2019.

Subjects with PD based on their diagnosis in the Danish National Hospital Register between 1996 and 2009 were eligible for the PASIDA study.[24] Subjects with PD, had to be diagnosed at age 35 or older, alive at the time of the scheduled interview (2008–2010), speak English or Danish, and be well enough to participate. For these patients, we requested complete medical records. Subjects were excluded when the extensive examination of their medical record revealed that they did not suffer from PD, when subject did not have a medical record, had pre-existing dementia, or when the diagnosis date was unknown. For each confirmed PD case, 5-10 potential controls were selected from the Danish Central Population Register, matched on birth year and sex. These controls were free of PD at the time of diagnosis of the case and were called in random order until one control agreed to participate in the study.

In both studies age at menarche and menopause were elicited from participants in interviews and they contributed blood or saliva samples for DNA extraction. See Supplemental figure 1 for more detailed information of the inclusion and exclusion criteria, and table 1 for a description of the characteristics of the final study population.

Genome-wide data processing and quality control (QC)

Extensive information regarding genome-wide data processing and QC is reported in supplemental note 1. Briefly, DNA samples from both studies were genotyped using the Global Screening Array (GSA; Illumina, Inc). Raw data processing provided annotations for a total of 696,375 variants. Genetic variants with GenTrain values <0.7, a GenCall Score <0.7, a call rate <95%, a missing genotype rate >2%, a Hardy-Weinberg equilibrium (HWE) tests P-value <5e-6, or minor allele frequency (MAF) of <1% were excluded. Subjects with a genotyping call rate <95% were removed. Overall, pre-imputation QC yielded 495,338 QC-filtered SNPs in 1,866 PEG and 3,486 PASIDA samples suitable for imputation.

Haplotype phasing was performed using SHAPEIT2, and imputation using Minimac3 based on a precompiled Haplotype Reference Consortium (HRC) reference panel. In our post-imputation QC, we retained autosomal SNPs with a minimac3 R-square ≥0.3. Genotyped variants were hard-called using a 90% probability imputation. Variants with MAF <1%, a HWE test of P-value$<1\times10^{-7}$, or variants with a call-rate <98% were excluded. This left a total of 4,592,660 SNPs in PEG and 4,843,960 SNPs in PASIDA. We restricted our subsequent analysis to 4,360,393 SNPs available in both datasets. All post-imputation quality control was performed using PLINK 2.0.[26]

Subjects with >2% missing genotypes among all variants were removed (N=9). We established familial kinship based on the estimated IBD.[26] If the IBD between two individuals was >12% (reflecting second degree relatives or closer relations), one individual was randomly selected to remain in the study. Fractional ancestry among all individuals was estimated using hidden Markov modeling and clustering (Structure 2.3.4).[27] We restricted

the analysis to individuals classified as the European Super-population and in the analysis we adjusted using fractional ancestry identified by Structure.

After quality control, the effective samples size comprised 1,737 females (523 in PEG, and 1,214 in PASIDA) with an average age of 68 years at interview, and 2,430 male (626 in PEG, and 1,804 in PASIDA) participants with an average age of 67 years at interview of European descent with genotyping and disease status. For more detailed information about the number of subjects lost due to exclusion criteria, see supplemental figure 1).

Mendelian randomization analysis

For each genetic variant in the MR analysis two effect estimates need to be available to estimate the association between exposure and outcome; the effect estimates for the association between the genetic variant and exposure; and the effect estimates for the association between the genetic variant and outcome/disease status.

For the association between the genetic variants and the exposure of interest (age at menopause and age at menarche, respectively), we used the summarized results from two large, external GWAS analyses in the UKBB.[28] The genetic variants were ‘clumped’ to take linkage disequilibrium into account, using a R-squared threshold of 0.001 and a distance of 1 million base pairs using Plink 2.0. For the main analysis, we restricted to the genetic variants with a minimal P-value $<5\times10^{-8}$ and an F-statistic of >100 in the UKBB-GWAS analysis to ensure only very strong instruments were used, leading to the selection 19 SNPs for the age at menarche and 8 SNPs for the age at menopause.

The association between each of these genetic variants and Parkinson’s disease was then calculated in the combined PEG/PASIDA dataset, stratified by sex. A logistic regression analysis was performed in plink 2.0. We adjusted for study, age at interview, fractional ancestry, and 10 principal components as potential confounders using a single propensity score. If age at interview was missing, this was imputed based on multiple imputation and information from other covariates to avoid loss of subjects in the logistic regression analysis.

MR analysis was performed using the MendelianRandomization package (v.0.5.0) in R 4.0.1. We performed a standard inverse variant weighted (IVW) MR regression analysis using a multiplicative random effects model when more than three variants were included in the model. Pleiotropic variants were identified using Mendelian Randomization Pleiotropy RESidual Sum and Outlier (MR-PRESSO). All MR analyses were performed separately by sex.

Sensitivity and validation analysis

As sensitivity analyses, we performed IVW MR analyses for age at menopause and PD status for various subgroups, including restricting to women with a natural menopause; restricting to women who were interviewed after age 60 (to eliminate those who potentially were still pre-menopausal); and stratifying by study. We also analyzed various subsets of SNPs by changing the P-value and F-statistics thresholds. In addition to the IVW analysis, we also performed MR-Egger as a sensitivity analysis.

Replication analysis and meta-analysis

After the initial analysis established an association with age at menopause and PD status, a replication of the main IVW MR analysis was performed using the recent sex-stratified GWAS analysis for PD using data from the IPDGC consortium excluding UKBB subjects.[21] This dataset included 19,773 women (7,384 PD cases and 12,389 controls) and 24,053 men (12,054 PD cases and 11,999 controls).

Finally, to calculate an overall effect estimate, we performed a meta-analysis using fixed-effects to combine the summary statistics for PD by combining the effect estimates from the consortium with those from our study populations (PEG&PASIDA), stratified by sex. This was followed by IVW MR analysis using the effect estimates from the meta-analysis for PD.

Power analysis

Based on our data, the R^2 for the instruments and age at menopause and age at menarche was estimated to be around 0.012 and 0.023, respectively, explaining approximately 1.2 and 2.3% of the variance. However, this is likely to be an underestimation due to measurement error of the estimated age of menopause/menarche in this older study population. The GWAS had an estimated heritability of 11.8% and 21.4% for age at menopause and menarche, respectively.[29,30] Therefore, estimating that the true R^2 is around 2 to 3%, given our sample size, and an estimated standard deviation of 5 years (based on the UKBB as well as in our dataset), the initial analysis had enough power (>80%) to detect an OR of 0.84 – 0.86; increasing the population to that of the IPDGC consortium, there was enough power to detect an OR of 0.94-0.95.[31]

Even though it appears there is more genetic heritability for age at menarche than age at menopause, the distribution is narrower and power for this study was more limited. For age at menarche, estimating that the true R^2 is around 4 to 6% and assuming a standard deviation of 1.6 years, there was enough power to detect an OR of 0.66-0.71 in our initial analysis, and an OR of 0.86-0.90 when using the data from the consortium.

Data availability and informed consent

Access to de-identified data related to this study will be made available based on material transfer agreements. Requests can be made to B.R. and J.H. for clinical data from PEG and PASIDA, and to C.L. and B.R. for genetic data from PEG and PASIDA. All study protocols regarding human subjects have been approved by their local Institutional Review Board and informed consent was given by all participants.

Results

Our initial study consisted of 1,737 women (523 PEG; 1,214 PASIDA) and 2,430 men (626 PEG; 1,804 PASIDA). Among the females, the average age at diagnosis was 64.3 years (standard deviation (SD): 9.6), and the average age at interview was 68.4 years (SD: 9.2). See more characteristics in table 1 and supplemental tables 1&2. The PEG and PASIDA studies differed slightly in terms of educational level and reproductive history, for example,

females in the PEG study were more likely to have had a bilateral oophorectomy and to have used some form of hormone therapy.

Age at menarche

For the main analysis, we used nineteen SNPs for the age at menarche. Using inverse variant weighted MR analysis, there was no indication for an association between age at menarche and PD status among females (OR: 0.75; 95%CI: 0.44 – 1.29, P: 0.29), nor among males (OR: 0.80; 95%CI: 0.49 – 1.32, P: 0.39). The I^2_{GX} was 94.1% and there was no indication for pleiotropy using MR-PRESSO analysis (P: 0.25) among females. Even though there was some limited evidence for pleiotropy among men (MR-PRESSO global test for pleiotropy, P: 0.05), no outliers were identified. In addition, there was no evidence for an association using the summary statistics from women of the PD consortium (using the 19 SNPs, OR: 1.08; 95%CI: 0.92 – 1.27, P: 0.34).

Age at menopause

We used eight SNPs as genetic instruments for the age at menopause. The age at menopause was associated with PD risk among females (OR: 0.85; 95%CI: 0.73 – 0.98, P: 0.03), while there was no effect among males between a hypothetical age at menopause and PD (OR: 0.98; 95%CI: 0.85 – 1.11, P: 0.71) in our population study. In various sensitivity analyses, the estimated effects were similar, see table 2. The MR-Egger analysis indicated potential directional pleiotropic effects based on the MR intercept. However, there was no evidence for pleiotropy using MR-PRESSO in any of the analyses. The I^2_{GX} was calculated to be 94.9% and indicated no evidence for weak instrument bias. For more detail about the eight independent SNPs associated with age at menopause, including the individual effect estimates for the SNPs, see supplemental table 3 to 5.

Replication and meta-analysis for age at menopause

We repeated our analysis using summary statistics provided by the PD consortium (IPDGC).[21] The association between age at menopause and PD status was replicated among females from the consortium (OR: 0.94; 95%CI: 0.90 – 0.99, P: 0.01), with the meta-analysis supporting an average 7% decrease in PD risk for each year of increase in age at menopause (OR: 0.93; 95%CI: 0.89 – 0.98, P: 0.003). A visual representation of our main finding is shown in figure 1 and a forest plot in figure 2. The leave-one-out analysis indicated a consistent effect where the overall effect was not depended on one specific SNP (see supplemental table 6 and figure 2).

When changing the P-value threshold and the number of SNPs used to construct as instruments for age at menopause the overall effect estimates attenuated when more variants are included. When including all SNPs with a P-value less than 5e-8, the estimated OR per year diminished and the confidence interval touches the null value of 1.00 (OR: 0.97; 95%CI: 0.94 – 1.00, P: 0.06).

Discussion

Previous studies analyzing the association between age at menopause and PD status have been inconclusive,[1,5–15] and this is the first study reviewing the association between age at menopause/menarche and PD status using the MR method, which is known to reduce certain biases common in observational studies and has the potential to provide evidence in support of causal associations. Our study results suggest that age at menopause is inversely associated with PD risk.

It has been suggested that previous studies might have been inconclusive due to confounding bias, as studies that identified and adjusted for various potential confounders reported conflicting results.[32,33] Ultimately, no observational study can completely rule out residual confounding and it is difficult to determine which covariates should be considered confounders of the association between menopause and PD status. In addition, most covariates that influence age at menopause such as body mass index, physical activity, caffeine/alcohol intake, or sex hormone supplementation are time-varying and difficult to assess with self-report many years after a woman's menopausal transition. Using Mendelian randomization, there is less of a risk that confounding bias occurs when assessing the association between the genetic instrument for age at menopause and PD status. The most important potential confounder between genetic variants and PD status would be population stratification. We adjusted for population stratification by restricting to European ancestry and by adjusting for fractional ancestry and principal components in our main analysis. In addition, the fact that our findings were replicated using a large meta-analysis for PD consisting of various subpopulations strengthens our findings.

Our initial findings were replicated using the summary statistics from a recent meta-analysis that combined various PD studies. The effect estimates were attenuated when using the larger dataset of the PD consortium, possibly due to an overestimation in our initial study population caused by the small sample size (type I error). However, it is also possible that this difference is (partially) caused by variations in the study populations. The meta-analysis was mainly based on clinical PD studies. Therefore, the meta-analysis included a slightly different and younger PD population. Although the meta-analysis did not provide an overall average age at diagnosis or interview, some of these individual studies have indicated that their study population had an average age of onset or interview of 55 years.[34–36] Preferably, premenopausal women would have been excluded from our analysis. Including a significant proportion of premenopausal women with PD or as controls could lead to a bias towards the null. Therefore, we hypothesize that the effect estimates from the PD consortium may be biased towards the null and could underestimate the overall effect estimates for age at menopause on PD risk.

One assumption of MR is that the effects of the genetic variants only occur through the exposure (age at menopause). Here this would mean that the genetic variants associated with an increase in menopausal age should not be associated with other risk factors for PD. There is some indication that the genetic variants for age at menopause are also associated with other reproductive characteristics based on a previous analysis that compared the menopause GWAS with other GWAS findings within the GWAS catalog,[29] and reported

a genetic correlation with age at menarche (rg: 0.11, P-value:4.7e-3), parity (rg: −0.16, P-value 1.5e-5), the use of hormone replacement therapy (rg: −0.67, P-value: 1.2e-84), and hysterectomy (rg:−0.28, P-value: 8.4e-7).[29] In addition, previous studies have also indicated associations between the specific SNPs that were used in our main analysis and other reproductive characteristics, e.g. premature ovarian failure, polycystic ovary syndrome, ovarian follicle numbers and hormone therapy usage.[37–41] Hence, it appears that these variants might have pleiotropic effects involving other reproductive characteristics, all of which are related to hormones among women. In our study, we only identified associations between these genetic variants and PD status among women, not among men, supporting the hypothesis that our findings are driven by hormonal changes among women.

Among the eight SNPs that were used in our main analysis, seven were within the gene-region while one SNP was near a down-stream gene. The GTEx Portal database[42] showed that several of these SNPs are associated with gene-expression of the (nearby) genes, some of which have differential expression within the basal ganglia (e.g. the rs34962991 and the *BRSK1*-gene expression; rs353473 and *FGFR4* expression; rs6854739 and *MRPS18C* expression; rs11075027 with *NPIPB2* and *GSPT1* expression; and rs2277339 and *HSD17B6* gene expression). The majority of the genes are related to DNA repair,[37,43] and they are known to be involved in common GO pathways such as double-strand break repair (*HELB, HELQ, MCM8, UIMC1* within GO:0006302), and DNA metabolic process (*MCM8, HELB, UIMC1, FGFR4, HELQ, PRIM1, STAT6* within GO: 0006259).

Our study did not find an association between age at menarche and PD status. There are several potential explanations for our findings: Besides the possibility of a true null effect, this could be due to limited statistical power in our study or the relatively narrow distribution of menarche ages. Even though there is a genetic component to the age at menarche, external influences likely influence both the age at menarche and PD risk. External influences, such as changes in lifestyle (diet) and socioeconomic factors, most likely are the cause for the decline in average age at menarche in the last few decades.[44,45] This is consistent with the genetic correlations with other GWAS findings within the GWAS catalog.[30] Different from the menopause GWAS, the menarche GWAS is not correlated with other female reproductive characteristics. Instead, it is strongly associated with anthropometric measurements; e.g. BMI (rg −0.30; P-value: 7.9e-77), bioelectrical impedance (rg: 0.27, P-value: 3.7 e-57), body fat percentage (rg: −0.20; P-value: 3.0e-25), and height (rg: −0.33; P-value: 1.5e-17).[30] Overall, based on our findings, there is currently no indication for an association between age at menarche and PD.

Lastly, our study only analyzed individuals of European descent as the UKBB genetic estimates for age at menarche/menopause were derived from European ancestry women only. Also, both the original PD study (PASIDA and PEG) as well as the PD consortium consist primarily of individuals of European ancestry. Therefore, further studies need to evaluate how well these genetic variants perform as genetic instruments in other ethnicities.

Conclusion

This study provides evidence that an earlier menopausal age is associated with an increased risk of PD among women of European ancestry. Each year of delay in age at menopause in our study was associated with a 7% decrease in PD risk. This effect was seen only among women and not among men for whom we generated a hypothetical age at menopause using the genetic variants employed in MR analyses. Thus, biological factors related to a later age at menopause – possibly a longer exposure to female sex hormones or other biologic causes of later menopause – might be neuroprotective and prevent or delay a PD diagnosis in women.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank all the participants in this study.

We thank Dr. Valerija Dobricic and Fabian Kilpert, Lübeck Interdisciplinary Platform for Genome Analytics (LIGA), University of Lübeck, for sample processing and GWAS data pre-processing, quality control, and imputation, respectively, and Mrs. Tanja Wesse, Mrs. Sanaz Sedghpour Sabet, Dr. Michael Wittig, and Dr. Andre Franke at the Institute of Clinical Molecular Biology, Christian-Albrechts-University of Kiel, Kiel, Germany for assistance with GSA genotyping. The LIGA team acknowledges computational support from the OMICS compute cluster at the University of Lübeck.

Funding sources:

C.M.L. and L.B. received funding from the German Research Foundation for the genome-wide SNP data generation and processing of the PEG and PASIDA GWAS data (DFG; FOR2488/1: LI 2654/2-1 and BE 2287/5-1). C.M.L. received support by a “habilitation grant” (H01-2019) from the University of Lübeck. J.S.S. was partially funded by NIH/NHGRI grant HG009120.

This project has been made available with funding from National Institutes of Health (NIH) grants R01-ES010544, R01-ES013717, U54-ES012078, P01-ES016732, and P50-NS038367; initial pilot funding from NIH P30-ES07048; a Community Fast Track grant by the MJFox Foundation; a pilot grant by the American Parkinson Disease Association; and from the Parkinson’s Alliance. CK is supported by the NIH grant F32AG063442.

Financial Disclosures of all authors (for the preceding 12 months)

Cynthia Kusters: is supported by the NIH grant F32AG063442, and employed in the department of Human Genetics as a Ruth Kirchstein postdoctoral fellow (under the NIH F32 grant) at the University of California, Los Angeles.

Kimberly Paul: is supported by the NIH grant R21ES030175 and the Department of Defense grant W81XWH1910697. She is employed in the department of Epidemiology as a postdoctoral fellow at the University of California, Los Angeles.

Aline Duarte Folle: was employed in the UCLA Department of Epidemiology as a Graduate Student Researcher and Public Administration Analyst, paid by Dr. Beate Ritz’s general funds and a grant by NIH (R01AG063969). She is currently a postdoctoral employee, funded by a Multiple System Atrophy Coalition grant CoreE-2020-08-001 and by NIH R01 ES31106-01A1.

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European Research Council. He has given expert testimony for the Research Council of Norway. He also has a contract with Massachusetts General Hospital / Harvard Medical School.

Johnni Hansen: is supported by the following grants: NORDFORSK, RT-10; Nordic Cancer Union. No number; NIEHS 1 R21 NS099910; NIEHS 1 R21 ES028472-01A1; UCLA, Alpha-1 Foundation, 611519. He is employed by the Danish Cancer Society.

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Janet Sinsheimer: has been or is supported by NIH grants HG007703, HG009120, GM053275, and ES023451 and employed as a Professor by the Department of Human Genetics, David Geffen School of Medicine at the University of California, Los Angeles. She is a member of the Data Safety and Monitoring Board for the MPS 1 Intrathecal Research Collaborative Project. She has received royalties from US Patent App. 14/119,145. J.S.S. was partially funded by NIH/NHGRI grant HG009120.

Christina Lill: is employed by the University Hospital Schleswig-Holstein, Lübeck, Germany; and the Imperial College London, London, UK. She is supported by grants from Michael J Fox Foundation, Deutsche Forschungsgemeinschaft, Cure Alzheimer’s Fund, and the European Research Council.

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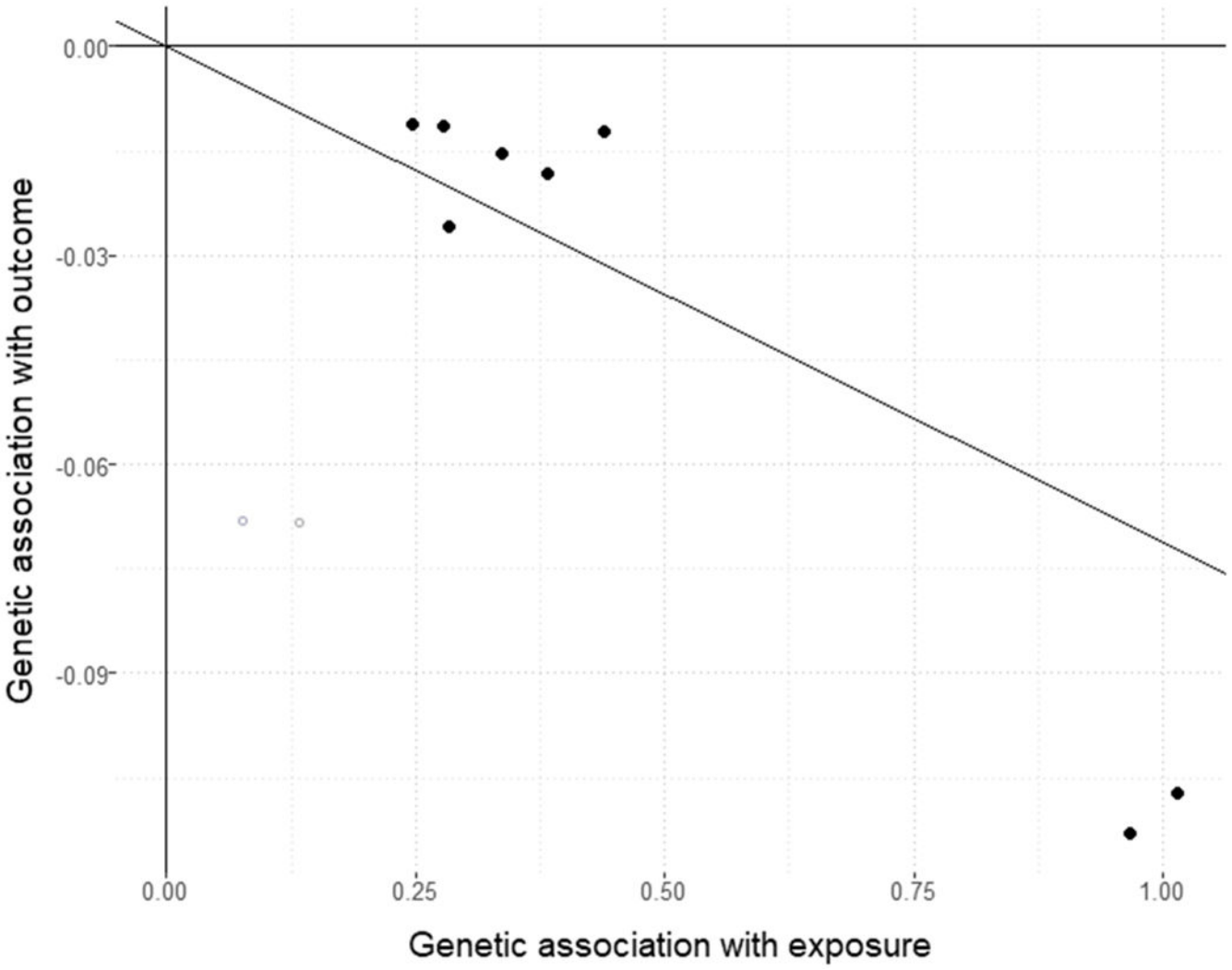

Figure 1.
Visual representation of Mendelian Randomization analysis for age at menopause and PD status among women for the top eight independent SNPs based on the combined summary statistics for PD (using the consortium and the PEG&PASIDA study).

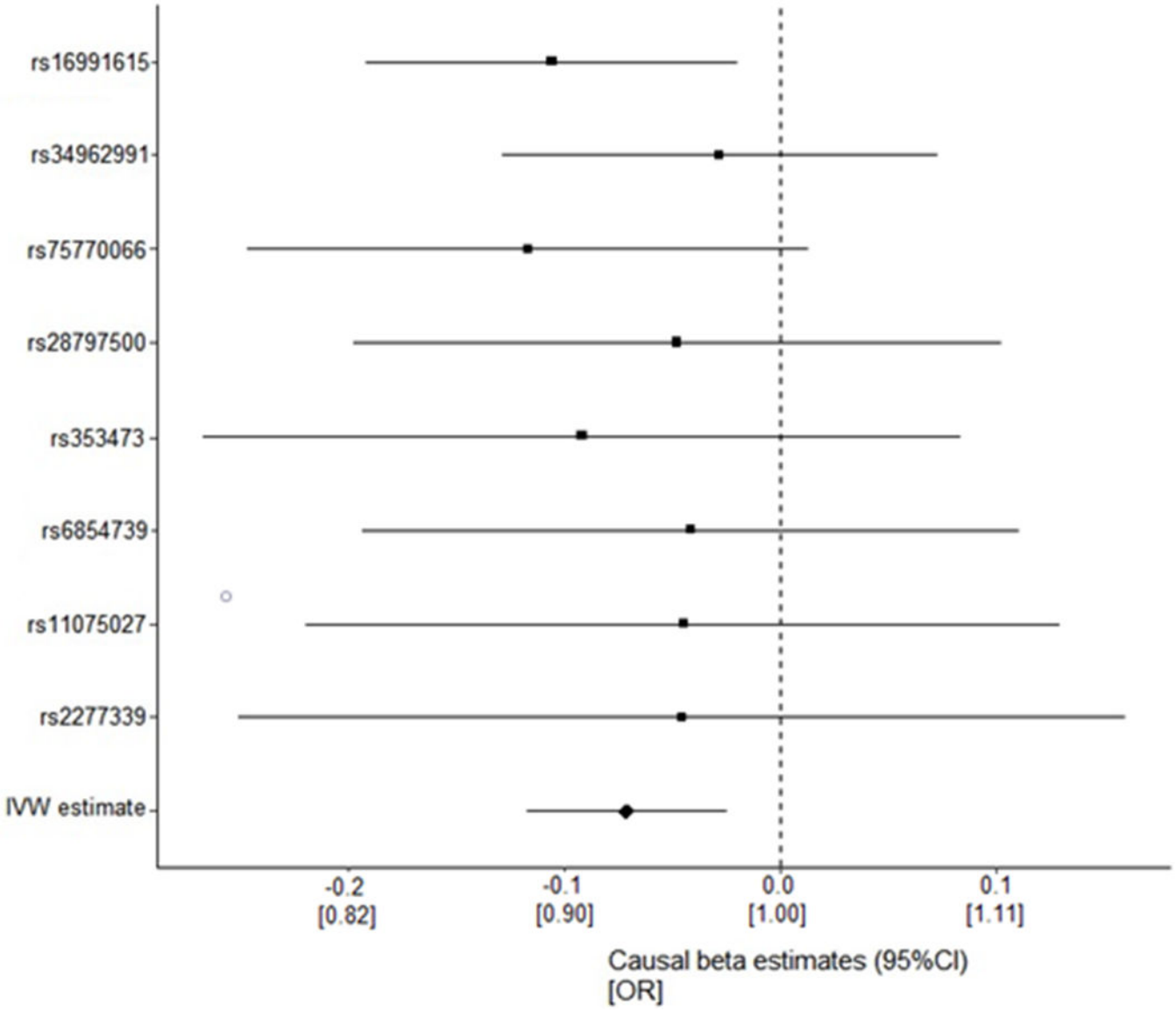

Figure 2.

Forest plot for the associations between age at menopause and PD status among women for the top eight independent SNPs that were genome-wide statistically significantly associated with age at menopause in the UKBB, using the combined summary statistics for PD (using the consortium and the PEG&PASIDA study).

Table 1.

Characteristics of the female subjects in our total study population (PEG & PASIDA) and stratified by study.

	PEG		PASIDA		Total	
	N/avg	SD/%	N/avg	SD/%	N/avg	SD/%
Total number of subjects	523		1,214		1,737	
Number of PD patients	208	39.8	597	49.2	805	46.3
At baseline						
Age at diagnosis	68.1	9.9	62.9	9.1	64.3	9.6
Age at interview	67.7	11.4	68.7	8.4	68.4	9.2
Smoking status						
Never smoker	292	55.8	671	55.4	963	55.5
Former smoker	188	36	408	33.7	596	34.4
Current smoker	43	8.2	133	11.0	176	10.1
Education level						
Up to high school	258	55.1	367	30.2	625	37.2
Vocational / short	69	14.7	543	44.7	612	36.4
College	95	20.3	252	20.8	347	20.6
University	46	9.8	52	4.3	98	5.8
Family history of PD	31	12.6	216	18	247	17.1
Caffeine intake (avg cups per day)	1.2	1.5	3.6	2.4	2.8	2.4
Reproductive characteristics						
Age at menarche	12.6	1.6	13.7	1.6	13.4	1.7
Age at menopause	46.5	8.9	49.2	6.7	48.4	7.6
Age at natural menopause	48.3	8.5	49.6	6.4	49.3	7.0
Parity – Number of children delivered	2.6	1.7	2.2	1.2	2.3	1.4
Bilateral oophorectomy	147	30.1	112	9.4	259	15.4
Birth control hormone usage	296	63.5	527	52.4	823	56.0
Postmenopausal hormone usage	244	50.7	225	20.0	469	29.2

Abbreviations: N: Number; avg: average; SD: Standard Deviation; %: percentage; PD: Parkinson's disease

Table 2.

Mendelian randomization analysis results for PD status and the estimated age at menopause in our study population (PEG & PASIDA), including various sensitivity analyses

Primary analysis	**OR**	**95% CI**	**P**
age at menopause among females	0.85	0.73 - 0.98	0.03
age at (hypothetical) menopause among males	0.98	0.85 - 1.11	0.71
Sensitivity analysis – Subgroups among females			
PASIDA study only	0.81	0.66 - 1.00	0.05
PEG study only	0.78	0.58 - 1.04	0.09
Among women older than 60 years	0.86	0.72 - 1.03	0.11
Among women with a natural menopause	0.86	0.71 - 1.03	0.11
Sensitivity analysis – using MR-Egger instead of IVW			
using MR-Egger	0.62	0.44 – 0.86	0.005
MR-Egger intercept	1.15	1.01 – 1.32	0.04

*I^2_{GX}=94.9% among females, main analysis

Heterogeneity test statistic (Cochran's Q)= 6.68, P-value: 0.46

Abbreviations: OR: Odds Ratio; 95%CI: 95% Confidence Interval; P: P-value

Table 3.

Replication and meta-analysis using the summary statistics from the PD consortium for PD status and estimated age at menopause. The original study (PEG & PASIDA) consisted of 1,737 females and 2,430 males, the PD consortium provided summary statistics for 19,773 females and 24,053 males, and the meta-analysis consisted of summary statistics for PD with a total of 21,510 females and 26,483 males.

				PEG&PASIDA			Consortium			Meta		
P-value Thresh.	**Min F**	**Subgroup**	**Nr SNPs**	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**
Main analysis												
2e-23	100.8	Females	8	0.85	0.73 - 0.98	0.03	0.94	0.90 - 0.99	0.01	0.93	0.89 - 0.98	0.003
2e-23	100.8	Males	8	0.98	0.85 - 1.11	0.71	1.00	0.96 - 1.05	0.82	1.00	0.96 - 1.04	0.94
Sensitivity analysis with differed subsets of SNPs												
5e-8	29.9	Females	56	0.91	0.82 - 1.01	0.07	0.98	0.94 - 1.01	0.18	0.97	0.94 - 1.00	0.06
1e-10	42.5	Females	31	0.89	0.80 - 1.00	0.05	0.97	0.93 - 1.01	0.16	0.96	0.98 - 1.00	0.05
1e-15	65.2	Females	19	0.86	0.76 - 0.98	0.02	0.97	0.93 - 1.01	0.11	0.95	0.92 - 0.99	0.02
1e-16	73.3	Females	15	0.86	0.75 - 0.98	0.02	0.96	0.92 - 1.00	0.05	0.95	0.91 - 0.99	0.01
5.1e-20	84.0	Females	10	0.84	0.73 - 0.97	0.02	0.95	0.90 - 0.99	0.02	0.94	0.89 - 0.98	0.003
1e-42	188.5	Females	5	0.82	0.68 - 0.99	0.04	0.94	0.89 - 0.99	0.02	0.92	0.88 - 0.97	0.003
1e-55	267.6	Females	3	0.77	0.63 - 0.93	0.01	0.94	0.88 - 1.00	0.04	0.92	0.87 - 0.98	0.01

P-value Thresh.: The P-value threshold used for restricting the SNPs from the UKBB GWAS for menopause; Min F: is the minimal F-statistic for the individual SNPs used for this analysis, this statistic is based on the original UKBB GWAS for menopause.

Abbreviations: Thresh: Threshold; OR: Odds Ratio; 95%CI: 95% Confidence Interval; P: P-value