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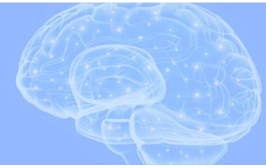
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# Reproductive characteristics, use of exogenous hormones and Parkinson disease in women from the E3N study

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Despite experimental studies suggesting a disease-modifying role of oestrogens, results from epidemiological studies on the relation of reproductive characteristics and hormonal exposures with Parkinson disease in women are conflicting.

We used the data from the E3N cohort study including 98 068 women aged 40–65 years in 1990 followed until 2018. Parkinson disease was ascertained using a validation process based on drug claim databases and medical records. Reproductive characteristics and hormonal exposures were self-reported (11 questionnaires). Associations of exposures with Parkinson disease incidence were investigated using time-varying Cox proportional hazards regression with a 5-year exposure lag and age as the timescale adjusted for confounders. We identified 1165 incident Parkinson disease cases during a mean follow-up of 22.0 years (incidence rate = 54.7 per 100 000 person-years). Parkinson disease incidence was higher in women with early (<12 years, HR = 1.21, 95% CI = 1.04–1.40) or late age at menarche (≥14 years, HR = 1.18, 95% CI = 1.03–1.35) than in women with menarche at 12–13 years. Nulliparity was not associated with Parkinson disease, but Parkinson disease incidence increased with the number of children in parous women (*P*-trend = 0.009). Women with artificial (surgical, iatrogenic) menopause were at greater risk than women with natural menopause (HR = 1.28, 95% CI = 1.09–1.47), especially when artificial menopause occurred at an early age (≤45.0 years). Postmenopausal hormone therapy tended to mitigate greater risk associated with artificial or early menopause (≤45.0 years). While fertility treatments were not associated with Parkinson disease overall, ever users of clomiphene were at greater Parkinson disease risk than never users (HR = 1.81, 95% CI = 1.14–2.88). Other exposures (breastfeeding, oral contraceptives) were not associated with Parkinson disease. Our findings suggest that early and late age at menarche, higher parity, and artificial menopause, in particular at an early age, are associated with increased Parkinson disease incidence in women. In addition, there was some evidence that use of exogenous hormones may increase (fertility treatments) or decrease (postmenopausal hormone therapy) Parkinson disease incidence. These findings support the hypothesis that hormonal exposures play a role in the susceptibility to neurodegenerative diseases. If confirmed, they could help to identify subgroups at high risk for Parkinson disease.

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**Keywords:** Parkinson disease; hormonal exposure; cohort study; women

## Introduction

Over 6 million people suffer from Parkinson disease worldwide,<sup>1</sup> and its prevalence has more than doubled in the last 30 years. Given that Parkinson disease is more frequent in the elderly, its burden is expected to rise dramatically in the future due to increased life expectancy.<sup>1</sup>

Parkinson disease pathogenesis involves complex interactions of genetic and environmental factors.<sup>2</sup> Parkinson disease incidence is 1.5-times higher in men than women<sup>3,4</sup> and the origin of this difference is unclear. Animal and cellular studies have shown that oestrogens have anti-inflammatory, neurotrophic and neuroprotective effects and can upregulate nigrostriatal dopaminergic activity.<sup>5</sup> Therefore, sex steroids might exert a protective role against Parkinson disease-related neurodegenerative processes.<sup>6</sup>

Reproductive life factors, such as puberty, pregnancies or menopause, involve natural variability of sex-hormone levels, while exogenous hormonal treatments [e.g. hormonal fertility treatments, oral contraceptives (OC), postmenopausal hormone therapy (HT)] artificially modulate hormonal levels. The association between hormonal exposure and Parkinson disease risk has been investigated in several case-control<sup>7–14</sup> and cohort studies,<sup>15–20</sup> with inconsistent results that may be explained by differences in study design, but also by intrinsic study limitations, in particular small sample sizes that did not exceed 500 cases in most studies (Supplementary Tables 1 and 2).<sup>7–9,11–19</sup> In addition, some studies did not include a complete assessment of important characteristics, such as type of menopause<sup>11,15,20</sup> or exogenous hormonal treatment,<sup>17</sup> that may modify the association between some reproductive factors and Parkinson disease. Finally, in studies based on electronic records, Parkinson disease diagnoses were not validated.<sup>18,20</sup>

In this study, we investigated the association of several reproductive life characteristics and use of exogenous hormones with Parkinson disease incidence over 24 years of follow-up in E3N, a large population-based prospective cohort study of ~100 000 French women.

## Materials and methods

### Study design and participants: the E3N cohort

E3N is an ongoing cohort study initiated in 1990 in order to investigate risk factors of non-communicable diseases in women. E3N included 98 995 women born between 1925 and 1950 (age at baseline: 40–65 years; approximately 20% of invited women) and enrolled in the Mutuelle Générale de l'Éducation Nationale (MGEN), a health insurance system covering people within the national education system, mostly teachers.<sup>21</sup> Self-administered questionnaires were sent every 2–3 years after baseline and addressed general and lifestyle characteristics as well as medical events. At the present time, 11 waves of data collection (Q1–Q11) are available until 2014, with response rates around 80–85% throughout the follow-up. In addition, women were passively followed between 2004 and 2018

through administrative databases provided by MGEN that include drug claims and information on medical contacts. Death certificates are available for women who died during follow-up and linkage to the French National Service on Causes of Death (CépiDC) provides causes of death coded according to the Tenth Revision of the International Classification of Diseases (ICD-10).

E3N obtained ethical approval from the French National Commission for Data Protection and Privacy (Commission Nationale de l'Informatique et des Libertés) and all participants gave written informed consent. The protocol is registered at clinicaltrials.gov (NCT03285230).

### Identification of Parkinson disease cases

A detailed description of the ascertainment of Parkinson disease cases in E3N is available elsewhere.<sup>22</sup> Briefly, potential Parkinson disease patients were identified through a self-reported doctor diagnosis of Parkinson disease in the follow-up questionnaires, antiparkinsonian drug claims from the MGEN databases, and death certificates.<sup>22</sup> Starting in 2010, potential Parkinson disease patients were contacted by mail to confirm the diagnosis. For women who confirmed a diagnosis of Parkinson disease or parkinsonism and for potential cases who could not be contacted (e.g. deceased, contact refusal), we contacted their treating neurologists or GPs in order to obtain detailed medical documentation, including year of Parkinson disease onset/diagnosis, cardinal motor signs and other neurological symptoms, use of neuroleptics, treatment, responsiveness to treatment and diagnosis. Finally, based on the medical documentation available, Parkinson disease status was adjudicated by an expert panel.<sup>22</sup>

Parkinson disease status of potential cases for whom medical documentation was not available was adjudicated using a validated algorithm based on antiparkinsonian drug claims and medical visits with neurologists and GPs (94% sensitivity, 88% specificity; area under the curve, 0.957).<sup>22,23</sup>

Among women who were confirmed to have Parkinson disease, diagnosis was based on medical records for 62% and the algorithm for 38%.<sup>22</sup>

Year of Parkinson disease diagnosis was set as the year of diagnosis available in medical records or, in decreasing order of priority, self-reported year of diagnosis, year of first use of antiparkinsonian drugs and year of the first questionnaire where Parkinson disease was self-reported. We previously showed that Parkinson disease incidence rates in E3N are in agreement with those observed in women from Western Europe between 1992 and 2018 according to the Global Burden of Disease, which supports the validity of our case finding strategy.<sup>22</sup>

### Characteristics of reproductive life and use of exogenous hormones

#### Reproductive life characteristics

Information on women's reproductive characteristics were self-reported and collected at baseline and updated throughout the

follow-up questionnaires where appropriate. Information on age at first menstruation (menarche) was collected at baseline (Q1–1990). Information on regularity and duration of menstrual cycles in midlife were collected in Q2–1992. Number of children was defined as the number of live births, which was collected in Q1–1990 and updated in Q5–1997. Information on breastfeeding duration was collected at baseline; a mother was considered to have breastfed if she cumulatively breastfed for 1 month or longer. Menopausal status was collected at each questionnaire until Q9–2008, when 99.9% of the women were menopausal. Women were considered menopausal if they had 12 consecutive months without menstrual periods, underwent surgical menopause, used HT for menopause, or self-reported being menopausal. Menopause was further classified as natural or artificial, if it occurred after radiotherapy or chemotherapy (iatrogenic menopause) or after bilateral oophorectomy or hysterectomy (surgical menopause). Age at menopause was set as the age when women had bilateral oophorectomy, hysterectomy, radiotherapy or chemotherapy in case of artificial menopause, or the age of the last menstrual period in case of natural menopause; if this information was missing, age at menopause was set, in decreasing order of priority, at self-reported age at menopause, age of first use of HT or when menopausal symptoms began. We were unable to define age at menopause for 9% of participants, for whom age at menopause was imputed based on the median age at menopause, i.e. 51 years for natural and 47 years for artificial menopause. Duration of reproductive life was computed as the difference between age at menopause and at menarche.

### Use of exogenous hormones

Information on ever use of OC (all preparations including progestogens with or without oestrogens used to prevent pregnancies) was collected at baseline and throughout the follow-up until 2008. Information on use and type of hormonal fertility treatments was collected in 1990 and 1992. From Q2–1992 until Q8–2005 information on HT use, i.e. any non-vaginal use of oestrogens (except oestrin) alone or combined with progestogens or tibolone, was collected. At each questionnaire, a woman was considered an ever user of HT if she reported any use of HT.

### Covariates

Rural and urban communes (i.e. the smallest administrative division in France) of residence were defined according to the French Institut national de la statistique et des études économiques classification.<sup>24</sup> Information on smoking status (never or ever) was collected at each questionnaire. Body mass index (BMI) was computed as self-reported weight, updated at each questionnaire, divided by height squared ( $\text{kg}/\text{m}^2$ ). The validity of self-reported anthropometric measures was previously assessed in E3N with high correlation coefficients for weight (0.94), height (0.89) and BMI (0.92).<sup>25</sup> Silhouettes in childhood and at 20 years were self-reported based on Sørensen's eight silhouettes.<sup>26</sup> Information on physical activity was collected in Q1–1990, Q3–1993, Q5–1997, Q7–2002, Q8–2005 and Q11–2014; as different questions were used to assess physical activity across questionnaires, we used latent-process mixed models to generate a latent physical activity variable that can be used in longitudinal analyses.<sup>27,28</sup> The highest degree of education was collected at baseline as a proxy of socioeconomic status.

### Statistical analyses

To minimize the potential effect of reverse causation due to the prodromal phase of Parkinson disease, we included a 5-year lag between

exposure assessment and Parkinson disease; follow-up started 5 years after the baseline and participants censored within the first 5 years of follow-up were excluded from the analyses. Women were followed until Parkinson disease diagnosis or end of follow-up for women who did not develop Parkinson disease (maximum of the date of the last questionnaire and last drug reimbursement).

The associations of reproductive life characteristics and use of exogenous hormones with Parkinson disease incidence were estimated through hazard ratios (HRs) with 95% CIs using Cox proportional hazards regression models with age as the time scale. Reproductive life characteristics and use of exogenous hormones were included in the models as time-fixed (age at menarche, regularity and duration of menstrual periods, breastfeeding, hormonal fertility treatments) or time-varying variables (parity, menopausal status, age at and type of menopause, use of OC and HT).

Age at menarche was categorized according to the quartiles of its distribution: <12 years (first quartile), 12–13 years (second and third quartiles) and  $\geq 14$  years (fourth quartile). Menstrual periods were classified as regular/irregular; regular periods were further categorized as short ( $\leq 24$  days), regular (25–31 days), or long ( $\geq 32$  days). Women were classified as nulliparous or parous; parity was further categorized into one child, two, and three or more children. Breastfeeding in parous women and use of hormonal fertility treatments and OC were considered as binary variables (ever versus never). Menopausal status was dichotomized as non-menopausal or menopausal; menopausal women were further classified according to the type of and age at menopause. Type of menopause was defined as natural or artificial (iatrogenic, surgical); among surgical menopause, with distinguished bilateral oophorectomy ( $\pm$  hysterectomy) and hysterectomy only. Age at menopause was categorized as premature ( $\leq 40.0$  years), early (40.1–45.0 years), normal (45.1–55.0 years) and late ( $> 55.0$  years) menopause. Owing to the small number of women with premature menopause and because there was no difference in the association of early and premature menopause with Parkinson disease, we combined participants with premature and early menopause in a single group for subsequent analyses. Use of HT was categorized as never or ever.

We first performed analyses adjusted for age (timescale) and rural living at baseline. Each exposure variable was considered individually, but models that included characteristics of menopause (e.g. type, age) and HT use were adjusted for menopausal status and models that included the number of children and breastfeeding were adjusted for nulliparity.

We then ran multivariable models including hormonal exposures associated with Parkinson disease at  $P < 0.05$  in the previous analyses. The directed acyclic graph (DAG) in [Supplementary Fig. 1](#) presents our hypotheses about the causal structure of the data and the temporal relation between the variables, and shows that parity and menopause may be mediators of the relation between menarche and Parkinson disease, while menarche and parity may be confounders of the association between menopause and Parkinson disease. Hence, multivariable analyses were performed separately and adjusted for different sets of confounders for menarche, parity, and menopause, given that these variables characterize different periods of women's reproductive life and are ordered in time. In Model A1, analyses for age at menarche were adjusted for baseline rural living, childhood silhouette (as a proxy of BMI). In model B1, analyses for parity were adjusted for baseline rural living, age at menarche, childhood silhouette, silhouette at 20 years and smoking status, and time-varying nulliparity and parity. In Model C1, analyses for menopause were adjusted for baseline rural living, age at menarche, childhood silhouette and silhouette



at 20 years, time-varying nulliparity, parity, smoking status, BMI, physical activity, menopausal status, type of menopause, and age at menopause.

We examined the role of HT using two complementary approaches. First, we restricted our analyses to women aged 55 years and older and examined Parkinson disease incidence in relation to menopausal status (age at menopause  $\leq 45.0$  or  $>45.0$  years; natural or artificial menopause) and HT use at the age of 50 years (5-year exposure lag). We distinguished non-menopausal women (reference group), women with menopause  $\leq 45.0$  years or artificial menopause, and those with a menopause  $>45.0$  years or natural menopause; among women with menopause  $\leq 45.0$  years or artificial menopause, we distinguished women who used HT from those who did not. In these analyses, the number of women who used HT did not allow us to examine the duration of treatment. Second, we restricted the analyses to menopausal women aged 55 years and older and examined Parkinson disease incidence in relation to time-varying menopausal status and HT use (also with a 5-year exposure lag). We distinguished women with menopause  $>45.0$  years or natural menopause (reference groups) from women with menopause  $\leq 45.0$  years or artificial menopause. Among women with menopause  $\leq 45.0$  years or artificial menopause, we distinguished women who used HT  $\geq 5$  years from those who used HT  $< 5$  years and those who did not use HT.

To examine whether specific fertility treatments were associated with Parkinson disease, we investigated the association between the two most frequent treatments (clomiphene, chorionic gonadotropin) and Parkinson disease; less-common hormonal treatments were grouped together. As fertility treatments were not mutually exclusive and some women may have been exposed to more than one treatment, HRs for the three types of fertility treatments were mutually adjusted and further adjusted for baseline rural living, age at menarche, childhood silhouette, silhouette at 20 years and smoking status.

We also created a *post hoc* composite score that included variables significantly associated with increased Parkinson disease incidence in previous analyses, and examined its association with Parkinson disease. The score ranged from 0 to 4, and was calculated as the count of the following characteristics: (i) menarche at  $< 12$  or  $\geq 14$  years; (ii) at least two children; (iii) artificial menopause; and (iv) early artificial menopause. This model was adjusted for baseline rural living.

For all variables (hormonal exposures and covariates), we created a missing value category to retain the same number of subjects in all analyses. Ordinal variables were tested for linear and quadratic trends using the median of each category. Likelihood ratio tests were used to test for homogeneity in nested models.

## Sensitivity analyses

Given the long prodromal phase of Parkinson disease, we performed sensitivity analyses for the relation between menopausal status and Parkinson disease, assuming longer exposure-response lags of 10 and 20 years to rule out reverse causation.

To investigate whether associations were different according to age at Parkinson disease diagnosis, we split the observation time according to the median age of Parkinson disease diagnosis (72.2 years) and tested whether associations were different in the two age groups.

Finally, to examine the influence of the case definition on our findings, we excluded Parkinson disease patients predicted by the algorithm and performed analyses restricted to cases validated based on medical documentation. Because we were concerned

that the reduced sample size would lead to insufficient statistical power, we performed additional analyses in which, in addition to validated cases, we also retained cases predicted based on a more specific version of the algorithm (90% sensitivity; 90% specificity).<sup>22</sup>

Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and Stata 15 (StataCorp, College Station, TX, USA). Two-sided *P*-values  $\leq 0.05$  were considered statistically significant.

## Data availability

Data on E3N cohort participants are available to *bona fide* researchers for all type of health-related research, which is in the public interest. Data are made available under managed access owing to governance constraints and need to protect the privacy of study participants. Raw data requests should be submitted through the E3N website ([www.e3n.fr](http://www.e3n.fr)) or sent to [contact@e3n.fr](mailto:contact@e3n.fr) and will be reviewed by the E3N Access Committee. Further information is provided at <https://www.e3n.fr/node/78>.

## Results

### Characteristics of the study population

Of 98 995 participants enrolled in E3N at baseline (Q1–1990), 2133 women were excluded from the present analyses (Fig. 1). Therefore, our analyses are based on 96 862 women (total follow-up time = 2 129 642 years), of whom 1165 developed Parkinson disease (incidence rate = 54.7/100 000 person-years) over an average follow-up of 22 years (SD = 4.0).

Baseline characteristics of these women are shown in Table 1 and Supplementary Table 4. Their average age at baseline was 49.3 years (SD = 6.6). About half of the women had their first menstruation between 12 and 13 years. Most women (94.4%) had regular periods with menstrual cycles of 25–31 days (82.3%). Nearly 13% of the women were nulliparous and most parous women had two children; only 80 women delivered a baby after the baseline assessment. Among parous women, 32% never breastfed. Over half of the participants ever used OC and 5% used fertility treatments. The proportion of menopausal women increased from 40.7% (natural, 77.0%; surgical, 19.9%; iatrogenic, 3.1%) at baseline to 99.3% (natural, 81.2%; surgical, 16.3%; iatrogenic, 2.5%) at the end of the follow-up. At baseline, 17.7% and 2.8% of menopausal women had early and late menopause, respectively, compared to 8.2% and 8.0% at the end of the follow-up. The average duration of fertile life was 36.0 years (SD = 4.7) at baseline and 37.7 years (SD = 4.1) at the end of the follow-up. The frequency of

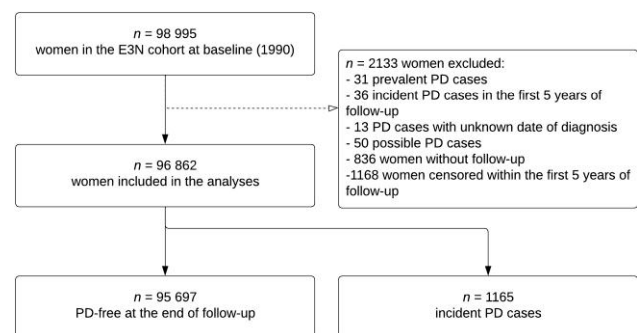


Figure 1 Flow chart of the study population for analyses using a 5-year lag. PD = Parkinson disease.

**Table 1** Reproductive characteristics and use of exogenous hormones at baseline

Characteristics		n = 96 862
Age at menarche <sup>a</sup> (MV = 2358)	<12 years	19 783 (20.9)
	12–13 years	47 700 (50.5)
	≥14 years	27 021 (28.6)
Menstrual cycle (MV = 1028)	Irregular	5399 (5.6)
	Regular	90 435 (94.4)
Menstrual cycle length <sup>b</sup>	≤24 days	8466 (9.3)
	25–31 days	74 399 (82.3)
	≥32 days	7570 (8.4)
Nulliparity (MV = 62)	Yes	12 370 (12.8)
	Non	84 430 (87.2)
Number of children <sup>c</sup> (MV = 42)	1 child	15 598 (18.5)
	2 children	40 753 (48.3)
	≥3 children	28 037 (33.2)
Breastfeeding <sup>c</sup> (MV = 10 585)	Never	23 721 (32.1)
	Ever	50 124 (67.9)
Hormonal fertility treatment	Never	92 440 (95.4)
	Ever	4422 (4.6)
	Clomiphene	2430 (2.5)
	Chorionic gonadotropin Other fertility treatment	1930 (2.0) 2698 (2.8)
Use of OC	Never	43 635 (45.0)
	Ever	53 227 (55.0)
Menopausal status (MV = 23)	Non-menopausal	57 379 (59.3)
	Menopausal	39 460 (40.7)
Type of menopause <sup>d</sup> (MV = 505)	Natural	29 991 (77.0)
	Artificial	8964 (23.0)
	Iatrogenic	1217 (3.1)
	Surgical	7747 (19.9)
	Bilateral oophorectomy <sup>e</sup> Hysterectomy only	4865 (12.5) 2882 (7.4)
Age at menopause <sup>d</sup> (MV = 23)	≤40.0 years	2198 (5.6)
	40.1–45.0 years	4758 (12.1)
	45.1–55.0 years	31 383 (79.5)
	>55.0 years	1121 (2.8)
Duration of reproductive life <sup>d</sup> (MV = 1020)	≤32 years	5453 (14.2)
	32–35 years	9163 (23.8)
	36–38 years	12 724 (33.1)
	39–41 years ≥42 years	8637 (22.5) 2463 (6.4)
Use of HT <sup>d,f</sup> (MV = 7209)	Never	19 389 (60.1)
	Ever	12 862 (39.9)

Data are expressed as mean ± standard deviation or n (%). MV = missing values.

<sup>a</sup>Includes 27 women who never menstruated.

<sup>b</sup>Among women with regular cycles.

<sup>c</sup>Among parous women.

<sup>d</sup>Among menopausal women.

<sup>e</sup>With or without hysterectomy.

<sup>f</sup>Assessed at Q2 (1992).

ever HT use among menopausal women increased from 39.9% at baseline to 65.1% by the end of the follow-up. By the end of the follow-up, women with artificial menopause (22.3%) more often had early menopause than women with natural menopause (5.6%), as well as younger age at menarche, greater probability of irregular menstrual cycles, lower number of children, and shorter duration of reproductive life (Supplementary Table 3).

Women who developed Parkinson disease were more likely to live in urban areas, were less often obese and ever smokers, and had lower physical activity levels than those who did not (Supplementary Table 4).

## Association of characteristics of reproductive life and use of exogenous hormones with Parkinson disease incidence

Table 2 shows crude incidence rates of Parkinson disease and HRs according to characteristics of reproductive life and use of exogenous hormones. In models adjusted for age (timescale) and baseline rural living (each exposure considered individually), Parkinson disease incidence was greater in women with both early and late menarche compared to those with menarche at 12–13 years ( $P$ -quadratic trend = 0.004). Parkinson disease incidence was similar in women with and without children, but Parkinson disease incidence increased with the number of children in parous women ( $P$ -linear trend = 0.007). Menopausal status was not associated with Parkinson disease incidence. Among menopausal women, Parkinson disease incidence was greater in those with artificial menopause compared to those with natural menopause (HR = 1.29, 95% CI = 1.12–1.49). The association was stronger for iatrogenic (HR = 1.43, 95% CI = 1.00–2.05) than surgical menopause (HR = 1.27, 95% CI = 1.12–1.48), but the difference was not significant ( $P$ -homogeneity = 0.551). Bilateral oophorectomy ( $\pm$ hysterectomy) was significantly associated with increased Parkinson disease incidence (HR = 1.36, 95% CI = 1.12–1.64), while hysterectomy only was not (HR = 1.18, 95% CI = 0.95–1.46), but the two groups were not statistically different ( $P$ -homogeneity = 0.314). Women with menopause ≤40.0 years and 40.1–45.0 had a greater risk of developing Parkinson disease (HR = 1.22, 95% CI = 0.88–1.71 and HR = 1.22, 95% CI = 0.98–1.51, respectively) compared to those with menopause between 45.1 and 55.0 years. There was no difference in Parkinson disease incidence between women with premature and early menopause ( $P$ -homogeneity = 0.984) and both groups were pooled together for subsequent analyses (≤45.0 versus 45.0–55.0 years, HR = 1.22, 95% CI = 1.01–1.48). There were no significant associations of duration and regularity of menstrual cycle, breastfeeding, ever use of fertility treatments or OC, duration of reproductive life, and use of HT with Parkinson disease.

Table 3 shows the results of multivariable analyses for hormonal exposures significantly associated with Parkinson disease in previous analyses. Early (HR = 1.21, 95% CI = 1.04–1.40) and late (HR = 1.18, 95% CI = 1.03–1.35) menarche remained significantly associated with higher Parkinson disease incidence compared to menarche at 12–13 years ( $P$ -quadratic trend = 0.004). Among parous women, Parkinson disease incidence increased with the number of children (≥3 versus 1, HR = 1.30, 95% CI = 1.08–1.57;  $P$ -linear trend = 0.009). Compared to natural menopause, artificial menopause was associated with increased Parkinson disease incidence (HR = 1.28, 95% CI = 1.09–1.47). Associations were not statistically different ( $P$ -homogeneity = 0.521) between iatrogenic (HR = 1.41, 95% CI = 0.99–2.02) and surgical (HR = 1.25, 95% CI = 1.07–1.46) menopause. The association was significant for bilateral oophorectomy (HR = 1.31, 95% CI = 1.07–1.61) but not for hysterectomy only (HR = 1.18, 95% CI = 0.95–1.47), without a significant difference between the two groups ( $P$ -homogeneity = 0.462). After adjusting for type of menopause, early menopause was no longer associated with Parkinson disease. However, there was suggestive evidence of an interaction between artificial and early menopause ( $P$ -interaction = 0.076, Fig. 2). Early menopause (≤45.0 years) was associated with Parkinson disease in women with artificial menopause (HR = 1.39, 95% CI = 1.06–1.82) but not in women with natural menopause (HR = 0.97, 95% CI = 0.73–1.30). The association between artificial menopause and Parkinson disease was stronger in women with early menopause

Table 2 Association of reproductive life characteristics and use of exogenous hormones with incidence of Parkinson disease

Characteristics		Cases n = 1165	IR	HR (95% CI)	P	P
Age at menarche (MV = 33 <sup>a</sup> )	<12 years	249	57.3	1.20 (1.03–1.40)	0.017	–
	12–13 years	517	49.1	Reference	–	–
	≥14 years	366	61.9	1.18 (1.03–1.35)	0.017	0.004 <sup>f</sup>
Menstrual cycle (MV = 11 <sup>a</sup> )	Irregular	67	57.3	0.93 (0.73–1.19)	0.574	–
	Regular	1087	54.6	Reference	–	–
Menstrual cycle length <sup>b</sup>	≤24 days	91	48.7	0.93 (0.75–1.15)	0.488	–
	25–31 days	903	55.2	Reference	–	–
	≥32 days	93	55.6	1.03 (0.83–1.28)	0.790	0.474 <sup>g</sup>
Nulliparity (MV = 1)	Yes	147	54.8	0.95 (0.80–1.13)	0.533	–
	No	1017	54.7	Reference	–	–
Number of children <sup>c</sup> (MV = 1)	1 child	152	44.2	Reference	–	–
	2 children	467	51.6	1.22 (1.02–1.47)	0.032	–
	≥3 children	397	65.2	1.31 (1.09–1.58)	0.005	0.007 <sup>g</sup>
Breastfeeding <sup>c</sup> (MV = 124)	Never	275	52.6	Reference	–	–
	Ever	618	55.9	1.06 (0.92–1.23)	0.390	–
Hormonal fertility treatment	Never	1118	55.0	Reference	–	–
	Ever	47	47.8	1.13 (0.85–1.52)	0.406	–
Use of OC	Never	581	68.7	Reference	–	–
	Ever	584	45.5	1.02 (0.90–1.15)	0.775	–
Menopausal status (MV = 2)	Non-menopausal	38	10.3	1.25 (0.76–2.05)	0.373	–
	Menopausal	1125	64.0	Reference	–	–
Type of menopause <sup>d</sup> (MV = 58)	Natural	819	61.3	Reference	–	–
	Artificial	248	77.8	1.29 (1.12–1.49)	<0.001	–
	Iatrogenic	31	77.7	1.43 (1.00–2.05)	0.051	–
	Surgical	217	77.9	1.27 (1.10–1.48)	0.002	0.551 <sup>h</sup>
	Bilateral oophorectomy <sup>e</sup>	124	89.0	1.36 (1.12–1.64)	0.002	–
Hysterectomy only	93	66.7	1.18 (0.95–1.46)	0.131	0.314 <sup>i</sup>	
Age at menopause <sup>d</sup>	≤40.0 years	36	76.8	1.22 (0.88–1.71)	0.238	–
	40.1–45.0 years	90	75.0	1.22 (0.98–1.51)	0.075	–
	45.1–55.0 years	926	62.4	Reference	–	–
	>55.0 years	73	67.8	0.93 (0.73–1.18)	0.561	0.035 <sup>g</sup>
Duration of reproductive life <sup>d</sup> (MV = 31)	<32 years	99	74.4	1.21 (0.97–1.51)	0.094	–
	32–35 years	191	56.3	0.96 (0.81–1.14)	0.648	–
	36–38 years	369	61.5	Reference	–	–
	39–41 years	315	65.6	1.00 (0.86–1.16)	0.986	–
	≥42 years	120	72.9	1.00 (0.81–1.22)	0.944	0.290 <sup>g</sup>
	Use of HT <sup>d</sup> (MV = 69)	Never	403	69.8	Reference	–
	Ever	653	66.8	1.00 (0.88–1.14)	0.978	–

IR = crude incidence rate (per 100 000 person-years); MV = missing values in Parkinson disease patients. HRs and 95% CIs computed using Cox proportional hazards regression with age as the timescale and a 5-year lag and adjusted for baseline rural living.

<sup>a</sup>Including two patients who never menstruated.

<sup>b</sup>Adjusted for cycle regularity.

<sup>c</sup>Among parous women, the model is adjusted for nulliparity.

<sup>d</sup>Among menopausal women, the model is adjusted for menopausal status.

<sup>e</sup>With or without hysterectomy.

<sup>f</sup>P-value for quadratic trend.

<sup>g</sup>P-value for linear trend.

<sup>h</sup>P-value for homogeneity: iatrogenic versus surgical menopause.

<sup>i</sup>P-value for homogeneity: bilateral oophorectomy versus hysterectomy only.

(HR = 1.70, 95% CI = 1.19–2.44) than in women with menopause after 45 years (HR = 1.19, 95% CI = 1.01–1.41).

The results of our analyses on the role of HT are shown in Table 4. In the first model with exposures assessed at 50 years, compared to non-menopausal women, women with menopause ≤45.0 years without HT had an increased incidence of Parkinson disease (HR = 1.41, 95% CI = 1.07–1.87), while there was no association for women with menopause >45.0 years (HR = 1.06, 95% CI = 0.92–1.22); the association was intermediate and not statistically significant for women with menopause ≤45.0 years who used HT (HR = 1.23, 95% CI = 0.69–2.20), but there was only a small number of women in this group. In addition, compared to women with

menopause >45.0 years, those with menopause ≤45.0 years without HT also had an increased incidence of Parkinson disease (HR = 1.33, 95% CI = 1.02–1.73, P = 0.034). Analyses based on type of menopause yielded similar findings, with a significant association between artificial menopause without HT and Parkinson disease (HR = 1.45, 95% CI = 1.15–1.82). In the second set of analyses in menopausal women with time-varying exposures, compared to women with menopause >45.0 years, HRs increased progressively (P-linear trend = 0.021) in women with menopause ≤45.0 years who used HT ≥5 years, those with menopause ≤45.0 years who used HT <5 years, and those with menopause ≤45.0 years who did not use HT, with a significant association in the latter group

**Table 3 Association of reproductive life characteristics and use of exogenous hormones with incidence of Parkinson disease: multivariable models**

Characteristics		Cases n = 1165	5-year lag HR (95% CI)	P	P
<b>Multivariable model A1</b>					
Age at menarche (MV = 33)	<12 years	249	1.21 (1.04–1.40)	0.016	–
	12–13 years	517	Reference	–	–
	≥14 years	366	1.18 (1.03–1.35)	0.018	0.004 <sup>b</sup>
<b>Multivariable model B1</b>					
Nulliparity (MV = 1)	Yes	147	1.16 (0.92–1.45)	0.219	–
	No	1017	Reference	–	–
Number of children among parous women (MV = 1)	1 child	152	Reference	–	–
	2 children	467	1.22 (1.01–1.46)	0.036	–
	≥3 children	397	1.30 (1.08–1.57)	0.006	0.009 <sup>c</sup>
<b>Multivariable model C1</b>					
Menopausal status (MV = 2)	Non–menopausal	38	1.39 (0.85–2.28)	0.0200	–
	Menopausal	1125	Reference	–	–
Type of menopause (MV = 58)	Natural	819	Reference	–	–
	Artificial	248	1.28 (1.09–1.47)	0.002	–
	Iatrogenic	31	1.41 (0.99–2.02)	0.060	–
	Surgical	217	1.25 (1.07–1.46)	0.005	0.521 <sup>d</sup>
	Bilateral oophorectomy <sup>a</sup>	124	1.31 (1.07–1.61)	0.009	–
Hysterectomy only	93	1.18 (0.95–1.47)	0.128	0.462 <sup>e</sup>	
Age at menopause	≤45.0 years	126	1.15 (0.95–1.40)	0.151	–
	45.1–55.0 years	926	Reference	–	–
	>55.0 years	73	0.93 (0.74–1.19)	0.574	0.115 <sup>c</sup>

MV = missing values in Parkinson disease patients. HRs and 95% CIs computed using Cox proportional hazards regression with age as the timescale and with 5-year lag. Multivariable model A1: adjusted for baseline rural living and childhood silhouette. Multivariable model B1: adjusted for baseline rural living, age at menarche, childhood silhouette, silhouette at 20 years, smoking status and time-varying nulliparity and parity. Multivariable model C1: adjusted for baseline rural living, age at menarche, childhood silhouette, silhouette at 20 years and time-varying nulliparity, parity, smoking status, BMI, physical activity, menopausal status, type of menopause and age at menopause.

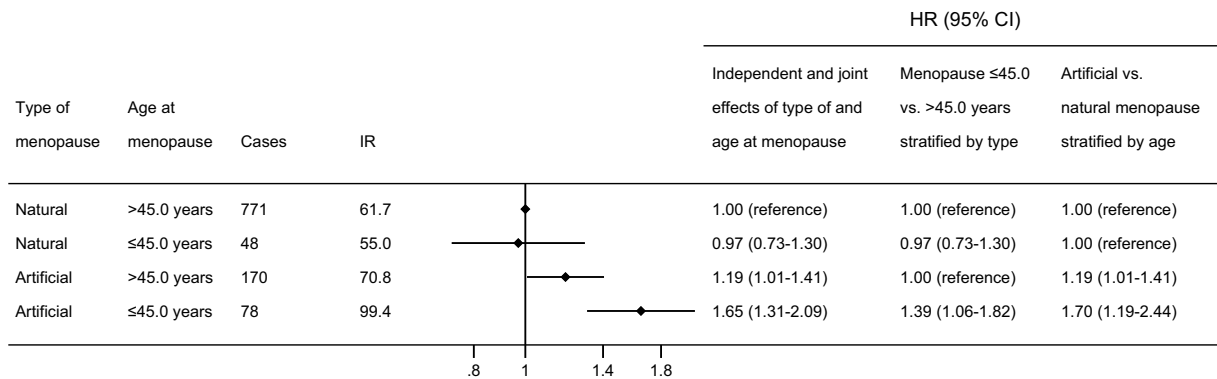
<sup>a</sup>With or without hysterectomy.

<sup>b</sup>P-value for quadratic trend.

<sup>c</sup>P-value for linear trend.

<sup>d</sup>P-value for homogeneity: iatrogenic versus surgical menopause.

<sup>e</sup>P-value for homogeneity: bilateral oophorectomy versus hysterectomy only.



**Figure 2 The interaction between type of menopause (natural, artificial) and age at menopause (≥45.0 years, <45.0 years) on the risk of Parkinson disease.** HRs 95% CIs were computed using Cox proportional hazards regression with age as the timescale and a 5-year lag. Models are adjusted for baseline rural living, age at menarche, childhood silhouette, silhouette at 20 years and time-varying nulliparity, parity, smoking status, BMI, physical activity, menopausal status, type of menopause and age at menopause. The P-value for the interaction between artificial menopause and early age at menopause is 0.076. HRs are first shown for the individual and joint effects of the two exposures. The two last columns show HRs for menopause ≤45.0 years versus >45.0 years stratified by type of menopause and for artificial versus natural menopause stratified by age at menopause. IR = crude incidence rate (per 100 000 person-years).

(HR = 1.33, 95% CI = 1.00–1.76). Analyses based on type of menopause yielded similar findings, with a significant linear trend (P-linear trend < 0.001) and a significant association between artificial menopause without HT and Parkinson disease (HR = 1.38, 95% CI = 1.12–1.71).

Figure 3 shows the incidence and risk of Parkinson disease in relation to the number of reproductive characteristics positively associated with Parkinson disease. Parkinson disease incidence increased progressively with the score (P-linear trend < 0.001); women with early or late age at menarche, high parity and early



**Table 4 Association of age at menopause and use of hormonal therapy with the incidence of Parkinson disease in women aged 55 years and older**

Characteristics of menopause and HT use	Cases n	HR (95% CI)	P	P	Multivariable model		
					HR (95% CI)	P	P
<b>Age at menopause and HT use</b>							
Exposures assessed at 50 years <sup>a,c</sup>	<b>1143</b>						
Non-menopausal	339	1.00 (Reference)	–	–	1.00 (Reference)	–	–
Age at menopause >45.0 years	676	1.07 (0.93–1.23)	0.365	–	1.06 (0.92–1.22)	0.400	–
Age at menopause ≤45.0 years and HT	12	1.20 (0.67–2.13)	0.544	0.698 <sup>g</sup>	1.23 (0.69–2.20)	0.476	0.610 <sup>g</sup>
Age at menopause ≤45.0 years and no HT	61	1.39 (1.06–1.84)	0.019	0.046 <sup>g</sup>	1.41 (1.07–1.87)	0.015	0.034 <sup>g</sup>
Time-varying exposures <sup>b,d</sup>	<b>1120</b>						
Age at menopause >45.0 years	994	1.00 (Reference)	–	–	1.00 (Reference)	–	–
Age at menopause ≤45.0 years and HT ≥5 years	39	1.15 (0.83–1.58)	0.406	–	1.15 (0.84–1.59)	0.380	–
Age at menopause ≤45.0 years and HT <5 years	18	1.21 (0.76–1.93)	0.419	–	1.24 (0.78–1.98)	0.363	–
Age at menopause ≤45.0 years and no HT	52	1.30 (0.99–1.73)	0.063	0.034 <sup>h</sup>	1.33 (1.00–1.76)	0.048	0.021 <sup>h</sup>
<b>Type of menopause and HT use</b>							
Exposures assessed at 50 years <sup>a,e</sup>	<b>1143</b>						
Non-menopausal	339	1.00 (Reference)	–	–	1.00 (Reference)	–	–
Natural menopause	574	1.01 (0.87–1.16)	0.917	–	1.00 (0.87–1.16)	0.965	–
Artificial menopause and HT	9	1.13 (0.58–2.19)	0.715	0.732 <sup>i</sup>	1.16 (0.60–2.25)	0.665	0.672 <sup>i</sup>
Artificial menopause and no HT	100	1.44 (1.14–1.81)	0.002	0.001 <sup>i</sup>	1.45 (1.15–1.82)	<0.001	<0.001 <sup>i</sup>
Time-varying exposures <sup>b,f</sup>	<b>1120</b>						
Natural menopause	816	1.00 (Reference)	–	–	1.00 (Reference)	–	–
Artificial menopause and HT ≥5 years	80	1.20 (0.95–1.51)	0.126	–	1.21 (0.96–1.52)	0.105	–
Artificial menopause and HT <5 years	44	1.30 (0.96–1.76)	0.092	–	1.32 (0.97–1.79)	0.076	–
Artificial menopause and no HT	98	1.37 (1.11–1.68)	0.004	<0.001 <sup>h</sup>	1.38 (1.12–1.71)	0.003	<0.001 <sup>h</sup>

HRs and 95% CIs computed using Cox proportional hazards regression with age as the timescale. Models include a 5-year exposure lag and follow-up starts at the age of 55 years. Of 1165 Parkinson disease patients, 22 with diagnosis <55 years were excluded, leaving 1143 cases; 23 further non-menopausal patients were excluded for the analyses restricted to menopausal women with time-varying exposures, leaving 1120 patients. The total number of cases in each subsection is highlighted in bold.

<sup>a</sup>Multivariable HRs adjusted for baseline rural living, age at menarche, childhood silhouette, silhouette at 20 years, nulliparity, parity, smoking status, BMI and physical activity.

<sup>b</sup>Multivariable HRs adjusted for baseline rural living, age at menarche, childhood silhouette, silhouette at 20 years, and time-varying nulliparity, parity, smoking status, BMI and physical activity.

<sup>c</sup>Characteristics of menopause and HT use are missing for 55 cases.

<sup>d</sup>Analyses restricted to menopausal women, with time-varying age at menopause and HT use. Characteristics of menopause and HT use are missing for 17 cases.

<sup>e</sup>Characteristics of menopause and HT use are missing for 121 cases.

<sup>f</sup>Analyses restricted to menopausal women, with time-varying type of menopause and HT use. Characteristics of menopause and HT use are missing for 82 cases.

<sup>g</sup>With age at menopause >45.0 years as the reference group.

<sup>h</sup>P-value for linear trend.

<sup>i</sup>With natural menopause as the reference group.

artificial menopause had over 2-fold greater risk of developing Parkinson disease than women with median age at menarche, less than 2 children and natural menopause (HR=1.97, 95% CI=1.28–3.02).

### Associations of specific fertility treatment with Parkinson disease incidence

Fertility treatments were not associated with Parkinson disease overall, but analyses for specific treatments showed some differences (Table 5). After adjusting for other fertility treatments, Parkinson disease incidence was 80% greater in women who used clomiphene compared with women never treated for infertility (HR=1.81, 95% CI=1.14–2.88), while there was no association between other treatments and Parkinson disease incidence (Table 5).

### Sensitivity analyses

The results of our main analysis for artificial menopause based on a 5-year lag were confirmed when using longer lags of 10 (based on 1081 Parkinson disease cases, of whom 222 had an artificial menopause; HR=1.24, 95% CI=1.06–1.45, P=0.006) and 20 years (based

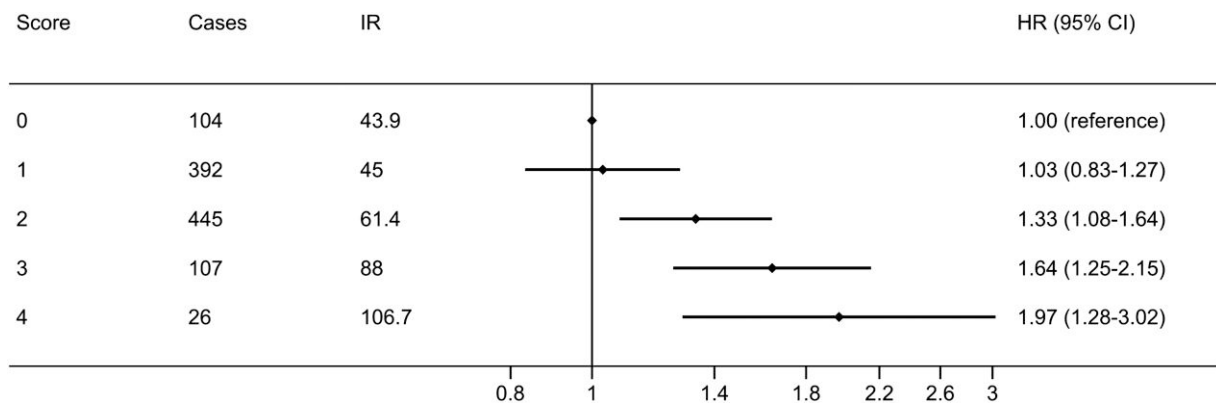
on 602 Parkinson disease cases, of whom 108 had an artificial menopause; HR=1.27, 95% CI=1.01–1.59, P=0.041).

Associations were similar after stratification by median age at Parkinson disease diagnosis (Supplementary Table 5).

Finally, in analyses using alternative definitions of Parkinson disease (restricted to cases validated by medical documentation, more specific version of the algorithm) the associations of age at menarche, parity, and artificial menopause with Parkinson disease were similar to those from our main analyses and statistically significant in both samples (Supplementary Tables 6 and 7).

## Discussion

We investigated Parkinson disease incidence relation to women's reproductive life history and use of hormonal treatments. Early and late age at menarche, a higher number of children, and artificial menopause, especially at an early age, were associated with greater risk of Parkinson disease. HT use appeared to mitigate the detrimental effects of early (<45.0 years) or artificial menopause on Parkinson disease, and there was some evidence that specific hormonal fertility treatments were associated with greater Parkinson disease incidence.



**Figure 3** Parkinson disease rates by reproductive characteristics score and associations between the score and Parkinson disease. The score was calculated as the sum of the following characteristics: (i) age at menarche <12 or ≥14 years; (ii) ≥ 2 children; (iii) artificial menopause; and (iv) artificial menopause before 45 years. HRs and 95% CIs were computed using Cox proportional hazards regression with age as the timescale and a 5-year lag and adjusted for baseline rural living. Sixty-five cases of Parkinson disease had missing or unknown values for at least one of the variables included in the score. IR, crude incidence rate per 100 000 person-years. Parkinson disease incidence increased progressively with the score (*P*-linear trend < 0.001).

**Table 5** Association between fertility treatments and incidence of Parkinson disease

Treatment	Cases		Model A2		Model B2		Model C2	
	n = 1165	IR	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Never	1118	55.0	Reference	–	Reference	–	Reference	–
Ever	47	47.8	1.13 (0.85–1.52)	0.406	1.14 (0.85–1.54)	0.386	–	–
Clomiphene	27	49.5	1.47 (1.00–2.15)	0.051	1.48 (1.01–2.17)	0.046	1.81 (1.14–2.88)	0.012
CGE	18	41.7	1.03 (0.64–1.64)	0.914	1.03 (0.65–1.65)	0.890	0.85 (0.48–1.48)	0.557
Other	23	38.4	0.88 (0.58–1.33)	0.544	0.88 (0.58–1.33)	0.549	0.74 (0.47–1.18)	0.208

IR = crude incidence rate (per 100 000 person-years); CGE = chorionic gonadotropin. HRs and 95% CIs computed using Cox proportional hazards regression with age as the timescale and a 5-year lag. The different types of treatments are not mutually exclusive because women may have used more than one type of treatment. Hence, the sum of women exposed to different treatments is higher than the number of ever users. Model A2: adjusted for baseline rural living (one separate model for each fertility treatment). Model B2: adjusted for baseline rural living, age at menarche, childhood silhouette, silhouette at 20 years and smoking status (one separate model for each fertility treatment). Model C2: adjusted for baseline rural living, age at menarche, childhood silhouette, silhouette at 20 years and smoking status. The model is mutually adjusted for use of clomiphene, chorionic gonadotropin and other hormonal fertility treatments.

We observed a U-shaped association between age at menarche and Parkinson disease, whereby women in the bottom and top quartiles had ~20% greater risk of developing Parkinson disease compared to women who first menstruated at 12–13 years. Most previous studies did not find a significant association between age at menarche and Parkinson disease.<sup>29</sup> In most studies, however, age at menarche was dichotomized at the median or treated as a linear variable, making it impossible to detect a U-shaped relation. In a nationwide cohort study of Korean women, later age at menarche (≥15 years) was associated with greater Parkinson disease risk compared to those who menstruated at 13–14 years, in agreement with our findings, but there was no association for the youngest group (≤12 years).<sup>20</sup> Conversely, in the UK Biobank, younger age at menarche was associated with greater risk.<sup>30</sup> Assuming that oestrogens display disease-modifying effects,<sup>5,6</sup> greater Parkinson disease risk in women with later age at menarche could be explained by shorter lifetime exposure to oestrogens, but this mechanism would not explain the positive association with early age at menarche. We hypothesize that earlier exposure to sex hormones might increase vulnerability to Parkinson disease by interfering with the fine-tuning of dopaminergic pathways. Indeed, puberty is a crucial time window for neural development and sex hormones play a role in the rewiring of the cortical

connectivity and dopaminergic circuitry.<sup>31,32</sup> It is also possible that the epigenetic age acceleration reported in women with early puberty could increase Parkinson disease incidence.<sup>33</sup>

Contrary to most studies that showed no association between parity and Parkinson disease,<sup>29</sup> we found that Parkinson disease incidence increased with the number of children among parous women, while nulliparity was not associated with Parkinson disease. Mechanisms involved in the increased risk of Parkinson disease among multiparous women remain unclear. Although pregnancies are associated with high plasma levels of oestrogens, some studies showed that, outside of pregnancy periods, women with more than one child present lower circulating oestradiol levels than nulliparous women.<sup>34,35</sup> Therefore, the lifetime oestrogen exposure of parous women could be lower than nulliparous and, under the hypothesis of a disease-modifying effect of oestrogens, multiparous women could be at higher Parkinson disease risk compared to nulliparous ones. Maternity is associated with important changes in women’s lifestyle,<sup>36</sup> but our analyses were adjusted for important health behaviours, including smoking, physical activity and BMI.

In agreement with previous studies,<sup>20</sup> we found no association between breastfeeding and risk of Parkinson disease. We also found

no association between menstrual cycle regularity or duration and Parkinson disease incidence.

Although there was no overall association between use of fertility treatments and Parkinson disease, some heterogeneity across specific treatments was present. Compared to women who did not use fertility treatments, Parkinson disease incidence was increased only in those who ever used clomiphene, a tissue-selective oestrogen-receptor modulator used for ovulation induction.<sup>37</sup> Because of its anti-oestrogenic effect, we speculate that use of clomiphene might reduce the disease-modifying effects of female sex hormones.<sup>5,6</sup> No previous publications on the association between fertility treatments and Parkinson disease are available, and our analyses are based on a small number of exposed women; hence, further studies with a larger number of women exposed to fertility treatments and long follow-ups are needed to replicate these findings.

Whereas menopausal status was not associated with Parkinson disease after adjusting for age, type of menopause played an important role since women with artificial menopause were at ~30% greater risk of developing Parkinson disease compared to women with natural menopause, which increased to over 60% when artificial menopause occurred before the age of 45. Both women with surgical or iatrogenic menopause were at increased Parkinson disease risk compared to women with natural menopause. Although the association was stronger for iatrogenic than surgical menopause, the difference was not statistically significant; however, few women had iatrogenic menopause. To our knowledge, this is the first study to report a greater Parkinson disease risk for iatrogenic menopause, and larger studies are needed to examine this issue. Among women with surgical menopause, those with bilateral oophorectomy had the greatest risk of Parkinson disease. However, there was no significant difference between different types of surgical menopause, and women with hysterectomy but without bilateral oophorectomy had an intermediate risk of Parkinson disease, perhaps reflecting an intermediate hormonal status characterized by menstrual cycle cessation without immediate ovarian insufficiency.<sup>38</sup> Our results for surgical menopause are consistent with some previous studies,<sup>7,8,17</sup> while other studies did not find an association<sup>12,16,19</sup> or even found an inverse association.<sup>13</sup> Women with artificial menopause suffer from a drastic reduction of endogenous oestrogen levels that is more marked than in women with natural menopause in whom there is a milder hormonal decline.<sup>39</sup> The oestrogenic drop is more pronounced when artificial menopause occurs at younger age, when natural oestrogenic levels are physiologically higher, which could explain the interaction observed between artificial menopause and early age at menopause. As a consequence of ovarian insufficiency, which frequently follows artificial menopause, the disease-modifying effect of female hormones may disappear, therefore increasing susceptibility to Parkinson disease. This hypothesis is further corroborated by our finding that HT use appeared to mitigate the deleterious effects of early ( $\leq 45.0$  years) or artificial menopause on Parkinson disease, thus suggesting that hormonal replacement after the sudden drop of oestrogens caused by early or artificial menopause might be beneficial. It is also possible that HT may improve motor symptoms,<sup>40</sup> so that the diagnosis of Parkinson disease would be delayed in HT users.

A recent nationwide cohort study of Korean women reported that early age at menopause was associated with greater risk of Parkinson disease,<sup>20</sup> but adjustment for type of menopause was not performed. In agreement with this study, we showed that Parkinson disease incidence was greater in women with younger

age at menopause; however, this association disappeared in analyses adjusted for artificial menopause. This finding is an agreement with those from a previous French case-control study in which early menopause was no longer associated with Parkinson disease while taking into account the type of menopause.<sup>8</sup> Therefore, the association between early menopause and Parkinson disease is likely due to artificial menopause, as both surgical and iatrogenic menopause cause early menopause.

Characteristics positively associated with Parkinson disease incidence had a cumulative effect; there was a significant linear association between the number of reproductive risk factors and Parkinson disease incidence, and women with early/late age at menarche, greater parity and artificial menopause at an early age had the highest incidence rates.

The main strengths of this study include the prospective cohort design, with a large sample size and long follow-up. The long follow-up allowed us to address the potential for reverse causation by performing lagged analyses. Prodromal symptoms, for example, might lead to more medical contacts and greater probability of being diagnosed with a condition leading to surgical menopause or being prescribed hormonal treatments. The consistency of results of our main analysis with a 5-year lag and of those using longer lags of up to 20 years allows us to rule out with reasonable confidence that the associations for time-varying exposures are due to reverse causation. Another strength is that we identified a larger number of Parkinson disease cases in women, over 1000, compared to previous studies that included 500 or fewer female Parkinson disease patients,<sup>7–9,11–19</sup> allowing us to examine the role of rare exposures with sufficient statistical power.<sup>22</sup> In addition, Parkinson disease patients were carefully ascertained, and Parkinson disease incidence rates in E3N are in agreement with those in women from Western Europe according to the Global Burden of Disease, in favour of the validity of our approach.<sup>22</sup> Contrary to previous cohort studies that used characteristics assessed at baseline only<sup>15–20</sup> or a case-control design,<sup>7–14</sup> an important strength of our approach is the use of time-varying exposures updated throughout the follow-up, allowing us to take into account change in exposures.

Our study also has limitations. First, exposures were self-reported, which could have led to measurement error and missing values for some of them (e.g. age at first menstruation or age at menopause). In particular, women with hysterectomy are classified as menopausal even though hysterectomy alone (with ovarian conservation) does not immediately lead to menopause. Nevertheless, uterus removal leads to the loss of an important part of the ovarian vascularization; these women experience a more rapid menopause onset than those with an intact uterus and can be considered as an intermediate group.<sup>38</sup> In addition, ovarian conservation during hysterectomy may not be accurately self-reported.<sup>41</sup> Therefore, we considered hysterectomy without oophorectomy as a separate group of surgical menopause, but we cannot exclude that this group included a small number of non-menopausal women. Nevertheless, exposure misclassification is unlikely to be differential and would therefore bias associations towards the null. Second, the cause of artificial menopause was not collected and we cannot rule out that, in some cases, the association between artificial menopause and Parkinson disease might be explained by indication bias, especially cancer; however, ovarian cancer is relatively rare and it has been suggested that it is inversely associated with Parkinson disease.<sup>42</sup> Other indications for oophorectomy and/or hysterectomy mainly include benign gynaecological diseases that are unlikely to influence Parkinson disease. Third, non-participation at the initial interview may have led to a selected sample

of participants who are mostly educated, health-conscious women not representative of the general population. Although E3N participants are educated and motivated women who provide high-quality information in follow-up questionnaires with a high response rate, results need to be generalized with caution. However, as Parkinson disease incidence rates were similar to expected rates, the selected nature of the cohort does not appear to have led to lower Parkinson disease rates, likely because Parkinson disease onset was long after the cohort started and because response rates have remained high throughout the follow-up (80–85%); we also replicated the inverse association between cigarette smoking and Parkinson disease, one of the most robust findings in Parkinson disease research.<sup>22</sup> In addition, it is generally considered that representativeness is not essential for estimating associations, and associations in occupational cohorts are not necessarily different compared to those in the general population.<sup>43,44</sup> Fourth, despite our efforts, we were unable to obtain medical records for all potential Parkinson disease patients, and determined disease status for those participants through an algorithm based on drug claims that has high sensitivity and specificity. We examined the robustness of our main findings using alternative and stricter definitions of Parkinson disease, and replicated our main conclusions. In addition, the diagnostic validation process was independent of vital status, as we obtained medical records both for women who were alive or dead. As shown elsewhere, the proportion of Parkinson disease patients validated based on medical records did not depend on the year of diagnosis.<sup>22</sup> Fifth, no precise information are available on the origin and ethnicity of the participants because their collection is restricted by law in France. Data on skin colour can be considered as a proxy of origin and ethnicity and indicate that the cohort participants are mostly White. Sixth, we were unable to examine the interaction between premature menopause ( $\leq 40$  years) and type of menopause due to an insufficient number of women with premature menopause. Last, reproductive characteristics and use of exogenous hormones are proxies of hormonal levels which were not objectively measured. The actual hormonal mediators of the observed associations need to be examined in further studies.

In conclusion, our findings suggest that specific reproductive life characteristics, namely age at menarche, greater number of children, and artificial menopause, as well as use of clomiphene, are associated with greater risk of Parkinson disease. Alternatively, HT use may mitigate the greater risk associated with early or artificial menopause, supporting the hypothesis that hormonal exposures play a role in the susceptibility to neurodegenerative diseases. These results could help identify groups at greater risk of Parkinson disease and hormonal treatments that could potentially affect the risk of developing Parkinson disease.

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## Competing interests

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## Supplementary material

Supplementary material is available at *Brain* online.

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