A case-control study of hormonal exposures as etiologic factors for ALS in women

Euro-MOTOR

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ABSTRACT

Objective: To investigate the role of hormonal risk factors for amyotrophic lateral sclerosis (ALS) among women from 3 European countries.

Methods: ALS cases and matched controls were recruited over 4 years in Ireland, Italy, and the Netherlands. Hormonal exposures, including reproductive history, breastfeeding, contraceptive use, hormonal replacement therapy, and gynecologic surgical history, were recorded with a validated questionnaire. Logistic regression models adjusted for age, education, study site, smoking, alcohol, and physical activity were used to determine the association between female hormones and ALS risk.

Results: We included 653 patients and 1,217 controls. Oral contraceptive use was higher among controls (odds ratio [OR] 0.65, 95% confidence interval [CI] 0.51–0.84), and a dose-response effect was apparent. Hormone replacement therapy (HRT) was associated with a reduced risk of ALS only in the Netherlands (OR = 0.57, 95% CI 0.37–0.85). These findings were robust to sensitivity analysis, but there was some heterogeneity across study sites.

Conclusions: This large case-control study across 3 different countries has demonstrated an association between exogenous estrogens and progestogens and reduced odds of ALS in women. These results are at variance with previous findings, which may be partly explained by differential regulatory, social, and cultural attitudes toward pregnancy, birth control, and HRT across the countries included. Our results indicate that hormonal factors may be important etiologic factors in ALS; however, a full understanding requires further investigation. ***Neurology*® 2017;89:1283–1290**

GLOSSARY

ALS = amyotrophic lateral sclerosis; **CI** = confidence interval; **HRT** = hormone replacement therapy; **OCP** = oral contraceptive.

Amyotrophic lateral sclerosis (ALS) is a fatal disease of variable phenotype and unknown etiology. There is evidence that disease manifestation during the second half of life is due to interactions between genetic and environmental factors over time.[1,2] Variants in at least 26 major genes are known to be associated with ALS, the most notable in European populations being the pathologic hexa-nucleotide repeat expansion of *C9orf72*.[3,4] However, definitive evidence of environmental factors has proven elusive. The Euro-MOTOR Consortium was established to undertake a systems biology approach toward ALS, which included generation of data on potential environmental etiologic factors from ALS cases and controls across 3 different countries (Ireland, Italy, and the Netherlands).[5]

A higher incidence in men has been observed across population-based ALS registries, and women are typically older at onset.[6–9] Furthermore, imaging studies of male and female ALS patients suggest that sexual dimorphism of the anatomic patterns of cortical and subcortical pathology in ALS contributes to disease heterogeneity.[10] A previous case-control study found

Supplemental data at Neurology.org

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that the ratio of index to ring finger length, a marker of in utero testosterone exposure, differs between ALS cases and controls.[11] Female ALS patients reportedly have fewer children than matched controls,[12] while studies of ALS cases and matched controls have demonstrated greater lifetime endogenous estrogen exposure to be a protective factor against ALS[13,14] and to be associated with improved survival among cases.[13] Moreover, we recently demonstrated that the effect of *C9orf72* on survival in ALS differs in male spinal-onset patients compared to female and bulbar-onset patients.[15]

Taken together, these observations indicate that sex hormones may play an important role in modulating ALS risk. To further investigate the role of hormones as etiologic factors in ALS, the Euro-MOTOR study recorded detailed hormonal histories from study participants.[5]

METHODS

Case ascertainment. Recruitment procedures of the Euro-MOTOR study were described in detail previously.[5] Briefly, incident cases and controls matched by age, sex, and location were recruited through population-based ALS registers and general practitioner practices, respectively, in Ireland, the Netherlands, and 3 regions of Italy, Apulia, Piedmont and Valle d'Aosta, and Lombardy, between 2011 and 2014. Controls were matched to cases for age at survey ±5 years. Individual matching was used in Ireland and Italy, while frequency matching was used in the Netherlands. All patients met the diagnostic criteria for definite, probable, or possible ALS (according to the revised El-Escorial criteria[16]).

Hormonal exposure assessment. Female participants were asked to answer additional questions pertaining to hormonal factors regarding age at menarche, age at menopause, monthly cycle length and cycle regularity, history of pregnancy (miscarriages and live births were included), breastfeeding history, oral contractive (OCP) and non-OCP use, details of surgical gynecologic history (oophorectomy and hysterectomy), and history of hormone replacement therapy (HRT). The full survey questions have been published previously.[5]

Calculation of lifetime unopposed estrogen exposure. While estrogen exerts a neuroprotective effect, progesterone may oppose such effects,[13] and estrogen levels are high and unopposed by progesterone only at certain times.[13,17] Therefore, calculation of lifetime unopposed endogenous estrogen exposure entailed several steps. First, because estrogen levels are low before menarche and after menopause, the reproductive span was calculated as the number of years from menarche to menopause. Next, because the ovulatory cycle consists of 2 phases, a high-estrogen preovulatory phase of varied length and a postovulatory phase of falling estrogen opposed by high progesterone more consistently 2 weeks in length, we adjusted the reproductive span by subtracting the last 2 weeks of every cycle.[13,17] Then, to calculate total endogenous lifetime exposure, 9 months were subtracted for each completed pregnancy, and 3 months were subtracted for each miscarriage (pregnancy invokes high estrogen levels, but they are opposed by high progesterone levels).[13,17] Most hormonal contraceptives contain combined estrogen and progesterone or progesterone only, thus interrupting natural hormonal cycles. Therefore, the total duration of OCP use was subtracted from the adjusted reproductive span. The above adjustments were used previously in studies on ALS and cardiovascular disease.[13,17] All exposure variables were truncated to 3 years before the survey date for both patients and controls in an attempt to remove exposures that may have occurred after ALS onset.[5]

Statistical analysis. Multivariate logistic regression was used to calculate odds ratios (ORs) for the risk of ALS from each hormonal factor, after adjustment for age, education, and study site. These models were then further extended to adjust for smoking history, alcohol use, and physical activity. To examine hormonal factors by study site, stratified models were constructed. OCPs were made legally available (by prescription) in Ireland in 1980 and in Italy in 1978 but had been available in the Netherlands since 1962. Therefore, the age at which OCPs were started is expected to be heterogeneous across sites and may present an important confounding factor. To mitigate expected heterogeneity in this variable across study sites, we categorized the age at first use of OCPs as never used OCP, started OCP at an age less than or equal to the control median age at starting OCP, and began OCP at an age greater than the control median age at starting OCP. This variable was used to model whether the age at which the OCP was started was a confounding factor.

Analysis was performed as a complete case analysis and after imputation of missing values. Imputation used multiple iterations (n = 30) of predictive mean matching for each region individually implemented with the R package mice.[18] As a sensitivity analysis, those experiencing menopause due to surgery (oophorectomy or hysterectomy), those on HRT, and those using OCPs at the time of menopause were excluded from the lifetime endogenous estrogen calculation.[13] In addition, stratification by education and by site of onset was performed. Finally, analyses were recalculated after exclusion of known *C9orf72* expanded cases. All analysis was carried out with *R* version 3.2.3[19] with additional packages.[18,20–25]

Standard protocol approvals, registrations, and patient consents. Overarching ethics approval for this study was obtained from the Medical Ethics Committee of University Medical Centre, Utrecht (reference AvG/sv/05/08022). All patients and controls provided informed consent.

RESULTS

Descriptive statistics. A total of 653 patients and 1,217 controls were included. Of the patients, 270 (42%) had bulbar-onset disease. Table 1 summarizes the demographic characteristics of patients and controls stratified by study site. Overall, cases were 1.2 years older than controls (p = 0.018). More controls were highly educated compared to patients (p = 0.001), a finding driven by the Lombardy and Piedmont and Valle d'Aosta cohorts. Dutch controls were marginally younger than patients (p = 0.047). Table e-1 at Neurology.org compares clinical characteristics of patients across study sites. Irish patients were more likely to have spinal onset and definite El-Escorial category, and there were differences in the rates of *C9orf72* testing across sites.

Table 1 Demographics of female Euro-MOTOR participants by study site

Study site	Case/control	n	Education				
			Age at survey, y Mean (SD)	*p* Value, *t* test	ISCED 0–4, n (%)	ISCED 5–6, n (%)	*p* Value, Fisher exact test
Apulia	Cases	60	63.8 (11.1)		54 (93.1)	4 (6.9)	
	Controls	100	63.7 (11.4)	0.939	92 (92.0)	8 (8.0)	1.000
Lombardy	Cases	86	65.7 (10.3)		73 (88.0)	10 (12.0)	
	Controls	89	65.8 (10.8)	0.971	62 (69.7)	27 (30.3)	0.005
Piedmont and Valle d'Aosta	Cases	124	67.1 (11.0)		112 (94.1)	7 (5.9)	
	Controls	137	65.2 (12.1)	0.191	116 (84.7)	21 (15.3)	0.017
Ireland	Cases	71	65.1 (11.6)		57 (80.3)	14 (19.7)	
	Controls	138	65.4 (11.7)	0.856	110 (79.7)	28 (20.3)	1.000
The Netherlands	Cases	312	64.8 (9.5)		244 (78.7)	66 (21.3)	
	Controls	753	63.6 (9.4)	0.047	564 (74.9)	189 (25.1)	0.206

Abbreviation: ISCED = International Standard Classification of Education.

Tables 2 and 3 summarize the endogenous and exogenous hormonal factors of patients and controls stratified by study site. Generally, there were minor differences between cases and controls within study sites with a lack of consistent direction across sites. Age at menarche and reproductive span were comparable across study sites and between cases and controls (table 2). The use of contraceptives was lower in cases compared to controls within all countries and reached statistical significance in Lombardy, in the Netherlands, and overall (table 3). Duration of OCP use was lower in cases compared to controls across all sites except Apulia, and with $p < 0.05$ in Ireland, in the Netherlands, and overall. In a comparison of controls across study sites, 25% of women from Apulia, 43% of women from Lombardy, 38% of women from Piedmont and Valle d'Aosta, 49% of Irish women, and 76% of Dutch women had a history of hormonal contraceptive use. Few participants used nonoral hormonal contraceptives; therefore, the remainder of our analysis of contraceptives is focused on OCPs.

Lifetime unopposed endogenous estrogen exposure was lower in the Dutch controls (mean 11.3 years) vs the Irish (mean 14.0 years) and the Italians (Apulia 15.3 years, Lombardy 15.8 years, Piedmont and Valle d'Aosta 15.2 years). Lifetime unopposed estrogen exposure was 14.2 years in all cases vs 12.9 years in all controls ($p < 0.001$).

Reproductive hormonal factors. Table 4 shows ORs for ALS each of the hormonal factors in turn after multivariate logistic regression. On complete case analysis, a history of ever having used OCP was significantly associated with reduced OR for ALS (OR = 0.65, 95% confidence interval [CI] 0.51–0.84). A dose-response relationship was evident between duration of OCP use and reduced OR (0.98, 95% CI 0.96–0.99 per year of use). These findings remained significant after multiple imputation of missing values and after correction for other suspected lifestyle etiologic factors, including lifetime physical activity, smoking, and alcohol consumption. Those who never used OCP were excluded; however, this had minimal effect on the apparent dose-response relationship, with an OR of 0.98 (95% CI 0.96–1.00) per year of use after exclusions.

Models stratified by education level found that history of OCP use (OR 0.61, 95% CI 0.46–0.81) and duration of OCP use (OR 0.97, 95% CI 0.95–0.99 per year) were significant in the lower education group but not in the higher education group (history of OCP: OR 0.80, 95% CI 0.45–1.45; duration of OCP: OR 0.99, 95% CI 0.96–1.03 per year). They also remained significant in models of spinal or bulbar patients only vs controls. Similarly, exclusion of women carrying the *C9orf72* expansion did not significantly alter results (table 4). ORs for ever use of OCP and duration of OCP stratified by cohort are shown in the figure. It is noteworthy that the ORs were in the same direction toward reduced OR in each study site (figure). Ever use of OCP was clearly associated with decreased OR in Ireland (OR 0.44, 95% CI 0.21–0.87) and Lombardy (OR 0.42, 95% CI 0.20–0.89), while duration of OCP use was clearly associated with decreased OR in Ireland only (OR 0.90, 95% CI 0.81–0.97). However, the mean ORs were <1 across all study sites (except Apulia) per year of OCP use (figure).

A reduced OR for ALS was found in those who started OCP at younger ages (OR 0.56, 95% CI 0.41–0.77) and those who started the OCP at older

Table 2 Summary of endogenous hormonal exposures factors by study site

Study site	n	Age at menarche, mean (SD), y	Reached menopause, n (%)	Age at menopause, mean (SD), y	History of irregular periods, n (%)	Mean reproductive span (SD), y[a]	Pregnancies, median (IQR), n	Live births, median (IQR), n	Has breastfed, n (%)
Apulia									
Cases	60	12.7 (1.5)	49 (84.5)	47.2 (5.9)	11 (18.3)	33.8 (5.9)	3.0 (2.0–4.0)	2.0 (2.0–3.0)	45 (77.6)
Controls	100	12.7 (1.8)	89 (89.0)	48.3 (5.5)	13 (13.0)	34.9 (6.0)	2.0 (2.0–3.0)	2.0 (1.8–3.0)	63 (63.0)
p Value		0.921 (t)	0.461 (F)	0.289 (t)	0.110 (F)	0.281 (t)	0.104 (M)	0.039 (M)	0.076 (F)
Lombardy									
Cases	86	12.7 (1.4)	69 (84.1)	50.6 (4.7)	16 (18.6)	38.1 (6.8)	2.0 (1.0–3.0)	2.0 (1.0–3.0)	49 (59.0)
Controls	89	12.8 (1.5)	74 (84.1)	51.4 (4.4)	19 (21.3)	37.6 (5.3)	2.0 (1.0–3.0)	2.0 (1.0–2.0)	56 (64.6)
p Value		0.756 (t)	1.000 (F)	0.272 (t)	0.747 (F)	0.610 (t)	0.309 (M)	0.613 (M)	0.529 (F)
Piedmont and Valle d'Aosta									
Cases	124	13.2 (1.6)	110 (90.9)	50.5 (4.1)	31 (25.0)	36.2 (5.3)	2.0 (1.0–3.0)	2.0 (1.0–2.0)	85 (71.4)
Controls	137	12.7 (1.4)	115 (84.6)	49.4 (5.3)	52 (38.0)	35.4 (6.4)	2.0 (1.0–2.0)	2.0 (1.0–2.0)	88 (64.2)
p Value		0.005 (t)	0.134 (F)	0.085 (t)	0.016 (F)	0.272 (t)	0.058 (M)	0.249 (M)	0.231 (F)
Ireland									
Cases	71	13.5 (1.5)	62 (87.3)	48.0 (6.4)	13 (18.3)	33.9 (7.1)	3.0 (1.5–5.0)	3.0 (1.0–4.0)	25 (35.2)
Controls	138	13.2 (1.5)	124 (89.9)	48.1 (6.5)	27 (19.6)	33.9 (7.1)	4.0 (2.0–5.0)	3.0 (2.0–4.0)	59 (42.8)
p Value		0.273 (t)	0.643 (F)	0.903 (t)	0.431 (F)	0.980 (t)	0.081 (M)	0.134 (M)	0.302 (F)
The Netherlands									
Cases	312	13.4 (1.5)	272 (89.2)	49.6 (5.8)	79 (25.7)	35.6 (6.2)	2.0 (2.0–3.0)	2.0 (1.0–3.0)	130 (56.5)
Controls	753	13.4 (1.60)	664 (88.8)	49.4 (5.70)	153 (20.4)	35.5 (6.1)	2.0 (2.0–3.0)	2.0 (2.0–3.0)	329 (62.7)
p Value		0.503 (t)	0.914 (F)	0.627 (t)	0.189 (F)	0.650 (t)	0.242 (M)	0.298 (M)	0.124 (F)
Overall									
Cases	653	13.2 (1.5)	562 (88.2)	49.5 (5.5)	150 (23.0)	35.7 (6.3)	2.0 (2.0–3.0)	2.0 (1.0–3.0)	334 (59.5)
Controls	1,217	13.2 (1.6)	1,066 (88.1)	49.3 (5.7)	264 (21.7)	35.4 (6.2)	2.0 (2.0–3.0)	2.0 (2.0–3.0)	595 (60.3)
p Value		0.432 (t)	1.000 (F)	0.471 (t)	0.782 (F)	0.284 (t)	0.394 (M)	0.190 (M)	0.787 (F)

Abbreviations: F = Fisher exact test; IQR = interquartile range; M = Mann-Whitney *U* test; t = Student *t* test.
[a] Truncated at 3 years before survey date to exclude exposure after onset.

ages (OR 0.74, 95% CI 0.55–0.99) compared to those who never used OCP. Broken down by study site, these results showed significant heterogeneity across sites with small numbers in some subgroups (table e-2). Imputation of missing values and correction for lifestyle factors did not change heterogeneity across sites meaningfully (table e-2), but it reduced the difference in ORs between those starting at younger ages (OR 0.54, 95% CI 0.41–0.72) and those starting at older ages (OR 0.67, 95% CI 0.53–0.86) overall (table e-2).

Lifetime endogenous estrogen exposure. Lifetime endogenous estrogen exposure was associated with a mildly increased OR for ALS with borderline significance even after imputation and correction for other lifestyle cofactors (OR 1.02, 95% CI 1.00–1.04) (table 4). After exclusion of women who experienced surgical menopause, lifetime endogenous estrogen exposure was no longer significant (OR 0.99, 95% CI 0.96–1.02). None of the remaining examined variables in table 4 revealed significant associations. A model was built including each term contributing to lifetime endogenous estrogen exposure as a separate term. In this model, only duration of OCP use remained significant, with an OR of 0.98 (95% CI 0.96–0.99) per year of OCP use.

HRT, hysterectomy, and oophorectomy. HRT use was low among cases and controls across all study sites except Ireland (table 3). Overall, the OR for HRT use after adjustment for age, education, and study site was 0.82 (95% CI 0.62–1.08). Stratified by study site, HRT use was significantly associated with ALS only in the Netherlands (OR 0.57, 95% CI 0.37–0.85). Age at the start of HRT use did not differ between cases and controls overall or for each study site. The duration of HRT use was not associated with ALS either overall (OR 1.03, 95% CI 0.99–1.08) or in the Netherlands only (OR 1.03, 95%

Table 3 Summary of exogenous hormonal factors

Study site	n	Hormonal contraceptive use, n (%)	OCP use, n (%)	Mean years taking OCPs[a] (SD)	Used HRT, n (%)	Hysterectomy, n (%)	Oophorectomy, n (%)
Apulia							
Cases	60	11 (19.0)	11 (19.0)	1.5 (5.6)	9 (15.5)	11 (19.0)	10 (18.9)
Controls	100	25 (25.0)	24 (24.0)	1.7 (4.4)	7 (7.0)	24 (24.0)	23 (24.0)
p Value		0.436 (F)	0.553 (F)	0.795 (t)	0.104 (F)	0.553 (F)	0.541 (F)
Lombardy							
Cases	86	22 (26.5)	22 (26.5)	1.86 (5.56)	12 (14.5)	13 (16.0)	6 (8.1)
Controls	89	38 (42.7)	38 (42.7)	3.68 (6.30)	16 (18.6)	18 (21.2)	10 (12.0)
p Value		0.037 (F)	0.037 (F)	0.051 (t)	0.538 (F)	0.431 (F)	0.443 (F)
Piedmont and Valle d'Aosta							
Cases	124	33 (27.7)	32 (26.9)	1.65 (4.36)	15 (12.6)	14 (11.8)	12 (10.4)
Controls	137	52 (38.0)	51 (37.2)	2.52 (5.11)	19 (14.0)	20 (14.6)	22 (16.3)
p Value		0.086 (F)	0.083 (F)	0.148 (t)	0.384 (F)	0.581 (F)	0.199 (F)
Ireland							
Cases	71	25 (35.2)	24 (33.8)	1.37 (3.00)	25 (35.2)	16 (22.5)	7 (15.2)
Controls	138	67 (48.6)	64 (46.4)	3.24 (6.24)	45 (32.6)	33 (24.3)	15 (20.3)
p Value		0.078 (F)	0.104 (F)	0.022 (t)	0.758 (F)	0.864 (F)	0.629 (F)
The Netherlands							
Cases	312	215 (68.9)	212 (67.9)	8.82 (9.59)	32 (10.5)	59 (19.0)	31 (10.2)
Controls	753	574 (76.3)	565 (75.1)	10.36 (9.96)	120 (16.0)	141 (18.8)	76 (10.3)
p Value		0.014 (F)	0.019 (F)	0.023 (t)	0.020 (F)	0.931 (F)	1.000 (F)
Overall							
Cases	653	306 (47.6)	301 (46.8)	5.1 (8.3)	93 (14.6)	113 (17.7)	66 (11.1)
Controls	1,217	756 (62.2)	742 (61.0)	7.5 (9.3)	207 (17.1)	236 (19.5)	146 (13.0)
p Value		<0.001 (F)	<0.001 (F)	<0.001 (t)	0.051 (F)	0.349 (F)	0.316 (F)

Abbreviations: F = Fisher exact test; HRT = hormone replacement therapy; M = Mann-Whitney *U* test; OCP = oral contraceptive pill; t = Student *t* test.
[a] Truncated at 3 years before survey date to exclude exposure after onset.

CI 0.97–1.08). A history of hysterectomy (OR 0.85, 95% CI 0.66–1.10) or oophorectomy (OR 0.77, 95% CI 0.56–1.06) was not associated with ALS.

Fertility. Overall, there was little difference in fertility between cases and controls (table 2). After adjustment for study site, education, age, and contraceptive use via multivariate logistic regression, the number of pregnancies or number of live births was not significantly associated with case/control status overall. However, ever being pregnant was associated with a reduced OR in Ireland (OR 0.35, 95% CI 0.14–0.84) but with an increased OR in Apulia (OR 3.43, 95% CI 1.15–13.0).

DISCUSSION The analysis of sex hormones, pregnancy, the use of oral contraception, and disease risk is complicated and prone to hidden confounders, particularly across different countries with different levels of access to contraception and different cultural attitudes toward fertility. Nevertheless, this large case-control study across 5 different sites has demonstrated a negative association between ALS and hormonal contraception use in women. We found reduced odds of ALS for a history of ever using OCP and that the risk decreased further with longer duration of OCP use, even among OCP users only.

This study did not confirm previous findings from the Netherlands that showed a negative association between lifetime endogenous estrogen exposure and ALS risk[13] or findings from Italy showing a negative association between reproductive span and ALS risk.[26] Conversely, our data suggest a weak association of increased risk with lifetime endogenous estrogen exposure and found no effect for reproductive span (table 4). By modeling the individual terms contributing to lifetime endogenous estrogen exposure, we determined that this finding was driven by subtraction of the duration of OCP use in the calculation of lifetime endogenous exposure.

Table 4 ORs for reproductive hormonal factors after multivariable logistic regression

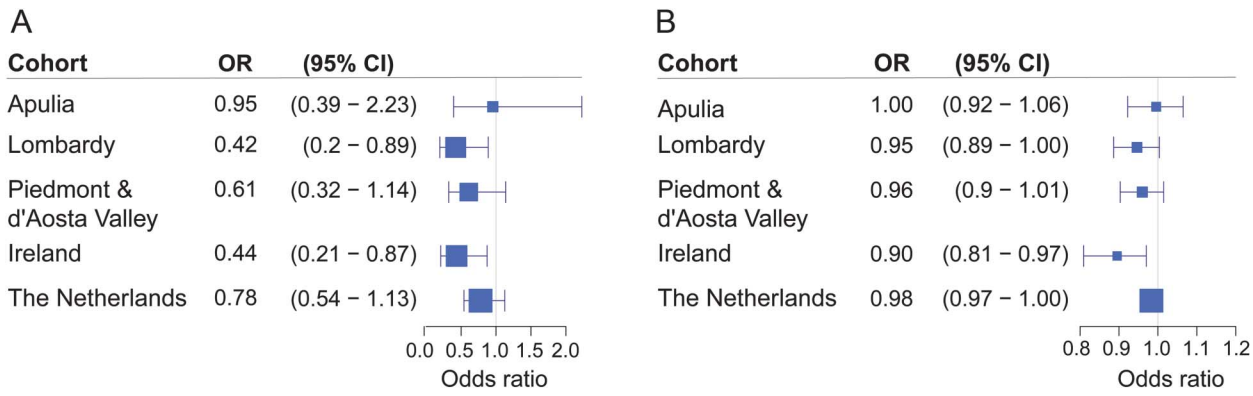
	Complete case analysis (n = 1,467)		Complete case analysis excluding known *C9orf72* expanded cases (n = 1,366)		After imputation of missing values (n = 1,870)		After imputation and adjusted for additional lifestyle factors (n = 1,870)	
Reproductive factor	OR (95% CI)[a]	*p* Value	OR (95% CI)[a]	*p* Value	OR (95% CI)[a]	*p* Value	OR (95% CI)[b]	*p* Value
Age at menarche	1.06 (0.99–1.14)	0.113	1.06 (0.99–1.14)	0.115	1.05 (0.99–1.12)	0.106	1.05 (0.99–1.12)	0.123
Reproductive span (per year)	1.00 (0.99–1.02)	0.702	1.00 (0.98–1.02)	0.941	1.00 (0.98–1.02)	0.849	1.00 (0.98–1.02)	0.978
Ever pregnant	0.96 (0.70–1.33)	0.813	0.92 (0.66–1.28)	0.602	0.92 (0.68–1.25)	0.589	0.92 (0.67–1.25)	0.582
Time spent pregnant (per year)	0.99 (0.88–1.10)	0.220	0.98 (0.88–1.09)	0.701	0.97 (0.88–1.07)	0.594	0.98 (0.89–1.08)	0.714
Ever breastfed	0.91 (0.73–1.14)	0.410	0.89 (0.71–1.12)	0.329	0.94 (0.76–1.14)	0.518	0.96 (0.78–1.17)	0.678
Time spent breastfeeding (per year)	1.08 (0.95–1.23)	0.220	1.07 (0.94–1.21)	0.330	1.11 (0.99–1.24)	0.072	1.12 (1.00–1.26)	0.049
Ever used OCP	0.65 (0.51–0.84)	<0.001	0.64 (0.50–0.84)	<0.001	0.66 (0.53–0.83)	<0.001	0.67 (0.53–0.84)	<0.001
Duration of OCP use (per year)	0.98 (0.96–0.99)	0.001	0.98 (0.96–0.99)	0.002	0.98 (0.97–0.99)	0.001	0.98 (0.96–0.99)	0.001
Lifetime endogenous estrogen exposure (per year)	1.03 (1.01–1.05)	0.017	1.03 (1.00–1.05)	0.036	1.02 (1.00–1.04)	0.051	1.02 (1.00–1.04)	0.049

Abbreviations: CI = confidence interval; OCP = oral contraceptive; OR = odds ratio.
[a] Adjusted for age, education, and cohort (n = 5).
[b] Adjusted for age, education, cohort (n = 5), lifetime physical activity, smoking, and alcohol status.

We found that HRT use was strongly associated with reduced risk of ALS in the Netherlands only. This discrepancy might be due to the relatively low prevalence of HRT users in others sites compared to the Netherlands or the use of different formulations of HRT. We were unable to explore this possibility because of poor response rates to questions about HRT drug names. This finding also contrasts findings from the United States that associated HRT use with increased ALS risk (OR 1.9, 95% CI 0.9–3.8).[14] However, our finding for HRT is compatible with our finding that OCP use is associated with lower ALS risk because both are forms of exogenous estrogens and progestogens. Stratifying by age when OCP was started suggests that it may be an important confounding factor, although we interpret this result with caution because of the heterogeneity between sites and the smaller difference between age groups after imputation of missing values and correction for lifestyle covariates (table e-2). Still, these findings are interesting in light of the "timing hypothesis" of neuroprotection due to HRT started at younger ages in Alzheimer research,[27,28] although we did not observe any differences in age when HRT was started in ALS cases vs controls. It has been reported that the past use of OCPs and HRT associates differently with long-term reductions in circulating sex hormones[29]; therefore, both the timing and formulation of exogenous hormones may be important.

None of the other hormonal parameters examined were associated with ALS risk, although it was not possible to determine other factors of likely importance such as progesterone exposure, androgen levels, cyclic fluctuation in estrogen and progesterone levels,

Figure Forest plot showing OR for history and duration of OCP use stratified by study site



(A) Odds of ALS among women who ever used OCP. (B) Odds of ALS per year of contraceptive pill use. ALS = amyotrophic lateral sclerosis; CI = confidence interval; OCP = oral contraceptive; OR = odds ratio.

or follicle-stimulating hormone and luteinizing hormone levels. Nevertheless, our findings were robust to sensitivity analysis and after exclusion of those who underwent surgical menopause.

The differences between our findings and those of others may be explained in part by the relative complexity of hormonal profiles throughout life and confounding factors. While the previous Dutch study argued that endogenous estrogens may exert neuroprotective effects,[13] our current findings indicate that exogenous hormones might exert such effects. While there is evidence from in vitro studies for neuroprotective mechanisms of estrogen[30,31] and that estrogens may slow progression in female hSOD1 G93A transgenic mice,[32] it is also the case that estrogens exhibit complex interactions with androgens,[33] and we note that higher prenatal testosterone determined by digit length has been linked to increased ALS risk in a case-control study.[11] Moreover, testosterone has recently been associated with brain maturation in puberty,[34] an age range that significantly overlaps the age range at which OCP was started in our study. This is consistent with observations from animal studies that a long preclinical period lasting decades may exist in ALS[35] and with the recently proposed idea that ALS results from a multistep process.[2]

Our analysis was complicated by different use rates of contraceptives in the 5 study sites. The age when OCP was started was lower and duration of use was longer in Dutch cases and controls compared to Italian and Irish cases and controls (table 2 and table e-2). Contraceptive use has been found to associate with age, marital status, and social class in Ireland,[36] and contraceptive practices are known to vary with sociodemographic factors across Europe (and between North and South Italy).[37,38] Therefore, the differences in contraceptive use we observed are likely due to different cultural/religious attitudes toward birth control between the 5 study sites, along with the late availability of hormonal contraceptives in Ireland and Italy. In addition, there are other potential sources of confounding such as compliance with medication instructions or the prescription of OCPs for indications other than birth control (e.g., treatment of hormonal conditions such as polycystic ovary syndrome). We cannot exclude the possibility that the formulations of OCPs and HRT drugs vary across countries.

The Euro-MOTOR survey of hormonal etiologic factors for ALS in women is the largest study on this topic to date. It benefits from prospective recruitment of incident patients and matched controls across 3 countries from a source population of 34.1 million individuals and the application of standardized protocols across all sites. Although our findings are at variance with previous work, there is sufficient evidence to support a role between sex hormones and risk of ALS in women. Our study shows a negative association between OCP use and the risk of ALS, along with an apparent dose-response effect of the OCP. These findings need replication and should be reinforced by prospective studies.

AUTHOR CONTRIBUTIONS

J.P.K.R., A.E.V., F.D., E.B., A.C., J.H.V., G.L., L.H.v.d.B., and O.H. contributed to the study concept and design and participated in data collection and processing. J.P.K.R., A.E.V., and F.D. performed the statistical analyses. J.P.K.R., A.E.V., F.D., R.V., E.B., A.C., J.H.V., G.L., L. H.v.d.B., and O.H. contributed to the analysis and interpretation of data. J.P.K.R wrote the manuscript. A.E.V., F.D., R.V., E.B., A.C., J.H.V., G. L., L.H.v.d.B., and O.H. revised the manuscript for important intellectual content. R.V., E.B., A.C., J.H.V., G.L., L.H.v.d.B., and O.H. provided study supervision. E.B., A.C., G.L., O.H., J.H.V., and L.H.v.d.B. obtained funding.

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