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ARTICLE

Ovarian aging is associated with gray matter volume and disability in women with MS

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Abstract

Objective

To determine if ovarian aging as measured by levels of anti-Müllerian hormone (AMH) is associated with pattern of multiple sclerosis (MS) progression in women.

Methods

Women with MS and healthy controls were included from a longitudinal research cohort with up to 10 years follow-up. Plasma AMH levels were measured by ELISA for baseline and years 3, 5, and 8–10. Mixed effects logistic and linear regression models were employed, with adjustments for age, disease duration, and other covariables as appropriate.

Results

AMH levels were similar (0.98-fold difference, 95% confidence interval [CI] 0.69–1.37, p = 0.87) in women with MS (n = 412, mean age 42.6 years) and healthy controls (n = 180, mean age 44 years). In a multivariable model of women with MS, including adjustments for age, body mass index, and disease duration, 10-fold lower AMH level was associated with 0.43-higher Expanded Disability Status Scale (EDSS) score (95% CI 0.15–0.70, p = 0.003), 0.25-unit worse MS Functional Composite z score (95% CI −0.40 to −0.10, p = 0.0015), and 7.44 mm^3 lower cortical gray matter volume (95% CI −14.6 to −0.30; p = 0.041) at baseline. In a multivariable random-intercept–random-slope model using all observations over time, 10-fold decrease in AMH was associated with a 0.27 increase in EDSS (95% CI 0.11–0.43, p = 0.006) and 5.48 mm^3 (95% CI 11.3–0.33, p = 0.065) and 4.55 mm^3 (95% CI 9.33–0.23, p = 0.062) decreases in total gray and cortical gray matter, respectively.

Conclusion

As a marker of ovarian aging, lower AMH levels were associated with greater disability and gray matter loss in women with MS independent of chronological age and disease duration.

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Coinvestigators are listed at links.lww.com/WNL/A126.

Glossary

AMH = anti-Müllerian hormone; **BMI** = body mass index; **CI** = confidence interval; **DMT** = disease-modifying therapy; **EDSS** = Expanded Disability Status Scale; **FSH** = follicular stimulating hormone; **GM** = gray matter; **MS** = multiple sclerosis; **MSFC** = MS Functional Composite; **PPMS** = primary progressive multiple sclerosis; **RA** = rheumatoid arthritis; **SPMS** = secondary progressive multiple sclerosis.

The pathologic processes underlying progression in multiple sclerosis (MS) are not fully understood. Two fundamental patient characteristics, however, are associated with rate of progression—sex and age.[1,2] Men often exhibit earlier or faster disability accumulation,[1–3] but phenotypic sex dimorphism diminishes after age 50.[4] The mean age at onset of secondary progressive MS (SPMS) and primary progressive MS (PPMS) is 45.[5] Interestingly, this age period coincides with the early phases of ovarian aging but before the mean age at menopause. Significant changes in immune reactivity are associated with ovarian aging.[6] We hypothesize that this perimenopausal, early biological aging period in women influences MS phenotype.

Plasma anti-Müllerian hormone (AMH) level is an ideal biomarker for ovarian aging in women with chronic illness. While estrogen and progesterone levels may not drop until the late perimenopausal or postmenopausal period, plasma AMH level begins to decrease early in ovarian aging. AMH levels correlate strongly with antral follicle counts and oocyte and leukocyte telomere lengths and are predictive of the age at menopause.[6,7] AMH levels are associated with aging-related changes in immune responses to infections and increased risk of cardiovascular disease.[7–10] These observations suggest that AMH may be useful not only as an indicator of follicular reserve, but also as a potential biomarker of age-related neurologic phenotypes in women. An additional advantage of using AMH as a biomarker of ovarian aging in cycling women is that unlike estrogen, progesterone, or follicular stimulating hormone (FSH), AMH levels fluctuate only minimally over the ovulatory cycle. Thus, collection of samples is not restricted by date within the menstrual cycle.

We sought to determine if ovarian aging, as evidenced by the level of AMH, is associated with disease progression in MS.

Methods

Study participants

A cohort of participants meeting well-established diagnostic criteria[11,12] for MS or clinically isolated syndrome were enrolled in a longitudinal study of the genotype and phenotype of MS at the University of California, San Francisco (msepicstudy.com/) (table 1).[13] All 415 women from this cohort were included in our analyses. For baseline cross-sectional case–control analysis, 180 age-similar healthy controls free of autoimmune disease as determined by health history questionnaire were included for comparison of AMH levels in the diseased vs healthy state. For longitudinal analyses of the case participants, the year 3, year 5, and combined years 8–10 time points were included for both serologic studies and clinical and radiologic outcomes. The previously reported overall retention at years 8–10 in the EPIC study is 91%[13] and for the women specifically is 90%. As EPIC is an ongoing cohort, there were more participants with available baseline data than longitudinal data. Some patients are only a few years into the study and contributed only to the cross-sectional analyses (n = 149). For the longitudinal analyses, 269 women contributed at least 2 time points and 149 women contributed to all 4 time points.

Standard protocol approvals, registrations, and patient consents

This study was approved by the UCSF Committee on Human Subjects Research. Written consent was obtained from all participants.

Demographic and clinical variables

Race and ethnicity are self-reported according to NIH guidelines and are confirmed and refined using ancestry informative genetic markers. Trained research staff performed the Expanded Disability Status Scale (EDSS) and components of the MS Functional Composite (MSFC) during all visits. Time on and off MS disease-modifying therapy (DMT)

Table 1 Baseline characteristics

	Cases (n = 415)	Controls (n = 180)
Age, y, mean	42.6	44
Age, y, median (range)	42 (23–63)	45 (22–65)
AMH undetectable, n (%)	114 (27.5)	64 (36)
AMH, pg/mL, median (range)	1198.3 (9.13–11,795)	955.6 (15.56–11,704)
MS duration, y, median (range)	6 (0–34)	—
EDSS, median (range)	1.5 (0–6.5)	—
BMI, mean (SD)	24.9 (5.6)	24.4 (4.7)
Birth control or HRT, n (%)	110 (26.5)	52 (33)
Current smoker, n (%)	44 (10.6)	14 (7.8)

Abbreviations: AMH = anti-Müllerian hormone; BMI = body mass index; EDSS = Expanded Disability Status Scale; HRT = hormone replacement therapy; MS = multiple sclerosis.

and type of therapy were recorded. Height and weight were recorded to calculate the body mass index (BMI). Use of sex steroid medications including hormonal contraception and hormone replacement therapy were also recorded. At baseline, no women were pregnant, and during follow-up, samples were not included from women during pregnancy. Smoking status—never, former, or current smoker—was recorded.

AMH measurement

Participants provided blood samples in acid citrate dextrose tubes. Plasma was prepared and stored at −80°C. AMH levels were measured with a highly sensitive immunoassay (lower limit of detection 6 pg/mL; pico AMH, Ansh Labs, Webster, TX). The assay has been previously validated[14] and provides reliable AMH measurement in frozen samples. Plasma levels were measured in duplicate for each sample. Levels were accepted with coefficient of variation <10% and mean values generated. Commercially provided and pooled patient controls were used to standardize results across assay plates.

Since the samples available were not collected at a consistent time in the menstrual cycle, additional measurements of FSH and estrogen were not pursued.

MRI metrics

High-resolution 3D T1-weighted (1 mm isotropic voxels), T2-weighted, and proton density volumes were acquired on a 3T MRI scanner. T2-weighted volumes were used to determine T2 lesion volumes and T1-weighted volumes for normalized brain, white matter, and gray matter (GM) using SIENAX. Degree of whole brain atrophy was determined using SIENA.

Statistical analysis

Because AMH values are nonlinearly distributed and exponentially decline over time, we applied logarithmic transformation before analysis. When using AMH as a predictor of clinical and radiographic outcomes, we set levels that were undetectable to 6 pg/mL, the lowest limit of detection, and modeled them concurrently with a dichotomous variable that indicated undetectable levels. This statistical method for including undetectable levels of exponentially decaying markers was previously described,[15] allowing all data to be included in the analyses.

For cross-sectional analyses with the logarithm of AMH as the outcome, we considered undetectable AMH levels as left-censored at 6 pg/mL[15] and applied linear regression. We flexibly controlled for chronological age by using linear splines with knots at age 35, 40, 45, 50, and 55, which allowed the effect per year to change at each knot. The knots were chosen a priori based on expected differences in the rate of change of AMH level due to reproductive aging. This approach reduced the risk of residual confounding from chronological age. For the longitudinal analyses, we employed multivariable mixed effects models with random effects for the intercept and time in study. Because the random intercepts can account for between-woman differences that are not otherwise explained, and because AMH was a time-updated covariate measured concurrently with each outcome measurement, we interpret the resulting AMH effects as primarily being changes in the outcome associated with changes in AMH, although some of the effect could be due to between-woman differences.[16] Estimation of covariates' effects on rates of change by modeling interactions with time on study was beyond the scope of this study. These models used all observations over time, and we adjusted for current age at each observation time with linear splines. We evaluated in our models the potential covariates disease duration, DMT use, ancestry, vitamin D level, smoking status, use of birth control or hormone replacement therapy, and BMI. Except for ancestry and smoking status, these were time-updated covariates in the longitudinal models. We chose multivariable models by forward stepwise selection, until no remaining candidate covariates had $p < 0.05$.

Results

Case vs control comparison of AMH level

The baseline characteristics of cases and controls were similar (table 1), including mean age (42.6 and 44 years, respectively) and BMI (24.9 and 24.4, respectively). As expected, AMH levels decreased with age, but each year older had different magnitudes of effect on AMH depending on the a priori defined 5-year periods through middle age (table 2). AMH levels were largely undetectable in women over age 55.

BMI and use of any type of estrogen medication (contraceptive or hormone replacement) were both associated with AMH level (table 2). In a multivariable linear regression model controlling for age, BMI, hormonal medication use, and smoking status, AMH levels were similar in women with MS and healthy controls (2% lower in cases; 95% confidence interval [CI] 31% lower to 37% higher; $p = 0.86$).

Baseline association of AMH level with MS clinical and MRI outcomes

In analyses adjusted only for chronological age (modeled flexibly as linear splines to allow different age ranges to have different effects), a 10-fold lower AMH level was associated with 0.53-point higher EDSS score (95% CI 0.24–0.80, $p = 0.0002$), 0.29-unit worse MSFC z score (95% CI −0.43 to −0.14, $p = 0.001$), 19.3 mm^3 lower total brain volume (95% CI −32.7 to −5.88, $p = 0.005$), 12.2 mm^3 lower total GM volume (95% CI −21.2 to −3.26, $p = 0.008$), and 10.3 mm^3 lower cortical GM volume (95% CI −17.6 to −3.06, $p = 0.005$). In multivariable models with other selected covariates of disease duration and BMI, evidence of association remained for most of these outcomes (table 3). DMT, smoking, 25(OH) vitamin D level, hormonal medications (birth control/hormone replacement therapy), race, and ethnicity were evaluated in statistical models but did not appear to confound the association of AMH with MS outcomes and were not included in final models. No statistically significant associations were observed between AMH level and T2 or white matter volumes (table 3).

Table 2 Multivariable regression analysis of anti-Müllerian hormone (AMH) level

	Estimated fold difference[a]	95% CI	*p* Value
Case vs control	0.98	0.69–1.37	0.87
Birth control/HRT use	0.53	0.37–0.75	0.0004
BMI (per unit)	0.95	0.93–0.98	0.0009
Current smoker (y/n)	0.59	0.35–0.99	0.044
Age, y[b]			
<35	0.94	0.88–1.00	0.064
35–40	0.76	0.68–0.87	<0.0001
40–45	0.77	0.68–0.88	<0.0001
45–50	0.62	0.53–0.72	<0.0001
50–55	0.54	0.44–0.67	<0.0001
<55	0.81	0.58–1.13	0.22

Abbreviations: BMI = body mass index; CI = confidence interval; HRT = hormone replacement therapy.
[a] Point estimates are fold differences in AMH level, with values less than 1.0 indicating that predictor variable is associated with lower AMH level.
[b] Age is modeled with a linear spline; effects shown are per year within each age range. As expected, AMH declined over time in all age ranges. The estimated rate for the effect per year in the age 55 plus range is highly uncertain due to very few levels' being above the detection threshold in that age range. Shown are results with mutual adjustment for all variables. No women were pregnant.

Table 3 Multivariable analyses of association of anti-Müllerian hormone (AMH) level with baseline clinical and MRI outcomes

	β[a]	95% CI	*p* Value
EDSS	0.43	0.15 to 0.70	0.0028
MSFC *z* score	−0.25	−0.40 to −0.10	0.0015
Cortical GM, mm^3	−7.44	−14.6 to −0.30	0.041
Total GM, mm^3	−8.21	−17.0 to 0.60	0.068
Total brain, mm^3	−12.5	−25.5 to 0.50	0.059
Total WM, mm^3	−4.32	−10.2 to 1.59	0.15
T2 lesion volume, mm^3	789.3	−1209 to 2787	0.44

Abbreviations: CI = confidence interval; EDSS = Expanded Disability Status Scale; GM = gray matter; MSFC = MS Functional Composite; WM = white matter.
[a] Per 10-fold decrease in AMH pg/mL adjusted for chronological age, disease duration, and body mass index. Disease-modifying therapy, smoking, vitamin D level, hormonal medications, race, or ethnicity were evaluated but did not appear to confound the association of AMH with multiple sclerosis outcomes and were not selected for inclusion in the multivariable models.

Longitudinal associations of AMH level and MS outcomes

In a random-intercept/random-slope model using all observations over time and adjusted for chronological age, decline in AMH level was associated with increasing EDSS scores. For a 10-fold decrease in AMH level, EDSS increased by 0.30 (95% CI 0.16–0.47, $p < 0.0001$). Decline in AMH was also associated with worsening of the MSFC *z* score (per 10-fold decrease in AMH $\beta = -0.096$ units, 95% CI −0.19 to 0.000, $p = 0.049$). Ten-fold decrease in AMH level adjusted for chronological age was also associated with 7.74 mm^3 loss of total GM (95% CI −14.0 to −1.46, $p = 0.016$) and 6.71 mm^3 loss of cortical GM (95% CI −11.9 to −1.52, $p = 0.011$). In a multivariable model adjusting for chronological age, BMI, and disease duration, 10-fold decrease in AMH was associated with a 0.27 increase in EDSS (95% CI 0.11–0.43, $p = 0.006$) and 5.48 mm^3 (95% CI −11.3 to 0.33, $p = 0.065$) and 4.55 mm^3 (95% CI −9.33 to 0.23, $p = 0.062$) decreases in total GM and cortical GM, respectively (table 4). There was no evidence of association of AMH level with changes in white matter or T2 lesion volumes over time (table 4).

Discussion

The consistently reported association of chronological age 45–50 years with onset of progressive disease in MS has yet to be explained. We identified a marker of ovarian aging, AMH, which is associated with both clinical and radiographic metrics of MS severity. These associations persist after adjustments for chronological age and disease duration. The results imply that ovarian aging in women has a deleterious effect on disease course. The menopausal transition is associated with overall decline in sex steroid production as well as immunosenescence, including decreases in lymphocyte telomere lengths, alteration of cellular, humoral, and innate immune responses, and increases in age-related illnesses including cardiovascular disease.[17,18] We propose the concept that the progressive forms of MS (PPMS and SPMS), with mean age at onset in the fifth decade, are aging-related diseases, and that in women ovarian functional decline is associated with accumulation of disability.

Research on the role of menopause on the immune system and phenotype in patients with MS has been limited to date. In a reproductive questionnaire-based study, there was suggestion of change in slope of EDSS over time at menopause.[19]

The role of the menopausal transition in another autoimmune disease with similar genetic susceptibility factors, rheumatoid arthritis (RA), has been studied. RA incidence overall increases after menopause[20] and women with early menopause (before age 45) have a higher risk for RA.[21] Postmenopausal women tend to experience greater joint damage.[22] Hormone replacement therapy was reported to alleviate symptoms and improve progression of RA.[23] While MS risk does not appear to increase after menopause, the association of worsened phenotype after ovarian functional decline may be shared between RA and MS.

Biological aging is associated with significant changes in the immune system, which could in part mediate an association of ovarian aging with MS phenotype. There are shifts in T-cell

Table 4 Multivariable longitudinal analyses of clinical and MRI outcomes

	β[a]	95% CI	*p* Value
EDSS	0.27	0.11 to 0.43	0.0006
MSFC *z* score	−0.083	−0.18 to 0.013	0.096
Cortical GM, mm³	−4.55	−9.33 to 0.23	0.062
Total GM, mm³	−5.48	−11.3 to 0.33	0.065
Total brain, mm³	−7.64	−16.8 to 1.56	0.10
Total WM, mm³	3.09	−8.84 to 2.66	0.29
T2 lesion volume, mm³	176.4	−762 to 1115	0.71

Abbreviations: AMH = anti-Müllerian hormone; CI = confidence interval; EDSS = Expanded Disability Status Scale; GM = gray matter; MSFC = MS Functional Composite; WM = white matter.
[a] Per 10-fold decrease in AMH pg/mL adjusted for chronological age with linear splines, body mass index, and disease duration. Disease-modifying therapy, smoking, vitamin D level, hormonal medications, race, or ethnicity were evaluated but did not appear to confound the association of AMH with multiple sclerosis outcomes.

populations with notable decreases in T-helper and naive CD4+ cells and relative increases in memory CD4+ cells.[24] Reduction of T-helper cells has further implications for control of humoral immunity with shifts in cytokine production. Immunosenescence is one of the fundamental processes of aging and menopause is further characterized by chronic low-grade inflammation with increased interleukin-6 and tumor necrosis factor–α production and oxidative stress.[18,25] Postmenopausal administration of estrogen was shown to partially reduce the rise in interleukin-6 associated with the immunosenescence phenotype and reduce increases in natural killer cell activity associated with aging.[26,27]

Evidence that ovarian sex steroids contribute to phenotype in MS is extensive.[28–30] Another potential mechanism for ovarian functional decline to influence MS progression is through loss of neuroprotective effects of sex steroid hormones. Data from both human and animal studies have demonstrated protective effects of estrogen on neuronal survival.[31–34] A critical window for potential estrogen treatment effects for neuroprotection has been proposed[35,36]; after prolonged period of estrogen withdrawal, it may be too late for successful intervention.[36] If the hypothesis of a critical window is correct, and if this neuroprotective mechanism plays a role in MS phenotype, then a marker such as AMH that captures the early phase of ovarian functional decline long before the last menstrual cycle may be helpful in designing interventional studies in progressive MS.

As an additional observation, we found that there were no substantial differences in cross-sectional AMH level between cases and healthy women after adjustment for age, smoking, hormonal medications, and BMI. While AMH levels may not exclusively determine normal fertility, these results support the overall thesis that MS does not cause significant infertility or loss of ovarian follicular reserve. A prior study of 76 women with MS and 58 controls suggested AMH levels may be lower in women with MS.[37] Notable differences in our work compared to this study include significantly larger sample size, greater age range, adjustment for BMI and smoking in the final models, and more sensitive AMH assay. Another recent study of 25 MS cases and 25 controls with both AMH and ovarian ultrasound data found no overall differences in AMH levels between cases and controls.[38]

We acknowledge limitations of the study. MRI data were not available for the healthy controls to determine if the association of AMH with GM volume is specific to patients with MS or enhanced in patients with MS when compared to healthy women. Since the samples available were not collected at a consistent time in the menstrual cycle, measurement of FSH and estrogen was not pursued. In future studies, it will be desirable to examine how well FSH, estrogen, or progesterone predict MS outcome measures and whether they explain in part the associations found here.

The strengths of our work include a robust sample size of women with MS and up to 10 years of longitudinal follow-up, including both clinical and MRI outcomes. Rigorous statistical methods were employed including use of both cross-sectional and longitudinal analyses, the latter using mixed effects models to account for both within- and between-patient variation. Our findings were consistent for both clinical and structural outcomes. We adjusted for chronological age using linear splines, a method that allows for different age ranges to have different effects in the model. This is important because a 1-year increment in age is not expected to have the same effect in the third decade of life as the fifth decade. We adjusted for disease duration and BMI. We also collected and considered other critical covariates such as DMT, smoking, race and ethnicity, hormonal medication use, and 25(OH) vitamin D levels. These variables did not appear to confound the association of AMH level with MS outcomes in our models.

Next steps in this work include identifying biological mechanisms that may explain the relationship of female biological age to disease course in MS. The study of male reproductive and immune senescence is also of interest. Reframing the conceptualization of progressive MS as an aging-related disease driven by biological aging processes may help to explain the phenomena of this phase of the disease and identify new targets for therapy.

Author contributions

Jennifer S. Graves: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data, statistical analysis, study supervision, and obtaining funding. Roland G. Henry: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of

research and final approval, acquisition of data, statistical analysis, and obtaining funding. Bruce Anthony Campbell Cree: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients, acquisition of data, and study supervision. Geralyn Lambert-Messerlian: drafting/revising the manuscript, study concept or design, accepts responsibility for conduct of research and final approval, acquisition of data, and study supervision. Ruth M. Greenblatt: drafting/revising the manuscript, study concept or design, accepts responsibility for conduct of research and final approval, comentoring of key author. Emmanuelle Waubant: drafting/revising the manuscript and accepts responsibility for conduct of research and final approval. Marcelle I. Cedars: drafting/revising the manuscript, analysis or interpretation of data, and accepts responsibility for conduct of research and final approval. Alyssa Zhu: analysis or interpretation of data, accepts responsibility for conduct of research and final approval, and contribution of vital reagents/tools/patients. Peter Bacchetti: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, and statistical analysis. Stephen L. Hauser: drafting/revising the manuscript, study concept or design, accepts responsibility for conduct of research and final approval, study supervision, and obtaining funding. Jorge R. Oksenberg: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, contribution of vital reagents/tools/patients, acquisition of data, and study supervision.

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Ovarian aging is associated with gray matter volume and disability in women with MS

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Study question

Are anti-Müllerian hormone (AMH) levels related to multiple sclerosis (MS) progression in women?

Summary answer

Lower AMH levels were associated with greater levels of disability and gray matter loss in women with MS regardless of age or disease duration.

What is known and what this article adds

The mean ages at onset for primary and secondary progressive MS coincide with the early phases of ovarian aging. This study provides evidence that AMH levels, which reflect ovarian aging, are related to disease progression in MS.

Participants and setting

The study included 415 women with MS and 180 age-matched healthy control (HC) women in San Francisco.

Design, size, and duration

This longitudinal study involved collecting clinical, AMH, and MRI data at baseline, year 3, year 5, and year 8–10 timepoints. Linear regression was used for cross-sectional analyses, and multivariable mixed effects models were used for longitudinal analyses.

Main results and the role of chance

Compared to the HCs, the patients had similar AMH levels (98% of HC levels; 95% confidence interval [CI] 69%–137%; $p = 0.86$) after adjusting for factors including age. In cross-sectional analyses with adjustments for age, body mass index, and disease duration, AMH levels in participants with MS were associated with clinical outcomes (Expanded Disability Status Scale [EDSS], Multiple Sclerosis Functional Composite [MSFC]) and gray matter MRI outcomes. In a multivariable longitudinal analysis, lower AMH levels were similarly associated with worse clinical and MRI outcomes.

Outcome	Cross-sectional analysis, β (95% CI); *p* value	Longitudinal analysis, β (95% CI); *p* value
EDSS score	0.43 (0.15, 0.70); 0.003	0.27 (0.11, 0.43); 0.006
MSFC, *z* score	−0.25 (−0.40, −0.10); 0.0015	−0.083 (0.18, 0.013); 0.096
Total gray matter, mm^3	−8.21 (−17.0, 0.60); 0.068	−5.48 (−11.3, 0.33); 0.065
Cortical gray matter, mm^3	−7.44 (−14.6, −0.30); 0.041	−4.55 (−9.33, 0.23); 0.062

The table presents the changes associated with a 10-fold reduction in AMH levels.

Bias, confounding, and other reasons for caution

MRI data for the HCs were unavailable, so it is unclear whether the observed association between AMH levels and gray matter volume changes is specific to patients with MS. Blood samples were not collected at a consistent point in the menstrual cycle, so levels of progesterone, estrogen, and follicular stimulating hormone were not compared to the MS outcomes.

Generalizability to other populations

The study involved a large group of women, factors such as age were adjusted for, and it was confirmed that variables such as race and hormonal therapy did not confound the results. The study's results are therefore probably generalizable.

Study funding/potential competing interests

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A draft of the short-form article was written by M. Dalefield, a writer with Editage, a division of Cactus Communications. The authors of the full-length article and the journal editors edited and approved the final version.