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A comparative study of cognitive impairment in sporadic and familial cases of multiple sclerosis

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Genetics plays a significant role in Multiple Sclerosis (MS), with approximately 12.6% of cases occurring in familial form. While previous studies have demonstrated differences in disease progression and MRI findings between familial and sporadic MS, there has been no comparison of cognitive impairment between them. In this study, we evaluated cognitive performance among patients with sporadic and familial MS, along with a healthy control group. A total of 130 individuals, matched for age, sex, and education, were recruited for each group. The mean age of participants was 37.8 ± 9.8 years, and 77.6% of them were female. Cognitive performance was assessed using the Brief International Cognitive Assessment for MS (BICAMS) across the three groups. Both familial and sporadic MS patients showed poorer cognitive performance in the Symbol Digit Modalities Test (SDMT) (Familial: 46.96 ± 12.59, Sporadic: 45.88 ± 14.13, Normal: 56.48 ± 11.89), California Verbal Learning Test (CVLT) (Familial: 66.90 ± 14.01, Sporadic: 68.19 ± 16.49, Normal: 75.18 ± 13.02), and the Brief Visuospatial Memory Test-Revised (BVMT-R) (Familial: 24 (12), Sporadic: 24 (12), Normal: 35 (4)) compared to healthy controls. Meanwhile, no significant differences in cognitive impairment were observed between the familial and sporadic MS groups in the SDMT ($p = 1.000$), CVLT ($p = 0.775$), and BVMT-R ($p = 0.733$). Furthermore, this study found significant relationships between education, depression, age, and sex with different aspects of cognitive performance in MS. Overall, both familial and sporadic MS patients demonstrated similar levels of cognitive impairment.

Keywords Multiple sclerosis, Familial MS, Cognitive impairment, BICAMS

Multiple Sclerosis (MS) is a chronic inflammatory autoimmune disorder that affects the central nervous system and is estimated to impact around 2.8 million individuals globally[1]. Genetics plays a crucial role in MS, serving as a major predisposing factor for the development and prognosis of the disease[2].Studies suggest that around 12.6% of individuals with MS fall under the category of familial MS, where the disease is present in their first, second, or third-degree relatives[3]. While research has explored the impact of genetics on various aspects of the disease such as age at disease onset[4], onset phenotypes[2], and disease progression[5], there is a gap in the literature regarding the comparison of cognitive impairment (CI) between familial and sporadic MS cases.

Interest in studying familial MS has grown, aiming to evaluate the genetic contributions to disease presentation and progression[6]. Recent studies suggest that the age at disease onset is lower in patients with familial MS, and there are differences in symptom patterns compared to non-familial cases[4,7,8], with the familial group exhibiting a higher frequency of multifocal presentations[5]. Additionally, patients with familial MS experience more frequent exacerbations and greater disability scores[7]. Differences in imaging data are also noted, with T1 brain MRI revealing larger lesion volumes in those with familial MS[9]. Furthermore, lesions are observed to occur more frequently in the brainstem, cerebellum, and cervical regions in the familial group, while fewer lesions are found in the subcortical area in these patients[7,8].

While the primary symptoms of MS mainly involve motor and sensory deficits, recent findings highlight the marked presence of CI and psychiatric disturbances in MS patients[10]. Key cognitive domains including memory, attention, executive function, and visuospatial performance could be affected by MS[11]. A significant percentage of MS patients, ranging from 43 to 70%, struggle with CI[12,13], which can substantially impact their daily functioning and overall quality of life[14,15]. CI in MS is closely linked to the disease's inflammatory and neurodegenerative aspects and could be used as a valuable indicator of disease progression over time[16,17].

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Given the differences observed in the profiles of patients with familial MS, we aim to investigate potential differences in CI between these two groups. Comparison of CI across these two groups could provide valuable insights into the influence of genetic factors on the development of CI in MS. Ultimately, these findings could enhance patient care and lead to more targeted interventions for individuals affected by MS.

Materials and methods

Patient recruitment

Familial MS patients were identified from 1929 MS referral cases to Imam Reza Clinic, affiliated with Shiraz University of Medical Sciences. Data collection was conducted between January and October 2022. The diagnosis of MS was confirmed by an expert neurologist using the McDonald criteria for all patients. The familial group consisted of individuals with relapsing-remitting MS (RRMS) who had 1st/2nd/3rd degree relatives diagnosed with MS. Following evaluation, patients with an Expanded Disability Status Scale (EDSS)[18] score greater than 4.5 were excluded to ensure that participants were sufficiently capable of completing the required tests. Ultimately, 130 patients were included in the familial MS group. The sporadic MS group was subsequently selected on a rolling basis from the same pool of patients, pair matched for age, sex, and education with the familial group. We pair matched the sporadic MS group using 10-year brackets for age and categorizing education as up to high school graduation, up to a bachelor's degree, and above a bachelor's degree. To assemble a diverse and representative control group we publicly called for volunteers without any neurological or psychiatric disorders who did not have a family history of MS in 1st/2nd/3rd- degree relatives. This control group underwent the same matching process as the sporadic MS group. 130 patients were eventually included in the sporadic MS and control groups. The patient recruitment procedure is illustrated in Fig. 1.

Demographic and disease-related data

Comprehensive demographic data, encompassing age, sex, and education level were collected via participant-completed questionnaires across all three groups. The participants were also evaluated for the presence of underlying diseases including malignancies, other auto-immune disorders, diabetes, migraine, hyperthyroidism, and hypothyroidism. Moreover, additional information pertinent to MS, including the disease duration, age at disease onset, and EDSS score, was obtained through an interview with a neurologist from the two MS groups.

Neuropsychological assessments

Brief International Cognitive Assessment for MS (BICAMS) was used for assessing CI in the participants. This test battery comprises three subtests: the oral version of Symbol Digit Modalities Test (SDMT) for assessing information processing speed, the California Verbal Learning Test-II (CVLT-II) for evaluating verbal memory function, and the Brief Visuospatial Memory Test-Revised (BVMT-R) for measuring visuospatial memory function[19]. This test is specially designed for MS patients and can effectively assess cognitive status even during MS relapses. We utilized the Persian version of BICAMS which is validated and standardized for the Iranian population[20]. All participants underwent the BICAMS test under similar and standardized conditions. Moreover, the Persian version of the Beck Depression Index (BDI) was used to assess depression in the three groups[21]. Patients with a BDI score equal to or above 14, indicating a range from mild to severe depression, were classified as experiencing depression.

Statistical analysis

Summary data were computed for both demographic and disease-related factors. Normally distributed data were presented as Mean ± Standard Deviation (SD), while non-normally distributed data were represented by the median and interquartile range (IQR). The chi-square test was used to compare the distribution of categorical data across groups. Assessment of normality and homogeneity of variance for quantitative data was conducted using the Shapiro- Wilk test and Levene's test, respectively. For variables conforming to a normal distribution, analysis of variance (ANOVA) was employed to compare the three groups (familial MS, sporadic MS, and control group), followed by Bonferroni post-hoc tests to determine pairwise differences. In cases where the assumption of homogeneity of variance was violated, Welch's ANOVA was utilized, with Games-Howell as the post-hoc test. For non-normally distributed data, the Kruskal- Wallis test was employed to compare groups, with Dunn's test used for pairwise comparisons. Additionally, disease-related factors between the familial and sporadic MS groups were compared using the Mann-Whitney U test. Multiple linear regression was conducted to evaluate the relationship between demographic and disease-related variables and cognitive performance in MS. In this analysis, the BICAMS subtests served as the dependent variables, and sex, age, education, BDI, EDSS, disease duration, and familial MS were used as independent variables. All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 27.

Results

A total of 390 participants were included in this study, comprising 130 patients with familial RRMS, 130 patients with sporadic RRMS, and 130 healthy controls. The mean age of the participants was 37.8 ± 9.8, and the majority of them were female (77.6%). In the familial group, 40 (30.8%), 32 (24.6%), and 58 (44.6%) patients had first-degree, second-degree, or third-degree relatives affected by MS, respectively. There were no significant differences between the three groups (familial MS, sporadic MS, and controls) in terms of education, age, BDI score or gender. Additionally, there were no significant differences in the prevalence of underlying diseases or depression among the groups. The median disease duration (time since symptom onset) was 4 years for both the familial MS group and the sporadic MS group. The mean age at disease onset was 30.3 and 31.8 for the familial
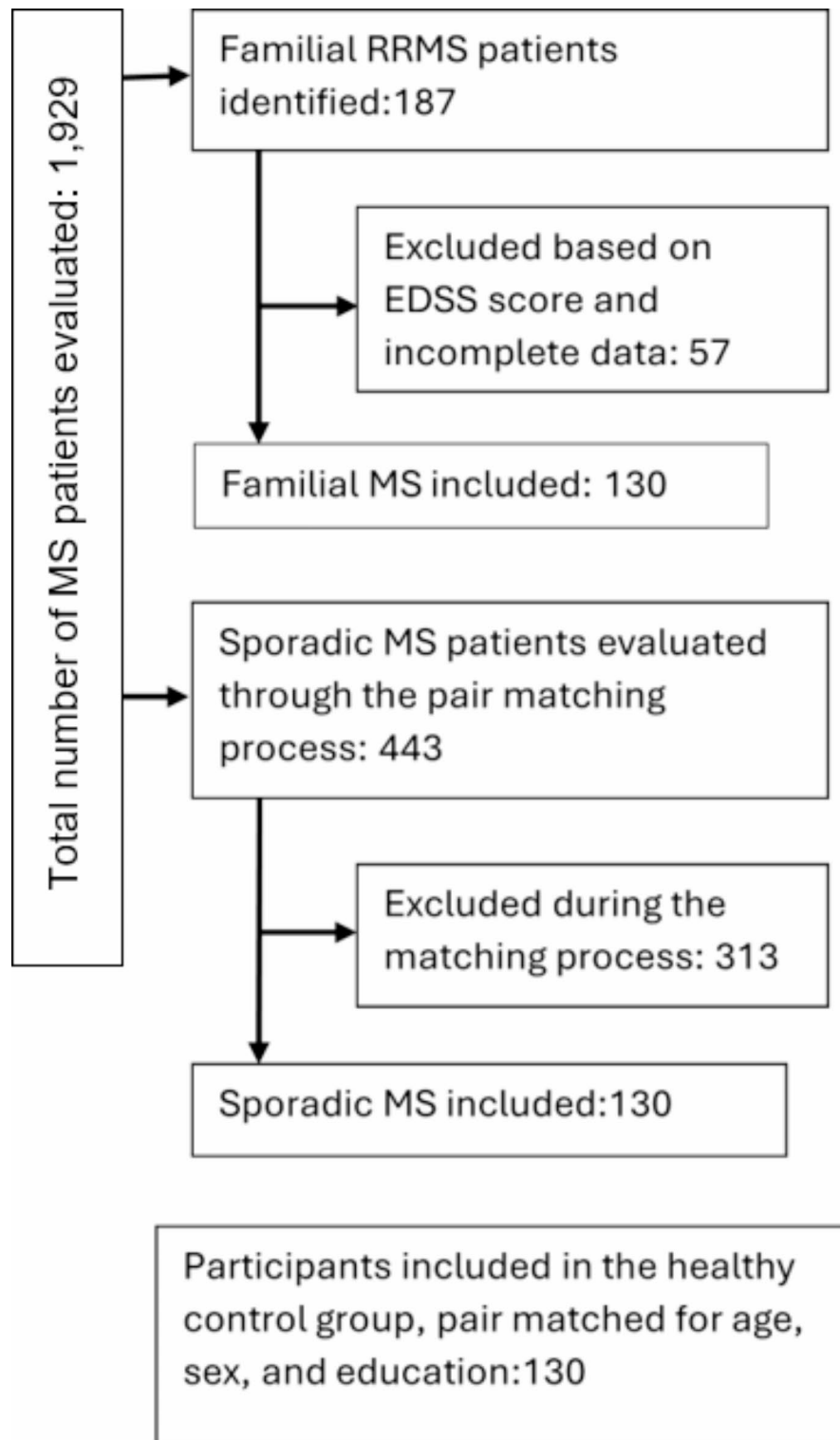
Fig. 1. Patient recruitment procedure.

and sporadic groups respectively, with no significant difference between the two groups. The EDSS scores were also similar between the two MS groups (Table 1).

In terms of the BICAMS subtests, there were significant differences observed among the groups in all three tests (Fig. 2). In post-hoc comparisons, the test scores were significantly higher in the healthy control group compared to both the familial MS group and the sporadic MS group.

However, no significant difference was observed between the familial and sporadic MS groups (Table 2).

Variable	Group			P-Value
	Familial MS	Sporadic MS	Control	
Sex				
Male	29	29	29	1.000
Female	101	101	101	
Age (Mean ± SD)	38.2 ± 10.4	37.6 ± 9.7	37.6 ± 9.4	0.861
Education (Years, Median (IQR))	16 (4)	16 (4)	16 (4)	0.608
Underlying disease	64	60	65	0.881
Depression	50	62	47	0.134
BDI (Median (IQR))	10 (15)	12 (12)	13 (16)	0.331
EDSS (Median (IQR))	1 (0.5)	1 (0.5)	–	0.770
Disease Duration (Median (IQR))	4 (9)	4 (7)	–	0.222
Age at disease onset (Mean ± SD)	30.3 ± 8.7	31.8 ± 8.3	–	0.170

Table 1. Demographic and disease-related factors across groups.
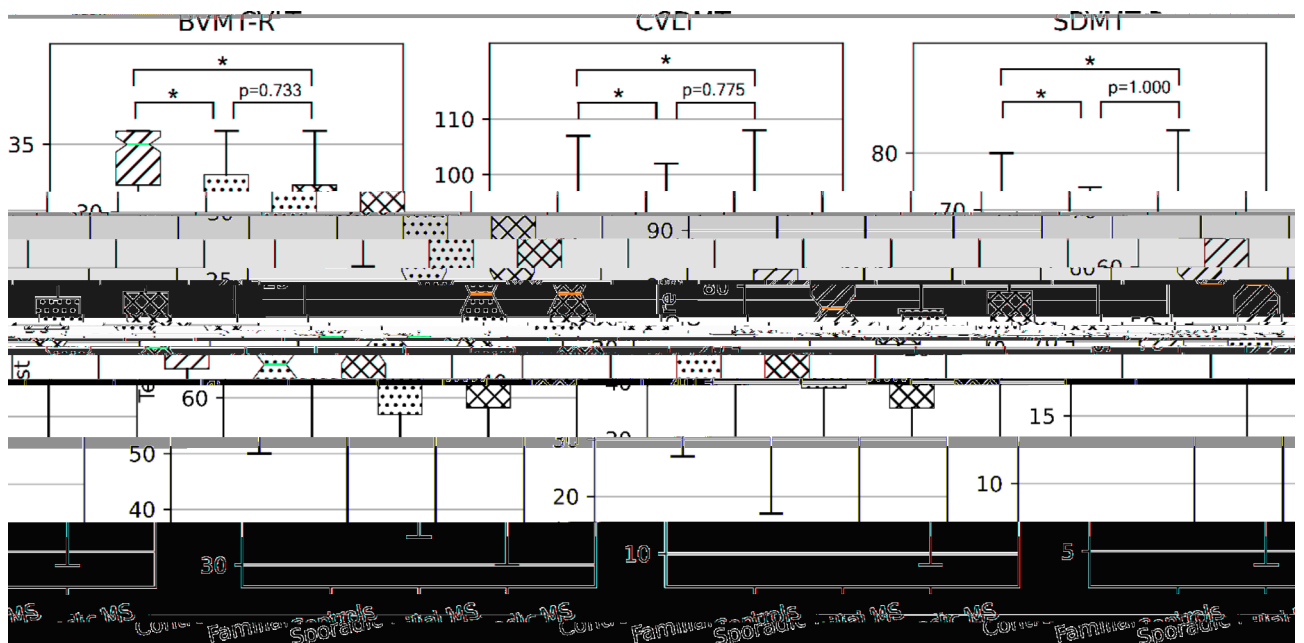
Fig. 2. Distribution of BICAMS subtests' scores and their comparison across groups. * P-value ≤ 0.001.

Variable	Groups			Group Analysis	Post-hoc result		
	Normal	Familial	Sporadic	P-Value	Normal vs. Familial	Normal vs. Sporadic	Familial vs. Sporadic
CVLT (Mean ± SD)[a]	75.18 ± 13.02	66.90 ± 14.01	68.19 ± 16.49	< 0.001	< 0.001	0.001	0.775
SDMT (Mean ± SD)	56.48 ± 11.89	46.96 ± 12.59	45.88 ± 14.13	< 0.001	< 0.001	< 0.001	1.000
BVMT-R (median (IQR))[b]	35 (4)	24 (12)	24 (12)	< 0.001	< 0.001	< 0.001	0.733

Table 2. BICAMS subtests across groups. [a]CVLT was compared between groups by Welch's test followed by games-Howell post-hoc comparisons. [b]BVMT-R was compared between groups by Kruskal-Wallis non-parametric test followed by Dunn's as post-hoc.

In the multiple linear regression analysis, a significant positive relationship was observed between education and cognitive performance across all BICAMS subtests. BDI scores showed a negative correlation with both the CVLT and BVMT scores. Additionally, age and male sex were found to be negatively associated with the SDMT and CVLT scores, respectively. Other variables did not significantly contribute to the prediction of cognitive performance (Table 3).

Independent variables	Dependent variables		
	CVLT	SDMT	BVMT-*R*
Sex	−6.625 (1.661)*	−1.057 (1.661)	−0.428 (1.109)
Age	−0.168 (0.081)	−0.390 (0.081)*	−0.081 (0.054)
Education	0.964 (0.204)*	1.688 (0.204)*	0.347 (0.136)*
BDI	−0.177 (0.066)*	−0.087 (0.066)	−0.095 (0.044)*
EDSS	−1.211 (0.719)	−0.612 (0.719)	0.202 (0.48)
Disease duration	0.078 (0.138)	−0.137 (0.138)	−0.122 (0.092)
Familial MS	1.990 (1.374)	−0.545 (1.374)	−0.363 (0.918)
Constant	64.363(5.960)	40.989(4.487)	25.437(2.997)
R-squared	0.121	0.349	0.083

Table 3. Results of multiple Linear regression analysis for BICAMS subtests. Unstandardized coefficients are reported with standard errors in parentheses. *P-value < 0.05.

Discussion

We compared CI between patients with familial and sporadic MS for the first time. The results illustrated that MS patients exhibited poorer performance in cognitive tests including CVLT, SDMT, and BVMT-R compared to healthy controls. This outcome pointed towards deficits in information processing speed, verbal memory, and visuospatial memory in MS patients. Meanwhile, the comparison of cognitive performance between familial and sporadic MS showed similar outcomes in the test battery.

CI in MS is a well-researched area, with numerous studies exploring factors influencing cognitive function in MS patients[11]. Variations between sexes have been noted, with male MS patients displaying poorer cognitive performance[22], potentially due to increased white matter damage compared to females[23]. We found a negative relationship between male sex and verbal memory, consistent with prior research on sex differences in cognitive performance among MS patients[24]. Aging is another factor impacting cognitive function, particularly by reducing processing speed in both healthy individuals and those with MS[25]. Our findings confirm the negative relationship between age and processing speed in MS patients, as measured by the SDMT test. Additionally, a negative correlation has been reported between education and CI in MS patients in previous studies[26]. Higher levels of education were associated with better cognitive performance across all domains in the patients included in our study.

Our study collected a significant sample matched for age, sex, and education—factors known to influence cognitive performance in MS patients. We also compared other factors such as depression, physical disability, age at disease onset, and disease duration between the groups. While depression is more prevalent[27] and can negatively affect cognitive performance in MS patients[28], our study found similar depression rates among the MS groups and healthy individuals studied. However, the multiple linear regression analysis revealed a negative relationship between depression and both visuospatial memory and information processing speed in MS patients.

Moreover, a positive correlation has been observed between physical disability and cognitive performance in MS patients in previous studies[26,29]. We included patients with an EDSS below.

4.5 in our study to ensure that the tests were suitable for this patient population and because of the limited accessibility to patients with higher EDSS scores considering our data-gathering method. The EDSS score was comparable between the two MS groups included in the study.

The duration of the disease has been also identified as a factor influencing CI in MS[30], yet the study by Ruano et al. indicates that its impact may be attributed to increased disability and older age among patients[29]. Additionally, younger age at disease onset has been proposed as a potential influencer of cognitive performance, particularly in pediatric MS patients, as it may hinder the development of cognitive abilities[31]. Notably, our study detected no significant difference in disease duration or age at disease onset between the two MS groups.

The key innovation in our study was considering the genetic aspect of MS by comparing familial and sporadic MS patients. To the best of our knowledge, no study has yet explored attributes of CI in familial MS patients. Significantly, our study was conducted on two patient groups that were comparable in various disease-related aspects. While we found no significant differences in CI between the two groups, comprehensive genetic studies are needed to further evaluate the influence of genetic risk factors on CI in MS.

It is important to recognize some limitations within our study. We only included RRMS patients with an EDSS score below 4.5, which could limit the generalizability of our findings. The impact of the MS subtype on CI is a subject of ongoing debate[29,30], therefore we decided to focus on patients with RRMS for this research study. The treatment regimen of patients and the severity of fatigue are two other important factors that were not assessed in the current study. Additionally, we were unable to compare CI between familial multiple sclerosis patients with different degrees of affected relatives due to our limited sample size. Including a wider variety of participants and addressing these additional features in future studies can lead to more comprehensive outcomes on this topic.

Conclusion

CI was evident in both familial and sporadic MS patients across all assessments, indicating impairment in information processing speed, verbal memory, and visuospatial memory. Meanwhile, there were no significant differences between the two groups in any of the cognitive tests, suggesting that familial MS is not associated with worse cognitive performance compared to sporadic MS. Demographic factors, disease-related variables, and prevalence of depression were similar between the two MS groups, which reduces the possible effect of confounding factors on the study findings. However, for a better understanding of the impact of genetics on CI in MS, genetic studies should be conducted to investigate the relationship between the presence of specific genes and cognitive performance in MS.

Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

References

1. Walton, C. et al. Rising prevalence of multiple sclerosis worldwide: insights from the Atlas of MS, third edition. *Mult Scler.* **26**, 1816–1821. https://doi.org/10.1177/1352458520970841 (2020).
2. Boles, G. S. et al. The familial risk and heritability of multiple sclerosis and its onset phenotypes: a case-control study. *Mult Scler.* **29**, 1209–1215. https://doi.org/10.1177/13524585231185258 (2023).
3. Harirchian, M. H., Fatehi, F., Sarraf, P., Honarvar, N. M. & Bitarafan, S. Worldwide prevalence of familial multiple sclerosis: a systematic review and meta-analysis. *Mult Scler. Relat. Disord.* **20**, 43–47. https://doi.org/10.1016/j.msard.2017.12.015 (2018).
4. Rojas, J. I. et al. Disease onset in familial and sporadic multiple sclerosis in Argentina. *Mult Scler. Relat. Disord.* **6**, 54–56. https://doi.org/10.1016/j.msard.2016.01.004 (2016).
5. Wellek, A. et al. Sibling disability risk at onset and during disease progression in familial multiple sclerosis. *Multiple Scler. J.* **17**, 1060–1066 (2011).
6. Balcerac, A. & Louapre, C. Genetics and familial distribution of multiple sclerosis: a review. *Rev. Neurol. (Paris)* **178**, 512–520. https://doi.org/10.1016/j.neurol.2021.11.009 (2022).
7. Andrijauskis, D., Balnyte, R., Keturkaite, I. & Vaitkus, A. Clinical and diagnostic features of patients with familial multiple sclerosis. *Med. Hypotheses* **131**, 109310. https://doi.org/10.1016/j.mehy.2019.109310 (2019).
8. Katsavos, S. et al. Familial multiple sclerosis in Greece: distinct clinical and imaging characteristics in comparison with the sporadic disease. *Clin. Neurol. Neurosurg.* **173**, 144–149. https://doi.org/10.1016/j.clineuro.2018.08.021 (2018).
9. Tipirneni, A. et al. MRI characteristics of familial and sporadic multiple sclerosis patients. *Mult Scler.* **19**, 1145–1152. https://doi.org/10.1177/1352458512469697 (2013).
10. Shinoda, K. et al. Contribution of cortical lesions to cognitive impairment in Japanese patients with multiple sclerosis. *Sci. Rep.* **10**, 5228. https://doi.org/10.1038/s41598-020-61012-3 (2020).
11. Grzegorski, T. & Losy, J. Cognitive impairment in multiple sclerosis - a review of current knowledge and recent research. *Rev. Neurosci.* **28**, 845–860. https://doi.org/10.1515/revneuro-2017-0011 (2017).
12. Chiaravalloti, N. D. & DeLuca, J. Cognitive impairment in multiple sclerosis. *Lancet Neurol.* **7**, 1139–1151. https://doi.org/10.1016/S1474-4422(08)70259-X (2008).
13. Rao, S. M., Leo, G. J., Bernardin, L. & Unverzagt, F. Cognitive dysfunction in multiple sclerosis. I. frequency, patterns, and prediction. *Neurology* **41**, 685–691. https://doi.org/10.1212/wnl.41.5.685 (1991).
14. Lanzillo, R. et al. Quality of life and cognitive functions in early onset multiple sclerosis. *Eur. J. Paediatr. Neurol.* **20**, 158–163. https://doi.org/10.1016/j.ejpn.2015.08.005 (2016).
15. Amato, M. P. et al. Cognitive impairment in early-onset multiple sclerosis. Pattern, predictors, and impact on everyday life in a 4-year follow-up. *Arch. Neurol.* **52**, 168–172. https://doi.org/10.1001/archneur.1995.00540260072019 (1995).
16. Carotenuto, A. et al. Associations between cognitive impairment at onset and disability accrual in young people with multiple sclerosis. *Sci. Rep.* **9**, 18074 (2019).
17. Rocca, M. A. et al. Clinical and imaging assessment of cognitive dysfunction in multiple sclerosis. *Lancet Neurol.* **14**, 302–317. https://doi.org/10.1016/S1474-4422(14)70250-9 (2015).
18. Thompson, A. J. et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol.* **17**, 162–173. https://doi.org/10.1016/S1474-4422(17)30470-2 (2018).
19. Dusankova, J. B., Kalincik, T., Havrdova, E. & Benedict, R. H. Cross cultural validation of the Minimal Assessment of cognitive function in multiple sclerosis (MACFIMS) and the brief International Cognitive Assessment for multiple sclerosis (BICAMS). *Clin. Neuropsychol.* **26**, 1186–1200. https://doi.org/10.1080/13854046.2012.725101 (2012).
20. Eshaghi, A. et al. Validity and reliability of a persian translation of the Minimal Assessment of cognitive function in multiple sclerosis (MACFIMS). *Clin. Neuropsychol.* **26**, 975–984. https://doi.org/10.1080/13854046.2012.694912 (2012).
21. Ghassemzadeh, H., Mojtabai, R., Karamghadiri, N. & Ebrahimkhani, N. Psychometric properties of a persian-language version of the Beck Depression Inventory–Second edition: BDI-II-PERSIAN. *Depress. Anxiety* **21**, 185–192. https://doi.org/10.1002/da.20070 (2005).
22. Beatty, W. W. & Aupperle, R. L. Sex differences in cognitive impairment in multiple sclerosis. *Clin. Neuropsychol.* **16**, 472–480. https://doi.org/10.1076/clin.16.4.472.13904 (2002).
23. Schoonheim, M. M. et al. Sex-specific extent and severity of white matter damage in multiple sclerosis: implications for cognitive decline. *Hum. Brain Mapp.* **35**, 2348–2358. https://doi.org/10.1002/hbm.22332 (2014).
24. Donaldson, E., Patel, V. P., Shammi, P. & Feinstein, A. Why sex matters: a cognitive study of people with multiple sclerosis. *Cogn. Behav. Neurol.* **32**, 39–45. https://doi.org/10.1097/WNN.0000000000000188 (2019).
25. Bodling, A. M., Denney, D. R. & Lynch, S. G. Cognitive aging in patients with multiple sclerosis: a cross-sectional analysis of speeded processing. *Arch. Clin. Neuropsychol.* **24**, 761–767. https://doi.org/10.1093/arclin/acp076 (2009).
26. Sadigh-Eteghad, S., Abbasi Garravnd, N., Feizollahi, M. & Talebi, M. The expanded disability status scale score and demographic indexes are correlated with the severity of cognitive impairment in multiple sclerosis patients. *J. Clin. Neurol.* **17**, 113–120. https://doi.org/10.3988/jcn.2021.17.1.113 (2021).
27. Patten, S. B., Beck, C. A., Williams, J. V., Barbui, C. & Metz, L. M. Major depression in multiple sclerosis: a population-based perspective. *Neurology* **61**, 1524–1527. https://doi.org/10.1212/01.wnl.0000095964.34294.b4 (2003).
28. Nunnari, D. et al. Impact of depression, fatigue, and global measure of cortical volume on cognitive impairment in multiple sclerosis. *Biomed. Res. Int.* **2015** (519785). https://doi.org/10.1155/2015/519785 (2015).
29. Ruano, L. et al. Age and disability drive cognitive impairment in multiple sclerosis across disease subtypes. *Mult Scler.* **23**, 1258–1267. https://doi.org/10.1177/1352458516674367 (2017).

6

30. Achiron, A. et al. Modeling of cognitive impairment by disease duration in multiple sclerosis: a cross-sectional study. *PLoS One* **8**, e71058. https://doi.org/10.1371/journal.pone.0071058 (2013).
31. Hosseini, B., Flora, D. B., Banwell, B. L. & Till, C. Age of onset as a moderator of cognitive decline in pediatric-onset multiple sclerosis. *J. Int. Neuropsychol. Soc.* **20**, 796–804 (2014).

Author contributions

Maryam Poursadeghfard and Ahmad Ali Abin designed the study and evaluated the results. Vida Niakosari, Ali Namjoo-Moghadam and Sana Hashemi conducted the analyses and wrote the manuscript with input from all authors. All authors provided critical feedback and helped shape the research and analysis.

Declarations

Competing interests

The authors declare no competing interests.

Ethical standards

Approval was duly granted by the Ethics Committee of Shiraz University of Medical Sciences in Shiraz, Iran (IR.SUMS.MED.1401.399). Every facet of the study, encompassing test administration and data collection from participants was performed with strict adherence to the Declaration of Helsinki.

Consent to participate

Written informed consent was obtained from all participants before entering the study.

Additional information

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