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Maternal multiple sclerosis is not a risk factor for neurodevelopmental disorders in offspring

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Abstract

Background: Childhood neurodevelopmental disorders (NDDs), including specific learning disorders (SLD), attention deficit/hyperactivity disorder (ADHD) and autism spectrum disorder (ASD), are pathogenically linked to familial autoimmunity and maternal immune-mediated diseases during pregnancy.
Objective: We studied maternal MS as a potential risk factor for NDDs occurrence in offspring.
Methods: MS and control mothers were subjected to questionnaires to ascertain NDD diagnosis in their progeny and the occurrence of both autoimmune and neurodevelopment disorders in their families. Suspected NDD cases were evaluated to confirm or rule out the diagnosis.
Results: Of the 322 MS women, 206 (64%) have 361 children; of these, 27 (7.5%) were diagnosed with NDD (11% ADHD; 22% ASD; 67% SLD). NDD-risk in offspring was associated to family history of autoimmunity and to NDDs both in MS and non-MS mother families ($r = 0.75$; $p = 0.005$) whereas it was not associated to maternal MS.
Conclusions: For the first time, we demonstrate that maternal MS does not predispose children to higher risk for NDD. On a mechanistic view, we suggest that the intrinsic organ-specific nature of MS does not impair the mother–child cross-talk in decidua nor does it influence fetal neurodevelopment.

Keywords: Neurodevelopmental disorders, multiple sclerosis, autism spectrum disorder, attention deficit hyperactivity/impulsivity disorder, specific learning disorders

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Introduction

Neurodevelopmental disorders (NDDs) are early childhood disorders varying from specific learning disabilities (SLD) up to impairment of cognitive and social functioning.[1] The most frequent NDDs are SLD, the attention deficit with hyperactivity/impulsivity disorder (ADHD) and the autism spectrum disorder (ASD). According to a recent survey (Center for Disease Control and Prevention, USA), NDDs prevalence increased by 17% (https://www.cdc.gov/) compared to a decade earlier.[2] ADHD has an estimated prevalence of around 3.4% while that of ASD is around 1–2%.[3] The two conditions frequently co-occur and overlap.[4] SLD has a varying prevalence between 2–10% in school-age children.[5]

Although NDDs etiology is still unclear, large cohort studies showed a significant prevalence of autoimmunity in families with children affected with NDD (reviewed on reference[6]). High odds ratio (OR) are found in mothers with type-1 diabetes mellitus (T1DM) systemic lupus erythematous (SLE), rheumatoid arthritis (RA) and thyroiditis for giving birth to children affected with autism, particularly when autoimmunity is on active phase during pregnancy.[6] A systematic review indicated, with moderate-high level of evidences, that maternal SLE is significantly linked to SLD, ASD and ADHD in offspring[7] confirming the idea that a gestational inflammatory state can negatively influences the developmental trajectory of the fetal brain.

Multiple sclerosis (MS) is a chronic immune-mediated demyelinating disease of the CNS, particularly frequent in our area.[8] We evaluated, for the first time, whether maternal MS, gestational

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MS-treatments and other family health disorders (autoimmunity and NDD in relatives) may influence the risk of NDD in offspring of MS mothers.

Patients and methods

We conducted a retrospective observational study in the province of Sassari, an area of about 492.000 population, Sardinia, insular Italy. Ethical approval (2423-CE) was previously obtained from local authorities. All procedures were in accordance with the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from each participant. The study is the result of a collaborative action between the northern-Sardinia MS Centre and the Unit of Child Neuropsychiatry of the University Hospital of Sassari.

Mothers with and without MS

The study was retrospectively conducted between January and December 2019 on consecutive MS women diagnosed according to current criteria,[9] and followed at the referral MS Centre for the northern Sardinia. Inclusion criteria were: definite MS and motherhood. Exclusion criteria were the absence of children and a suspected genetic syndrome. Information included: personal and family history, age at MS onset and MS diagnosis, personal and family comorbidity with other immunological diseases, MS-specific therapies before and during pregnancy, and pregnancy-related problems (e.g. miscarriage).

Comparable data of a supplementary group of 55 non-MS mothers of children with NDDs were used. All children with NDDs of this subgroup belong to a comprehensive database included in a previous research on the mother-child immunogenetic interactions in pregnancy and the risk of ASD in the progeny.[6] Clinical information were collected both from children with NDDs and their parents and healthy siblings. Personal and family history, personal and family comorbidities with immunological disorders and/or other NDD, the use of drugs before and during pregnancy and pregnancy-related problems were also investigated. Exclusion criteria were the presence of an ascertained genetic syndrome.

Screening questionnaires for MS and non-MS mothers also included: number, age and sex of offspring; age at NDD diagnosis, family history of NDDs or other neuropsychiatric problems; suspicion of NDD in one of their children.

Children and adolescents with NDDs

ASD, ADHD and/or SLD diagnoses were considered and registered if neuropsychological and cognitive testing fulfilled the international indications based on DSM5; 1 ASD diagnosis was supported by the Autism Diagnostic Observation Schedule – second edition (ADOS-2),[10] a semi-structured assessment tool for collecting standardised information about social communication skills, restricted interests, and repetitive behaviours. ADHD diagnosis was supported by the Conners Parent Rating Scale – Long Edition (CPRS),[11] a broadly used instrument, administered to both parents and teachers to assess core ADHD symptoms and symptoms of other behavioural and emotional disorders commonly associated with ADHD. SLD was assessed using specific reading and mathematics tasks[12] to evaluate current learning level. All suspected NDD cases were evaluated according to the above and below procedures.

Cognitive measures were preferably assessed by Wechsler Intelligence Scale for Children (WISC-IV). lternatively, we administered the Leiter-3,[13] a nonverbal measure of intelligence that evaluates analogy and perceptual reasoning irrespective of language and formal schooling. The Raven's Coloured Progressive (CPM) and Standard Progressive Matrices[14] were also used for nonverbal assessments of intelligence.

Data analysis

To test whether our sample is representative of the general population, we preliminarily evaluated the normal distribution of our data by comparing the prevalence of ADHD in our sample population with that of the Statistical Office of the National Register (Italy) for the years 2007–2016 (http://old.iss.it//). We selected the ADHD model as it is the unique dataset available in the Italian registry of health.

Comparison of the two populations (comparative index = 2.14) allows us to consider our research population suitable for our purpose.

We used contingency table procedures to analyse MS and NDDs frequency data. Fisher's exact tests were performed and prevalence differences calculated to compare estimated frequency both within and across NDDs children and between mothers. Fisher's chi-square tests were performed to test the OR. We also evaluated the association between mothers with MS, exposed and not exposed to MS treatments during pregnancy, and the risk to give birth to

children with NDDs. A logistic regression model was employed to predict the diagnosis of NDDs in children of MS mothers. We used simple regressions to determine the others specific research questions. Analyses were conducted by using Statgraphics Centurion XVI software (StatPoint Technologies, Warrenton, USA) and GraphPad Prism 5.0 (GraphPad Software Inc. San Diego, CA, USA). Relationships were calculated at the 95% confidence level. Significance level was set for $p < 0.05$.

Results

Clinical-demographic features

Demographic characteristics of the whole cohort are summarised on Table 1. The initial sample included 798 participants: 421 children and 377 women; 322 women out of 377 (85.4%) were diagnosed with MS. Of these, 206 (64%) have children ($n = 361$) and 13 (6.3%) were on active MS-specific therapy during pregnancy. Consistently with inclusion criteria, we selected a final sample of 727 participants: 261 mothers (206 with MS and 55 without MS) and their 466 children (361 from MS mothers and 105 from non-MS mothers). Of the 466 children, 78 were NDDs children of non-MS mothers. We analysed 167 male (46.2%; mean age 22.4 ± 11.3 years) and 194 female children (53.7%; mean age 23.5 ± 12.1 years) of MS mothers and found 27 individuals (7.5%) diagnosed with NDDs after the revaluation of suspected cases (n. 5).

a. *Is maternal MS a risk factor for a NDDs diagnosis in offspring?*

To answer this question we firstly calculated the OR between mothers with and without MS (Table 2); the factors under study were children affected or not-affected by NDDs who had mothers with MS vs. children affected or not-affected with NDDs who had mothers without MS. The corrected chi-square test showed a significant negative association ($p = 0.0001$).

Secondly, we performed a logistic regression to describe the relationship between NDDs diagnoses in children and five independent variables: non-MS mothers, offspring gender, familiarity for NDDs and other disorders (including autoimmunity) and therapies while on pregnancy (Table 4). We found that maternal MS is not associated to NDDs in offspring (OR for the absence of MS in mothers = 19.9).

b. *Is maternal age at pregnancy associated to NDDs in offspring from MS mothers?*

We evaluated the relationship between the age of mothers during pregnancy and the likelihood of NDD diagnoses in their offspring through a linear regression model. Mother's age was selected as dependent variable while the diagnosis of NDDs in

Table 1. Demographic features of the individuals (total 727 out of the initial 798) included in the study.

Characteristic	Mothers with MS	Mothers without MS
Mothers (*n*)	55	206
Mean age and range (y)	42.9 (29–57)	51.8 (26–77)
Standard deviation	6.9	9.8
Children with NDDs (*n*)	78	27
Children without NDDs (*n*)	27	334
On treatment during pregnancy	0	13

NDDs: neurodevelopmental disorders; MS: multiple sclerosis; *n*: number; y: years.

Table 2. Contingency table by categorical data with Yate's correction: OR between mothers with MS and mothers without MS during pregnancy.

	Mothers with MS	Mothers without MS	*p*	OR	95%CI	Chi-square
NDD children	27	78				
No-NDD children	334	27				
Total	361	105	<0.0001	0.28	0.20–0.38	204.2

OR: odds ratio; NDDs: neurodevelopmental disorders; MS: multiple sclerosis; *p*: *p* value.

their children was selected as independent variable. The correlation coefficient $r^2 = 0.079$, indicated a weak and not significant correlation between the variables ($p = 0.15$; not shown).

c. *Are MS-specific immune treatments associated to NDDs in offspring?*

Only 13 women underwent MS immunotherapies during pregnancy. In five (38%) cases, treatment was administered during the first few months of gestation (from 2 up to 12 weeks). One MS mother, on natalizumab treatment during the first 12 weeks of a twin-gestation, gave birth to two children later diagnosed with severe ASD (see discussion section). The other four MS mothers were on azathioprine (one case) and beta-interferon (three cases) and gave birth to children later diagnosed with SLD. Correlation coefficient $r = 0.19$ indicated a relatively weak relationship between MS-therapy of the pregnant mother and the presence of NDDs in children ($p < 0.001$; Figure 1). We calculated the OR between exposed and unexposed offspring from MS mothers under specific treatment during pregnancy. The difference of children who received ($n = 5$) or not received ($n = 11$) a NDD diagnosis born to treated pregnant mothers, compared to children of mothers that had withdrawn their treatment before pregnancy and who gave birth to children with ($n = 22$) or without ($n = 206$) NDDs diagnosis, is statistically significant ($p = 0.02$; OR = 4.25; Table 3).

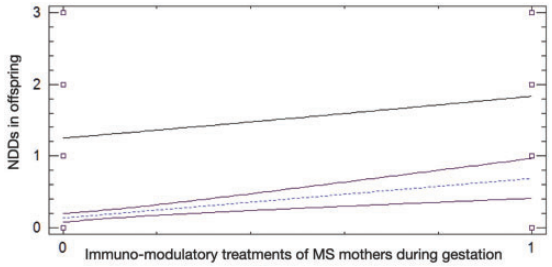

Figure 1. Simple regression between MS treatments during pregnancy and NDDs diagnosis in offspring. The figure shows a weak association between MS-therapy of the pregnant mother and the presence of NDDs in children. The inner bounds show 95% confidence limits, the outer bounds show 95% prediction limits for new observations (black lines). Dotted line (blue): simple regression; $r^2 = 0.04$; *X*-axis: treatments during pregnancy = 1 (azathioprine; glatiramer acetate; beta-interferon; natalizumab); *Y*-axis = NDDs: 1 = ADHD; 2 = SLD; 3 = ASD.

d. *Are NDDs associated with other family health disorders?*

We investigated the correlation between NDD and autoimmune disorders within the family and the NDD diagnosis in offspring from both healthy and MS mothers. Overall, we found a weakly significant association between familial immune-mediated conditions and NDDs in children from MS mothers ($r = 0.13$; $p = 0.01$) and a significant relationship between NDD family history and NDD diagnosis in off spring at the 95% CI ($r = 0.75$; $p = 0.005$; Figure 2). The OR = 3.13 ($p = 0.0000$) confirms familial NDDs as a significant risk factor for NDD diagnosis in offspring at the 95% CI (Table 4).

Discussion

A healthy pregnancy requires a fine balance of the maternal immunity to maintain a protective environment and to ensure a tolerance state to avoid rejection of the semi-allogeneic fetal-placental unit.[15] In contrast, mothers with T1DM, SLE, RA and thyroiditis have a high risk for giving birth to children affected with autism and other NDDs, particularly when maternal autoimmunity is on active phase during pregnancy.[6,16] This strongly suggests that a gestational inflammatory state is detrimental for the neurodevelopmental trajectory of the fetus. Subsets of healthy mothers are found to produce anti-foetal brain antibodies able of inducing NDDs-like pathology and behavior in offspring of animal models.[6] Several other studies have linked maternal infections

Table 3. Contingency table by categorical data with Yate's correction: OR for mothers with MS exposed and not exposed to MS-specific treatment during pregnancy.

	NDD diagnosis	No NDD	*p*	OR	95%CI	Chi-square
Treated MS mothers	5	11				
Untreated MS mothers	22	206				
Total	27	217	0.02	4.2	1.3–7.4	5.06

NDDs: neurodevelopmental disorders; MS: multiple sclerosis; *p*: *p* value.

with neurological and psychiatric disorders in descendants, the strongest association being with ASD.[17] At the experimental level, progeny of rodent mothers injected with viral RNAs or bacterial lipopolysaccharides displays structural brain modification and behavioral anomalies explicitly evocative of human NDD disorders,[16] which can persist into adulthood.[18] One mainstay of the experimental NDDs is the combination of maternal chemokines and cytokines (e.g. IL-6, IL-17, IL-4) which, by crossing the placenta and acting directly on the developing fetal brain or altering its epigenetic transcript regulation, have detrimental actions in plasticity, neuronal precursors migration, and synaptic pruning.[16,19]

A large variety of decidual leukocytes play a vital role in the control of immunosurveillance and foetal growth, including the innate natural killer (NK) cells, the largest immune cell population at the maternal-fetal interface during early pregnancy.[20]

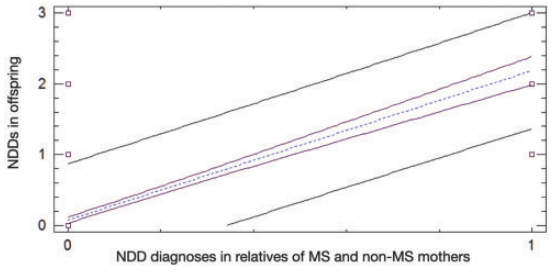

Figure 2. Simple regression between NDDs in families of MS and non-MS mothers and NDD in their offspring. The inner bounds show 95% confidence limits for the mean NDD of many observations at given values of familiarity. The outer bounds show 95% prediction limits for new observations. The correlation coefficient = 0.75 and p value = 0.005 (Durbin–Watson) indicated a relationship between the variables. Black lines: prediction and confidence intervals; dotted line (blue): simple regression; $r^2 = 0.57$. X-axis: Familiarity = 1 (presence in our dataset). Y-axis = NDDs: 1 = ADHD, 2= SLD; 3 = ASD.

However, conditioned by a particular killer-cell immunoglobulin-like receptors (KIR)-HLA ligand regulation, activated NK cells can produce proinflammatory cytokines and induce detrimental immune responses. We previously found that proinflammatory KIR/HLA patterns are increased whereas tolerogenic KIR/HLA complexes are reduced in ASD children and, more significantly, in their mothers. We hypothesised that a pro-inflammatory immunogenetic background contributes to the chronic uterine inflammatory state which persists throughout foetal development and interferes with the typical brain development.[6,21,22]

In the present study we analysed, for the first time, the possible interaction between maternal MS and the risk of NDD development in the progeny. Firstly, we showed that children from MS mothers, even considering the mother's age at gestation,[23] are not at higher risk of being diagnosed with NDDs in early childhood or later in life. On the contrary, a significant risk factor lies in the familiarity for NDDs in close members of the same family.

Although no particular MS-therapies seem to influence NDDs appearance in the progeny, MS treatments during pregnancy may influence the OR for a NDD diagnosis in offspring. Although some controversies exist on the detrimental impact of natalizumab during gestation on the neurodevelopment of the fetus,[24,25] we suggest that ongoing immunomodulating MS therapies during the first weeks of gestation, and not MS itself, may interfere with the typical neurodevelopment trajectory of offspring. A larger, more accurate and longitudinal study should be carried out to definitely address this particular point.

We acknowledge the limit that our observational study is not linked to standardized research protocols and has an intrinsic retrospective nature, thereby

Table 4. Results from logistic regression analyses predicting NDD diagnosis from mothers with MS.

Estimated regression model (maximum likelihood)	OR	95% CI
Mother without MS[a]	19.9	7.9–49.9
Gender of the offspring	0.5	0.2–1.0
Familiarity for NDDs	3.1	1.1–8.5
Familiarity for other diseases	0.0[b]	0.0–∞

Notes: Dependent variable: NDDs (Y/N); factors: MS (multiple sclerosis); gender of the offspring; familiarity for NDDs; familiarity for other diseases. All reported values are odd ratios (OR) with 95% CI.
[a]Mother without MS (absence of MS; estimated value: 2.99).
[b]$p < 0.05$.

suffering from potential selection biases. However, the study design allowed us to analyse a real-life observational cohort of children with variables that could be available for all. Other potential biases are the different numerosity of sample between mothers with MS and those without MS, as well as between the number of children and adolescent with NDDs compared to their healthy controls.

In conclusion, and in contrast with other autoimmune diseases, maternal MS seems not to represent a risk factor for a NDDs diagnosis in childhood or adolescence from our representative sample. On a phenomenologic view, we suggest that the intrinsic organ-specific nature of MS does not impair the mother-child cross-talk in decidua nor does it influence fetal neurodevelopment.[6]

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Declaration of conflicting interests

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