

Polymorphism in the serotonin transporter gene polymorphisms (5-HTTLPR) modifies the association between significant life events and depression in people with Multiple Sclerosis

Alice Saul¹, Bruce Taylor¹, Steve Simpson¹, Anne-Louise Ponsonby², Leigh Blizzard¹, Terence Dwyer³, Brendan McMorran⁴, Brenda Wood¹, Ingrid van der Mei¹

1. Menzies Institute for Medical Research, Hobart, Australia
2. Murdoch Childrens Research Institute, Melbourne, Australia
3. **International Agency for Research on Cancer, Lyon, France**
4. The John Curtin School of Medical Research, The Australian National University, Canberra

Running head: Interaction between 5-HTTLPR, significant life events and depression in people with MS

Please address all correspondence to:

I.A.F. van der Mei, Menzies Institute for Medical Research, Private Bag 23, Hobart, Tasmania, Australia 7001; Email: Ingrid.vanderMei@utas.edu.au; Tel +61 3 6226 7700, Fax +61 3 62267704

Key words: Multiple Sclerosis, 5-HTTLPR, Serotonin-transporter-linked polymorphic region, significant life events, stress, depression, anxiety.

Abstract

Background: In the general population, variation in the serotonin-transporter-linked polymorphic region (*5-HTTLPR*) has been shown to modify the association between stressful events and depression/anxiety. This has not been examined in people with MS.

Objective: We examined the interaction between significant life events (SLE), *5-HTTLPR* and depression/anxiety.

Methods: A population-based longitudinal cohort of 198 people with MS was followed biannually for 2.5 years. Depression and anxiety symptoms were measured at each review using the Hospital Anxiety and Depression Scale (HADS). SLEs were assessed using a questionnaire based on the Social Readjustment Rating Scale.

Results: We found an interaction between SLE load in the previous 12 months and functional variation in the *5-HTTLPR* allele type in predicting depression, with the association between SLE load and depression being stronger for those with S/S allele type ($\beta=0.21$ (95% CI: 0.09-0.33) per 10 unit increase) and S/L ($\beta=0.14$ (95% CI: 0.05-0.24)) compared to L/L allele type ($\beta=0.04$ (95% CI: -0.05-0.24)) ($p_{\text{interaction}} < 0.001$). No convincing evidence of an interaction was found with anxiety.

Conclusion: We found that the association between SLE load and MS depression severity was stronger among those with one or two copies of the short allele of the *5-HTTLPR*. The identification of a gene-environment interaction between SLEs and depression in a population where depression is partly disease-driven is novel.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory autoimmune disease of the central nervous system.¹ Individuals with MS have both physical and emotional symptoms that change everyday living dramatically, with onset generally at a time where young adults are starting relationships, families and beginning careers.² With varied disease presentation and aetiology, individuals may experience employment losses, strain on family life with future goals threatened.²

The co-occurrence of mental health issues, particularly depression and anxiety, in MS has been well documented since initial descriptions by Charcot in the late 1800s.^{3, 4} Our group previously reported a prevalence of 44.5% for anxiety and 18.5% for depression in the Southern Tasmanian MS Longitudinal Study,⁵ which was far higher than what is seen in geographically similar non-MS populations (anxiety 11%, depression 8.3%).⁶

In the general population, it is thought that both anxiety and depression vary in severity and onset by the interplay of genetic and environmental factors, such as the occurrence of significant life events (SLE) and the serotonin transporter gene (*SLC6A4*).^{7, 8} *SLC6A4* controls the expression of the serotonin transporter protein (5-HTT), and the 5-HTT protein transports serotonin from the synaptic cleft to the presynaptic neuron.^{8, 9} In the promoter region of the *SLC6A4*, a polymorphism exists classified as serotonin-transporter-linked polymorphic region (*5-HTTLPR*).⁹ Genetic variation within *5-HTTLPR* leads to altered expressivity of 5-HTT, with the shorter-allele version (S) less efficiently transcribing the protein when compared to the longer-allele version (L).^{10, 11} Research into the aetiology of psychiatric disorders has

focused in this area as selective serotonin reuptake inhibitors are known to be effective in treatment of people with depression and anxiety.^{12, 13} While there is no direct association between variation in the *5-HTTLPR* allele type and depression^{14, 15} or anxiety^{16, 17}, a number of studies have shown strong associations between SLE and the severity of depression¹⁴ or anxiety^{18, 19}. Moreover, studies have demonstrated interactions with *5-HTTLPR* allele, finding a stronger association between SLE and depression^{20, 21} or anxiety^{22, 23} in individuals with the S/S allele type when compared to L/S or L/L allele type. There are no studies that have examined this interaction in people with MS, however. Therefore the aim of this paper is to examine the interaction between SLEs in the previous 6 and 12 months and *5-HTTLPR* allele type in predicting depression or anxiety in a cohort of 198 participants with established MS. Based on the previous literature, it can be hypothesised that we will find a significant interaction between SLEs, variation in the *5-HTTLPR* allele type and depression or anxiety in people with MS.

Methods

Study Design and Population

A prospective sample of adults with MS was followed for an average of 2.3 years (SD=0.5) between January 2002 and December 2005 as part of the Southern Tasmanian Multiple Sclerosis Longitudinal Study. Medical records, media, MS clinics and the MS Society of Tasmania were used in order to recruit the cohort. Definite MS was established by a single neurologist based on the 2001 McDonald diagnostic criteria for MS.²⁴ Participants were reviewed and completed questionnaires biannually (winter and summer) and blood samples were taken at each review. The study retention rate was 90% (183/203), with 4% (8/203) withdrawing early and 6% (12/203) lost to follow-up because they moved or died. While 203 participants were recruited, only 198 were used for the present study as four participants did not have definite MS and one completed the baseline review only. Ethical Approval of this research was provided by the Southern Tasmanian Human Research Ethics Committee, and all participants provided written informed consent.

Measures

Outcome Measures (Anxiety and Depression)

Depressive symptoms and anxiety symptoms were measured at each summer and winter review using the Hospital Anxiety and Depression Scale (HADS-D and HADS-A respectively). This seven-item scale is designed to assess the severity of depression/anxiety by which participants rated each item from 1-3. These scores were added to establish an overall HADS-D/HADS-A score in the range of 0-21. A score of 0-7 represents a lack of disorder within participants, while 8-10 indicates mild

depression/anxiety and 11-21 severe depression/anxiety. A validation study in MS patients found a sensitivity of 90% and a specificity of 87.3% for the >7 cut-point when comparing against a diagnosis of depression, and 88.5% sensitivity and 80.7% specificity when compared with the diagnosis of generalised anxiety disorders.²⁵

Significant Life Events (SLE)

SLE were assessed retrospectively at the last review using a questionnaire based on the Social Readjustment Rating Scale by Holmes & Rahe²⁶ detailing different types of life events with varied weights of emotional impact. The present study used an updated weight system by Scully and colleagues, who developed new weights from the original SRRS 30 years after the original weightings were developed to allow for changes in culture in a non MS population.²⁷ The presence/absence and month/year of 16 types of SLE were recorded. These events related to the participants employment or financial issues, gaining/losing family members, changing personal circumstances (habits, lifestyle, relationships, employment), experiencing personal achievement/disappointment, health related issues, problems with the law or having a close friend/relative experience the same.

The total number of SLE in each 6 and 12 month period were summed for the 16 SLEs. We included the 12-month period to allow a longer window through which SLEs could have an effect on depression and anxiety. To take into account that some events are more significant than others, a total SLE load was calculated using weights from the Social Readjustment Rating Scale by Holmes & Rahe.²⁶

Genotyping

At study completion, 5-HTTLPR genotypes were determined using a protocol described by Turker and colleagues.²⁸ Product lengths were established using 15pmol of forward primer 5-GGCGTTGCCGCTCTGAATGCC and backwards primer 5-

GGCGTTGCCGCTCTGAATGCC to nucleotide positions -1416 to -1396 and -1170 to -1149, respectively.²⁸ The product lengths were 221 base pairs for the shorter allele and 265 base pairs for the longer allele. A 7% polyacrylamide gel next to a DNA size standard were used in order to resolve amplified fragments.²⁸ We used two categorisations: one categorisation was only based on whether participants had the longer (L) or a shorter (S) allele type (LL vs LS vs SS) (bi-allelic model). The second and preferred categorisation also takes into account the presence of an A-G single nucleotide polymorphism of rs25531 that divides the L allele into different fragment lengths: L_A or L_G. As recent evidence shows that L_AL_G is functionally more similar to S_AS_A (L_AL_A vs L_AS + L_AL_G vs SS + S_LL_G + L_GS) it is represented within the analysis as a tri-allelic model.⁸

Other Measures

Study participants reported information on their lifestyle (including time in the sun in each season, highest education level achieved, and other demographic covariates) at each biannual review. Physical activity was assessed using a modified version of the International Physical Activity Questionnaire²⁹, and blood samples were taken at each review. From biannual blood samples, serum vitamin D levels (25-hydroxyvitamin D; 25(OH)D) was measured at the end of the study using a commercially available radioimmunoassay (DiaSorin, Stillwater, MN). Expanded Disability Status Scale Score (EDSS)³⁰ was assessed annually by the study neurologist. Disease type and age of symptom onset were assessed by the study neurologist at study entry.

Statistical analysis

All analysis was completed STATA/IC 12.1. Descriptive statistics were used in order to examine the characteristics of the cohort at entry/duration of study. To allow for

repeated measured observations for each individual, multilevel mixed effects linear regression was used to assess the relationships between SLE number or load or 5-*HTTLPR* allele type, and anxiety & depression score. The interaction between SLE number/load, variation in the 5-*HTTLPR* allele type and anxiety/depression score, was assessed by including the cross-product of the 5-*HTTLPR* allele type and SLE.

Based on a review of the possible causal mechanisms, potential confounders were age, sex, 25(OH)D level, education, physical activity, sleep wakefulness, EDSS and MS duration/course. Covariates for these factors were retained in the models if they were statistically significant predictors or if their inclusion changed the estimated coefficient of the principal study factor by more than 10 percent.³¹

In order to show that the gene-environment interaction is environmentally determined, the possibility of confounding by gene-environment correlations (rGE) must be eliminated.³² rGE were examined using multilevel mixed linear regression (SLE load variables) and multilevel mixed effects Poisson regression (SLE number variables). These outcome variables were transformed due to the presence of a right skewed distribution and back-transformed to report the results in their original units.

Results

Table 1 shows the characteristics of the cohort. The mean age was 48.2 years, mean age of symptom onset was 34.2 years, and the mean EDSS level was 3.7 at study entry. All results in the following sections are unadjusted because adjustment for the potential confounding factors did not change materially the estimated coefficients of study factors or satisfied the rules for confounding.

Association between 5-HTTLPR allele type and significant life events

We first assessed whether the SLEs that people experienced were influenced by 5-HTTLPR allele type, but we found no associations. For example, using the tri-allelic 5-HTTLPR categorisation, no associations were observed with either SLE *number* (SLEs in the preceding 6 months: $\beta = 0.09$ (-0.09, 0.27), $p = 0.34$; SLEs in the preceding 12 months: $\beta = 0.11$ (-0.12, 0.33), $p = 0.31$) or SLE *load* (test for trend: 6 months: $\beta = 0.02$ (-0.06, 0.09), $p = 0.63$, 12 months: $\beta = 0.77$ (-0.75, 0.28), $p = 0.32$). Similar findings were observed for the bi-allelic genotype (data not shown).

Depression

Association between 5-HTTLPR allele type and depression

Variation in the 5-HTTLPR type was associated with HADS-D score in a dose-dependent fashion, which was significant for the bi-allelic categorisation (**Error! Reference source not found.**). There was no difference in this association by sex (bi-allelic categorisation: $p_{interaction} = 0.86$; tri-allelic categorisation: $p_{interaction} = 0.47$).

Association between significant life events and depression

Both SLE number (2 or more events) and load in the previous 12 months were associated with a higher level depression score on HADS-D (Table 2). SLE number and load in the previous 6 months were not associated with HADS-D, however.

Interaction between 5-HTTLPR allele type, significant life events in the previous 12 months and depression

We found a significant interaction between the variation in the 5-HTTLPR allele type and SLE load in the previous 12 months in predicting HADS-D score with both allelic models where the association between SLE load and depression was stronger for those with S/L and S/S compared to L/L. For the tri-allelic categorisation, the associations between SLE load (per 10-unit increment) and HADS-D were L/L: $\beta=0.04$ (-0.05, 0.24); S/L: $\beta=0.14$ (0.05, 0.24); S/S: $\beta=0.21$ (0.09, 0.33); $p_{interaction} < 0.001$).

For the bi-allelic categorisation, the associations between SLE load (per 10-unit increment) and HADS-D were L/L: $\beta=0.03$ (-0.09, 0.14); S/L: $\beta=0.16$ (-0.06, 0.25); S/S: $\beta=0.22$ (-0.09, 0.35), $p_{interaction} < 0.001$).

The association between SLE number and HADS-D was also stronger for those with S/L and S/S compared to L/L in the tri-allelic assumption, but this interaction was not statistically significant. For the tri-allelic categorisation, the associations between SLE number in the previous 12 months (per 10-unit increment) and HADS-D were L/L: $\beta=1.57$ (-3.21, 6.37); S/L $\beta=4.73$ (0.40, 9.06); S/S $\beta=4.68$ (-0.39, 9.76); $p_{interaction}=0.32$. For the bi-allelic categorisation, the associations were L/L: $\beta=0.25$ (-4.09, 4.60); S/L $\beta=6.28$ (-1.65, 10.91); S/S $\beta=4.76$ (-0.55, 10.07); $p_{interaction}=0.15$).

Anxiety

Association between 5-HTTLPR allele type and anxiety

Similarly to depression, when using the bi-allelic categorisation, there was a significant association between the 5-HTTLPR allele type and HADS-A score, with those with the S/S allele type reporting higher levels of anxiety when compared to L/L allele types (**Error! Reference source not found.**). The association was less strong and non-significant when using the tri-allelic categorisation. The association was stronger in females ($\beta=0.79$ (-0.06, 1.66), $p=0.07$) than males ($\beta=0.19$ (-1.02, 1.41), $p=0.74$), but the interaction by sex was not statistically significant ($p_{interaction}=0.09$).

Association between significant life events and anxiety

SLE number and load in the previous 12 months was associated with HADS-A, but the association was driven by the highest category only (Table 2). We found no association between SLE number or SLE load in the previous 6 months and HADS-A (**Error! Reference source not found.**). Potential confounders factors such as age, sex, vitamin D level, education, physical activity, sleep wakefulness, EDSS, MS disease duration and MS course did not satisfy the causal criteria for confounding.

Interaction between 5-HTTLPR allele type, significant life events and anxiety

We found a significant interaction between variation in the 5-HTTLPR allele, SLE load in the previous 12 months and HADS-A using a bi-allelic categorisation ($p_{interaction}=0.04$). However, there was no dose-response, with the associations between SLE load and HADS-A being L/L: $\beta=0.15$ (0.04, 0.26) per 10-unit increase; S/L $\beta=-$

0.04 (-0.14, 0.07); S/S $\beta=0.27$ (0.12, 0.43); $p_{interaction}=0.04$. In addition, no interactions were observed using the tri-allelic categorisation for either SLE load ($p_{interaction}=0.13$) or SLE number ($p_{interaction}=0.77$ bi-allelic; $p_{interaction}=0.74$ tri-allelic).

Discussion

In this prospective cohort study of people with established MS, we found that the association between SLE load in the preceeding 12 months and depression severity was stronger among those with one or two copies of the short allele of the *5-HTTLPR*. No convincing evidence of an interaction was found with anxiety.

The significant gene-environment interaction between the *5-HTTLPR*, SLE load and depression aligns with studies conducted in the general population, including a meta-analysis of 54 studies^{20, 21} and fits with the knowledge that the shorter allele version (S) is less efficient in transcribing the serotonin transporter protein compared to the longer allele version (L).^{10, 11} For depression, the interaction was identified irrespective of which allelic model we used (bi-allelic or tri-allelic), and a similar interaction with SLE *number* was also found, although not significant. The fact that this interaction was observed in a population where depression is partly disease-driven is novel. In people with MS, depression severity has been positively correlated with right frontal lesion load ($r=0.23$, $p=0.03$), total temporal brain volume ($r=0.26$, $p=0.01$), and right hemisphere brain volume ($r=0.25$, $p=0.02$),³³ and a lack of amygdala-prefrontal cortex connectivity has been observed in people with MS with depression when compared to the general population.³⁴

The impact of this interaction is substantial. Among people with S/S, the HADS depression score (range 0-16 in our sample) was 2.2 times higher for an increase of 100 units of the SLE load (SLE load in the previous 12 months ranged from 0-250). The increase was 1.4 for those with S/L and only 0.4 for those with L/L. Thus, SLEs

substantially increased the depression score among people with MS with S/S, but it did not increase the depression score much among those with L/L.

We found no convincing evidence of an interaction between SLE variables and anxiety. While we did find a significant interaction using a bi-allelic categorisation between variation in the *5-HTTLPR*, SLE load in the previous 12 months and anxiety score, there was no dose-response, and no association was seen using the preferred tri-allelic categorisation or for SLE number. The absence of a clear interaction is somewhat surprising given there is some evidence of an interaction in the general population.^{22, 23} These results suggest that anxiety in MS is driven more by the disease rather than environmental factors.

While the study was conducted prospectively, the assessment of SLE was retrospectively assessed, possibly reducing the reliability of this measure, particularly for events beyond 12 months. We also calculated SLE load using referenced weights from a non-MS population in the SRRS, it is not known if standardised SRRS weights are influenced by the presence of MS. The sample size was of moderate size and may enhance the chance of false negative findings. While we were able to take into account the severity of SLE, we were unable to distinguish between desirable/undesirable events. Because previous literature has suggested that positive/negative events are processed via different transitional brain routes, it would be beneficial to explore this in future studies.³⁴

Given that depression and anxiety are known as complex traits, future studies would benefit in examining gene-gene interactions as it is likely that many genes may play a role in their development with personality traits also being associated with a higher

risk of depression.^{35, 36} It would also be interesting for future studies to examine fatigue as an outcome in this interaction as previous literature as suggested that MS fatigue and depression can overlap in this cohort.³⁷

In conclusion, we found that the association between SLE load and depression severity was stronger among those with one or two copies of the short allele of the 5-*HTTLPR*, while there was no convincing evidence of an interaction with anxiety.

References

1. Keegan BM and Noseworthy JH. Multiple sclerosis. *Annu Rev Med.* 2002; 53: 285-302.
2. Dennison L and Moss-Morris R. Cognitive-behavioral therapy: what benefits can it offer people with multiple sclerosis? *Expert review of neurotherapeutics.* 2010; 10: 1383-90.
3. Haussleiter IS, Brune M and Juckel G. Psychopathology in multiple sclerosis: diagnosis, prevalence and treatment. *Therapeutic advances in neurological disorders.* 2009; 2: 13-29.
4. Charcot JM. *Lectures on the Diseases of the Nervous System.* London: La Salpe'trie're, 1977.
5. Wood B, van der Mei IA, Ponsonby AL, et al. Prevalence and concurrence of anxiety, depression and fatigue over time in multiple sclerosis. *Multiple sclerosis.* 2013; 19: 217-24.
6. Simpson S, Jr., Pittas F, van der Mei I, Blizzard L, Ponsonby AL and Taylor B. Trends in the epidemiology of multiple sclerosis in Greater Hobart, Tasmania: 1951 to 2009. *J Neurol Neurosurg Psychiatry.* 2011; 82: 180-7.
7. Klauke B, Deckert J, Reif A, et al. Serotonin transporter gene and childhood trauma--a G x E effect on anxiety sensitivity. *Depression and anxiety.* 2011; 28: 1048-57.
8. Calapoglu M, Sahin-Calapoglu N, Karacop A, Soyoz M, Elyildirim UY and Avsaroglu S. Serotonin transporter bi- and triallelic genotypes and their relationship with anxiety and academic performance: a preliminary study. *Neuropsychobiology.* 2011; 63: 103-11.
9. Heils A, Teufel A, Petri S, et al. Allelic variation of human serotonin transporter gene expression. *J Neurochem.* 1996; 66: 2621-4.
10. Jasinska AJ, Lowry CA and Burmeister M. Serotonin transporter gene, stress and raphe-raphe interactions: a molecular mechanism of depression. *Trends in neurosciences.* 2012; 35: 395-402.
11. Canli T and Lesch KP. Long story short: the serotonin transporter in emotion regulation and social cognition. *Nature neuroscience.* 2007; 10: 1103-9.
12. Caspi A, Sugden K, Moffitt TE, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science.* 2003; 301: 386-9.
13. Tamminga CA, Nemeroff CB, Blakely RD, et al. Developing novel treatments for mood disorders: accelerating discovery. *Biological psychiatry.* 2002; 52: 589-609.
14. Risch N, Herrell R, Lehner T, et al. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA.* 2009; 301: 2462-71.
15. Kenna GA, Roder-Hanna N, Leggio L, et al. Association of the 5-HTT gene-linked promoter region (5-HTTLPR) polymorphism with psychiatric disorders: review of psychopathology and pharmacotherapy. *Pharmacogenomics and personalized medicine.* 2012; 5: 19-35.
16. Munafo MR, Brown SM and Hariri AR. Serotonin transporter (5-HTTLPR) genotype and amygdala activation: a meta-analysis. *Biological psychiatry.* 2008; 63: 852-7.
17. Minelli A, Bonvicini C, Scassellati C, Sartori R and Gennarelli M. The influence of psychiatric screening in healthy populations selection: a new study and

- meta-analysis of functional 5-HTTLPR and rs25531 polymorphisms and anxiety-related personality traits. *BMC psychiatry*. 2011; 11: 50.
18. Mitchell SJ and Ronzio CR. Violence and other stressful life events as triggers of depression and anxiety: what psychosocial resources protect African American mothers? *Maternal and child health journal*. 2011; 15: 1272-81.
 19. Zavos HM, Wong CC, Barclay NL, et al. Anxiety sensitivity in adolescence and young adulthood: the role of stressful life events, 5HTTLPR and their interaction. *Depression and anxiety*. 2012; 29: 400-8.
 20. Karg K, Burmeister M, Shedden K and Sen S. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. *Arch Gen Psychiatry*. 2011; 68: 444-54.
 21. Zannas AS, McQuoid DR, Steffens DC, Chrousos GP and Taylor WD. Stressful life events, perceived stress, and 12-month course of geriatric depression: direct effects and moderation by the 5-HTTLPR and COMT Val158Met polymorphisms. *Stress*. 2012; 15: 425-34.
 22. Stein MB, Schork NJ and Gelernter J. Gene-by-environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2008; 33: 312-9.
 23. Armbruster D, Moser DA, Strobel A, et al. Serotonin transporter gene variation and stressful life events impact processing of fear and anxiety. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum*. 2009; 12: 393-401.
 24. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol*. 2001; 50: 121-7.
 25. Honarmand K and Feinstein A. Validation of the Hospital Anxiety and Depression Scale for use with multiple sclerosis patients. *Multiple sclerosis*. 2009; 15: 1518-24.
 26. Holmes TH and Rahe RH. The Social Readjustment Rating Scale. *Journal of psychosomatic research*. 1967; 11: 213-8.
 27. Scully JA, Tosi H and Banning K. Life event checklists: Revisiting the social readjustment rating scale after 30 years. *Educ Psychol Meas*. 2000; 60: 864-76.
 28. Turker T, Sodmann R, Goebel U, et al. High ethanol tolerance in young adults is associated with the low-activity variant of the promoter of the human serotonin transporter gene. *Neuroscience letters*. 1998; 248: 147-50.
 29. Craig CL, Marshall AL, Sjostrom M, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003; 35: 1381-95.
 30. Bowen J, Gibbons L, Gianas A and Kraft GH. Self-administered Expanded Disability Status Scale with functional system scores correlates well with a physician-administered test. *Mult Scler*. 2001; 7: 201-6.
 31. Greenland S. Modeling and variable selection in epidemiologic analysis. *Am J Public Health*. 1989; 79: 340-9.
 32. van Os J, Rutten BP and Poulton R. Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. *Schizophr Bull*. 2008; 34: 1066-82.
 33. Zorzon M, de Masi R, Nasuelli D, et al. Depression and anxiety in multiple sclerosis. A clinical and MRI study in 95 subjects. *Journal of neurology*. 2001; 248: 416-21.

34. Passamonti L, Cerasa A, Liguori M, et al. Neurobiological mechanisms underlying emotional processing in relapsing-remitting multiple sclerosis. *Brain : a journal of neurology*. 2009; 132: 3380-91.
35. Montag C and Reuter M. Disentangling the molecular genetic basis of personality: from monoamines to neuropeptides. *Neurosci Biobehav Rev*. 2014; 43: 228-39.
36. Klein DN, Kotov R and Bufferd SJ. Personality and depression: explanatory models and review of the evidence. *Annu Rev Clin Psychol*. 2011; 7: 269-95.
37. Sindermann C, Saliger J, Nielsen J, et al. Personality and Primary Emotional Traits: Disentangling Multiple Sclerosis Related Fatigue and Depression. *Arch Clin Neuropsychol*. 2017: 1-10.

Table 1. Characteristics of the participants in the MS Longitudinal Study

	n/N	%
Female sex	138/198	69.6
MS course* **		
Relapsing-Remitting	148/198	74.7
Secondary Progressive	40/198	20.2
Primary Progressive	9/198	4.6
Prevalence of depression (HADS-D>7)*	36/195	18.2
Prevalence of anxiety (HADS-A>7)*	86/193	44.5
Use of anti-depressive medication*	24/198	15.7
Use of anxiety medication*	35/198	22.9
5-HTTLPR allele frequencies		
Bi-allelic categorisation		
L/L	63/186	33.9
L/S	84/186	45.1
S/S	39/186	21.0
Tri-allelic coding		
L _A L _A	45/186	27.2
L _A L _G + L _A S	92/186	49.5
SS + L _G S + SL _G	49/186	26.3
	n	Mean (SD; Range)
Age (Years)*	198	48.5 (11.2; 21-77)
Age of symptom onset* **	198	34.5 (9.8; 14-65)
Duration of disease from diagnosis (Years)*	198	9.1 (8.6; 0-43)
EDSS*	198	4.1 (2.2; 0-9.5)
HADS depression score*	198	4.5 (3.3; 0-16)
HADS anxiety score*	198	6.4 (4.0; 0-20)
SLE number (over total study)	178	2.2 (2.0; 0-11)
SLE load (over total study)	178	103.2 (92.8; 0-504)
*Measurement at study entry. **One case had a baseline MS course that was undefined.		
HADS: Hospitality Anxiety and Depression Scale; EDSS: Expanded Disability Status Scale.		

Table 2 Association between 5-HTTLPR allele type, SLE number/load and depression or anxiety.

	<i>Depression score</i>		<i>Anxiety score</i>	
	β^{**} (95% CI)	P	β (95% CI)	P
5-HTTLPR variables				
<u>5-HTTLPR allele type (Bi-allelic)</u>				
L/L (L _A L _A + L _A L _G)	3.98 (3.28, 4.67)	Ref	5.95 (5.10, 6.81)	Ref
L/S (L _A S _A + L _G S _A + S _G L _G)	+0.80 (-0.12, 1.72)	0.09	+0.63 (-0.50, 1.76)	0.27
S/S (S _A S _A)	+1.25 (0.13, 2.38)	0.03	+1.65 (0.27, 3.03)	0.02
<i>Test for trend</i>		0.02		0.02
<u>5-HTTLPR allele type (Tri-allelic)</u>				
L/L (L _A L _A)	4.21 (3.38, 5.04)	Ref	6.09 (5.07, 7.10)	Ref
S/L (L _A L _G + L _A S _A)	+0.36 (-0.65, 1.38)	0.49	+0.35 (-0.89, 1.60)	0.58
S/S (S _A S _A + L _G S _A + S _G L _G)	+0.81 (-0.34, 1.97)	0.17	+1.23 (-0.18, 2.64)	0.09
<i>Test for trend</i>		0.16		0.08
SLE variables				
<u>SLE number category in previous 12 months</u>				
0 events	4.06 (3.54, 4.61)	Ref	6.17 (5.52, 6.82)	Ref
1 event	+0.25 (-0.43, 0.93)	0.48	-0.26, (1.00, 4.48)	0.50
2 or more events	+1.23 (0.44, 2.02)	<0.001	+1.05 (0.19, 1.91)	0.02
<i>Test for trend</i>		<0.001		0.29
<u>SLE number in previous 12 months</u>	+0.39 (0.12, 0.67)	0.00	+0.31 (0.02, 0.61)	0.04
<u>SLE load category in previous 12 months</u>				
0 (No SLE)	4.09 (3.55, 4.63)	Ref	0.19 (5.54, 6.84)	Ref
≤57	+0.02 (-0.68, 0.71)	0.96	-0.27 (1.04, 0.50)	0.49
>57	+1.36 (0.62, 2.10)	<0.001	+0.87 (0.06, 1.69)	0.04
<i>Test for trend</i>		<0.001		0.07
<u>SLE load in previous 12 months – per 10 unit increase</u>	+0.15 (0.08, 0.21)	<0.001	+0.11 (0.04, 0.18)	<0.001

**Results presented as the mean (95% CI) of reference level of the predictor, then coefficient (95% CI) of other levels relative to reference.

Formatting note: figures in bold denote statistical significance (p<0.05). Italicisation denotes p-values.