Department of Clinical Neurology, School of Medicine

The relation of familial longevity and progression of disability in multiple sclerosis

Xin Gao

Dissertation submitted to the University of Nottingham for the degree of

Master of Science (by research)

September 2018

Abstract

Background: Multiple sclerosis (MS) is an immune-mediated demyelinating disease with immune system attacking the protective myelin sheath that covers nerve fibres and causes neurological deficits. Ageing is a factor that is related to the progression of disease disability. Previous studies have shown an inheritance of longevity. The genetic or familial effect on disability progression in MS has not been studied in detail previously.

Aims: This study aims to explore the association between familial longevity and Multiple sclerosis severity score (MSSS), which is a measurement of the rate of disability progression in MS. Another aim of this study is to evaluate the frailty index (FI) of patients and family members, and its relationship with the MSSS.

Methods: A self-reported questionnaire was designed based on the literature review. The MSSS was measured by disease duration and Expanded Disability Status Scale (EDSS). In total, 323 patients were recruited from the Nottingham cohort of the MS Registry and responded to the questionnaire in the MS clinic or by post. The relation of familial longevity and individual frailty was assessed by multiple linear regression. The effect of familial longevity, frailty and neurological degenerative disease history was extracted by principal component analysis (PCA).

Results: Multiple regression showed that while adjusted for significant covariates, the relation between MSSS and interaction of 'parental age, neurological degenerative disease history, and frailty' was significant but relatively weak (β = -0.304, p<0.000). We also showed that the association between MSSS and current patients' frailty was significant but relatively weak (β= 0.169, p<0.000). Cox regression showed a significant association between risk of reaching EDSS 6.5 (adjusted HR=1.119, p<0.000) / transition to the secondary progressive stage (adjusted HR=1.123, p<0.000) and current patients' frailty index, but not for the extracted interaction of 'parental age, neurological disease history and frailty'.

Conclusion: We found that familial longevity alone may not be associated with any change in the progression rate of MS disability when controlled by other covariates in this exploratory study. Nevertheless, the parental ageing (defined as the interaction of 'parental age, neurological disease history and frailty' in the current study) may weakly relate to MSSS. Patients' frailty, but not that of parents or grandparents correlated with faster progression of MS disability. Further study may provide more definitive results on the familial influences on MS progression rate.

Acknowledgements

I gratefully acknowledge the help of my supervisors, Dr Nikos Evangelou and Prof. Cris Constantinescu, who have offered me valuable suggestions in the academic studies. I also would thank Dr Chris Tench for his advice regarding the statistical analysis. Last, my thanks would go to the patients, research nurses, and my colleagues in this study for data collection.

List of abbreviation

AGE	Advanced glycation end-products
ANOVA	Analysis of variance
APOE	Apolipoprotein E
ATP	Adenosine triphosphate
BBB	Blood-brain barrier
CIS	Clinically isolated syndrome
CMV	Cytomegalovirus
CSF	Cerebrospinal fluid
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
DNA	Deoxyribonucleic acid
DMT	Disease-modifying therapies
EBV	Epstein–Barr virus
EDSS	Expanded Disability Status Scale
EGF	frailty growth factor
FDA	Food and Drug Administration
FGF	Fibroblast Growth Factor
FI	Frailty index

GFAP	Glial fibrillary acidic protein
GM	Grey matter
GWAS	Genome-wide association study
HA	Hyaluronan
HERV	Human endogenous retroviruses
HLA	Human leukocyte antigen
ICH-GCP	International Council for Harmonisation-Good Clinical Practice
ISC	Immune senescence
IRAS-REC	Integrated Research Application System-Research Ethics Committee
LS-OCMB+	Lipid-specific oligoclonal IgM bands+
MHC	Major Histocompatibility complex
MS	Multiple sclerosis
MSRV	Multiple sclerosis-associated retroviruses
MTR	Magnetized transfer ratio
NFH	Neurofilament Heavy Chain
NFL	Neurofilament Light Chain
NHS	National Health Service
NO	Nitric oxide
OB	Oligoclonal bands
OCMB	Oligo clonal IgM bands

OPC	Oligodendrocyte precursor cell
PCA	Principal components analysis
PDGF	Platelet-derived growth factor
PPMS	Primary progressive MS
PMS	Progressive MS
PTPRC	Protein tyrosine phosphatase receptor-type C
RMS	Rostral migratory stream
RRMS	Relapsing-remitting MS
ROS	Reactive Oxygen Species
SES	Socioeconomic status
SPMS	Secondary progressive MS
SVZ	Subventricular zone
TGF	Transforming growth factor
TLR	Toll-like receptors
WM	White matter

List of Tables

Table 1. Clinical markers of disability progression in MS

Table 2. MRI markers of disability progression in MS

Table 3. CSF markers of disability progression in MS

Table 4. Pearson relation between different clinical variables and MSSS

Table 5. Model 1 (Patient age, disease onset age and disease type)

Table 6. Model 2 (EDSS, disease onset age)

Table 7. Relation of patients' frailty index and MSSS

Table 8. Comparison of mean MSSS score of the different number of grandparents over 90 years old

Table 9. Sub correlation analysis for possible confounder of both grandparents

Table 10. The rotation of principal component analysis (4 main components extracted)

Table 11. Partial correlation between father ageing and MSSS

Table 12. Relation of father ageing and MSSS

Table 13. Partial correlation between mother ageing and MSSS

Table 14. Relation of mother ageing and MSSS

Table 15. Relation of ageing level from both parents and MSSS

Table 16. The rotation of principal component analysis (2 main components extracted)

Table 17. Partial correlation between parental ageing and MSSS

Table 18. Relation of parental ageing and MSSS

Table 19. Association of patients' frailty level and risk of reaching EDSS 6.5

Table 20. Association of patients' frailty level and risk of transition to SPMS

Table 21. Association between patients' frailty level without mobility problems and MSSS

Table 22. Association of patients' frailty level without mobility problems and risk of reaching EDSS 6.5

Table 23. Association of patients' frailty level without mobility problems and risk of transition to SPMS

List of figures

Figure 1. List of 36 deficits contained in the eFI.

Figure 2. The scatter plot of patients' frailty and MSSS

Contents

Abstract **2**

Acknowledgements **4**

List of abbreviation **5**

List of Tables **8**

List of figures **10**

Introduction and hypothesis **14**

1.1 Background 14

1.2 Multiple sclerosis 14

1.2.1 Epidemiology 15

1.2.2 Infectious factors 16

1.2.3 Smoking 16

1.2.4 Genes of MS 18

1.2.5 Pathology 19

1.2.6 Pathophysiology 20

1.2.7 Immunology 21

1.2.8 Biochemistry 22

1.3 Treatment 23

1.4 Ageing and MS 24

1.4.1 Demyelination and remyelination 24

1.4.2 Macrophages, microglia, and astrocytes 26

1.4.3 Oligodendrocyte precursor cells 29

1.4.4 Disability and progression 31

1.4.5 Iron 32

1.4.6 HSCs-- hematopoietic stem cells 32

1.4.7 Gene expression 33

1.4.8 Subventricular zone 33

1.5 Inheritance of Ageing and Family longevity 34

1.6 MSSS (Multiple Sclerosis Severity Scores) and EDSS (Expanded Disability Status Scale) 36

1.7 Frailty Index 37

1.8 Clinical, MRI, and CSF parameters of Disability Progression in Multiple Sclerosis 38

1.9 Current Knowledge Gaps 42

Methodology 43

2.1 Ethical and regulatory aspects 43

2.2 Primary objective 43

2.3 Secondary objective 44

2.4 Feasibility/pilot study 44

2.4.1 Executive summary 44

2.5 The current study type 47

2.6 Reproducibility 50

Results 51

3.1 Comparison of responders to the postal questionnaires and questionnaires given in MS clinic 51

3.2 Clinical parameters 52

3.3 Possible Confounders for all the MS patients 55

3.4 For the questionnaires collected from both clinic and by post 59

3.4.1 Patients 59

3.4.2 Family members 60

Discussion 82

4.1 Principal findings 82

4.2 Possible explanation of our findings and comparison to other studies 82

4.2.1 Clinical markers of Multiple sclerosis 82

4.2.2 Factors related to the progression of Multiple sclerosis 84

4.2.3 Frailty, chronological age, and lifestyle 86

4.2.4 Patients' frailty and progression of disease disability 86

4.2.5 Familial longevity, frailty, and progression of disease disability 88

4.3 Considering alternative explanations of the findings 92

4.4 Clinical relevance 94

4.5 Strengths and weaknesses of the study 94

4.6 Suggestions for further research 99

Conclusion 102

Appendices 103

Appendix 1: Invitation letter 103

Appendix 2: Patient information sheet 106

Appendix 3: Questionnaire booklet 114

Appendix 4: Consent form 142

Reference 144

Introduction and hypothesis

1.1 Background

The course of MS is affected via interaction of environmental and genetic factors, although specific mechanisms are not fully understood. Understanding the factors that influence disease progression might help to know the disease course better and might affect treatment decisions.

This study will explore if familial factors and comorbidities, might be associated with the disability progression in MS patients. This project aims to analyse data of patients that have clinically diagnosed MS in a secondary care setting. Their health status (individual history, comorbidity, frailty) will be collected and combining this with their clinical data already in existence on health records. The research focus to be addressed includes their family history, the age of death of parents and grandparents, and their effect on clinical progression as assessed by EDSS which includes neurological assessment of 8 functional systems (e.g. mobility, cognitive function, bowel and bladder problem) (Kurtzke, 1983). It is anticipated that the findings of this prognostic study may provide new data that help explore more accurately disease severity and progression of disability among MS patients.

1.2 Multiple sclerosis

MS is an immune-mediated chronic inflammatory disease affecting the central nervous system causing demyelination, axonal degeneration, and gliosis (Compston and Coles, 2008, Fraussen et al., 2016). There are three clinical subtypes in MS: RRMS (relapsing-remitting MS), PPMS (primary progressive MS), and SPMS (secondary progressive MS). RRMS is characterised by fluctuated relapses following several months to years of relative quiet (remission) with no new disease activation. Deficits during attacks may either resolve or leave deficits, the latter occurs in 40% of attacks and becoming more common over time (Compston and Coles, 2008, Tsang and Macdonell, 2011). Usually, the RRMS starts with a CIS which shows demyelination but does not fulfil the criteria for MS (Compston and Coles, 2008, Miller

et al., 2005). 85% of patients with MS have a history of the clinically isolated syndrome (CIS) (Miller et al., 2005, Miller et al., 2012, Confavreux and Vukusic, 2006b). CIS means the attack only happens once. 85% of patients with MS start with the status of alternating episodes, which is regarded as RRMS. Following this, 60–70% of patients transit to SPMS which is characterized by progressive neurological disability. Some MS patients undergo a steady decrease in neurological function without recovery, and this type is recognised as PPMS (Dutta and Trapp, 2014). The dysfunction includes spinal, optic nerves, cerebrum, or brain stem (Compston and Coles, 2008). These are related to various long-term comorbidities including difficulty mobilisation, bowel and bladder problems, cognitive problems, sexual dysfunction, depression as well as some forms of severe neuropathic pains which have an enormous impact on the individual patient.

1.2.1 Epidemiology

MS is more prevalent among young adults in North America, Europe and Australasia, hence it appears to be influenced by temperate and latitudes (Trapp and Nave, 2008). Females show higher susceptibility to MS, but males tend to enter the progressive course from disease onset (Sadovnick and Ebers, 1993). Males show a shorter time to progression than females (Rovaris et al., 2006). The tendency of progression will be more conspicuous (3:1) if the disease onset is before age 16. The average age of onset ranged from 29 to 33. For women, this age was slightly younger. Similarly, it was found that childhood MS was more prevalent in females (75.2%) (Sadovnick and Ebers, 1993). Recently, a study described the pattern of evolution of incidence of MS over 60 years in Denmark, and the authors reported that disease onset age was 50 years and older (Koch-Henriksen et al., 2018). It was reported that females have a higher prevalence of relapsing-remitting MS (Sellner et al., 2011). Sex difference in MS maybe because of the influence on sex steroid hormones in the immune system, blood-brain barrier, and parenchymal CNS cells. It has been found that sex steroid supplementation may benefit patients with MS by ameliorating symptoms in both clinical and experimental studies (Mao and Reddy, 2010). Some other factors, for example, low sunlight, vitamin D deficiency, diet,

geomagnetism, air pollutants, radioactive rocks, cigarettes, and toxins may act as contributing factors for MS (Compston and Coles, 2008).

1.2.2 Infectious factors

Patients with MS have higher rates of the previous infections with measles, mumps, rubella, and Epstein-Barr virus (Compston and Coles, 2008). A significant correlation between a clinical history of infectious mononucleosis and enhanced MS susceptibility has been found (Mao and Reddy, 2010). The common childhood infections such as measles, mumps, rubella, and varicella are related to MS as reported. A case-control study by the Italian MS Study Group found that MS patients were reported possibly had at least one childhood illness after age six years. It was suggested that patients with MS were prone to have had at least one childhood infection at later ages (Filippini, 1989).

Meanwhile, some pathology studies suggest that B cells highly accumulate in chronic lesions, are associated with Epstein-Barr virus (EBV) (Compston and Coles, 2008). Other studies found the EBV accompanied by B cells that permeate the meninges and white matter, which showed the association of EBV and MS (Mao and Reddy, 2010, Serafini et al., 2007). The hypothesis of a viral origin in MS was supported by the possibility that demyelinating diseases could be induced via viruses in animals (Mao and Reddy, 2010, Miller et al., 2001, Denic et al., 2011). The discovered human endogenous retrovirus (HERV)-W family contains one extracellular retroviral particle-MSRV, which is generated by cultured meningeal cells and monocytes from MS patients initially as reported. The strong relation of MSRV and MS has been proved in independent studies (Sotgiu et al., 2004).

1.2.3 Smoking

Smoking increases the occurrence of MS possibly by elevating the frequency and continuance of respiratory infections. The relation between Chlamydia pneumonia infection and MS has been reported. Also, it has been shown that smokers may have higher C. pneumonia-specific antibodies. Moreover, in two prospective studies of US women, it was found that the incidence of MS was higher among cigarette smokers and increased with accumulative exposure to

smoking. The association between smoking and MS might rely on the immunomodulatory effects of tobacco (Hernan et al., 2001, Mikaeloff et al., 2007).

Cigarette smoke may lead to immunosuppression (e.g., nicotine) or immunostimulation (e.g., tobacco glycoprotein). It was reported that smokers were more susceptible to autoimmune responses and connected with the increased risk of autoimmune diseases (e.g., rheumatoid arthritis or systemic lupus erythematosus (Mikaeloff et al., 2007). The potential mechanism includes increased microvascular blood flow and permeability of the blood-brain barrier (BBB) which are directly caused by nicotine. Leakage of the BBB in humans initiates the progress of MS (Hernan et al., 2001, Mikaeloff et al., 2007, Hernan et al., 2005).

Another possibility is that CNS can be affected by the direct toxic. One of the elements in cigarette smoke is cyanide. Levels of its primary metabolite-thiocyanate may contribute to demyelination in the CNS of animals. The epidemics of tropical spastic paraparesis and optic neuropathy have shown this complex, which shares some clinical traits with MS (Hernan et al., 2001, Hernan et al., 2005).

Smoking may accelerate transforming from RRMS into SPMS as stated. It was estimated that smokers had the more than three times higher possibility of risk of transition. Experimental evidence proves that higher levels of nitric oxide (NO) metabolites in the cerebrospinal fluid (CSF) are related to clinical development of MS. Smoking increases NO in plasma and nicotine generates more NO in the CNS, which may correlate with increased NO concentration in MS inflammatory lesions. Enhanced NO levels lead to neurodegeneration and irreversible neuronal disabilities in the secondary progressive clinical course (Manouchehrinia et al., 2013, Hernan et al., 2005). It was suggested that patients with MS who quit smoking might delay the progression of MS (Manouchehrinia et al., 2013, Healy et al., 2009). The relation between smoking and conversion to secondary progressive process is also partially linked to older smokers with longer disease duration (Pittas et al., 2009).

Children with smoking parents are more susceptible to MS. The duration of smoking also increases the risk of MS. Parental smoking leads children to passive smoking. It indicates a shortage of interferon gamma-producing cells in the adenoids of children exposed to passive smoking. Previous researchers did a population-based, case-control study in children which provides evidence of these views (Mikaeloff et al., 2007).

1.2.4 Genes of MS

Genetic factors are mentioned after the first genetic relation found in 1972 for Human leukocyte antigen (HLA) class I antigens (Sotgiu et al., 2004, Kenealy et al., 2003). The class II HLA alleles have been showed to have a stronger relation to the HLA-DR2 haplotype (Kenealy et al., 2003).

As for the regions that harbour MS loci, the most finding for the linkage is chromosome 6p21, which locates in major histocompatibility complex (MHC) containing HLA, the correlation with the HLA-DR2 allele can explain this linkage signal. The MHC has been recognised to account for 10-50% of the genetic component of MS susceptibility, at least in the northern European Caucasian population. Also, one of the candidate genes in chromosome 19q13 identified by bioinformatics is Apolipoprotein E (APOE), which connects with neuroregeneration and remyelination after the lesions in the central and peripheral nervous system.

The DR4 and DR3 alleles are more important in Sardinians. It seems that both the DRB1*1501 and DQB1*0602 molecules, and probably the DRB5*0101 molecule, are related to the susceptibility of MS (Svejgaard, 2008). Additionally, the independent effect of HLA-DRB1*15 and HLA-A*02 on MS susceptibility is confirmed, and there is a critically statistical interaction between HLA DRB1*15 and HLA A*02 in the progress of MS as reported (Hedstrom et al., 2011).

Reduced apoE concentrations in CSF in patients with MS have been reported, which may affect the extent of MS progression. The link between the APOE-4 allele and severity of MS has been found and confirmed (Imitola et al., 2006, Kenealy et al., 2003).

Alteration in genes of transcriptional regulation and inflammation, changes in the distribution of Na+ and Ca2+ channels in pathological samples, and reduced expression of metabolism-related genes lead to the chronic dysfunction of neurons and oligodendrocytes in lesions outside as reported. The chronic oxidative damage in the susceptible genes probably mediates a reduction in the expression of neuronal genes, such as synaptosomal-associated protein as reported. The transaction of axon and neurodegeneration occur in animals with a loss of proteolipid protein and humans with a mutation in the proteolipid protein gene. Proteolipid protein and myelin-associated glycoprotein play vital roles in the delivery signals of the myelin-derived trophy to axons (Imitola et al., 2006).

Sizeable international research collaborations have proved the polymorphism of two cytokine receptor genes in MS pathogenesis: the interleukin 7 receptor alpha chain gene (IL7RA) on chromosome 5p13 and the interleukin 2 receptor alpha chain gene (IL2RA (=CD25)) on chromosome 10p15. The C allele of a single-nucleotide polymorphism, rs6897932, within the alternative spliced exon 6 of IL7RA is included in around 30% MS cases. Meanwhile, some pathways and network-based Genome-wide association studies (GWAS) have confirmed these results (such as HLA-DRB1 and IL2RA) (Mao and Reddy, 2010, International Multiple Sclerosis Genetics et al., 2011). In a recent review, authors demonstrated that multiple GWAS over the past ten years had revealed more than 200 loci that lead to disease pathogenesis independently (Baranzini and Oksenberg, 2017). Additionally, the mutation in protein tyrosine phosphatase receptor type C (PTPRC) is associated with the development of MS in some families (Jacobsen et al., 2000).

1.2.5 Pathology

The histopathological changes in MS lesions contain inflammation, demyelination, loss of oligodendrocytes, reactive astrogliosis, and axonal pathology (Bjartmar and Trapp, 2003). Axonal degeneration causes permanent disability (Trapp and Nave, 2008). The neurodegeneration partially independent from inflammatory course induces chronic brain damage in progressive MS as reported (Bradl and Lassmann, 2009). The axonal loss

contributes to the relation between tissue atrophy and the progression of functional decline (Bjartmar and Trapp, 2003). Progressive axonal loss logically explains the conversion from RRMS to SPMS and the progression of permanent neurological disability in SPMS (Trapp and Nave, 2008). While the CNS is incapable of compensating the loss of axons, the transition may occur (Dutta and Trapp, 2014). The axonal loss has been seen to be related to the irreversible neurological decline in MS (Lassmann and van Horssen, 2011). Pathologically, axonal transection and axonal end-bulb formation indicate continuous axon injury (Fitzner and Simons, 2010). It starts at the beginning and accumulates as the pathological substrate for progressive disability in MS. The degree of axonal damage can be affected by genes included in responses to inflammatory and demyelinating process (Bjartmar and Trapp, 2003). PMS can be pathologically characterised by brain atrophy. (Mahad et al., 2015). Increased cortical atrophy has been implicated in progressive disability and grey matter atrophy has been shown to predict neurological decline (Dutta and Trapp, 2014).

1.2.6 Pathophysiology

Executive and cognitive dysfunction may pathologically relate to grey matter demyelination. The lesions in the cortex and hippocampus, as well as the degree of neuronal injury, might help to understand the cause of dysfunction (Trapp and Nave, 2008). The symptoms of fatigue can be explained by the failure of transmitting electric impulses in partially demyelinated axons (Compston and Coles, 2008). Another possible contributing factor for SPMS is vascular dysfunction. The thrombotic expressions in MS patients may be part of the activation of innate immunity has been proposed (Koudriavtseva et al., 2015, Koudriavtseva, 2014). The demyelination in the brain cortex and diffuse degeneration through white and grey matter in PMS eventually lead to dilated cerebral ventricles and spinal cord atrophy with tissue loss (Lassmann and van Horssen, 2011, Bjartmar and Trapp, 2003). The influence of relapses on the progression of permanent disability is controversial (Confavreux et al., 2000).

1.2.7 Immunology

“A commonly proposed idea is that MS is an autoimmune disease where autoreactive T lymphocytes enter the CNS from the peripheral immune system in the initial courses of lesion formation (the outside-in hypothesis)’’ (Mahad et al., 2015). A T-lymphocyte subtype induces an inflammatory process by secreting interleukin-17, which disrupts the blood-brain barrier and allows Th17cells penetrate into the brain then kill neurons (Compston and Coles, 2008). An autoimmune response leads to the failure of suppression of inflammation; and produces antibodies-mediated axonal injury (Compston and Coles, 2008). B-lymphoid follicles accumulation causes cortical lesions and increases the products of intrathecal antibody. Antibodies specifically impair products of myelin-basic protein, which relate to MS (Compston and Coles, 2008). The chronically inflamed environment retains inflammatory cells and facilitates the abnormal formation of lymphatic structures. The secondary lymphoid organs form the conduit system, which allows the transport of signal molecules (especially chemokines CCL19 and CCL21). The absent conduits (as found in the ectopic lymph follicles of SPMS, Lassmann and Kerjaschki, unpublished observation) make these chemokines available to macrophages and microglial cells in the subpial cortex, and probably modify the function and behaviour of these cells during inflammation (Bradl and Lassmann, 2009). The pro-inflammatory T cell responses are stimulated together with proinflammatory molecules in MS are associated with B cells. B cells richly infiltrate in cortical lesions while B cells in CSF are related to rapid progression in SPMS. Ectopic B cell follicles are also implicated in cortical demyelination, loss of neurites and permanent disability (Fraussen et al., 2016). Although the increase of plasma blasts, DC-SIGN+ B cells, and CD83+ B cells are in the peripheral blood of patients with SPMS, DC-SIGN+ B cells activation and B-T cell interactions (CD83) are included in SPMS. Meanwhile, the increasing status of activated B cells is shown in patients with neurodegeneration. Thus, the presence of autoantibodies has been found to be correlated with the disease progression and axonal loss in PMS (Fraussen et al., 2016). OCMB (oligoclonal IgM bands) in RRMS associates with increased EDSS, progression index and the possibility to convert to SPMS. LS-OCMB+MS patients have a shorter time to convert

to SPMS and show an increased MSSS. All these studies provide evidence that the lipid-specific IgM antibodies involve neurodegeneration and progression. Meanwhile, B cells and antibodies are regarded as promotion factors of CNS inflammation in progressive (Fraussen et al., 2016). The most lymphocytes of inflammatory infiltration are MHC Class I restricted CD8+ T-cells, which expand in lesions apparently (Lassmann and van Horssen, 2011).

1.2.8 Biochemistry

Demyelinated axons vary extents of neurofilament fragmentation, with a decreased numbers of mitochondria and microtubules (Dutta and Trapp, 2014). Mitochondrial dysfunction is associated with progression, which can produce tissue injury by three mechanisms: energy failure, the induction of apoptosis and increased production of reactive oxygen species (Lassmann and van Horssen, 2011, Plantone et al., 2016). Respiratory-deficient mitochondria contribute to demyelination and neurodegeneration (Bradl and Lassmann, 2009, Lassmann and van Horssen, 2011). In hypoxic lesions, reduced COX1 (the haem-containing protein of Complex IV of the respiratory chain) consist of mitochondrial alterations. Mitochondrial proteins and mRNAs have also shown similar changes in active lesions of chronic MS (Dutta and Trapp, 2014, Bradl and Lassmann, 2009). Meanwhile, the mitochondrial injury may amplify oxidative damage (Lassmann and van Horssen, 2011). Diffusible factors released from reactive oxygen species are available to hyperactive microglia cells and produce excessive neurotoxic factors, which contribute to mitochondrial dysfunction (Bradl and Lassmann, 2009). The central hypotheses of degeneration of chronically demyelinated axons include a broken balance between demand and supply of energy (Dutta and Trapp, 2014). Increasing numbers and more activation of mitochondrial in chronic inactive lesions reflect the higher energy demand (Lassmann and van Horssen, 2011). It is said that the mitochondria which reach chronically demyelinated axoplasm become reconciled and capacity of ATP production is reduced (Dutta and Trapp, 2014, Trapp and Nave, 2008). Accumulation of Na+ produced by energy failure reverses the Na+/Ca2+ exchanger (an energy-independent antiporter) and exchanges exoplasmic Na+ for extracellular Ca2+. If the concentration

exceeds the threshold, the Na+/Ca++ exchanger and activated Ca++ channels will incite Ca2+ excess load then axon degenerate (Dutta and Trapp, 2014, Bradl and Lassmann, 2009, Trapp and Nave, 2008). Finally, axons accumulate intracellular Ca2+ which triggers many metabolic pathways leading to apoptosis (Dutta and Trapp, 2014, Plantone et al., 2016). Overloaded Ca2+ may excite a vicious cycle of degradative enzymes and lead to mitochondrial dysfunction and failure of energy production (Trapp and Nave, 2008). Loss of the Na+/K+ ATPase protein may influence this cycle and accumulation of Ca2+; it indicates that chronically demyelinated axons lose function before degeneration (Dutta and Trapp, 2014, Trapp and Nave, 2008). Axons lack voltage-gated Na+ channels and/or Na+/K+ ATPase. The reduction of ATP impairs Na+/K+ ATPase function. Then the axons that lack voltage-gated Na+ channels are impossible to exchange Na+ for K+. Thus, failure of axolemma repolarisation causes axonal swelling, another characteristic of chronic MS lesions (Dutta and Trapp, 2014, Trapp and Nave, 2008).

1.3 Treatment

MS is currently incurable. Current treatments primarily aim to speed symptoms recovery from attacks, to mitigate the frequency and severity of relapses, to slow the progression of the disease and to manage MS. For patients who develop severe disabilities and impairments, treatments for MS signs and symptoms include physical therapy (together with using mobility aid if need is also helpful to manage leg weakness and other MS-associated gait problems), muscle relaxants (for painful or uncontrollable muscle stiffness or spasms, especially in legs), medications to alleviate fatigue, etc. Other drugs targeting depression, pain, sexual dysfunction, and bladder or bowel control problems that caused by MS may be prescribed.

For MS attacks, corticosteroids, such as oral or intravenous methylprednisolone, are prescribed to reduce nerve inflammation. Plasma exchange (plasmapheresis) given up to 1 month after onset can usefully mitigate persistent deficits although not following disease activity (Weinshenker et al., 1999, Keegan et al., 2002). To modify progression, ocrelizumab (Ocrevus) is the only FDA-approved disease-modifying treatment (DMT) slows worsening of

disability in people with primary-progressive MS, several DMTs are available for relapsing-remitting MS. Treatment options for relapsing-remitting MS include Beta interferons, Ocrelizumab (Ocrevus), Glatiramer acetate (Copaxone), Dimethyl fumarate (Tecfidera), Fingolimod (Gilenya), Teriflunomide (Aubagio), Natalizumab (Tysabri), Alemtuzumab (Lemtrada) and Mitoxantrone. β interferons are prescribed medications to treat MS with mild side effects of flu-like symptoms and injection-site reactions. For oral pills like Tecfidera, common side effects are flushing, stomach pain, diarrhoea, nausea, and vomiting. The side effects of Fingolimod include a headache, diarrhoea, back pain, and abnormal liver tests, for Tysabri involve a headache, feeling tired, and joint pain.

1.4 Ageing and MS

Age of disease onset is a predictor for the likelihood of transforming to SPMS and the severity of disability accumulated. It takes older patients a shorter time to convert to the progressive phase (Rovaris et al., 2006). Ageing relative factors, like hypoxia, mitochondrial dysfunction, and iron accumulation may lead to neuronal damage and disability (Geraldes et al., 2017). Increasing iron load reaches a peak at the time of average evolution to PMS (Lassmann and van Horssen, 2011). In the aged brain, microglia cells activate and express more antigen-presenting molecules, this cytokine together with astrocytes, both activated by regional changes in the expression of inflammatory genes. These phenomena can affect responses to pathological stimulation and may significantly relate to the progressive MS (Bradl and Lassmann, 2009).

1.4.1 Demyelination and remyelination

Age appears to influence the chronic diffuse neurodegeneration of MS as well as capacity for remyelination (Confavreux and Vukusic, 2006a). The age of the patient reduces the efficiency of remyelination, breaking the threshold of a destructive process and repair process (Hampton et al., 2012, Rist and Franklin, 2008). Age determines axonal damage following demyelination as reported (Hampton et al., 2012). Older axons may be essentially more liable to focal immune-mediated demyelinating injury. It is coherent with the proof of fundamental cellular

changes with ageing that involves the accumulation of nuclear and mitochondria mutations followed by damaged repairment of DNA and cellular and organ deterioration (Hampton et al., 2012). In the previous study, both axons of young and aged animals were remyelinated at the recovery stage, but it was evident that the corpus callosum was thinner in the aged animals, as was the magnitude of the macrophage and astrocyte responding demyelination. This phenomenon showed the axon loss in these animals, and it was more conspicuous in the aged animals at both stages. Moreover, the results indicated aged animals had increased vulnerability to demyelination-related axon transection. The young animals were continuing to remyelinate when still on cuprizone (cuprizone is a toxin that induces myelin damage by killing oligodendrocytes resulting in demyelination in both white and grey matter of the CNS) while the aged mice were not, which suggested slower remyelination with age may affect axon degeneration. The consequence of which made demyelinated axons to be exposed to inflammation for a long time and hence cause increased damage. Delayed remyelination and age-related changes may explain the increased axon degeneration (Irvine and Blakemore, 2006).

“Mutual activation of astrocytes and macrophages contribute to oligodendrocyte remyelination and thereby create an environment for the regenerative process. Nevertheless, with the ageing of CNS, these events are out of control, such as the delayed expression of certain elements and dysregulation of the signalling network.” This concept is termed the dysregulation hypothesis (Franklin et al., 2002).

It is apparent that both oligodendrocyte and, to some extent, Schwann cell remyelination in older animals is relatively more persistent, but it can still complete. The remyelination needs time to be extensive and reach completion as the previous experiment results showed. Regarding inflammation and remyelination, this might reflect the retardation of inflammatory response behind delayed remyelination in old animals. Thus, it may suggest that modification of the inflammatory response need alternation of remyelinated rate (Shields et al., 1999).

In a previous study, the researchers reported a focal model of immune-mediated demyelinating damages that causes primary demyelination and axonal injury. The model indicated enhanced susceptibility to axonal damage and insufficient remyelination in aged adult animals. In aged animals, the remyelination was Schwann cell-mediated, comparing to the oligodendrocyte-mediated remyelination in younger rodents. (Hampton et al., 2012).

Another feature of ageing is the accelerated deposition of advanced glycation end-products (AGE) in MS. The receptor for AGE (RAGE) produces pro-inflammatory cytokines and free radicals then maintain the cycle of damage. In MS, concomitant with declined ability to repair the injury, weak antioxidant defence mechanism increases the susceptibility of damage in axons and neurons. Additionally, immune senescence (ISC) reflects an inefficient ability to respond to inflammation. Ageing relates to the alternation of innate immune responses such as dysregulated Toll-like receptors (TLR) responses and production of cytokines and other inflammatory mediators. Moreover, the immune suppression decreases, which suggests that it is more difficult for ageing neurons to keep microglia in a static state. That age impacts neuroinflammation shows the importance of ISC in repair and synaptogenesis environment. (Amor et al., 2014). Chronic CMV infection is regarded as a critical role in ISC (Sanai et al., 2016). Dysfunction and irregular production of T cell upon RRMS and similar changes in PPMS prove early thymic involution and consequently the premature stage of ISC. Similar changes from juvenile MS patients demonstrate the early thymus-related physiological change and may indicate atypical ISC. Age influence on the pathogenesis of MS intrinsically includes the adaptive immune system. The factors to explain early onset ISC may contain chronic immune stimulation which caused by repeated exposure to antigens, accumulation of senescent immune cells and genetic elements promoting primary differentiation (Bolton and Smith, 2018).

1.4.2 Macrophages, microglia, and astrocytes

The phagocytosis and chemotaxis process in senescent macrophages and microglia decline. The first line of defence against pathogens consists of CNS microglia and macrophages

(including CNS-infiltrating macrophages derived from circulating monocytes) by regulating innate and adaptive immunity and constitute (Yin et al., 2017). In a study testing the potential of microglia to engulf amyloid-β fibrils, ageing microglia lost the ability for phagocytosis. Microglia with ageing also showed reduced movement and cellular migration responding laser-induced injury and extracellular ATP (Rawji et al., 2016).

The ability to upgrade pro-inflammatory cytokines seems to decrease in ageing monocyte-derived macrophages but the increase in microglia. There is a phenomenon called microglia priming (Rawji et al., 2016, Amor et al., 2014). With ageing, it is easier to activate the immune system in the CNS. The priming of immune responses may cause the increased risk of neurodegenerative diseases in the aged humans and peripheral infections related to MS (Amor et al., 2014).

The macrophage response is delayed in old animals, indicating that it may determine the efficiency of remyelination (Ludwin, 1980, Graca and Blakemore, 1986). This hypothesis was examined by using clodronate liposomes to deplete circulating monocytes and reduce the macrophages, many of which were monocyte-derived, within the toxin-induced demyelinating lesions. This led to a reduced oligodendrocyte remyelinating process of lysolecithin lesions, further proving the proremyelination role for the macrophage (Franklin et al., 2002).

It is showed that the ability of aged macrophages and microglia to adopt a regulatory phenotype is harmed. There are several possible mechanisms: First, ageing microglia loses inhibitory ligand-receptor interactions. Second, the accumulation of misfolded proteins such as amyloid-β occurs during normal ageing (amyloid- β is found to elevate pro-inflammatory cytokines in microglia). Third, the increased expression of transforming growth factor-b with age may cause damage to the capacity of secreting anti-inflammatory cytokines in microglia. This transforming growth factor-b-mediated impairment can be attributed to the downregulation of interferon regulatory factor-7, a transcription factor in inducing microglia to an anti-inflammatory phenotype (Rawji et al., 2016).

With age, astrocytes lose some of their neuroprotective properties as well as obtain neurotoxic properties. Thus, senescent microglia release more NO and astrocytes ability to fight the increased oxidative stress decreased, which would result in axon injury by calcium-mediated mechanisms (Irvine and Blakemore, 2006).

Ageing influences several brain functions which are adjusted by astrocytes, e.g., synaptic plasticity, metabolic balance, and BBB permeability. Enhanced expression of the glial fibrillary acidic protein (GFAP) and vimentin are most common. Additionally, high-mobility group box-1 (HMGB1) protein produced by astrocytes facilitates the secretion of inflammatory factors. Inflammatory mediators generate a vicious age-associated cycle with lower chronic inflammation and aggravated neurodegenerative processes. These occur via NO-induced pathways, and ROS (Reactive Oxygen Species)-mediated dysfunction in mitochondria and endoplasmic reticulum (Correale and Farez, 2015).

Accumulated myelin debris in the aged brain may lead to failure of remyelination (Jadasz et al., 2012). Ageing connects with senescence and clearance dysfunction of microglia. The repeated senescence with a decreased self-renewal potential is shown in aged human brains. Ageing microglia are featured by the lipofuscin granules, reduced processes complexity, altered granularity and increased mRNA expression of pro-inflammatory cytokines. In an experiment, older rats showed slower recruitment of phagocytic cells and insufficient clearance of myelin debris after a toxin-induced demyelination lesion, which reflected the delayed remyelination. It was suggested that inefficient remyelination associated with inhibition of neuronal precursors differentiation and impairment of neuroregeneration related to uncleared myelin debris (Neumann et al., 2009, Sellner et al., 2011). The accumulation of glycosaminoglycan hyaluronan (HA) in demyelinated lesions in MS has been found and that HA can impede remyelination by blocking oligodendrocyte precursor cell (OPC) maturation. Removing such cytokines and myelin debris might promote the differentiation of OPCs and provide an environment for an intrinsic regenerative process to succeed remyelination of damaged axons. These processes are absent in aged animals (Neumann et al., 2009, Mao

and Reddy, 2010, Rist and Franklin, 2008). In a model of focal white matter demyelination, except for insufficient clearance of myelin debris, the delayed remyelination with age is correlated with the change of the retinoid X receptor pathway in macrophages. The clearance of myelin debris and remyelination are enhanced while young macrophages are introduced to the ageing demyelinated CNS (Rawji et al., 2016).

1.4.3 Oligodendrocyte precursor cells

Remyelination is comprised of response to demyelination and restoration of myelin sheaths. The OPCs, one of the stem/precursor cells, mediate the process of remyelination (Jadasz et al., 2012, Rist and Franklin, 2008). On the cellular level of remyelination, the first phase includes the OPCs activation, which then proliferates and refills an area of demyelination. After the recruitment of OPC, the second phase of the repair process starts, in which the recruited cells differentiate into cells that undertake the demyelinated axons and differentiate into remyelinated oligodendrocytes. During demyelination, OPCs are mitotically active and induce the expression of oligodendrogenic genes. In the demyelination, the activated astrocytes and microglia release mitogens acting on OPCs which probably mediate the proliferation stimulus. Furthermore, the OPCs recruitment requires coordinated interaction between cell surface molecules and extracellular matrix. The successful recruitment allows OPCs to interact with demyelinated axons, differentiate, express myelin proteins and wrap around axons finally then create new myelin sheaths'' (Jadasz et al., 2012, Rist and Franklin, 2008).

Ageings influence the efficiency of remyelination in the recruitment and differentiation of OPCs. With ageing, the reduced rates of remyelination reflect decelerated recruitment and differentiation of OPC, or a decreased population of OPC (Simpson et al., 2007, Rist and Franklin, 2008, Hampton et al., 2012, Franklin et al., 2002, Sim et al., 2002). Efficient remyelination requires to improve both OPC recruitment and OPC differentiation. One explanation for delayed differentiation is the inhibitory factors or incapability of response to these factors. The axons with remyelinating OPCs are likely to be the source of such elements (Franklin et al., 2002, Sim et al., 2002).

The delayed onset of platelet-derived growth factor (PDGF) mRNA expression may be responsible for the impaired recruitment of OPCs (Franklin et al., 2002). The relation between the delayed PDGF expression and accumulation of OPCs occurs in aged animals is coherent with PDGF-mediated OPC recruitment. Additionally, the mitogen-induced proliferation of OPCs is inhibited by transforming growth factor-β1 (TGF-β1), and this molecule might initiate differentiation. In aged animals with impaired differentiation, the peak expression of Insulin-like growth factor (IGF1) and TGF-β1 facilitate OPC differentiation is delayed (Franklin, 2002, Sim et al., 2002, Franklin et al., 2002). Additionally, the changes of axonal expression in Notch ligands such as jagged may be an inhibitor to OPCs differentiation by activating Notch receptors. That is, differentiation-inducing signals may disappear. In other words, the demyelinated axons with the aged CNS are becoming less sensitive to remyelination (Sim et al., 2002, Franklin et al., 2002).

Disease proceeds with age, repair, remyelination, and other physiological functions are weakened. The OPC recruitment of aged animal models is intact, but not the differentiation into myelination oligodendrocytes. Elderly humans display inefficient remyelinating capabilities with a decreased remyelination process (Mahad et al., 2015).

Remyelination is partially inhibited by preventing recruitment to the repairing lesions of blood-derived monocytes from the young parabiotic partner. Both youthful monocytes and other factors contribute to remyelination as reported. Reduced remyelinating activity may not only be caused by changed signals in the environmental but also implies epigenetic changes which limit differentiation (Mahad et al., 2015, Ruckh et al., 2012, Jadasz et al., 2012).

Ageing affects OPCs during the remyelination process. The delayed inflammatory response is seen in older animals. Moreover, the expression of different growth factors that are secreted in the lesion and influence the proliferation, migration, and differentiation of OPCs are also delayed in aged animals (Jadasz et al., 2012, Rist and Franklin, 2008).

Altered cell-intrinsic environment limits the ability of aged OPCs to remyelinate (Jadasz et al., 2012). Perinatal OPCs have different intrinsic properties from their adult counterparts. Some properties of OPCs change with age and differ from the adult OPCs phenotype, proving the existence of intrinsic changes in ageing OPCs. These age-related differences are revealed by the changes when ageing OPCs are transformed into the same susceptive environment (Rist and Franklin, 2008).

With the ageing of stem and progenitor cell, homeostatic regeneration reduces accompanied by the ageing of an organism. The neurogenic ability and the replicative function of neural stem/progenitor cells are established. Therefore, an age-related decrease in NSPCs leads to reduced neurogenesis (Jadasz et al., 2012).

1.4.4 Disability and progression

The older disease onset age is correlated with higher risk of primary progressive MS, earlier conversion to SPMS, also earlier reaching disability endpoints (Scalfari et al., 2011). In a study with 2156 MS patients, the demographic, clinical, and healthcare characteristics were compared for those over and under 65 years, disability was found to be critically higher among older patients. Regarding psychology, the frequency of MS-associated symptoms in older patients is higher (Sanai et al., 2016). Considered the activation, differentiation, and maturation of progenitor and stem cells, age-related restrictions in cellular homeostasis and plasticity might cause inefficient remyelination and impair clinical recovery (Jadasz et al., 2012).

The deficit in CD4+ T-regulatory cells is associated with increased autoimmune neuroinflammation. The age-related phenomenon may appear earlier in MS patients due to the already dysregulation of the immune system, thereby initiating the transition from a remitting-relapsing to the secondary progressive clinical phase (Sanai et al., 2016).

Magnetised transfer ratio (MTR) is an MRI tool to examine the macromolecule exchange in parenchymal tissue and is considered to reflect myelin status. The MTR is critically higher in

lesions with remyelination than demyelination (Schmierer et al., 2004). Ageing modifies the tissue response to inflammation by measuring MTR activity and comparing two age cohorts after correction of disease duration and lesion load as reported (Newbould et al., 2014). Furthermore, the remyelination of the compact white matter retards and terminates, consistent with progressive neurodegeneration as demonstrated in imaging studies (Sanai et al., 2016).

1.4.5 Iron

Accumulating iron in ageing CNS results from an increase of the ferritin protein in oligodendrocytes and microglia. This accumulation increases the intracellular deposition of ferrous iron (Fe2+) that gets catalysed to ferric iron (Fe3+) and leads to the oxidative stress and cell death, causing neurodegeneration. Moreover, in the inflammatory processes of MS, more deposition of iron is via increased iron-rich macrophage migration, free radical production, and cell death. The increased iron deposition is caused by the destroyed iron-rich oligodendrocytes (Sanai et al., 2016).

With ageing, the accumulated iron is stored in myelin, oligodendrocytes, and microglia in the form of non-toxic ferric (trivalent, $Fe^{3}+$) bound to ferritin. In MS lesions, damaged myelin and oligodendrocytes release iron which converts to the divalent ferrous form ($Fe^{2}+$) into the extracellular space, and then the toxicity of reactive oxygen species can be increased. Liberated iron then accumulates partly in microglia and macrophages as well as being transported to the perivascular space. In patients with the progressive clinical course, the accumulation of oxidised lipids is associated with the iron overload. Significant diffuse neurodegeneration and atrophy also appear in the healthy brain with iron accumulation, even at early stages of MS (Mahad et al., 2015).

1.4.6 HSCs-- hematopoietic stem cells

The reduced capacity to regenerate is one of the features of senescence. Stem and progenitor cells determine the potential of regeneration, which suggests that the decline of regeneration with age would partly be caused by damaged stem and precursor cell function. On the molecular level, mutations in nuclear and mitochondrial DNA accumulate with age lead to a

cascade of other changes which contribute to the aged phenotype. Two examples of cell types are hematopoietic stem cells (HSCs) and muscle satellite cells (Rist and Franklin, 2008). Gene expression studies comparing young and aged muscle satellite cells discovered differences in the transcriptional profiles in the cellular ageing (Bortoli et al., 2003).

Senescent HSCs have the insufficient regenerative ability. Both intrinsic factors and extrinsic factors affect the ageing of HSCs. Components of intrinsic features involve telomere shortening, epigenetic changes, and accumulation of DNA damage. Also, genomic preservation is essential for HSCs ageing as reported. Furthermore, decreased function of self-renewal and the loss of cell polarity might be interpreted as dysregulation of massive cell cycle regulatory proteins in senescence of HSCs (Rawji et al., 2016).

1.4.7 Gene expression

With ageing, there is a reduced gene expression for synaptic plasticity, vesicular transport, and mitochondrial function, which is a result of DNA damage by oxidative stress. Hence, it is possible that ageing and modification of genes can to some extent contribute to the rejuvenation of neurodegeneration. In a previous model for disease progression, neuronal dysfunction was early, and an adaptive state in the neuron appeared, figured by altered expression of genes and function with age (Imitola et al., 2006). Other types of changes include epigenetic modifications, which depend on the type of modification and their reversibility (Rist and Franklin, 2008).

1.4.8 Subventricular zone

Ageing subventricular zone (SVZ) cells lose the ability of proliferation and recruitment in experiments. Thus, aged neural precursors may be incapable of responding to demyelination-induced signals. With ageing, neural precursors function is changed by the extracellular environment. Chronic infusions of EGF, TGF-α, and FGF-2 facilitate the SVZ precursor proliferation and mobilisation in the proximal parenchyma and the differentiation into neurons, astrocytes, and oligodendrocytes. In MS, the failure in remyelination is likely to be influenced by modifications of growth factor expression.

There is data showing that proliferation and recruitment of the SVZ population following demyelination was inefficient in older animals. Furthermore, injecting growth factors like FGF-2 and TGF-α strengthened cell proliferation but not improve cell recruitment in old animals. The neural precursors from older animals proliferate slowly, and growth factors can restore this rate.

In vitro, young and aged neural precursors have similar potentials to migrate, while the migration rate and the recruiting process are less efficient in the aged one. It indicates that the recruitment of aged neural precursors may result from age-related modifications of signalling in the environment. Moreover, because the glial volume and extracellular space reduce in the ageing brain, the aged CNS medium comprises an environment not allowing the recruitment in the parenchyma via blocking cells from the rostral migratory stream (RMS) (Decker et al., 2002).

1.5 Inheritance of Ageing and Family longevity

It is suggested that 25-30% of the variation in human life expectancy may be under genetic control, and the rest is caused by environment, injuries, and accidents (Bozzini and Falcone, 2017). This view is also reported in a Danish twin study. The inheritance of life expectancy is 0.23 for men and 0.20 for women as estimated (Browner et al., 2004, De Benedictis et al., 2001). Meanwhile, the progeroid syndromes with expedited ageing and mortality at an early age are due to genetic effect. Therefore, longevity genes delay the rate of ageing and may slow cellular senescence, improving resilience, or increasing resistance to stress. These phenomena are accordant with the observation that offspring of centenarians have the less age-related disease and better health, which indicates that they inherit a longevity gene from their long-lived parent protecting them from illness (Browner et al., 2004).

Most centenarians and near-centenarians usually exist in families. This phenomenon is mainly due to cases of exceptional longevity (beyond age 90 years), which primarily has a genetic basis (Browner et al., 2004). A previous study has reported that excessive longevity clusters

in families and this pattern is in accordance with a relatively simple model of inheritance involving the additive effects of one or more loci. It also has been found that extreme longevity in a population of the Utah Population Database has a solid familial basis. These patterns are accordant with the Mendelian inheritance of longevity-related genes. First degree relatives of those living to the 95th percentile have higher possibility to live to the 95th percentile compared with a controlled group (Kerber et al., 2001).

Meanwhile, the proportion of age-specific death is critically lower in the children of long-lived parents. Previous study results showed an evident familial inheritance of longevity, which may be attributed to either a genetic or environmental component in the family (Gudmundsson et al., 2000, Caselli et al., 2006). The genetic factor of longevity can be interpreted as the absence of disease-related genes, or the presence of some protective genes that compromise senescence (Gudmundsson et al., 2000).

Most longevity exists in a maternal line of the family as reported. Nonetheless, other studies claim that the male branch is more important compared to the female line. The sex-dependent inheritance of lifespan may indicate mitochondrial DNA or sex chromosomes-linked components (Caselli et al., 2006). Some studies indicate that the familial longevity is transmitted at old ages, which among the Chinese is the same sex-linked between parents and offspring. In other words, there are father-son and mother-daughter pattern of longevity, but a non-significant or relatively weak inheritance of longevity among father-daughter or mother-son. It is also demonstrated that these long-lived elders are prone to have long-lived parents. It is pointed out same-sex inheritance, although having long-lived parents contributes to children's longevity. Nevertheless, no relation has been found between the life expectancies of elderly men and their mothers, and that of elderly women and their fathers. With the top 10% of age at death of parents, the exceptional life expectancy of a mother has an absolute positive but not significant impact on the longevity of a son as reported (You et al., 2010).

1.6 MSSS (Multiple Sclerosis Severity Scores) and EDSS (Expanded Disability Status Scale)

The MSSS is a useful tool for the disease progression comparisons by applying data from medical assessment. The Global MSSS can be considered as a measurement to compare the disability in the future. Although it is helpful for comparing different patients groups, fluctuated disease status prevents its use from predicting future disability in this disease.

Global MSSS was created from 9,892 European patients. The MSSS for an individual patient is verified by finding the corresponded column according to the patient's EDSS and the corresponded row according to the disease duration of patients. The colour-coded deciles represent the development of disease at different time points (Roxburgh et al., 2005).

	0	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	EDSS
1	0.67	2.44	4.30	5.87	7.08	7.93	8.64	9.09	9.35	9.50	9.63	9.74	9.84	9.90	9.94	9.97	9.98	9.98	9.99	
2	0.53	2.01	3.69	5.24	6.46	7.27	7.98	8.58	8.95	9.18	9.38	9.59	9.79	9.88	9.93	9.97	9.99	9.99	9.99	
3	0.45	1.77	3.34	4.82	6.00	6.81	7.54	8.14	8.55	8.83	9.07	9.35	9.63	9.77	9.86	9.92	9.97	9.98	9.99	
4	0.35	1.45	2.87	4.27	5.41	6.24	6.98	7.65	8.12	8.42	8.70	9.08	9.47	9.68	9.80	9.88	9.95	9.98	9.99	
5	0.30	1.28	2.60	3.90	4.95	5.79	6.58	7.26	7.75	8.08	8.38	8.83	9.32	9.60	9.76	9.86	9.95	9.98	9.99	
6	0.25	1.13	2.33	3.54	4.55	5.38	6.14	6.81	7.33	7.66	7.98	8.50	9.08	9.45	9.68	9.81	9.93	9.97	9.99	
7	0.24	1.04	2.10	3.17	4.13	4.96	5.75	6.46	6.98	7.32	7.65	8.24	8.91	9.33	9.59	9.76	9.90	9.95	9.99	
8	0.21	0.94	1.92	2.93	3.81	4.57	5.36	6.10	6.61	6.95	7.32	7.97	8.71	9.21	9.55	9.74	9.89	9.96	9.99	
9	0.21	0.88	1.76	2.65	3.45	4.17	4.93	5.64	6.14	6.50	6.90	7.65	8.53	9.09	9.47	9.70	9.87	9.95	9.99	
10	0.19	0.78	1.53	2.34	3.10	3.79	4.55	5.28	5.77	6.14	6.58	7.39	8.31	8.92	9.34	9.61	9.83	9.94	9.99	
11	0.17	0.71	1.40	2.13	2.82	3.46	4.21	4.94	5.42	5.82	6.30	7.18	8.15	8.79	9.24	9.52	9.78	9.92	9.98	
12	0.16	0.64	1.28	1.98	2.64	3.25	3.94	4.63	5.13	5.54	6.03	6.92	7.93	8.63	9.13	9.43	9.71	9.88	9.97	
13	0.13	0.57	1.14	1.80	2.44	3.05	3.70	4.38	4.91	5.32	5.80	6.74	7.83	8.55	9.03	9.34	9.65	9.85	9.96	
14	0.11	0.49	1.03	1.70	2.33	2.91	3.55	4.26	4.82	5.23	5.70	6.56	7.59	8.34	8.86	9.20	9.57	9.82	9.95	
15	0.10	0.45	0.99	1.64	2.26	2.82	3.44	4.14	4.68	5.09	5.51	6.33	7.41	8.17	8.70	9.11	9.51	9.78	9.94	
16	0.09	0.38	0.85	1.42	1.99	2.56	3.17	3.86	4.41	4.81	5.18	6.00	7.14	7.97	8.54	9.04	9.49	9.75	9.94	
17	0.05	0.32	0.76	1.28	1.77	2.30	2.95	3.65	4.17	4.55	4.94	5.74	6.89	7.77	8.38	8.99	9.52	9.79	9.96	
18	0.04	0.26	0.66	1.12	1.57	2.09	2.70	3.37	3.89	4.27	4.62	5.43	6.62	7.54	8.23	8.94	9.51	9.78	9.96	
19	0.05	0.28	0.63	1.00	1.39	1.89	2.50	3.19	3.72	4.12	4.49	5.35	6.59	7.51	8.22	8.98	9.57	9.81	9.96	
20	0.05	0.26	0.59	0.94	1.29	1.71	2.29	2.99	3.51	3.93	4.30	5.15	6.43	7.45	8.23	8.98	9.58	9.80	9.95	
21	0.05	0.30	0.66	1.02	1.39	1.77	2.34	2.97	3.43	3.83	4.21	5.09	6.35	7.33	8.08	8.87	9.49	9.77	9.96	
22	0.04	0.23	0.54	0.90	1.28	1.66	2.20	2.82	3.29	3.69	4.09	5.04	6.35	7.35	8.10	8.84	9.42	9.73	9.95	
23	0.05	0.27	0.58	0.91	1.26	1.64	2.19	2.78	3.21	3.69	4.19	5.16	6.47	7.46	8.20	8.87	9.43	9.75	9.95	
24	0.05	0.24	0.52	0.86	1.25	1.63	2.15	2.71	3.09	3.52	4.01	5.03	6.36	7.38	8.15	8.81	9.39	9.74	9.96	
25	0.05	0.23	0.47	0.77	1.15	1.56	2.05	2.53	2.84	3.21	3.74	4.88	6.26	7.24	8.00	8.73	9.35	9.75	9.98	
26	0.05	0.20	0.45	0.78	1.17	1.58	2.08	2.63	2.99	3.40	3.95	5.02	6.39	7.44	8.21	8.89	9.48	9.80	9.96	
27	0.05	0.22	0.48	0.78	1.15	1.56	2.03	2.56	2.91	3.29	3.86	4.93	6.33	7.38	8.14	8.91	9.56	9.85	9.98	
28	0.04	0.17	0.40	0.74	1.16	1.52	1.88	2.39	2.76	3.04	3.46	4.54	5.99	7.07	7.90	8.75	9.45	9.80	9.98	
29	0.03	0.18	0.47	0.80	1.19	1.51	1.79	2.27	2.68	3.01	3.41	4.35	5.68	6.76	7.66	8.62	9.38	9.75	9.96	
30	0.01	0.13	0.45	0.82	1.19	1.45	1.69	2.23	2.75	3.13	3.50	4.35	5.61	6.66	7.54	8.47	9.27	9.67	9.91	
Years																				

=1st Decile
=2nd Decile
=3rd Decile
= 4th Decile
= 5th Decile
= 6th Decile
= 7th Decile
= 8th Decile
= 9th Decile
= 10th Decile

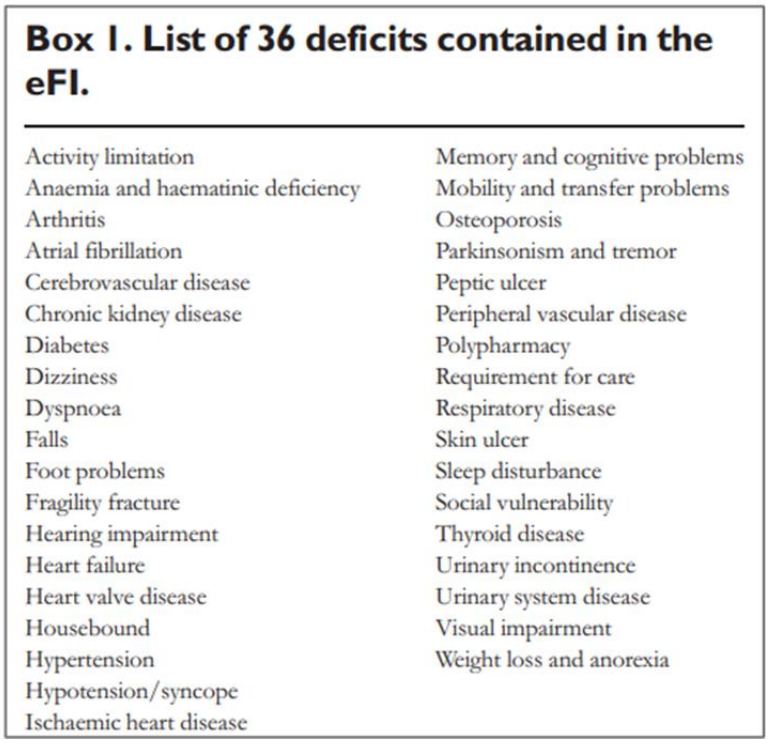
EDSS is a scale for quantifying disability in MS and monitoring changes in different disability levels over time. It is widely used for clinical trials and assessment. The scale starts from 0 to 10 and increases with 0.5-unit that stands for higher levels of disability. EDSS scores 1.0 to 4.5 refer to patients with MS who can walk without any aid and depends on measurements of impairment in 8 functional systems. EDSS scores 5.0 to 9.5 are based on the walking

impairment. The criticism of this scale is due to the reliability in walking as the primary measure of disability (Kurtzke, 1983).

1.7 Frailty Index

The frailty index (FI) is a scale to measure the health level for older people - as a proxy measurement of ageing and vulnerability to poor outcomes (Rockwood and Mitnitski, 2011).

It is defined by the proportion of individual deficits present out of the all age-related health parameters included (Mitnitski et al., 2001). An FI can be acquired by collecting health deficits from the routinely medical record in primary and secondary care settings. These data involve diseases, signs, symptoms, laboratory abnormalities, cognitive impairments, and disabilities in activities of daily living (Rockwood and Mitnitski, 2011).



Box 1. List of 36 deficits contained in the eFI.

Activity limitation	Memory and cognitive problems
Anaemia and haematinic deficiency	Mobility and transfer problems
Arthritis	Osteoporosis
Atrial fibrillation	Parkinsonism and tremor
Cerebrovascular disease	Peptic ulcer
Chronic kidney disease	Peripheral vascular disease
Diabetes	Polypharmacy
Dizziness	Requirement for care
Dyspnoea	Respiratory disease
Falls	Skin ulcer
Foot problems	Sleep disturbance
Fragility fracture	Social vulnerability
Hearing impairment	Thyroid disease
Heart failure	Urinary incontinence
Heart valve disease	Urinary system disease
Housebound	Visual impairment
Hypertension	Weight loss and anorexia
Hypotension/syncope	
Ischaemic heart disease	

Fig 1. List of 36 deficits contained in the eFI (Clegg et al., 2016).

FI = (number of health deficits present) ÷ (number of health deficits measured)

For example, a person with 20 of 40 deficits, the FI score will be 20/40 = 0.5. (Clegg et al., 2016). The FI makes the most of the high redundancy in the human organs. Therefore, it can

be replicated among different databases with different items and numbers of items (Pena et al., 2014). The standard procedures to create an FI are found in an open publication (Searle et al., 2008).

1.8 Clinical, MRI, and CSF parameters of Disability Progression in MS

The cited tables show the clinical parameters of MS disability progression reported in longitudinal natural history studies (Gajofatto et al., 2013).

Table 1. Clinical markers (Gajofatto et al., 2013)

Marker	*Findings*
Male gender	Shorter time to walking assistance need (i.e., EDSS score 6) and/or transition to the SP phase
Older age	Older age at onset associated with shorter time to EDSS 6 and/or to SP phase
Progressive course	PP MS patients reach relevant disability milestones in a significantly shorter time compared to RR MS cases
Multifocal presentation	Symptoms/signs of multiple CNS sites involvement at onset are associated with shorter time to EDSS 6 and/or SP phase compared to the monofocal presentation
Motor symptoms	MS patients with pyramidal and/or cerebellar involvement at onset reach EDSS 6 more rapidly compared to patients with optic nerve or sensory symptoms (no influence on time to SP MS)
Incomplete recovery	Shorter time to EDSS 6 and/or transition to the SP phase

Relapse rate	Shorter time to second attack and higher relapse number in first 2–5 years after onset are associated with more rapid progression to EDSS 6 and transition to the SP phase

EDSS: expanded disability status scale; PP: primary progressive; RR: relapsing-remitting; SP: secondary progressive.

Table 2. MRI markers (Gajofatto et al., 2013)

Marker	*Disease phase*	*Findings*
T2 lesion load	CIS	Good association with conversion to definite MS
	Later in the RR phase	Weak association with disability (EDSS) progression
T1 lesion load	RR	The change in T1 lesion volume correlated more strongly with disability progression than did T2 lesion volume change
Brain Atrophy	CIS, early RR, and PP	Whole brain and central atrophy were good predictors of EDSS at 10 years (R^2= 0.74)
GM atrophy	Early RR and PP	A good relationship with disability progression (definitely better than WM atrophy)
Regional GM atrophy	CIS, RR, and PP	Good correlation with disability progression and cognitive dysfunction: the rate of thalamic atrophy in MS subjects was correlated with changes in EDSS, and

		cerebellar cortical atrophy was indicated among the predictive parameters of progression in those RR MS patients who converted to the SP phase
GM lesion load	Early and long-standing RR	Good association with GM atrophy and progression of physical and cognitive disability
Spinal cord area	CIS and early RR	Cord area, rather than T2 lesion load, might have a role in predicting the accumulation of disability
GM magnetisation transfer ratio	CIS, RR, and SP	GM magnetisation transfer ratio histogram peak height and average lesion magnetisation transfer ratio percentage change after 12 months were independent predictors of disability worsening at 8 years (R^2= 0.28)
GM mean diffusivity and fractional anisotropy	RR and PP	Average GM mean diffusivity identified patients with high risk of disease progression over the following 5 years, and change in fractional anisotropy of normal appearing GM (β = 0.523) and disease duration (β = 0.342) were independent predictors of EDSS change

CIS: clinically isolated syndrome; EDSS: expanded disability status scale; GM: grey matter; PP: primary progressive; RR: relapsing-remitting; SP: secondary progressive; WM: white matter

Table 3. CSF markers of MS disability progression reported in longitudinal studies (Gajofatto et al., 2013).

Marker	*Findings*
14-3-3 protein	14-3-3 positive CIS patients group reached more frequently EDSS $\geqslant$ 2.0 at the end of the 32 months follow-up period compared to 14-3-3 negative CIS cases (57% versus 20.5%; $p = 0.05$)
	14-3-3 positive patients had higher EDSS progression rate over 8.5 months following lumbar puncture
Tau	RR MS patients with higher CSF tau experienced a more rapid one point increase in the EDSS score during a mean follow up of 3 years
NFL	A significant correlation between CSF NFL levels and EDSS progression over 10 years
	Higher MSSS and a higher rate of conversion from RRMS to SPMS at 14 years follow up in cases with high CSF NFL levels compared to those with undetectable or intermediate NFL levels
NFH	Correlation with EDSS at 3 years follow up in relapsing and progressive MS patients ($r = 0.54$; $p < 0.01$)
	Correlation with EDSS score 6–8 weeks after relapse onset ($r = 0.46$; $p = 0.04$)
IgM OB	MS patients with positive IgM OB recognising myelin lipids reached higher disability compared to cases with positive IgM OB not-recognising myelin lipids over a mean follow-up time of 61 months (mean EDSS score 2.2 ± 0.2 versus 1.2 ± 0.2; $p = 0.02$)

	Positive IgM OB was an independent predictor of the probability of reaching EDSS score of 3 or 4 in RR MS patients at 5 and 10 years follow up endpoints
Chitinase3-like1	High levels were associated with disability progression during follow-up in CIS patients who converted to MS
Cystatin C	Correlation with EDSS score at last visit (median follow-up = 6 years) in patients with recurrent myelitis and spinal onset MS ($r = 0.69$; $p = 0.03$)

CIS: clinically isolated syndrome; NFH: neurofilament heavy chain; NFL: neurofilament light chain; OB: oligoclonal bands.

1.9 Current Knowledge Gaps

Some susceptible genes and risk factors for disability progression and of MS have been described. Nevertheless, relatively little is explored concerning the effects of genetic or epigenetic predictors on disability progression. Genetic research aiming to find a prognostic marker for MS patients are relatively rare. We are aware of significant hereditary factors affecting longevity. Hence, we decided to explore that whether the inherited component in family longevity is influential to disability progression among MS patients. The priority in this study is investigating the relation between family longevity/medical history and disability progression of MS patients, and if a correlation exists, how disability measured with the EDSS, is affected by family longevity. We hypothesised that the longer patients' parents or grandparents live, the slower progression of disease disability will be.

Methodology

2.1 Ethical and regulatory aspects

The study was undertaken at Nottingham University Hospital. The MS service is a part of the National MS Register, which is a research-oriented register and patients have been consented to take part in research activities and consented to be contacted for MS-related clinical trials (11/SW/0160).

700 Patients in Nottingham are participants of the Nottingham iMed database out of the total of 4000 patients. As we wanted to explore a larger sample of patients, an ethics application to IRAS – REC has been prepared. That was approved by the NHS Research Ethics Committee NRES South-west-central Bristol.

As the study did not involve any potential harmful treatments or lifestyle alterations the central ethical issue was the confidentiality of data and adequate data storage. Confidentiality of data was addressed by coding all patient data and arranging appropriate secure storage of paper and electronic data (which were labelled with coded ID only). All participating researchers adhered to ICH-GCP and good clinical governance guideline.

The study regimen was executed in Nottingham University Hospital, and the University of Nottingham and all the paper data were kept in secure storage at the Division of Clinical Neurology, University of Nottingham. All the data analysis and electronic data were executed and stored at the University of Nottingham's computers and were password protected. The password was accessible to the chief investigator and the research members only.

2.2 Primary objective

The study collected and compared data from up to 700 patients with clinically definite MS on areas including: family history, individual history and disease deficits (36 comorbidities in the eFI from Fig 1.,which have some overlaps with the MS related comorbidities), estimated EDSS

score (from neurologist report based on eight functional systems), age at the onset, year of diagnosis, disease duration, any record of Disease Modifying Treatments (DMT) and type of MS.

2.3 Secondary objective

Relevant individual and family FI were calculated to measure their health status. In the end, 500 questionnaires were sent by post and 162 questionnaires collected from the MS clinic.

2.4 Feasibility/pilot study

2.4.1 Executive summary

Inclusion criteria: 1) clinically definite MS diagnosed at least 12 months duration.

2) Adults: aged between 18 and 80 years old who can complete the questionnaire with or without assistance.

Exclusion criteria: Inability to understand or complete the consent form and questionnaire information sheet.

a. Description of the structure, questions, and choices in the questionnaire

Patients were required to complete a self-reported questionnaire about their past medical history and their family history. The questionnaire consisted of 2 parts 7 sections in total. The investigated questions concentrated on two parts. Part I (6 sections) focused on family history: frailty condition of each patients' family parents and grandparents from the aspects of longevity (age at death) or current age, lifestyle, frailty conditions. Part II (7^{th} section) – Patients' History: patients' lifestyle and frailty status (symptoms and other comorbidities) were inquired.

Principal questions: 1) parents'/grandparents' chronological age. It is difficult for participants to recall their parents' and even more, grandparents' exact age. Missing data could be reduced if age groups are used instead of the actual age. The choices included several age ranges. According to epidemiology data, most MS patients were aged between 20-40 years old and only adults (>=18 years old) were included. The age choices were designed to start at 36 years old with intervals of 5 units/years for the parents and with a maximum of 100 years of age. As for grandparents, the age intervals were 10 units/years starting from less than 50

years old and ending with 100 years old. People who died before this minimum age range or live after this maximum age range are presumed sporadic cases, the data from the people of this part were merged in the minimum/maximum age. Because most of the participants were young and many of their parents/grandparents were presumably still alive; one question was, whether they were dead or alive, to separate the populations and record the answers as age at death or current age.

2) Lifestyle (smoking and drinking habits). Based on literature review, some lifestyles can affect human longevity as well as MS. For family members section, the choices were categorised for four levels, 'Never,' 'yes, but quit later,' 'not frequent,' 'frequent,' 'not known' was also included for both smoking and drinking habits. For patients, cigarettes per day and drinking units per week were collected. The smoking status (never smoke, ex-smoker or current smoker) was asked and those stopped smoking at the time they answered questionnaires were regarded as ex-smokers. The average drinking units/week were calculated by referencing different units of various kinds of alcohols from the website (https://www.drinkaware.co.uk/understand-your-drinking/unit-calculator).

3) FI (comorbidity or symptom). Considered the application of this index to this research, the 'none of above' and 'do not know' choices were added to avoid missing data to the greatest extent possible.

4) The BMI (body mass index) of patients was collected according to the findings from Anna K Hedström et al. study (2012). The weight and height of patients were asked to calculate.

5) Work status and exercise hours per week about patients were asked for excluding possible confounder in the statistical analysis.

b. Research data resources: Approximately 700 patients at the Nottingham university hospital MS clinic were recruited. All of them were consecutive outpatients and they were selected by age. The first patient was the oldest patients who were still alive. Because data of dead family members (longevity) was particularly important

in this study. All eligible patients were contacted by a letter from the chief investigator, who is a member of their MS clinical care team, to inform them of the study. Patients in this clinic have agreed to be contacted about future research and possible clinical trials. The patient information sheet, consent form, and questionnaire booklet were involved with the letter. The research team and principal investigator contact details were provided to patients for perusing and contacting the research members should they have any questions.

The MS service utilises the iMed system (https://www.imed.org/en/about-imed.html), a bioinformatics software for clinicians treating patients with MS. The clinicians can store and maintain a comprehensive, easily accessible database including histories of patients' medical and treatment. This system also allows clinicians to review, sort and analyse data from all their patients, and optimise their medical care and therapeutic results. In total, 4000 patients have been registered there. It also allows MS doctors and nurses to manage and display all the essential information on necessary demographic information, medical history, neurological status, relapses, treatments, neurological rating scores on various scales (EDSS, Scripps Neurological Rating Scale (SNRS)), and results on MRI, CSF and evoked-potentials tests, etc. Especially, interactive graphical charts in iMed allow clinicians to analyse data of multiple patients quickly, or to do a study on a patient specifically. The secure multi-user support enables clinicians to share a single patient database among different users, distribute proper rights and data access privileges to all users.

The data of non-responders was not collected or analysed, whether the non-responders were younger or less disabled was unknown. The data of adjusted variable for statistical analysis such as patient age, disease onset age/disease duration, current disease type/clinical course, treatment history was collected from medical records with patients consent. All the reviewed medical records were obtained from iMed database or NHS system.

c. Strategy and schedule (solution and time duration): Possible participants were recruited from the MS clinic to save the time of data collection, reduce postal costs and environmental impacts. Patients were sent the information sheet together with their clinic letter appointment. If they want to take part, they will ask the clinical team and be given the questionnaires to fill in while waiting for their consultation.

For the postal questionnaires, patients wish to take part in the study were required to sign the consent form, complete the questionnaires and return them in the prepaid envelope. The consent form was stored separately from the questionnaire on receipt. The patients' information sheet prepared by the research team gives participants adequate information regarding all aspects of the study and information about participation in this study. The investigator or their nominee, e.g., from the research team or a member of the participant's usual care team, informed the participant or their nominated representative (other individual or body with appropriate jurisdiction), of all aspects concerning participation in the study.

Since not all the questionnaires used have been validated in other languages or ethnic groups, the invitation pack was only provided to those who are able to read and understand the study information sheet and questionnaires.

The questionnaire booklet took approximately 10-15 minutes to complete. This project lasted for 3 months.

2.5 The current study type

This study is a single-centre, epidemiological, cross-sectional, and observational study. The information was recorded without manipulating the study environment. This study compared different age groups with many variables simultaneously. It only provided a snapshot of the specific time point and may not offer definite information about cause-effect relationships. Considered that longitudinal study is relatively time-consuming, this project started with a cross-sectional study to find a correlation of variables in most scenarios.

- Questionnaire: All the independent variables and dependent variables are based on literature review. The scale to measure the frailty condition was chosen from Andrew Clegg et al. study (2016), and score to rank the progression of disability in MS was referenced from Roxburgh et al. study (2005). The questionnaire was not tested for face validity with MS patients in this pilot study.
- Postal and non-postal questionnaire: In this study, some questionnaires were given to patients in the MS clinic. The benefits of the non-postal questionnaire are that researchers can supervise the participants and correct mistakes in time. However, the time for patients to finish the questionnaire is not enough sometimes. Therefore, the accuracy and validity of the answers may be affected. For the postal questionnaires, the responders did not indicate whether they required help to complete, participants had access to various connection and may provide completed data with any help. However, the response rate is not that optimal sometimes. The available sample size and the power of the study may be affected because of the low response rate. Without supervision, the reliability of their answers cannot be guaranteed, and the missing data cannot be avoided.
- Statistical analysis

  The association was analyzed using Person or Spearman correlation. The degree of relation was assessed by multiple regression and Cox regression. The independent variables were categorized using principal component analysis (PCA). The association without covariates was analyzed by partial correlation.

The data were summarised using means and standard deviations for normally distributed variables and medians and quartiles for skewed distributions. Pearson/Spearman correlation was used for assessing linear association between variables. For categorical variables, because the sample size was not enough to divide into different groups for ANOVA, all of them were treated as continuous variables by using dummy codes. Mann–Whitney U Test and Student's T-Test were used to compare the difference of EDSS and MSSS between

the clinic and postal questionnaires. Regression method was used to predict the relation between family longevity/FI and disability progression. Meanwhile, Cox regression was applied for assessing the relation between family history/individual frailty and time duration of specific EDSS score. Finally, data-transformations were not applied for the analysis of non-normal data (IBM SPSS_Statistics_24). *P* values of less than 0.05 would be considered as statistically significant. Finally, there was no interim analysis in this project, cases with missing data in the variables of interest were removed to avoid inaccuracy. For the possible confounder variables, the proportion of missing value less than 5% was acceptable. The cases with more than 5% missing data in any variable were deleted when testing the effect on the outcome variable and regression model. The multiple linear regression model was used to explore the relationship between the independent variable together with adjusted variables/confounders and dependent variables. Sub-analysis was applied to the data of each family members as well as participants to find the significant possible confounder variable for the final multiple regression model. Whether the confounders should be put in the regression model was not only depend on *p* values but also based on professional knowledge background. PCA was used for reducing the dimension of various confounders and integrate data, especially for variables of parents. As the information collected from grandparents include various missing data which is difficult to analyze by multiple imputation or extract by PCA, sub-group analysis was applied such as manually dimension reduction-create average age/longevity of grandparents, highest level of smoking/drinking/neurological disease history, highest/lowest FI, correlation and regression were used individually. Finally, for the measurement of longevity in the whole family, the average age of parents and grandparents (both maternal and paternal) were created to explore the familial factor effect on EDSS/MSSS. In addition, partial correlation analysis was utilised to exclude the effect of other clinical markers on MSSS and investigate the real correlation between longevity/ ageing and progression of disease disability.

Principle component analysis

Multivariate analysis sometimes involves several correlated variables. PCA is a powerful method for reducing dimension that can be used for downsizing a substantial set of variables but still keeps most of the information. It is a mathematical procedure that creates a (smaller) number of uncorrelated variables called principal components from several (possibly) correlated variables. The extracted components can explain the variability in the data as much as possible, among which the principal component has the highest correlations with the original variables. It is a useful method to compress data albeit sometimes the dimensions are difficult to interpret.

Partial correlation

Partial correlation is used to measure the association between two variables when controlling or adjusting the effect of one or more additional variables. Spurious correlations occur when two effects have no random relationship in fact but are linked by statistical association. These pseudo correlations are caused by an unobserved third variable that affects the two analysed variables. This hidden variable is also regarded as the confounding, hidden factor, suppressor, mediating variable, or control variable. Partial Correlation is a tool to correct for the overlap of the moderating variable. Usually, this method is only used in the models involving three or four variables, and the relationship is assumed linear.

2.6 Reproducibility

Due to the time, cost, and opportunity of various epidemiology studies, it is essential to test the reproducibility of epidemiology findings.

In this project, the dataset was accessible. The computer code of the figures, tables, and other principal results were created in a human-readable form. Also, the software environment necessary to conduct that code was available. Documentation of the computer code, software environment, and analytical data set allowed researchers to duplicate the analyses or execute in similar data sets. The methods of distribution were utilised for others to access the software, data, and documentation.

Results

3.1 Comparison of responders to the postal questionnaires and questionnaires given in MS clinic

For the post-based questionnaires, 161 questionnaires were sent back in a month, and the response rate was 32.4%. The average age of patients responding to the postal questionnaires was 57.45 years old (SD=9.12), which was higher than that from questionnaires collected in the clinic (49.31±11.25 years old). The average EDSS of patients posted back the questionnaires was 4.89±2.15, which was slightly higher than that of patients responded in MS clinic (4.2±2.35).

A Mann-Whitney U test showed that there was a significant difference (U =10733, *p*= 0.006) between the EDSS for the clinic group compared to the group responded by post. The median EDSS was 4.656 for the clinic group compared to 5.788 for those sent back by post. It seemed that the postal responders were relatively more disabled. However, there was a non-significant difference of MSSS score for clinic responders (M=4.71, SD=2.79) and postal responders (M=5.01, SD=2.59); t (321) = -0.998, *p*=0.319. All in all, there was no big difference between the disability of the postal responders and clinic responders.

The proportion of missing data for postal questionnaires was 20.69% (only 13 variables of interest were counted), which was lower than that from clinic questionnaires, 23.17%. Therefore, the completeness of questionnaires from postal responders was very similar to that of patients who completed the questionnaire in the MS clinic.

In total, 323 participants were recruited (Male=91, Female=232, female-to-male ratio was 2.55). Among the participants, 180 had RRMS (55.7%), 118 had SPMS (36.5%), and 25 had PPMS (7.7%). The average disease duration for patients in this project was 17.73 years (SD=10.82). The average patient age was 53.43 years old (SD=10.93). The average disease onset age of the participants was 35.71 years old (SD=10.49). Most patients had MS when they were 35 years old. The most common incidence age for women was 35 years old, for

men was 36 years old. The maximum incidence age in this project was 59 years (men) to 64 years (women).

The average EDSS for all the participants was 4.5 (4.5±2.3). The modal EDSS was 6.5. The average MSSS for all the patients was 4.86 (4.86±2.7). The mode of MSSS was 5.61.

The age of the patient and frailty score correlated (Spearman correlation coefficient, r=0.449, n=321, p<0.000). In terms of Spearman correlation coefficients of parents and grandparents, for mother it was r=0.374, n=315, p<0.000, for father it was r=0.44, n=296, p<0.000, for maternal grandmother it was r=0.053, n=202, p=0.451, for maternal grandfather it was r=0.168, n=165, p=0.031, for paternal grandmother it was r=0.317, n=137, p<0.000, for paternal grandfather it was r=0.157, n=116, p=0.093, these indicated that apart from maternal grandmother and paternal grandfather, the current age/age at death was positively correlated with frailty level. Also, it could be seen that relations of the FI with parents were slightly higher than grandparents.

3.2 Clinical parameters

The gender, treatment history, patient age, disease onset age, disease duration, disease type was examined and tested for correlations with EDSS and MSSS.

The Spearman correlation coefficients for EDSS were assessed, the significant results were found for patient age (r=0.414, n=323, p<0.000), disease duration (r=0.415, n= 323, p<0.000), and disease course (RRMS vs SPMS vs PPMS) (r=0.666, n=323, p<0.000). The results were found to be non-significant for gender (r=-0.067, n=323, p=0.232), treatment history (r=0.02, n=323, p=0.721), and disease onset age (r=0.021, n=323, p=0.712).

The results of clinical parameters and MSSS are summarised in Table 4.

The Pearson correlation coefficients of MSSS were assessed and results were reported to be significant for patient age (r=0.234, n=323, p<0.000), disease onset age (r=0.301, n=323, p<0.000), and disease course (RRMS vs SPMS vs PPMS) (r=0.493, n=323, p<0.000). The

non-significant results were found for gender (r=-0.059, n=323, *p*=0.29), treatment history (r=0.013, n=323, *p*=0.819), and disease duration (r=-0.054, n=323, *p*=0.329).

However, if only RRMS cases were considered in the statistical analysis, treatment history had a significant relation with MSSS (r=0.189, n=180, *p*=0.011). Multiple regression for disease treatment history and MSSS showed the significant result when adjusted for patient age, clinical course and disease onset age (R^2=0.349, F (4,318) =42.564). Therefore, treatment history could positively relate with MSSS regardless of disease course when other variables were controlled (*B*=1.081, *p*<0.000). It was not that treatment would lead to higher MSSS, it might be because patients with higher MSSS was on medication. It is also possible that the classification of the type of MS was not exact in the current study.

Cox model showed DMT had a significant effect on the risk of reaching EDSS 6.5 with adjustment of patient age, disease type and disease onset age (adjusted HR=2.011, 95% CI=1.196 to 3.383, *p*=0.008) but not the transition to secondary progressive phase (adjusted HR=1.406, 95% CI=0.863 to 2.290, *p*=0.172). Here, it was noted that treatment increased the twice risk of reaching EDSS 6.5. It might be due to that the patients reached EDSS 6.5 had more severe conditions which need treatment. Also, it is possible that the classification of the type of MS was not exact in the current study. Meanwhile, the selection bias and absence of data about non-responders may contribute to this finding. Cox model showed that disease onset age was significantly associated with 6.8 % increase of risk to reach EDSS 6.5 (HR=1.068, 95% CI=1.045 to 1.192, *p*<0.000) as well as the transformation of progressive course (HR=1.077, 95% CI=1.053 to 1.102, *p*<0.000). Cox model of gender and risk to reach EDSS 6.5 (HR=0.846, 95% CI=0.557 to 1.286, *p*=0.435) / secondary phase (HR=1.167, 95% CI=0.77 to 1.768, *p*=0.467) were shown to be non-significant.

When multiple regression was used for patient age, disease onset age and disease type, about 32% of the variance could be accounted by the model (R^2=0.316, F (3, 319) =49.028, *p*<0.000). When EDSS and disease onset age were put in the regression model, about 75%

of the variance could be explained R^2=0.754, F (2, 320) =491.301, p<0.000. The results are summarised in Table 5 and 6.

Correlations

		MSSS	yourGender	TreatmentHistory	PatientAge	DiseaseOnsetAge	DiseaseDuration	DiseaseType
MSSS	Pearson Correlation	1	-.059	.013	.234**	.301**	-.054	.493**
	Sig. (2-tailed)		.290	.819	.000	.000	.329	.000
	N	323	323	323	323	323	323	323
yourGender	Pearson Correlation	-.059	1	.009	-.007	.035	-.040	-.072
	Sig. (2-tailed)	.290		.877	.905	.534	.478	.196
	N	323	323	323	323	323	323	323
TreatmentHistory	Pearson Correlation	.013	.009	1	-.251**	-.239**	-.021	-.236**
	Sig. (2-tailed)	.819	.877		.000	.000	.703	.000
	N	323	323	323	323	323	323	323
PatientAge	Pearson Correlation	.234**	-.007	-.251**	1	.491**	.534**	.445**
	Sig. (2-tailed)	.000	.905	.000		.000	.000	.000
	N	323	323	323	323	323	323	323
DiseaseOnsetAge	Pearson Correlation	.301**	.035	-.239**	.491**	1	-.474**	.123*
	Sig. (2-tailed)	.000	.534	.000	.000		.000	.027
	N	323	323	323	323	323	323	323
DiseaseDuration	Pearson Correlation	-.054	-.040	-.021	.534**	-.474**	1	.332**
	Sig. (2-tailed)	.329	.478	.703	.000	.000		.000
	N	323	323	323	323	323	323	323
DiseaseType	Pearson Correlation	.493**	-.072	-.236**	.445**	.123*	.332**	1
	Sig. (2-tailed)	.000	.196	.000	.000	.027	.000	
	N	323	323	323	323	323	323	323

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 4. This table shows the Pearson relation between different clinical variables and MSSS.

Source	*B*	*SE B*	β	*t*	*p*
Patient Age	-.037	.015	-0.151	-2.539	.012
Disease Onset Age	.080	.014	.311	5.799	.000
Disease Course	2.209	.221	.522	10.015	.000

Table 5. This table shows the model 1 which include three main variables extracted from table 4

Source	*B*	*SE B*	β	*t*	*p*
Disease Onset Age	.068	.007	.265	9.539	.000

EDSS	.965	.033	.816	29.406	.000

Table 6. This table shows the model 2 which controlled for EDSS and disease onset age.

3.3 Possible Confounders for all the MS patients

BMI

The relations of BMI and FI, EDSS and MSSS were assessed as follows. Out of the 323 patients, 308 reported their weight and height. The mean value for BMI was calculated (26.74±6.14). The Spearman correlation was used for investigating the relation between BMI and FI/EDSS. For FI and BMI, the result was significant (r=0.174, n=307, *p*=0.002). For EDSS and BMI, the relation was not significant (r=0.081, n=308, *p*=0.155). The correlation between BMI and MSSS was found to be significant (Pearson r=0.117, n=308, *p*=0.04). When adjusted for patient age, disease onset age and disease type, the multiple regression model could explain about 34% of the variance (R^2=0.335, F (4, 303) =38.136, *p*<0.000). But it seemed that BMI could not significantly affect MSSS (*B* =0.035, *p*=0.093). By using cox model, BMI was not associated with increase of risk to reach EDSS 6.5 (HR=1.027, 95% CI=0.992 to 1.062, *p*=0.134) and transform to SPMS (HR=1.024, 95% CI=0.988 to 1.06, *p*=0.192).

Smoking

The relations of smoking and FI, EDSS and MSSS were measured as follows: Among all the 323 patients, 320 reported their different smoking status, 308 provided the amount of cigarette they smoke every day. The result showed the number of non-smokers was 174 (53.9%), ex-smokers was 104 (32.2%), and current smokers was 42 (13%). The participants stopped smoking at the time they answered questionnaires were recorded as ex-smokers. The mean level of cigarettes per day for current smokers was 10.363 (SD=5.916), for ex-smokers was 12.899 (SD=8.44). The Spearman correlation was found non-significant between different smoking status and individual FI (r=0.088, n=319, *p*=0.117). It was reported that the Spearman

correlation between cigarettes number per day and FI was significant for all the participants (r= 0.127, n=307, *p*=0.026) but not for current smokers (r= 0.104, n=40, *p*=0.523).

Examining the relation between smoking and EDSS, a significant correlation was found between different smoking status and EDSS (Spearman correlation r=0.123, n=320, *p*=0.028). However, the Spearman correlation of cigarettes per day and EDSS was non-significant (r= 0.137, n=42, *p*=0.398) for current smokers. Similar results were found for the correlation of different smoking status and MSSS although did not reach statistical significance (r= 0.107, n=320, *p*=0.056). The non-significant findings with a small sample size pointed to the same direction. The Pearson correlation between cigarette amount and MSSS (r=0.304, n=40, *p*=0.056) was found to be non-significant among current smokers.

When multiple regression model was adjusted for patient age, disease onset age and disease type, about 33% of MSSS variance could be explained (R^2=0.327, F (4, 315) =38.31, *p*<0.000). Still, it indicated that different smoking status might not affect MSSS (*B*=0.271, *p*=0.124) significantly. Here this result might be due to small samples. With adjustment of patient age, disease onset age and disease type, multiple regression could explain about 32% of the variance (R^2=0.323, F (4,303) =37.655, *p*<0.000). It was reported that the number of cigarettes might significantly affect the prediction of MSSS (*B* =0.034, *p*=0.033). So, it was estimated that one cigarette per day leads to 0.034 change of MSSS with adjustment for other covariates.

Cox model indicated the non-significant effect of different smoking status and cigarettes amount on the progression of disease disability. For reaching EDSS 6.5, the results for different smoking status was HR=1.143, 95% CI=0.855 to 1.527, *p*=0.366, for cigarettes amount of all participants was HR=1.016, 95% CI=0.993 to 1.039, *p*=0.184. As for conversion of secondary course, the findings for different smoking status was HR=1.169, 95% CI=0.886 to 1.544, *p*=0.269, for cigarettes count was HR=1.006, 95% CI=0.984 to 1.028, *p*=0.584.

Drinking

The associations of drinking and FI, EDSS and MSSS were examined as follows. In this project, only the current drinking level (alcohol units per week) was collected. The ex-drinkers or non-drinkers were coded as 0 for drinking amount. Out of the 323 patients, 321 reported their drinking habits. A significant negative correlation for current alcohol consumption and EDSS (r=-0.135, n=321, *p*=0.016) was found to be significant but not for FI (r=-0.086, n=320, *p*=0.124). Similarly, for alcohol units and MSSS, the Pearson correlation was showed to be non-significant (r=-0.066, n=321, *p*=0.237) but negative.

When multiple regression model was adjusted for patient age, disease onset age and disease type, about 32% of the variance could be explained (R^2=0.319, F (4, 316) =36.934, *p*<0.000). Still, it indicated that the effect of drinking amount on MSSS was not significant (*B* =-0.011, *p*=0.443) but negative.

Cox model was shown to be non-significant for alcohol consumption and risk to reach EDSS 6.5 (HR=0.986, 95% CI=0.963 to 1.009, *p*=0.228) / SPMS (HR=0.997, 95% CI=0.977 to 1.016, *p*=0.729).

Exercise

The associations of exercise and FI, EDSS and MSSS were explored as follows. Out of the 323 participants, 321 reported their different exercise status. Among all the patients in this project, 206 (63.8%) do exercise every week. On average, exercise time per week was 2.95 hours (SD=4.22). The correlation was found to be significant between different exercise status and individual FI (Spearman r=-0.209, n=320, *p*<0.000). Also, It was found that the correlation between exercise hours per week and FI was significant for all the participants (Spearman r=-0.154, n=320, *p*=0.006). For the relation of current exercise (yes-no) and EDSS, EDSS negatively correlates with different exercise status (yes-no) (r=-0.321, n=321, *p*<0.000) as well as exercise hours per week (r=-0.355, n=321, *p*<0.000). The Pearson correlation was found to be negative between different exercise status and MSSS (r=-0.265, n=321, *p*<0.000), the

relation of exercise hours per week and MSSS was also found to be negative (r=-0.273, n=321, *p*<0.000) among all participants.

For exercise or not, multiple regression could explain about 33% of the variance (R^2=0.334, F (4,316) =39.573, *p*<0.000) with adjustment of patient age, disease onset age and disease type. The model suggested that different exercise status was associated with lower MSSS (*B* =-0.754, *p*=0.006). Therefore, it was estimated that exercise might cause 0.754 reductions of MSSS when controlled for other variables. For exercise hours, multiple regression could explain about 35% of variance when adjusted for patient age, disease onset age and disease course (R^2=0.346, F (4,316) =41.73, *P*<0.000), it seemed that exercise time could make significant negative contribution to the change of MSSS (*B* =-0.11, *p*<0.000). Similarly, it was estimated that per hour exercise could reduce 0.11 of MSSS in this model.

Cox model of different exercise status and risk of reaching EDSS 6.5 (HR=0.596, 95% CI=0.404 to 0.879, *p*=0.009) / secondary course (HR=0.488, 95% CI=0.338 to 0.706, *p*<0.000) were reported to be significant. It seemed that doing exercise may reduce 43.1% risk of reaching EDSS 6.5 as well as 51.2% risk of converting to SPMS. Also, cox model of exercise hours and risk to reach EDSS 6.5 (HR=0.909, 95% CI=0.85 to 0.971, *p*=0.005) /secondary course (HR=0.888, 95% CI=0.831 to 0.949, *p*<0.000) were seen to be significant. It showed that one hour of exercise could lead to a 9.1% reduction of risk to reach EDSS 6.5 and 11.2% decrease of risk of transforming to SPMS.

Mood

The relations of mood disorder with EDSS and MSSS were assessed: among all the participants, 316 (97.8%) answered about their current mood status (yes-no), and 126 (39%) reported emotional issues (regardless of anxiety, depression, etc.). The result indicated a significant correlation was found between mood disorder and EDSS (Spearman r=0.168, n=316, *p*=0.003). Still, the association showed the significant result of mood disorder and MSSS (Pearson r=0.175, n=316, *p*=0.002). When multiple regression was adjusted for patient

age, disease onset age and disease course, about 34% of the variance could be explained (R^2=0.337, F (4,311) =39.519, *p*<0.000). It indicated that current emotional status might contribute to the prediction of MSSS significantly (*B* =0.703, *p*=0.006). It was estimated that having emotional issues may contribute to 0.703 increase of MSSS in the current model.

3.4 For the questionnaires collected from both clinic and by post

3.4.1 Patients

The clinical data together with postal data, 321 patients provided information regarding individual frailty level or other comorbidities. The average level of FI for patients in this research was 3.75 (SD=3.069). 16.9% participants reported no comorbidities/symptom. The highest score came from the patients with 16 comorbidity/symptom (3%). The significant correlation about frailty level and EDSS (Spearman r=0.46, n=321, *p*<0.000) and association about individual frailty status of patients and MSSS (Pearson r=0.312, n=321, *p*<0.000) could be seen. When adjusted for patient age, disease onset age and disease type, about 34% of the variance could be explained in multiple regression model (R^2=0.344, F (4, 316) =41.426, *p*<0.000). The frailty condition of patients correlated with MSSS significantly (*B* =0.169, *p*<0.000). It could be estimated that one unit change of FI might cause 0.169 increase in MSSS from this model. The results are summarised as below:

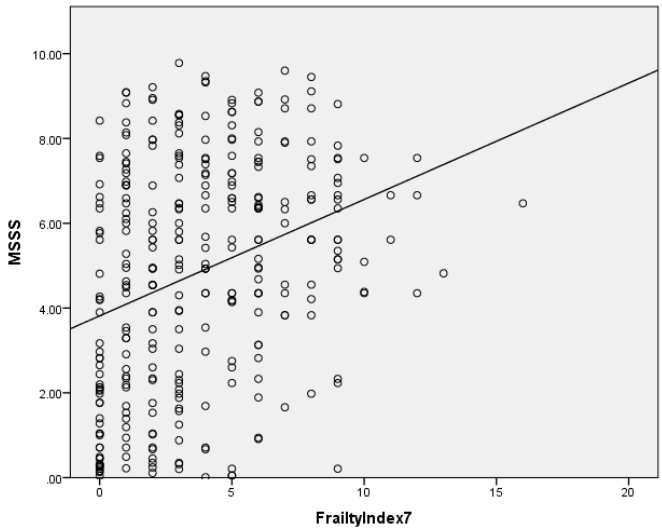


Fig 2. This graph shows a significant positive relation between frailty level (Frailty Index 7) of patients and MSSS.

Source	*B*	*SE B*	β	*t*	*p*
Patients' frailty	.169	.046	.192	3.694	.000
Disease Onset Age	.085	.014	.330	6.242	.000
Patients' age	-.056	.015	-.227	-3.665	.000
Disease Course	2.065	.223	.484	9.250	.000

Table 7. This table shows a significant relationship between patients' frailty and MSSS.

3.4.2 Family members

Current age/age at death

PARENTS

Mother

To find the effect of familial longevity/current age towards MSSS, out of the 323 patients responded, 174 their mothers are still alive.

Regarding that 149 dead cases, their mother age concentrated on group 8, which was 71-75 years old. The mean age group was 7.59±2.73, which is around 65-70 years old. The age groups of mothers age of death ranged from 1 to 12 (36-95 years old). The bivariate correlation was computed to assess the relationship between mothers age and EDSS/ MSSS of patients. For MSSS and mother age of death, the association was also found to be non-significant (Pearson r=0.156, n=149, p=0.058). For EDSS and mother longevity, a non-significant association was found (Spearman r=0.158, n=149, p=0.054). Multiple regression analysis was computed to test whether the mother age of death could affect the MSSS when adjusted for other controlled variables. The results indicated the predictors could explain about 76% of the variance (R^2=0.757, F (3, 145) =150.876, p<0.000) when adjusted for patient onset age and EDSS. It was found that mother longevity non-significantly may predict MS severity (B =-0.049, p=0.213) with an adverse effect.

As for the alive cases, results of multiple regression analysis indicated the predictors could explain about 85% of the variance (R^2=0.852, F (3, 170) =325.993, *p*<0.000) when adjusted for patient onset age and EDSS. It was found that current mother age significantly correlated with MS severity (*B* =-0.438, *p*<0.000) with an adverse effect.

Father

As for fathers age, the modal age range of 189 dead cases from 323 completed cases was group 8 (71-75 years old). The average age interval for father was group 7.72±2.63, which was about 70 years old. The age difference of father was 11 groups (1-12), which was the same as mothers age, 59 years. The father age variable was treated as same as mothers age, and the bivariate correlation was computed to assess the relationship between father age and EDSS/MSSS. For MSSS and father age of death, the correlation was also found to be non-significant (Pearson r=0.114, n=189, *p*=0.118). For EDSS and father longevity, the significant correlation was found (Spearman r=0.172, n=189, *p*=0.018). In the multiple regression model, when EDSS and disease onset age were adjusted, the predictors could explain about 77% of the variance (R^2=0.773, F (3, 185) =210.502, *p*<0.000). It was shown that father longevity had no significant relation with MSSS (*B* =-0.038, *p*=0.269) with an adverse effect.

As for the alive cases, results of multiple regression analysis indicated the predictors could explain about 82% of the variance (R^2=0.824, F (3, 126) =196.671, *p*<0.000) when adjusted for patient onset age and EDSS. It was found that current father age negatively correlated with MS severity (*B* =-0.438, *p*<0.000) with an adverse effect.

GRANDPARENTS

Maternal grandmother

For the effect of grandparents' longevity, 276 cases of the 300 completed data were dead in data from maternal grandmother. The distribution of age of death of the grandmother had a

mode of 81-90 years old. Their average age of death was in group 3 (3.22±1.247), which was 71-75 years old approximately. Only a minority of them died young before 50 years of age (4.7%), while 12.3% had the longevity of 91-100 years. The bivariate correlation was computed to assess the relationship between maternal grandmother age and EDSS/MSSS. For MSSS and maternal grandmother age of death, the negative correlation was also found to be non-significant (Pearson r=-0.001, n=273, *p*=0.987). For EDSS and maternal grandmother longevity, a non-significant correlation was found (Spearman r=0.034, n=273, *p*=0.581). The multiple regression model showed about 79% of the variance could be explained (R^2=0.789, F (3,269) =334.481, *p*<0.000) while adjusted for EDSS and disease onset age. It was reported that maternal grandmother longevity did not affect MSSS (*B* =-0.006, *p*=0.915) with an adverse effect.

Maternal grandfather

As for maternal grandfather, 273 of the 283 completed cases had died. The mode of maternal grandfather age was different from maternal grandmother age, which was group 3 (71-80 years old). The average level of maternal grandfather longevity was group 2.63±1.366, which was around 65-70 years. Apart from 8.8% of them lived less than 50 years, 6.6% could reach maximum longevity, 91-100 years. The bivariate correlation was computed to assess the relationship between maternal grandfather age and EDSS/MSSS. For MSSS and maternal grandfather age of death, the negative correlation was found to be non-significant (Pearson r=-0.027, n=268, *p*=0.661). Also, For EDSS and maternal grandfather longevity, the negative correlation was found to be non-significant (Spearman r=-0.008, n=268, *p*=0.898). 79% of the variance could be explained by multiple regression model with adjustment of EDSS and disease onset age (R^2=0.79, F (3,264) =330.413, *p*<.000). The analysis indicated maternal grandfather longevity did not significantly predict MSSS (*B* =-0.025, *p*=0.658) with an adverse effect.

Paternal grandmother

Regarding paternal grandmother, 261 dead cases (11 cases without information about the age of death) were provided out of 269 completed data. Most of the paternal grandmothers had the same longevity as maternal grandmothers, which was 81-90 years old. Average longevity for this family member was the same age group as a maternal grandfather, 65-70 years. The proportion for cases with 91-100 years was 12.3%, which was the same as that of maternal grandmother. The bivariate correlation was computed to assess the relationship between paternal grandmothers' age and EDSS/MSSS. For MSSS and paternal grandmother age, the correlation was also found to be non-significant (Pearson r=-0.102, n=250, *p*=0.109). For EDSS and paternal grandmother age, the non-significant correlation was found (Spearman r=-0.115, n=250, *p*=0.07). Multiple regression model could explain about 78% of the variance with adjustment of EDSS and disease onset age (R^2=0.782, F (3, 246) =294.79, *p<0*.000). It was noticed that in this model, paternal grandmother longevity could not predict the MSSS significantly (*B* =0.052, *p*=0.365).

Paternal grandfather

Finally, 241 dead cases (9 cases without information about the age of death) were shown among 257 completed data of paternal grandfather. According to the data from the sample in this study, it was found that the paternal grandparents had the same mode of longevity, which was 71-80 years. The mean value for paternal grandfather age was less than the other 3 grandparents, which was group 2.53 (around 65-70 years old). Current data showed only 6% paternal grandfather could live until 91-100 years old and 11.2% died before 50 years old. The bivariate correlation was computed to assess the relationship between paternal grandfathers' age and EDSS/MSSS. For MSSS and paternal grandfather age, the correlation was also found to be non-significant (Pearson r=-0.067, n=241, *p*=0.303). For EDSS and paternal grandfather age, no significant correlation was found (Spearman r=-0.095, n=241, *p*=0.142). Multiple regression for this variable shows that about 79% of the variance could be explained when

adjusting for EDSS and disease onset age (R^2=0.788, F (3, 237) =293.474, *p*<.000). The prediction of paternal grandfather longevity was not significant (*B* =0.026, *p*=0.669).

Grandparents over 90 years old

The number of grandparents over 90 years old for each participant was counted. The difference of mean for MSSS could be seen clearly in table 8. If group 2 was ignored, it seemed that more grandparents over 90 years old in the family, the lower average MSSS score would be. However, the Student t-test indicated that there was no significant difference of MSSS score for having no grandparent over 90 years old (M=4.91, SD=2.62) and having one grandparent over 90 years old (M=4.71, SD=2.88); t (290) = 0.574, *p*=0.566.

Grandparents Over 90	Mean	N	Std. Deviation
0	4.9147	219	2.62199
1	4.7062	73	2.87898
2	5.5222	9	2.40239
3	3.3700	2	3.43654
4	2.6367	3	1.33245
Total	4.8504	306	2.67508

Table 8. This table shows the mean level of MSSS with different number of grandparents over 90 years old.

Frailty

Mother

Investigating the relationship between the frailty of family members and MSSS, the data of comorbidities was collected. For the frailty condition of the mother, 315 completed cases were

analysed. The mean level of mother frailty was 2.89±2.988. It was shown that 19.2% of patient's mothers reported no disease in the list. Most of them had one deficit/ symptom. The bivariate correlation was computed to assess the relationship between mother frailty level and EDSS/MSSS. For EDSS and mother frailty, the significant correlation was found (Spearman r=0.166, n=315, *p*=0.003). However, for MSSS and mother frailty, the correlation was found to be non-significant (Pearson r=0.062, n=315, *p*=0.274). While adjusted for disease onset age, disease type, patient age, and mother age regardless of dead or alive, approximate 32% of the variance could be explained by multiple regression model (R^2=0.324, F (5,309) =29.672, *p*<.000). The contribution of mother frailty was still non-significant (*B* =0.03, *p*=0.502).

Father

For fathers' frailty status, 27 cases were missing data. From the 296 completed data, the modal FI was 1, which is the same as that of the mother. It was reported that 18.3% of fathers had no deficits in this list. Mean value of father deficit was 2.47±2.746. The bivariate correlation was computed to assess the relationship between father frailty level and EDSS/MSSS. For EDSS and father frailty, a non-significant correlation was found (Spearman r=0.089, n=296, *p*=0.125). Also, for MSSS and mother frailty, the correlation was found to be non-significant (Pearson r=0.031, n=296, *p*=0.592). The multiple regression model with adjustment of patient age, disease type, disease onset age, and father age regardless of dead or alive could explain 34% of the variance (R^2=0.34, F (5,290) =29.894, *p*<.000). It was found that the effect of fathers' frailty was non-significant (*B* =-0.03, *p*=0.556).

Parents

When both parents were taken into consideration, 314 most and least frailty condition were extracted. The bivariate correlation was computed to assess the relationship between parents' frailty level and EDSS/MSSS. For EDSS and parent frailty level, the significant correlation was found for 'most frailty' (Spearman r=0.146, n=314, *p*=0.01) and 'least frailty' (r=0.15, n=314, *p*=0.008). However, for MSSS and parent's frailty, the correlation was found to be non-

significant for ‘most frailty’ (Pearson r=0.053, n=314, *p*=0.348) and ‘least frailty’ (r=0.043, n=314, *p*=0.448). When adjusted for disease onset age, disease type, the age of patients and age of both parents, approximate 33% of the variance could be explained in multiple regression model (R2=0.327, F (6,305) =24.705, *p*<0.000). The most frailty condition of parents did not contribute to the prediction of MSSS (*B* =0.014, *p*=0.739). As for least frailty of parents, multiple regression model could explain approximate 33% of the variance (R^2=0.327, F (6,305) =24.683, *p*<0.000) with adjustment of disease onset age, disease type, the age of patients and age of both parents. This effect of this variable on MSSS was non-significant (*B* =0.009, *p*=0.886).

Maternal grandmother

In total, 203 completed cases about the frailty of the maternal grandmother were provided to investigate frailty issue of grandparents. Same as parents’ frailty, most of the maternal grandmother only own one frailty problems/symptom from the list. 9.3 % of them reported no problem with frailty. The mean level of maternal grandmother frailty was 2.46±2.464. The bivariate correlation was computed to assess the relationship between father frailty level and EDSS/ MSSS. For EDSS and maternal grandmother frailty, the significant correlation was found (Spearman r=-0.179, n=203, *p*=0.011). Also, for MSSS and maternal grandmother frailty, the correlation was found to be significant (Pearson r=-0.188, n=203, *p*=0.007). After adjustment of disease onset age, disease type and age of patients, multiple regression model would explain approximate 32% of the variance (R^2=0.324, F (4, 198) =23.765, *p*<.000). It was shown that frailty condition of maternal grandmother affects MSSS significantly (*B* =-0.138, *p*=0.032). Here, it was estimated that one-unit change of FI from the maternal grandmother, the MSSS would decrease 0.138 in the current model.

Maternal grandfather

For maternal grandfather, 165 completed cases were collected. Consistent with maternal grandmother frailty, it was found that one disease/symptom was most common among

maternal grandfather. 13.6% of them reported no deficit in the list. Mean value of maternal grandfather was 1.5±1.83. The bivariate correlation was computed to assess the relationship between maternal grandfather frailty level and EDSS/ MSSS. For EDSS and maternal grandfather frailty, the non-significant correlation was found (Spearman r=-0.001, n=165, *p*=0.987). Also, for MSSS and maternal grandfather frailty, the correlation was found to be non-significant (Pearson r=-0.078, n=165, *p*=0.322). The disease onset age, disease type, and age of patients were adjusted for multiple regression model, and results indicate that approximate 32% of the variance can be explained (R^2=0.316, F (4,160) =18.455, *p*<0.000). It seemed that maternal grandfather frailty status could not predict MSSS significantly (*B* = -0.104, *p*=0.294).

Paternal grandmother

As for fathers' parents, 139 patients provided frailty information of paternal grandmother. Same as mothers' parents, most paternal grandmothers had one disease/symptom of the list. 8.7% of them reported no any symptom/deficit in the list. The mean level of paternal grandmother frailty was 1.99±2.061. A bivariate correlation was used to assess the association between father parents' frailty level and EDSS/MSSS. For EDSS and paternal grandmother frailty, a non-significant correlation was found (Spearman r=-0.14, n=139, *p*=0.101). Also, for MSSS and maternal grandmother frailty, the correlation was found to be non-significant (Pearson r=-0.097, n=139, *p*=0.256). While adjusting for disease onset age, disease type and age of patients, multiple regression model would explain an approximate 33% of the variance (R^2=0.333, F (4,134) =16.752, *p*<0.000). It is reported that frailty condition of paternal grandmother may influence MSSS non-significantly (*B* =-0.077, *p*=0.433).

Paternal grandfather

Regarding paternal grandfather, only 118 cases were provided. It seemed that among all the grandparents, most of them only suffer from one disease based on the data. Mean value of maternal grandfather was 1.52±1.54. The bivariate correlation was computed to assess the

relationship between paternal grandfather frailty level and EDSS/MSSS. For EDSS and paternal grandfather frailty, a non-significant Spearman correlation was found (r=-0.094, n=118, *p*=0.313). Also, for the correlation of MSSS and paternal grandfather frailty, the result was found to be non-significant (Pearson r=-0.08, n=118, *p*=0.39). With adjustment of disease onset age, disease type and age of patients, multiple regression model would explain approximate 34% of the variance (R^2=0.342, F (4, 113) =14.707, *p*<0.000). It seemed that frailty condition of paternal grandfather might not influence MSSS significantly (*B* =-0.057, *p*=0.697).

Possible confounders for grandparents

Sub correlation analysis for possible confounder of both grandparents:

Correlations

		MSSS	Smoke3	Alcohol3	Neurodisease3	Smoke4	Alcohol4	Neurodisease4	Smoke5	Alcohol5	Neurodisease5	Smoke6	Alcohol6	Neurodisease6
MSSS	Pearson Correlation	1	.008	-.006	-.007	.138*	-.066	.009	.104	-.009	-.115	.076	.021	-.040
	Sig. (2-tailed)		.899	.923	.919	.038	.337	.905	.141	.899	.135	.311	.780	.629
	N	323	261	252	226	227	212	191	203	193	169	178	182	146
Smoke3	Pearson Correlation	.008	1	.307**	-.044	.188**	.189**	-.120	.107	.181*	.023	.082	.154*	.025
	Sig. (2-tailed)	.899		.000	.523	.006	.007	.113	.145	.016	.778	.296	.047	.771
	N	261	261	245	216	212	201	176	188	178	152	164	167	138
Alcohol3	Pearson Correlation	-.006	.307**	1	-.182**	.167*	.492**	-.030	.113	.406**	-.019	.157*	.402**	-.068
	Sig. (2-tailed)	.923	.000		.008	.016	.000	.690	.127	.000	.816	.047	.000	.430
	N	252	245	252	208	207	202	173	185	178	148	161	165	138
Neurodisease3	Pearson Correlation	-.007	-.044	-.182**	1	-.001	-.171*	-.041	.073	-.029	-.073	-.002	-.141	.087
	Sig. (2-tailed)	.919	.523	.008		.990	.025	.600	.354	.723	.383	.977	.099	.319
	N	226	216	208	226	182	170	170	165	157	146	141	139	133
Smoke4	Pearson Correlation	.138*	.188**	.167*	-.001	1	.399**	-.084	.147	.163*	-.006	.306**	.127	-.082
	Sig. (2-tailed)	.038	.006	.016	.990		.000	.270	.053	.035	.948	.000	.112	.352
	N	227	212	207	182	227	204	174	174	169	141	155	157	130
Alcohol4	Pearson Correlation	-.066	.189**	.492**	-.171*	.399**	1	.017	.010	.372**	.092	.250**	.402**	-.020
	Sig. (2-tailed)	.337	.007	.000	.025	.000		.826	.898	.000	.287	.002	.000	.825
	N	212	201	202	170	204	212	167	169	164	135	147	151	122
Neurodisease4	Pearson Correlation	.009	-.120	-.030	-.041	-.084	.017	1	-.101	-.025	.107	-.059	-.054	-.064
	Sig. (2-tailed)	.905	.113	.690	.600	.270	.826		.213	.765	.212	.503	.532	.482
	N	191	176	173	170	174	167	191	153	148	139	130	134	122
Smoke5	Pearson Correlation	.104	.107	.113	.073	.147	.010	-.101	1	.408**	-.070	.312**	.340**	.007
	Sig. (2-tailed)	.141	.145	.127	.354	.053	.898	.213		.000	.383	.000	.000	.939
	N	203	188	185	165	174	169	153	203	188	156	161	159	132
Alcohol5	Pearson Correlation	-.009	.181*	.406**	-.029	.163*	.372**	-.025	.408**	1	.101	.149	.532**	.054
	Sig. (2-tailed)	.899	.016	.000	.723	.035	.000	.765	.000		.222	.068	.000	.547
	N	193	178	178	157	169	164	148	188	193	147	152	156	127
Neurodisease5	Pearson Correlation	-.115	.023	-.019	-.073	-.006	.092	.107	-.070	.101	1	.006	.128	.158
	Sig. (2-tailed)	.135	.778	.816	.383	.948	.287	.212	.383	.222		.949	.149	.083
	N	169	152	148	146	141	135	139	156	147	169	125	128	121
Smoke6	Pearson Correlation	.076	.082	.157*	-.002	.306**	.250**	-.059	.312**	.149	.006	1	.509**	-.051
	Sig. (2-tailed)	.311	.296	.047	.977	.000	.002	.503	.000	.068	.949		.000	.566
	N	178	164	161	141	155	147	130	161	152	125	178	164	127
Alcohol6	Pearson Correlation	.021	.154*	.402**	-.141	.127	.402**	-.054	.340**	.532**	.128	.509**	1	.002
	Sig. (2-tailed)	.780	.047	.000	.099	.112	.000	.532	.000	.000	.149	.000		.986
	N	182	167	165	139	157	151	134	159	156	128	164	182	127
Neurodisease6	Pearson Correlation	-.040	.025	-.068	.087	-.082	-.020	-.064	.007	.054	.158	-.051	.002	1
	Sig. (2-tailed)	.629	.771	.430	.319	.352	.825	.482	.939	.547	.083	.566	.986	
	N	146	138	138	133	130	122	122	132	127	121	127	127	146

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 9. ('3' means variables from maternal grandmother, '4' means from maternal grandfather, '5' means from paternal grandmother, '6' means from paternal grandfather)

According to this Pearson correlation matrix, these possible confounder variables were proved to be non-significantly correlate with MSSS and would associate with each other. Because these factors were not the variables of interest in this project, these were excluded for final regression analysis to reduce the noise.

Principal Component Analysis about parent level:

Without the settings about a number of factors from extraction, the scree plot result extracted 4 components after rotation was applied for 263 completed cases. They were ‘father ageing level’ (interaction of father age, father neurological disease history and father frailty condition), ‘mother ageing level’ (interaction of mother age, mother neurological disease history and mother frailty condition), ‘parental smoking level’ (interaction of mother and father smoking habits), ‘parental drinking level’ (interaction of mother and father drinking habits). The results are summarised as follows:

Rotated Component Matrix[a]

	Component			
	1	2	3	4
FrailtyIndex2	.840			
Neurodisease2	.671			
FatherAge	.586			
Alcohol2		.870		
Alcohol1		.816		
Neurodisease1			.758	
MotherAge			.691	
FrailtyIndex1			.592	
Smoke1				.795
Smoke2				.737

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 5 iterations.

Table 10. This table shows the variables in different components extracted from the rotation (‘1’ means from the mother side, ‘2’ means from father side).

The bivariate and partial correlations were computed to assess the relationship between these components with EDSS and MSSS.

Father ageing

For EDSS and father ageing, a significant correlation was found (Spearman r=0.197, n=263, *p*=0.001). For MSSS and father ageing, the association was also found to be significant (Pearson r=0.149, n=263, *p*=0.016). Multiple regression analysis of father ageing and MSSS indicated the predictors explained approximate 79% of the variance (R^2=0.788, F (3,259) =320.543, *p*<0.000) when adjusted for patient onset age and individual EDSS. It was found that father ageing significantly affects MSSS (*B* =-0.181, *p*=0.028). It could be estimated that one unit change of father ageing level may contribute to 0.181 reductions of MSSS in the current model. If the living status was considered for 263 completed cases, the multiple regression model could explain about 81% of the variance (R^2=0.809, F (4,258) =272.846, *p*<0.000) with adjustment of disease onset age and individual EDSS would still predict MSSS significantly (*B* =-0.177, *p*=0.025). Therefore, the relation of father ageing and MSSS would not be changed by dead or alive. Partial correlation showed that when EDSS and disease onset age were controlled on the relation between father ageing and MSSS, it was found that the following partial correlation r=-0.136, n=259, *p*=0.028. The results are summarised in table 11 and 12.

Correlations

Control Variables			FatherAgeing	MSSS	DiseaseOnsetAge	EDSS
-none-[a]	FatherAgeing	Correlation	1.000	.149	.168	.200
		Significance (2-tailed)	.	.016	.006	.001
		df	0	261	261	261
	MSSS	Correlation	.149	1.000	.322	.845
		Significance (2-tailed)	.016	.	.000	.000
		df	261	0	261	261
	DiseaseOnsetAge	Correlation	.168	.322	1.000	.069
		Significance (2-tailed)	.006	.000	.	.262
		df	261	261	0	261
	EDSS	Correlation	.200	.845	.069	1.000
		Significance (2-tailed)	.001	.000	.262	.
		df	261	261	261	0
DiseaseOnsetAge & EDSS	FatherAgeing	Correlation	1.000	-.136		
		Significance (2-tailed)	.	.028		
		df	0	259		
	MSSS	Correlation	-.136	1.000		
		Significance (2-tailed)	.028	.		
		df	259	0		

a. Cells contain zero-order (Pearson) correlations.

Table 11. This table shows the comparison of correlation between father ageing and MSSS in different orders.

Source	*B*	*SE B*	β	*t*	*p*
Disease Onset Age	.072	.008	.274	9.441	.000
EDSS	.991	.035	.839	28.708	.000
Father Ageing	-.181	.082	-.065	-2.211	.028

Table 12. This table shows that relation between father ageing and MSSS.

Mother ageing

As for EDSS and mother ageing, a non-significant correlation was found (Spearman r=0.11, n=263, *p*=0.076). For MSSS and mother ageing, the association was also found to be non-significant (Pearson r=0.062, n=263, *p*=0.316). In the multiple regression model, when EDSS and disease onset age were adjusted, the predictors could explain about 79% of the variance (R^2=0.792, F (3, 259) =328.561, *p*<0.000). It was shown that mother ageing had a significant

prediction for MSSS (*B* =-0.257, *p*=0.002). It could be estimated that one unit change of mother ageing would cause 0.257 reductions of MSSS. If the living status was controlled for 224 completed cases, the multiple regression model could explain about 81% of the variance (R^2=0.808, F (4,258) =271.7, *p*<0.000) with adjustment of EDSS and disease onset age, mother ageing could predict MSSS significantly (*B* =-0.279, *p*<0.000). Thus, the relation of mother ageing and MSSS would not be changed by dead or alive. When EDSS and disease onset age were controlled, partial correlation suggested that mother ageing was correlated with MSSS (r=-0.194, n=259, *p*=0.002).

The results are summarised in table 13 and 14.

Correlations

Control Variables			MSSS	MotherAgeing	DiseaseOnsetAge	EDSS
-none-[a]	MSSS	Correlation	1.000	.062	.322	.845
		Significance (2-tailed)	.	.316	.000	.000
		df	0	261	261	261
	MotherAgeing	Correlation	.062	1.000	.216	.112
		Significance (2-tailed)	.316	.	.000	.070
		df	261	0	261	261
	DiseaseOnsetAge	Correlation	.322	.216	1.000	.069
		Significance (2-tailed)	.000	.000	.	.262
		df	261	261	0	261
	EDSS	Correlation	.845	.112	.069	1.000
		Significance (2-tailed)	.000	.070	.262	.
		df	261	261	261	0
DiseaseOnsetAge & EDSS	MSSS	Correlation	1.000	-.194		
		Significance (2-tailed)	.	.002		
		df	0	259		
	MotherAgeing	Correlation	-.194	1.000		
		Significance (2-tailed)	.002	.		
		df	259	0		

a. Cells contain zero-order (Pearson) correlations.

Table 13. This table shows the comparison of correlation between mother ageing and MSSS in different orders.

Source	*B*	*SE B*	β	*t*	*p*
Disease Onset Age	.074	.008	.284	9.758	.000
EDSS	.987	.034	.836	29.273	.000
Mother Ageing	-.257	.081	-.093	-.093	.002

Table 14. This table shows that relation between mother ageing and MSSS.

When both father ageing and mother ageing were put in the multiple regression model with adjustment of EDSS and disease onset age, about 80% of the variance could be explained (R^2=0.797, F (4,258)=252.648, *p*<0.000). The father (*B* =-0.197, *p*=0.015) and mother (*B* =-0.268, *p*=0.001) ageing level could affect MSSS significantly. If the living status of parents were controlled for 263 completed cases, the multiple regression model could explain about 82% of the variance (R^2=0.821, F (6,256) =195.803, *p*<0.000) with adjustment of EDSS and disease onset age, father ageing (*B* =-0.184, *p*=0.016) and mother ageing (*B* =-0.234, *p*=0.003) could still affect MSSS significantly. Still, the relation of interaction between father and mother ageing with MSSS would not be changed by dead or alive. Also, the parameters showed that the relation of MSSS with mother ageing level was relatively higher than father ageing. The results are summarised as follows:

Source	*B*	*SE B*	β	*t*	*p*
Disease Onset Age	.078	.008	.295	10.120	.000
EDSS	1.003	.034	.850	29.457	.000
Mother Ageing	-.268	.080	-.097	-3.343	.001
Father Ageing	-.197	.081	-.071	-2.444	.015

Table 15. This table further shows the relationship between the ageing level of both parents and MSSS.

Parental drinking and smoking

For the parental drinking/smoking and EDSS/MSSS, the Spearman correlation of EDSS and parental lifestyle were found to be non-significant for parental drinking (r=-0.074, n=263, *p*=0.234) and smoking (r=0.105, n=263, *p*=0.091). The Pearson correlation of MSSS and parental lifestyle were found to be non-significant for parental drinking (r=-0.101, n=263, *p*=0.103) and parental smoking (r=0.116, n=263, *p*=0.061). In the multiple regression model, when EDSS and disease onset age were adjusted, the predictors could explain about 79% of the variance (R^2=0.785, F (4, 258) =235.23, *p*<0.000). It was shown that parental drinking (β =-0.087, *p*=0.283) and smoking (β =0.016, *p*=0.842) might have no significant effect on MSSS. However, if the patient age, disease type, and disease onset age were controlled for 263 completed cases, the multiple regression model could explain about 37% of the variance (R^2=0.369, F (5,257) =30.116, *p*<0.000), parental drinking (*B* =-0.282, *p*=0.041) and smoking (*B* =0.331, *p*=0.018) could negatively affect MSSS significantly.

In order to reduce dimension further, the second principle analysis was applied to extract 2 component. Finally, two main factors were carried out, which were 'parental ageing (interaction of age, neurological disease history and frailty status from both parents)' and 'parental lifestyle (interaction of smoking and drinking of both parents).' The results are summarised as follows:

Rotated Component Matrix[a]

	Component	
	1	2
FrailtyIndex2	.777	
FrailtyIndex1	.637	
FatherAge	.581	
Neurodisease2	.574	
MotherAge	.560	
Neurodisease1	.365	
Alcohol1		.752
Alcohol2		.682
Smoke2		.510
Smoke1		.497

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.

a. Rotation converged in 3 iterations.

Table 16. This table shows the variables in two main components extracted from the rotation ('1' means from the mother side, '2' means from father side).

The bivariate correlation was computed to assess the relationship between these components and EDSS/MSSS.

Parental ageing

For EDSS and parental ageing, a significant correlation was found (Spearman r=0.245, n=263, p<0.000). For MSSS and parental ageing, the correlation was also found to be significant (Pearson r=0.158, n=263, p=0.01). Multiple regression analysis of parental ageing and MSSS indicated the predictors explained about 80% of the variance (R^2=0.795, F (3,259) =333.826, p<0.000) when adjusted for patient onset age and individual EDSS. It was found that parental ageing could negatively predict MSSS (B =-0.304, p<0.000). It could be estimated that one unit change of parental ageing level may lead to 0.304 decreases of MSSS, this relation was slightly higher than the analysed parental ageing separately. If the living status was considered for 263 completed cases, the multiple regression model could explain 82% of the variance (R^2=0.82, F (5,257) =234.054, p<0.000) with adjustment of patient onset age and individual EDSS, parental ageing would still predict MSSS significantly (B =-0.277, p<0.000). Therefore, the relation of parental ageing and MSSS would not be changed by dead or alive. The partial correlation results suggested that parental ageing was related to MSSS, r=-0.223, n=259, p<0.000 when controlling for EDSS and disease onset age. The analysis is summarised as follows:

Correlations

Control Variables			MSSS	ParentalAgeing	DiseaseOnsetAge	EDSS
-none-[a]	MSSS	Correlation	1.000	.158	.322	.845
		Significance (2-tailed)	.	.010	.000	.000
		df	0	261	261	261
	ParentalAgeing	Correlation	.158	1.000	.255	.228
		Significance (2-tailed)	.010	.	.000	.000
		df	261	0	261	261
	DiseaseOnsetAge	Correlation	.322	.255	1.000	.069
		Significance (2-tailed)	.000	.000	.	.262
		df	261	261	0	261
	EDSS	Correlation	.845	.228	.069	1.000
		Significance (2-tailed)	.000	.000	.262	.
		df	261	261	261	0
DiseaseOnsetAge & EDSS	MSSS	Correlation	1.000	-.223		
		Significance (2-tailed)	.	.000		
		df	0	259		
	ParentalAgeing	Correlation	-.223	1.000		
		Significance (2-tailed)	.000	.		
		df	259	0		

a. Cells contain zero-order (Pearson) correlations.

Table 17. This table shows the comparison of the correlation between parental ageing and MSSS in different orders.

Source	*B*	*SE B*	β	*t*	*p*
EDSS	1.003	.034	.850	29.384	.000
Disease Onset Age	.076	.008	.291	9.974	.000
Parental Ageing	-.304	.083	-.110	-3.675	.000

Table 18. This table shows the effect of parental ageing on MSSS.

Parental lifestyle

For the relation of parental lifestyle and EDSS/MSSS, the correlation of EDSS and parental lifestyle were found to be non-significant for parental lifestyle (Spearman r=0.018, n=263, *p*=0.775). The correlation of MSSS and parental lifestyle was found to be non-significant (Pearson r=-0.005, n=263, *p*=0.93). In the multiple regression model, when EDSS and disease onset age were adjusted, the predictors could explain about 78% of the variance (R^2=0.784, F (3, 259) =313.702, *p*<0.000). It seemed that parental lifestyle (*B* =-0.054, *p*=0.503) did not have an adverse effect on MSSS. However, if the patient age, disease type, and disease onset

age were controlled for 263 completed cases, the multiple regression model could explain about 35% of the variance (R^2=0.345, F (4,258) =34.001, *p*<0.000), parental lifestyle (β =0.036, *p*=0.799) could not affect MSSS significantly.

Cox regression of EDSS 6.5 and MS transformation for variables of interest

For patients' frailty level and EDSS 6.5:

According to the data in this project, 63 cases (19.5%) had reached EDSS 6.5 among all the participants. The patients that first presented to the clinic with an established EDSS 6.5 were removed for analysis. As frailty was coded as a continuous variable in this research, the cox model could only reflect a tendency. The results showed that it could significantly affect the risk to reach EDSS 6.5. The patients' frailty was not a risk factor for reaching EDSS 6.5 (HR=1.062, 95% CI=1.000 to 1.129, *p*=0.052) when it was tested individually in this study, and it seemed that patients' frailty level was not associated with the risk of reaching EDSS 6.5. When the model was adjusted for patient age and disease onset age, patients' frailty level affected the risk to reach EDSS 6.5 more prominently (adjusted HR=1.119, 95% CI=1.051 to 1.191, *p*<0.000). Then, patients' frailty level was related to an 11.9 % increase in risk to reach EDSS 6.5. The results are summarised in table 19.

Source	*B*	*Exp (B)*	*p*	*95%CI for Exp(B)*
Patients' frailty	.112	1.119	.000	1.051-1.191
Patients' age	-.143	.867	.000	.830-.905
Disease Onset Age	.167	1.182	.000	1.132-1.234

Table 19. This table shows the association between patients' frailty level and risk of reaching EDSS 6.5

For parent level and EDSS 6.5:

The parental ageing was assessed as possible risk factors to reach EDSS 6.5 in this research. The cox model results were summarised. For the parental ageing, it was reported that parental ageing might be a protective factor for reaching EDSS 6.5 (adjusted HR=0.856, 95% CI=0.776 to 1.234, *p*=0.856) when it was considered alone, but the effect was not significant. While the model was adjusted for patient age, disease type, disease onset age and patients' FI, the parental ageing was still carried out to be a protective factor for reaching EDSS 6.5 (adjusted HR=0.867, 95% CI=0.667 to 1.126, *p*=0.283), which indicated that 13.3% risk to reach EDSS 6.5 could be reduced non-significantly.

For patients' frailty level and disease transition to SPMS:

According to the data in this project, 118 cases (39.6%) had reached EDSS 6.5 out of 298 previous RRMS participants. The cases with PPMS coursed were removed for analysis because the transition to SPMS is only applicable for previous patients with RRMS. Considered that frailty condition was coded as a continuous variable in this research, the cox model could only reflect a tendency of frailty level and risk of transformation. The results showed that it was a significant factor in the risk of reaching SPMS, patient frailty may be a factor for the transition to the secondary progressive course (HR=1.064, 95% CI=1.003 to 1.128, *p*=0.038) when it was tested individually. When the model was adjusted for patient age and disease onset age, patients' frailty level became a significant risk factor to transform to SPMS (adjusted HR=1.123, 95% CI=1.056 to 1.193, *p*<0.000), which indicated that patients' frailty level was associated with a 12.3% increase in the risk of reaching secondary progressive course. The results are summarised in table 20.

Source	*B*	*Exp (B)*	*p*	*95%CI for Exp(B)*
Patients' frailty	.116	1.123	.000	1.056-1.193
Patients' age	-.116	.891	.000	.858-.924
Disease Onset Age	.152	1.164	.000	1.121-1.208

Table 20. This table shows the association between patients' frailty level and risk of converting to secondary clinical phase.

For parent level and disease transition to SPMS:

This Cox model could only reflect a tendency of parental ageing and risk of conversion to SPMS. As for parental ageing level, Cox model indicated that it might not be a risk factor to reach SPMS (HR=1.123, 95% CI=0.905 to 1.392, p=0.292) when it was put in the model alone. When the model was adjusted for patient age and disease onset age, a parental ageing level still could not become a significant risk factor to the conversion of SPMS (adjusted HR=1.057, 95% CI=0.855 to 1.307, p=0.606).

Additional FI without mobility problem

The item 'mobility problems' was removed, and new patients' frailty was created because that EDSS was assessed based on mobility. Extra analysis results are summarised as follows.

In terms of the relation of MSSS and this new FI, when adjusted for patient age, disease onset age and disease type, about 33% of the variance could be explained in multiple regression model (R2=0.333, F (4, 316) =39.461, p<0.000). The new frailty condition of patients still correlated with MSSS significantly (B =0.142, p=0.004). It could be estimated that one-unit change of new FI might cause 0.142 increase in MSSS from this model. The results are summarised in table 21.

Source	*B*	*SE B*	β	*t*	*p*
Patients' frailty without mobility problems	.142	.050	.149	2.875	.004
Patients' age	-.052	.015	.211	-3.380	.001
Disease Onset Age	.084	.014	.327	6.128	.000
Disease course	2.124	.224	.498	9.502	.000

Table 21. This table shows the association between patients' frailty level without mobility problems and MSSS.

As for the risk of reaching EDSS 6.5 and SPMS, the results were still significant.

Only testing the new patients' FI, it was not a risk factor for reaching EDSS 6.5 in this case (HR=1.043, 95% CI=0.977 to 1.114, *p*=0.208), it seemed that new patient frailty level was not associated with the risk of reaching EDSS 6.5. When the model was adjusted for patient age and disease onset age, new patients' frailty level affected the risk to reach EDSS 6.5 significantly (adjusted HR=1.101, 95% CI=1.029 to 1.178, *p*<0.000). Then, patients' frailty level was related to a 10.1 % increase of risk to reach EDSS 6.5. The results are summarised in table 22.

Source	*B*	*Exp (B)*	*p*	*95%CI for Exp(B)*
Patients' frailty without mobility problems	.096	1.101	.006	1.029-1.178
Patients' age	-.138	.871	.000	.834-.909
Disease Onset Age	.165	1.180	.000	1.130-1.231

Table 22. This table shows the association between patients' frailty level without mobility problems and risk of reaching EDSS 6.5

The new patients' frailty may not be a factor in the transition to the secondary progressive phase (HR=1.053, 95% CI=0.989 to 1.122, *p*=0.107) while it was tested only. When the model

was adjusted for patient age and disease onset age, new patients' frailty level became a significant risk factor to transition of SPMS (adjusted HR=1.114, 95% CI=1.043 to 1.189, *p*<0.000), which indicated that patients' frailty level was associated with an 11.4% increase in the risk of reaching secondary progressive course. The results are summarised in table 23.

Source	*B*	*Exp (B)*	*p*	*95%CI for Exp(B)*
Patients' frailty without mobility problems	.108	1.114	.001	1.043-1.189
Patients' age	-.113	.893	.000	.860-.927
Disease Onset Age	.151	1.162	.000	1.120-1.207

Table 23. This table shows the association between patients' frailty level without mobility problems and the risk of converting to secondary clinical phase.

Discussion

4.1 Principal findings

In the current study, we examined the influence of familial longevity and the association with the rate of disability progression in MS as measured by MSSS and rate of progression to SPMS. We have found in this pilot, relatively small observational study, some indications that familial longevity might affect the disease progression. This association has been weak, and our findings are of course preliminary requiring study with more population to support or refute this hypothesis. Also, the analysis confirmed that frailty/comorbidity level of patients with MS is related to a risk of disability progression by affecting MSSS and risk of reaching EDSS 6.5 or SPMS.

4.2 Possible explanation of our findings and comparison to other studies

4.2.1 Clinical markers of MS

Gender

In the current study, the responders' female-to-male ratio was 2.55, which is similar to a Sweden study which stated the average female-to-male ratio for MS of 2.62 (Mackenzie et al., 2014). This is accordant with the findings that females are more likely to have MS (Sadovnick and Ebers, 1993). This ratio is similar to a previous descriptive study reporting the incidence and prevalence of MS in the UK 1990–2010 (Mackenzie et al., 2014). In that UK study, the average female-to-male ratio for MS was 2.4. That study also reported that the peak incidence of MS was at the age of 40 years in female and 45 years in the male. The maximum age at incidence in this project was 59 years (men) to 64 years (women), which was because that sample was small and older patients were more willing to respond in the current study. In addition, it was found that the maximum prevalence rates were at the ages of 50 years in female and 55 years in the male. These were lower than the findings of that UK study, peak prevalence rates were at the ages of 56 years (female) and 59 years (male).

According to a previous study, the average age of onset ranges from 29 to 33 (Sadovnick and Ebers, 1993). The average disease onset age was 35.71 years old in the current study which is relatively older than previous findings. Recently, a Denmark 60-year study described an increased MS incidence age, the researchers reported that disease onset age was 50 years old and older (Koch-Henriksen et al., 2018). In the current study, most patients had MS when they were 35 years old which is younger than that Denmark study in 2018.

It was reported that Males show a shorter time to progression than females based on previous studies (Rovaris et al., 2006, Broman et al., 1981, Muller, 1951). In the current study, it is noticed that Cox regression model showed no significant difference in terms of transition to a secondary progressive phase and risk of reaching EDSS 6.5, which is accordant with findings of other studies (Riise et al., 1992, Trojano et al., 1995, Amato and Ponziani, 2000).

Treatment

In a previous study, the disease-modifying drugs were reported to delay disability progression for patients with RRMS (Brown et al., 2007). According to a previous large observational study about the influences of treatment with interferon beta or glatiramer acetate on disability in RRMS patients, the models indicated that patients with DMT had a slower progression of EDSS than predicted for untreated controls (Palace et al., 2015).

The results in the current study cannot be compared to the larger epidemiological studies. The practice in the UK until recently was to start DMT only in patients with disease activation and in this sense not surprising in the older cohort reported here, the patients on DMT were the more severely affected. Treatment history was significantly related with MSSS in the patients with RRMS (r=0.189, p=0.011). Also, in the current study, the positive association between DMT and MSSS was found when patient age, clinical course, and disease onset age were considered without regard to disease duration (B =1.081, p<0.000), this relation was opposite to previous studies. Similarly, the cox model showed DMT history had a significant influence on the risk of reaching EDSS 6.5 with adjustment of patient age, disease type and disease

onset age (HR=2.011, *p*=0.008) but not the conversion of secondary phase in the whole population of this study.

Previous researchers demonstrated that the DMT might change the clinical course with the study for 2 to 3 years, but the effect of DMTs on the expected lifespan of patients with MS is unclear (Sanai et al., 2016). Specifically for interferon, a previous 21 years treatment cohort study, found a survival advantage of patients on early IFN β -1b treatment at any dose which together with confirmatory findings strengthened the IFN β -1b benefit on all-cause mortality (Goodin et al., 2012).

Disease onset age

In a 25-year cohort study, the researchers found that younger onset age was a factor significantly indicating a favourable long-term prognosis for adult MS (Runmarker and Andersen, 1993). Also, a prospective study compared the childhood and adult-onset MS patients in their adulthood based on the clinical and MRI findings and cognitive impairment, and the researchers reported that the prognosis of adult-onset MS seems to be better than that of early onset MS (Ozakbas et al., 2012). So, considering that the participants were all adults in the current study, the results could confirm that disease onset age was associated with MSSS (r=0.301, *p*<0.000) positively, in other words, older onset was associated with higher MSSS. The data from the earlier study (Koch et al., 2007a) also supported that SPMS patients with higher disease onset age have significantly shorter time to progression. The Cox model in the current analysis showed that disease onset age was significantly related to an increased risk of reaching EDSS 6.5 (HR=1.068, *p*<0.000) as well as the transition to the progressive course (HR=1.077, *p*<0.000), which further proved conclusion in previous studies.

4.2.2 Factors related to the progression of MS

BMI

In a cross-sectional study, it was found that patients with higher Body mass index (BMI) had significantly higher disease severity scores, but the effect was modest (Richter et al., 2016).

However, it was found no significant relationship in the current study (*B* =0.035, *p*=0.093) with adjustment of patient age, disease onset age and disease type.

Smoking

The earlier study proved that smoking had a significant influence on progression, on reaching specific disability landmark and risk of secondary progression (Manouchehrinia et al., 2013, Hernan et al., 2005). In this smaller study, a non-significant relation was found between smoking status and risk of reaching EDSS 6.5/SPMS even if other covariates were adjusted, which were in accordance with the findings of a previous study (Koch et al., 2007b). The dissociation of smoking status and MSSS, in this case, was consistent with previous findings (Manouchehrinia et al., 2013).

Alcohol

In a previous study, consumption of alcoholic beverages was found to be associated with favourable prognosis regarding the disability progression of RRMS, but not PMS (D'Hooghe M et al., 2012). Here, in the current study, it seemed that data could not show a significant association between alcohol consumption and risk to reach EDSS 6.5 for patients with RRMS.

Exercise

The relation between exercise and progression of disease disability among all patients with MS is difficult to define due to different clinical stages and residual symptoms. From the previous review, it was concluded that there was no apparent evidence of detrimental or adverse effects of exercise training (Motl and Pilutti, 2012). In the current study, the data confirmed that doing exercises (*B* =-0.754, *p*=0.006) or more exercise time (*B* =-0.11, *p*<0.000) may lead to reduction about the progression of disease disability even adjusted for other covariates. Also, it seemed that more exercises hours might contribute to the decrease of risk to reach EDSS 6.5 (HR=0.909, *p*=0.005) and transition to the secondary progressive clinical course (HR=0.888, *p*<0.000).

Mood

According to a new study about psychiatric comorbidity and MS, it was found that psychiatric issues increased the severity of subsequent neurologic disability (McKay et al., 2018). It seemed that the result from current data also supported this finding (*B* =0.703, *p*=0.006 controlled for patient age, disease onset age and clinical course).

4.2.3 Frailty, chronological age, and lifestyle

Chronological age

Apart from maternal grandmother and paternal grandfather, the FI of all the other family members was positively correlated with their chronological age or age at death. This finding is in accordance with a previous ageing study (Mitnitski et al., 2002), which concluded that the FI could affect predict survival sensitively. Also, the FI used in this study supported the validity of the items in the electronic FI, which was created by using data from the medical record in UK primary care (Clegg et al., 2016).

Smoking

A previous study found smoking to be positively associated with frailty status (Hubbard et al., 2009, Wang et al., 2013, Kojima et al., 2015). However, the data showed that smoking was not related to frailty, which differs from earlier findings. The difference in sample size and statistical methods could contribute to this difference.

Exercise

A previous review summarised the positive effect of physical activity on physical frailty (Marzetti et al., 2017). The data analysis of exercise status in the current study also confirmed this conclusion (r=-0.209, *p*<0.000).

4.2.4 Patients' frailty and progression of disease disability

With regards to analysis about patients' frailty level, results are accordant with the findings of a most recent multicentre epidemiology study (Hanlon et al., 2018). It was found that the MS

patients had the longest frail condition. Also, it was reported mortality in all age groups for men and women was related to pre-frailty and frailty significantly (except in female aged 37–45 years) with controlling for confounding variables. In the current study, the FI was closely correlated with chronological age without regarding of 'dead or alive.' For MS patients, their FI was correlated with EDSS as well as MSSS. Regression analysis not only showed the significant effect of patients' frailty on MSSS but also the effect on risk of reaching EDSS 6.5 and transition to secondary progressive clinical phase even with adjustment of other clinical variables, which further strengthen the conclusion from earlier studies. These findings further strengthened the relationship between comorbidities and progression of disease disability reported in earlier studies, because some items of FI in the current study were overlapped with the comorbidity list in a previous study (Horton et al., 2010).

It is noted that the r-value of correlation analysis for FI and EDSS was higher than frailty level and MSSS, which might imply that frailty was associated with EDSS score more closely than progression index which was the essence of MSSS. These three statistical models showed that frailty level of patients contributed to the MSSS score as well as clinical conversion even controlled for patient age and disease onset age. That means more comorbidities or clinical symptoms may increase the progression index, which is the progression velocity to reach a specific disability score. Also, frailty might increase the risk of transition to SPMS or reaching EDSS 6.5 in the current study. The biological age might explain these two effects of frailty. According to previous studies, the FI was created to measure biological age, which is a different concept from chronological age (Mitnitski et al., 2001). In the model of the current study, the effect of patient age and disease onset age equals to disease duration statistically, which reflect the influence of chronological ageing. Thus, it seems that frailty plays the role as the acceleration of chronological ageing, which is biological ageing, makes patients less resilient and much easier to progress when having MS. It may also explain why patients with the same disease duration reach specific EDSS score at the different time. Since it is difficult to clarify whether the comorbidity or residual symptoms can accelerate the progression of

EDSS indeed and whether age-related diseases play a more important role or not, further proof by following patients with and without comorbidities in the longitudinal study and compare the progression of MS disability is needed. This cross-sectional study could only provide a kind of possibility. After all, the influence of ageing on the progression of MS was come up not for a long time not mention to figure out how ageing affects MS. An earlier study raised the possibility that age influences MS in different ways and different degree/velocity (Sanai et al., 2016). The findings in the current case support this hypothesis to some degree. All in all, individual frailty may affect not only the degree of disability but also the velocity of progression of disability.

4.2.5 Familial longevity, frailty, and progression of disease disability

After analysis of familial markers from the current result, some weak relations could be seen between MSSS and parental ageing level defined in the current study when controlling for relevant confounders. However, for the risk of reaching EDSS 6.5 and transforming into the progressive course, it was found that parental ageing level had no significant effect.

Parental age and MSSS

In terms of parents' age, the simple correlations between mother's or father's age at death and EDSS or MSSS were positive although non-significant, it seemed that with age going up, disability or severity score also goes up. However, regression and partial correlations showed that once the effect of EDSS and disease onset age were controlled, the parental age may negatively influence MSSS score and reduce the progression index. Also, based on the sign of regression coefficients, the longer the patients' parents live, the slower progression velocity may be. Comparing the parameters of the model with and without parental living status, β value and *p*-value changed significantly. Considered the small population in the current study, it is relatively difficult to figure out whether it will still significantly affect MSSS in larger populations if without adjustment for being dead or alive. However, both negative sign of regression coefficient and partial correlation coefficient show a tendency of negative influence on MSSS (i.e., older age of parents is associated with lower MSSS).

By comparing the regression coefficient and the partial correlation coefficient from table 11-14, it seemed that mother's age played a more critical role in the model to predict MSSS than father's age. These may also support the assumption of a previous longevity study (Vagero et al., 2018), which concluded that parents could transmit longevity to both daughters and sons in a modest, but robust way. From these findings, it is possible that patients in the current study could inherit something from their parents. Regarding the importance of the mother's age in the prediction model of MSSS, it seems that the previous study could explain this (Hawkes, 2004). It was mentioned that for the offspring, genes controlling cellular maintenance and repair are inherited from the mother. However, this needs further study.

All in all, these findings imply that some weak relationship exists between parental age and progression velocity of disability even the effect of EDSS and disease onset age were controlled.

Grandparental age and MSSS

It is noticed that the sign of regression coefficients from the age of paternal grandparents was opposite to the age of maternal grandparents and parents. Here, it is difficult to conclude the specific direction of effect from grandparental age on MSSS. However, based on a previous meta-analysis of grandparental and grandchild survival, the researchers only found significant positive relations for both the maternal grandparents (Strassmann and Garrard, 2011). It could be used to explain the difference in age of maternal and paternal grandparents in the current study. Also, the most recent publication reported consistent findings (Vagero et al., 2018).

Parental and Grandparental frailty and MSSS

For parents, the frailty of mother and EDSS were significantly related in a positive way. Also, for grandparents' side, all the analysis from the frailty of maternal grandmother were significant. The results of frailty from all the other family members were not significant.

Also, it is noticeable that the relations between EDSS and most/least frailty were positively significant, but not the relations with MSSS. The *p* values of the regression model were

relatively high. These findings implied that the frailty of either parent might only relate to EDSS degree but not progression velocity.

'Parental ageing' and 'Parental lifestyle' and MSSS

Mother and father ageing

The PCA was applied to reduce dimensions, in this case, the main components extracted from rotation were interpretable. The created variable 'mother/father ageing' selectively synthesised the effect of current age/longevity, frailty and neurological disease history. In the model for prediction of MSSS, either putting mother and father ageing together with control for EDSS and disease onset age or putting their ageing separately with/without adjustment of EDSS and disease onset age, both models showed significant influences on MSSS no matter whether 'dead or alive' status was considered. These indicated that something that comes from the interaction of these three markers was passed to patients and contributed to the change of MSSS although relatively weakly, which was compatible with previous findings in the current study. Also, the partial correlation was consistent with multiple regression regarding the sign and value of the coefficients. Meanwhile, comparison of coefficients in the correlation matrix in mother and father ageing level suggested that mother's ageing relates progression velocity more than father's ageing (table 11 and 13). Strictly speaking, the role of familial environmental factors or socio-economic status cannot be excluded.

The rotated component matrix (table 16) showed that the frailty of father/mother plays a most crucial role in the final components, the previous significant relations only exist in parents' age, not frailty. Thus, it is difficult to confirm which variable affects MSSS most, only from rotation matrix, because that mathematical transformation is defined as a way that the first principal component has the maximumly possible variance, which explains the variability in the data as much as possible. So, the extracted variables can only be taken as markers to show the rough effect of 'parents' ageing' on progression velocity.

Parents' ageing

Parental ageing defined in the current study represented the combination of parental age, parental frailty level, and parental neurological degenerative disease history. The relation of this variable and MSSS reflected the rough effect of chronological ageing and biological ageing on disability of progression. Since the choices of question about history with the degenerative neurological disease was overlapped with some items in the list of FI, and they were used here for measurement of biological ageing (Mitnitski et al., 2002). These two variables together could explain the biological age. The parents' current age or age of death was to measure chronological ageing. In the current study, the parental age and longevity could not affect MSSS individually may be because they are too weak to change MSSS.

When they were merged in the second PCA model together with neurological degenerative disease history, the model synthesised the influence of ageing from both parents. The relation of parents' ageing and MSSS was significant with/without adjustment of EDSS and disease onset age regardless of living status. Also, no big difference was found between the coefficients of parental ageing and mother/father ageing, which further strengthened the influence from either of parents without losing much information. All in all, these findings supported the previous hypothesis that the inheritance of longevity should take into account of general susceptibility, frailty, and a new understanding of resilience from the perspective of epigenetics and social epidemiology (Vagero et al., 2018). After all, longevity is a concept determined by multifactorial influences.

Smoking and drinking

The analysis results from possible confounder variables showed almost no effect on velocity and degree of progression in disease disability. Even the second principle analysis still appeared to be non-significant. Although the influence of living habits on human longevity or frailty level was not entirely understood (Hubbard et al., 2009, Rajpathak et al., 2011), current study could not integrate lifestyle and ageing together to test effect because of small sample

size and unlimited missing data. Also considered that these variables are not mainly interested in measuring MSSS, they were removed from prediction model once they were found to be non-affected for progression of disease disability.

Parental ageing and risk of reaching EDSS 6.5 and conversion to SPMS

At last, two Cox models of average parental age and reaching EDSS 6.5 or converting to a progressive course reported no significance even adjusted for other variables. According to the statistical parameters, the predicted model could not successfully fit to predict the risk of reaching any disability stage, so it is difficult to get any confirmative conclusion about whether ageing can contribute to the change of risk. However, at least in this study, no significant relation it was found. In terms of statistical parameters, the model did not fit current data well. Also, it is likely that if MS was classified more precisely (RRMS vs SPMS), the sample size was larger, and EDSS scores were given accurately, the results for MSSS, reaching EDDS 6.5 and conversion to SPMS would have been similar.

4.3 Considering alternative explanations of the findings

1. Model to assess dead or alive cases separately:

Statistically speaking, because MSSS is affected by EDSS and disease duration, by analysing the MSSS table, high MSSS or progression index can only be obtained by high EDSS and short disease duration. In other words, once EDSS is fixed, disease duration negatively relates to progression velocity in the MSSS table. In the current study, a difference in patient age and disease onset age is the disease duration. Patient age is significantly associated with parental age, upon which the parental age may replace the effect of patient age mathematically. Thus, in a multiple regression model adjusted for EDSS and disease onset age, there is no significant difference in sign and value of coefficients no matter patient age or parents' age is measured.

When dead and alive cases were analysed separately for the age of both parents, the value of significance and regression coefficients changed obviously, but the tendency of the

relationship between parental age and MSSS remained stable. The relation in alive cases was significant but not in dead cases. It could be explained by the positive association between patient age and current parents' age.

It is also important to point out parents' age differs from patient age. Differences in these two variables could contribute to the clinical significance of these findings. Although both β values of multiple regression model for dead and alive cases were negative in this study, it is still relatively difficult to get a confirmative answer for the direction or degree of relationship between parental age and progression velocity to speak strictly. Because EDSS is closely related to MSSS, parents' age is positively correlated with EDSS, and there is another possibility that MSSS would increase gradually with the ageing of parents. This is still acceptable due to the decline of regeneration when patients get old. The difference between this interpretation and the previous one is that the parents' age seems to influence EDSS more. However, parental age negatively associated with MSSS in previous interpretation. All in all, in which direction parents' age influence MSSS is unclear by comparison of these two interpretations, but something significantly exists between these.

Also, together with the findings in the regression model of the variables extracted from the PCA, it might be concluded that successful lifespan or normal lifespan at least, could help to reduce the velocity of progression to some extent. By reviewing death reason of patients' parents in this case, some reported that they died young due to cancer, heart attack or some disease lead to sudden death. Instead, these parents lived without any other health deficits before they die. Thus, it is difficult to clarify whether parents who live longer with some chronic diseases can successfully pass their longevity.

2. Another model with EDSS to assess frailty

When the regression model with control for EDSS and disease onset age was applied to measure the relation of frailty and MSSS, almost no significant change of *p*-value can be seen.

In the current study, the changed signs of regression coefficient for frailty level of the mother, maternal grandmother, and paternal grandmother can be seen but not the significance. The effect of father frailty and most frailty levels of parents became significant. However, these differences could not reflect anything meaningful regarding statistics or clinics.

4.4 Clinical relevance

The relation of ageing and MS was mentioned in recent decades. In terms of clinical relevance, clinicians should be cautious about any comorbidities in patients with MS especially those in the progressive course and provide timely treatment.

4.5 Strengths and weaknesses of the study

First study to explore the relationship between familial longevity and MS

There are many longevity or ageing studies associated with specific disease especially heart disease (Brand et al., 1992, Spann and Ottinger, 2018, Gubbi et al., 2017), but this project is unique clinical research to discover family factor and progression of MS by questionnaires. The results may provide new insight to understand the effect of ageing on disability progression also might exclude some possible risk factors for disease development.

The first application of FI in MS patients

This FI has been used to manage disease in earlier studies. The previous researchers used modified FI, which is a dominant measurement of risk before operation by utilising data of routinely available health record to predict morbidity and mortality of patients after neurosurgery for intracranial neoplasms in the various national cohort study (Youngerman et al., 2018). In another study, the researchers used a frailty deficit index to assess how frailty affect the prognosis of advanced epithelial ovarian cancer after primary debulking surgery (Kumar et al., 2017). This is the first study applying the FI to MS patients for investigating comorbidities or symptoms. Also, the current study proved the validity of the electronic FI (Clegg et al., 2016), which was created by utilising data of routinely electronic medical record in primary care. In the current study, with the limitation of time and ethics, the items of FI were

asked directly in the questionnaires given to patients in the clinic or by post. However, by reviewing the completed data and results from the statistical analysis, this index still worked well, and the final findings are compatible with a previous epidemiology study (Hanlon et al., 2018).

In a previous study, researchers found the relation of vascular comorbidity and faster progression of disability in MS (Marrie et al., 2010). In another epidemiology study, a self-report questionnaire was created, and its use was validated for capturing comorbidities affecting MS patients (Horton et al., 2010). In addition, frailty phenotype was defined by five criteria (weight loss, exhaustion, grip strength, low physical activity, slow walking pace) (Hanlon et al., 2018). The FI used in the questionnaire in the current study was a combination of comorbidity as well as a symptom, which consists of MS clinical symptoms and comorbidities. The items in this index selectively included some items that were missing in the Horton et al. study, which was more concise and included more details than in the Hanlon et al. study. In the previous Horton M et al. study, their researchers explored the relation of comorbidity and disability progression in MS. The findings in the current study also measured that relation but with different predictors and outcome variable.

Reproducibility and validity

The research tool used in this study is the distribution of a printed questionnaire which is cheap and easy to replicate. Also, any statistical methods applied are the models commonly used. Every variable or index is chosen from related literature to avoid subjective bias.

However, the reliability of responded answers needs a further repeated measure for the same questions in the same population. One of the disadvantages of the questionnaire study is that participants possibly give different answers at different times even when the same questions are asked. Hopefully, participants can offer approximately consistent answers no matter when they fill in questionnaires. In the current study, some answers are not precisely known (e.g., grandparental age and frailty), and there may be other possible confounders. During the clinic,

participants usually reported that they could not offer any accurate details especially in the section of grandparents. Also, some patients mentioned in the questionnaires that their family members are not living together with them, some of their grandparents died in accidents when they or their parents were young.

Questionnaires and index

Comparing the data collected from the MS clinic and that sent back by post, the response rate in one month was relatively acceptable. Because of the summer holiday, some patients may need more time to send back the questionnaires. Giving questionnaires by post and in clinic simultaneously could save time and the rates of missing data were similar.

Another scale, Charlson comorbidities index is also commonly used in the clinical study, which is created to evaluate the relative risk of death from prognostic clinical covariates (Charlson et al., 1994). The most recent research used this index to study the influence of multiple comorbidities on functional baseline of patients with MS upon initial presentation to the neuroimmunology clinic at a tertiary referral centre, and the found that a higher comorbidity score may be related to a more severe clinical course in patients with MS (Alsallom et al., 2018). Also, there are various versions of the FI. Although in the current study, time is not enough to compare these different kinds of frailty or comorbidity index. There is no difference in terms of a conclusion.

Limitation

For the epidemiological study, it is relatively difficult to collect large longitudinal data in the current MRes project. Due to time limitations, the current study is only a pilot study for formal epidemiology research regarding sample size. Because of these small samples, some possible confounders were excluded for a regression model to keep the stability of the model.

However, in the current study, some MS-related factors were proven to be significant in analysis results. On the one hand, some confounders are correlated with each other. To avoid multicollinearity or violation of assumption in multiple regression, they were excluded in the

final regression model. On the other hand, the relation of MSSS and most of these variables exists based on the relation with EDSS, but their effects are relatively weak as well. To make the model fit current data statistically to measure variables of interest, EDSS was put in the model. So strictly speaking, the predictors of interest may contribute to the difference between EDSS and MSSS. Because MSSS is created by EDSS and disease duration, the predictors in the current study may have a similar effect as disease duration regarding statistics.

Also, missing data is another problem, especially in clinic questionnaire study. In this case, missing data mainly exist in the grandparental sections. For imputation, there are various procedures for different missing mechanisms, but the main disadvantage is that these methods need lots of work to create interpolation sets. Meanwhile, sensitivity analysis and some pitfalls should be considered to avoid misleading conclusions (Sterne et al., 2009). To achieve multiple imputation, which is created for dealing with the problem of increased noise due to imputation, it is better to use relatively macro data to avoid overly precise results and errors in any conclusions drawn.

Bias

Some bias may arise in this questionnaire-based study with regards to the data collection and management. Primarily, there is recall bias on behalf of the MS patients. If the patients could not remember exact information or provide incomplete data that may lead to inaccurate statistical results especially that involve factors of interest. Then is the investigation bias, which comes from both researchers and participants. In this case, the self-designed questionnaire has not been validated before. Also, the comparison of different combinations of the index was not made. So, the quality of this questionnaire needs a further test. For report bias, an alternative explanation of the conclusion is mentioned previously. The data analysed finally was raw data in this study to avoid diagnosis or exposure suspicion bias. However, when participants answered questions about frailty or death reason regardless of specific family members, in the clinic, they reported that due to their parents or grandparents old age, they might die of some disease. It is unknown that whether postal responders also did this when

answering because they fill in questionnaires without the supervision of a researcher. In addition, the data of the non-responders such as demographics and disability status were not extracted from the database. Thus, it is difficult to know whether the acquired data of these 323 responders can represent the whole sample (700).

Statistical models/methods

It is difficult to find the perfect model to measure the relation of family longevity and disability progression of MS. Finally, multiple regression with adjustment was applied to detect linear relation, and the Cox model was performed for measuring risk to reach EDSS score of 6.5 and secondary progressive phase. In this case, building statistical models is not the principal objective and one-year time is not enough to run the detailed analysis for different controlled variables. So, for the measurement of the outcome variable (MSSS) and building a relatively appropriate model, EDSS and disease onset age were chosen for fitting the current data statistically.

The data from an earlier study (Koch et al., 2007a) supported that progression of MS was an age-dependent process without relation with relapses. However, previous researchers reported that more frequent relapses in the first 2 years indicated reaching hard disability points more rapidly. More early relapses and shorter first inter-attack intervals lead to faster deterioration by interacting with the neurodegeneration characterising secondary progression (Scalfari et al., 2010). In other previous studies, it was concluded that early available clinical parameters contribute to the time from disease onset to the EDSS 4, but not the consecutive progression to the permanent disability (Confavreux et al., 2000, Confavreux et al., 2003). Similarly, a previous study also reported that early inflammatory attacks might not affect the transition to the secondary progressive course (Scalfari et al., 2013). Here, with the limitation of time to review all the medical records, the information of relapses was not collected.

Lack of repeated measurement and subgroup/other datasets

Considering that in a cross-sectional questionnaire study, it is relatively difficult to perform repeated measure only in one year to confirm the results current study was not found by chance. Also, even if the repeated measure is applied to the same population, the conclusion may change at different times. So, all the current conclusions could be significant by chance regarding quality and precision, because old patients were selectively recruited ant first to get certain longevity/lifespan of their parents (longevity is defined for dead people and sometimes meant to refer only to exceptionally long-lived members of a population). For the generalisation of this hypothesis, due to a single centre and limited research duration, it is relatively difficult to get more datasets to test validity in other populations. Thus, any significant findings from this data exploration should be treated conservatively.

For this exploring study, no other methodological limitations were considered to influence the results including research cites or statistical software. If there is, that may be retrospective design for data used in the cox model or inadequate controls for confounding variables. For instance, the precision and reliability of medical records about reaching EDSS 6.5 or converting to SPMS. It is difficult to build a model with many adjusted covariates in the current study with a small sample size.

4.6 Suggestions for further research

The repeated measure for the same questions in the same population is needed to thoroughly investigate the relationship between familial longevity and progression of disease disability. For testing the validity of findings in the current project, the priority is to use larger population data at least much larger than the current one. If possible, collecting data from multiple, ideal multi-country centres are helpful for increasing the power statistically, and generalising the conclusions. Also, the validity of the FI in predicting hospital admissions can be tested by analysing data in the primary care.

Longevity or lifespan is caused by multiple factors, like the progression of MS. To find a relatively appropriate model to assess how familial longevity is affecting disease progression, the choice of parameters and control of possible confounders are important. Also, it would be important to collect every patient blood samples and other biomarkers to test if any relationship found has a genetic component or it is related to socioeconomic factors. Notably, a previous study reported the association of SES and risk of comorbidities in this disease (Marrie et al., 2008). The lower SES is related to reduced access to medical care, lower quality of care, or adoption of poor health behaviours, the future related study may need to consider the effect of SES and include this confounder.

Thus, clinical marker or MRI marker become more helpful. More statistical tests are necessary to build the prediction model. The further researcher can test more predictors and compare different statistical models. Also, the generalisation of applying MSSS to monitor the progression of disability needs further confirmation by independent studies.

With regards to statistics, if sample size allows, more advanced models or analysis can be used. Sub-analysis for other comorbidities could be tested, previously such as vascular disease, psychiatric comorbidity and diabetes were proved to be connected with faster disability progression in MS (Marrie et al., 2010, McKay et al., 2018). A most recent study also reported that anxiety and diabetes adversely affect cognition in MS (Marrie et al., 2018). Additionally, age-related diseases can also be measured to see if there is any relation to disability progression in secondary clinical phase. For example, in a previous study, the authors reported that hypertension, diabetes mellitus, and obstructive lung disease impacted clinical course in MS patients regarding walking speed and self-reported disability (Conway et al., 2017).

If necessary, cross-validation can be used to evaluate the generalisation of current findings to other independent population. Avoiding statistical problems like overfitting (the production of an analysis that corresponds too closely or precisely to a particular set of data, therefore may not be able to fit additional data or predict future observations precisely), an independent

analysis can be applied to guarantee variables are statistically independent of each other. For future exploration study should follow the procedure, plan analysis-descriptive analysis-diagnosis analysis-prediction analysis-explanation analysis.

If the relation is found to be significant, comparison of progression of patients with long-lived parents (e.g., over 85 years old) and a short-lived one can further strengthen the hypothesis. As there are many definitions of exceptional longevity (Gudmundsson et al., 2000, Deluty et al., 2015, Kim et al., 2013), the specific threshold was not set in current case but could be considered in the future study.

It is necessary for future researchers to collect data from the medical and demographic data to avoid inaccurate data. Cross-referencing some of these data will confirm data accuracy and reduce noise for analysis.

Also, future researchers can further confirm the relation of ageing and MS by exploring patients in the secondary progressive stage. According to previous studies, progressive course mainly caused by neuro-degeneration (Fitzner and Simons, 2010). Also, it was reported that reduced antioxidant activity of lipoproteins and shortened telomere length are related to the severity of MS (Guan et al., 2015). To figure out how ageing influences progression of MSSS is essential to understand the progression of disability in the secondary stage. Some participants reported that their parents are healthy without any disease and live a long time, but their disease conditions progress faster than other patients, or they have a more severe disability. These phenomena may occur just because of coincidence, or they might fail to achieve healthy ageing. After all, whether ageing can be programmed is still a controversial topic. With respect to brain ageing, a previous study indicated that telomeric DNA length in cerebral grey and white matter are related to longevity in people aged 70 years or older (Nakamura et al., 2007). Therefore, the effect of brain ageing and longevity on MS could be investigated to help fully understand ageing and MS.

Conclusion

The current study explored the exploration of the relationship between familial longevity and progression of disability in MS. We demonstrated that in the current population, familial longevity was found not to be associated with the progression rate of MS disability (MSSS) when measured separately. Nevertheless, once adjusted for other covariates, the synthesised effect of parental lifespan, parental frailty level and their neurological degenerative disease history on MSSS was significant, albeit weak. It is concluded that there may be a relationship between familial ageing and progression rate of MS, but the power of effect is not entirely clear. Additional studies can measure not only longevity but also consider other comorbidities. Especially for patients in the progressive clinical course, such researches can clarify the influence of familial factors on progression of disability in this disease.

Appendices

Appendix 1: Invitation letter



INVITATION LETTER

Title of Study: THE EFFECT OF FAMILY LONGEVITY ON THE DISABILITY PROGRESSION IN PATIENTS WITH MULTIPLE SCLEROSIS: AN EPIDEMIOLOGICAL EVALUATION

Dear……

You are being invited to take part in the above research study. Before you decide it is essential for you to understand why the research is being done and what it will involve. Please take time to read the enclosed information sheet carefully. Talk to others about the study if you wish. This study will be investigating the effect of family longevity on the disability progression in patients with Multiple Sclerosis (MS).

Some studies have found that ageing appears to influence the chronic diffuse neurodegeneration of MS as well as capacity for remyelination, and it is suggested that 25-30% of the variation in human life expectancy may under genetic control.

The study has been approved by North West - Liverpool Central Research Ethics Committee and is being carried out in the Division of Clinical Neurology, C Floor, South Block, Queen's Medical Center, and Nottingham, NG7 2UH. We are looking to recruit patients with MS who are over 18 years old. You will be asked to complete a questionnaire booklet and sign the consent form and return it back to us if you choose to participate in this study.

If you wish to know more about the study, please take time to read the enclosed information sheet. Alternatively, you can contact us should you require more information, or you have any concern about the study.

Contact Details;

Dr Nikos Evangelou

Division of Clinical Neurology, C Floor. South Block, Queen's Medical Center, Nottingham, NG7 2UH

Phone: +44 (0)115 82 31192.

Email: mzxxg@nottingham.ac.uk

Yours sincerely,

Dr Nikos Evangelou

Clinical Associate Professor

University of Nottingham

Appendix 2: Patient information sheet



University of Nottingham
UK | CHINA | MALAYSIA

Participant Information Sheet

Title of Study: The effect of family longevity on disability progression in patients with Multiple Sclerosis: An Epidemiological Evaluation

Name of Researcher(s): Dr Nikos Evangelou, Dr Xin Gao

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. If you want, one of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?

This study is being undertaken as part of an MSc qualification. In Multiple sclerosis (MS) the immune system attacks the protective myelin sheath that covers nerve fibres, and the disease causes the nerves degeneration or loss. Many patients after a few years develop progressive symptoms due to the nerve degeneration. We don't fully

understand why that affects some patients more than others. Several genes and other risk factors for disability progression have been described.

We are aware of inherited factors which affect how long people live. Some families have members that live until they are old and some not. Hence, we decided to explore the relation of family longevity and disability progression among MS patients by using a patient self-reported questionnaire. Information about family history, personal history and current health condition of MS patients will be included in this research, along with information from your clinic notes. We anticipate that the results of this prognostic study may add in the future in our prediction of disease severity and progression of disability in MS.

Why have I been invited?

You are being invited to take part because you have a diagnosis of MS made by the referring neurologist and have been registered with MS Register indicating that you would like to be contacted for MS research purposes. We are inviting 2000 participants like you to take part. If you feel unable to complete the questionnaire (e.g., reading disorder, visual problems or dexterity difficulties) and would like us to help you, please contact a research member of the team, and we will try to help you.

Do I have to take part?

It is up to you to decide whether or not to take part in this questionnaire study. If you do decide to take part in you will keep this information sheet, and we will ask you to sign a consent form which would appreciate if you could return to us with the questionnaire in the self-stamped envelope or return to us when you come to the clinic.

If you decide to take part in you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights or medical care.

What will happen to me if I take part?

This research involves completing selected questions from a patient self-reported questionnaire about your family history and individual history, which may take you 10 minutes to complete. You will be asked to complete the questionnaire at home and return it to the team by a prepaid envelope provided or during your clinic visit; you will also be given a choice to complete the questionnaire with the assistance of a member of your family or a carer. In addition, the completion and subsequent return of the questionnaires will be assumed to be informed consent for this particular questionnaire study. If you are not willing to take part in this study, you do not need to send back the envelope.

This study will last for 6 months, but your participation will be limited to the one questionnaire. If we do not hear from you within one month, we will telephone you to check you have received the questionnaire. As you are participating at the MS register, we will also review your medical notes to estimate your level of disability based on a neurological examination by neurologists.

Expenses and payments

Participants will not be paid to participate in the study.

What are the possible disadvantages and risks of taking part?

This study involves a single questionnaire, so there are no risks, discomfort or inconvenience expected as a result of participation in this study. Despite our efforts not to upset any participants, it is possible that some may be upset by answering questions about various health conditions experienced by themselves or their family members. Participants can of course not take part in this study and not fill in the questionnaire. If the questionnaire raises any concerns for you, please contact your doctor.

If the information is disclosed during the study that could pose a risk of harm to you or others, the researcher will discuss this with the Chief Investigator and where appropriate, report to the university, the general practitioner or the MS clinical team and inform you accordingly.

What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get from this study may help provide a new perspective of predicting the prognosis of MS

What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers' contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting the Patient Advice and Liaison Service (PALS) at Nottingham University Hospital NHS Trust (01159249924 ext.65412).

In the event that something does go wrong, and you are harmed during the research, and this is due to someone's negligence then you may have grounds for legal action for compensation against the University of Nottingham, but you may have to pay your

legal costs. The normal National Health Service complaints mechanisms will still be available to you.

Will my taking part in the study be kept confidential?

Yes. We will follow the ethical and legal practice, and all information about you will be handled in confidence.

We will follow the ethical and legal practice, and all information about you will be handled in confidence.

If you join the study, we will use the information collected from you [and your medical records] during the course of the research. This information will be kept strictly confidential, stored in a secure and locked office, and on a password-protected database at the University of Nottingham. Under UK Data Protection laws the University is the Data Controller (legally responsible for the data security), and the Chief Investigator of this study (named above) is the Data Custodian (manages access to the data). This means we are responsible for looking after your information and using it correctly. Your rights to access, change or move your information are limited as we need to manage your information in specific ways to comply with specific laws and for the research to be reliable and accurate. To safeguard your rights, we will use the minimum personally – identifiable information possible.

You can find out more about how we use your information and read our privacy notice at:

The data collected for the study will be looked at and stored by authorized persons from the University of Nottingham who are organizing the research. They may also be looked at by authorized people from regulatory organizations to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant, and we will do our best to meet this duty.

Where possible information about you which leaves the hospital will have your name and address removed) and a unique code will be used so that you cannot be recognized from it, however sometimes we need to ensure that we can recognize you to link the research data with your medical records so in these instances we will need to know your name and date of birth.

Your contact information will be kept by the University of Nottingham for 12 months – 3 years after the end of the study so that we are able to contact you about the findings of the study and possible follow-up studies (unless you advise us that you do not wish to be contacted). This information will be kept separately from the research data collected, and only those who need to will have access to it. All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team given permission by the data custodian will have access to your personal data.

Individual participant medical or personal information obtained as a result of this study are considered confidential, and disclosure to third parties is prohibited with the exceptions noted above. Participant confidentiality will be further ensured by utilizing identification code numbers to correspond to treatment data in the computer files. Such medical information may be given to the participant's medical team and all

appropriate medical personnel responsible for the participant's welfare. If the information is disclosed during the study that could pose a risk of harm to the participant or others, the researcher will discuss this with the CI and where appropriate report accordingly. Data generated as a result of this study will be available for inspection on request by the participating physicians, the University of Nottingham representatives, the REC, local RandD Departments and the regulatory authorities.

What will happen if I don't want to carry on with the study?

Your participation is voluntary, and you are free to withdraw at any time, without giving any reason, without your legal rights or medical care being affected. If you withdraw, we will no longer collect any information about you or from you, but we will keep the information about you that we have already obtained as we are not allowed to tamper with study records and this information may have already been used in some analyses and may still be used in the final study analyses. To safeguard your rights, we will use the minimum personally-identifiable information possible.

Involvement of the General Practitioner/Family doctor (GP)

We will not need to notify your GP (or other health care practitioner) for this study.

What will happen to the results of the research study?

Data will be analyzed, written up and sent for publication in medical journals or international conferences, and you will not be identified in any report/publication. Some part of this study will be written up as part of a Master dissertation. We will also inform you by letter of any significant findings if you wish, and we can send you copies of the published papers at your request.

Who is organizing and funding the research?

This research is being organized by the University of Nottingham and is being funded by the Division of Clinical Neurology, C Floor, Medical School, University of Nottingham, Queen's Medical Centre, and Nottingham, NG7 2UH.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given a favourable opinion by the Research Ethics Committee.

Further information and contact details

If you have any queries about the study, please contact Dr Nikos Evangelou, Division of Clinical Neurology, C Floor, Medical School, University of Nottingham, Queen's Medical Centre, and Nottingham, NG7 2UH. Phone: 0115 82 31192.

Email: nikos.evangelou@nottingham.ac.uk

Appendix 3: Questionnaire booklet

Confidential

Family History and Multiple Sclerosis Questionnaire

We are aware of inherited factors which affect how long people live. Some families have members that live until they are old and some not. Hence, we decided to explore the relation of family longevity and disability progression among MS patients by using self-reported questionnaires. Information about family history, personal history and current health condition of MS patients will be included in this survey, along with information from your clinic notes. We anticipate that the results of this prognostic survey may add in the future in our prediction of disease severity and progression of disability in MS. Please answer ALL the questions below by merely choosing the answer which you think applies to you the most.

If you have any questions regarding the completion of this questionnaire, please contact the research team by email: mzxxg@exmail.nottingham.ac.uk.

Thank you very much for your time.

1) Have you registered in the MS clinic?

- Yes
- No

2) Your MS Register ID. _____ (If you haven't registered, just put "N/A".)

3) Your email address _____ (if you don't have email, just put N/A or provide one your family members' email address)

4) Gender

- Male
- Female

5) Initials _____

6) Your date of birth _____

7) Current Age of Biological MOTHER (if died, please choose the Age of death)

36-40 41-45 46-50 51-55 56-60 61-65 66-70 71-75 76-80 81-85 86-90 91-95

8) If your Biological MOTHER died, please provide the reason of death. If she is still alive, please note "alive". _____

9) Previous smoking (Biological MOTHER)

- Never
- Yes, but quit later
- Yes, but not frequent
- Frequent
- Don't know

10) Lifelong average alcohol consumption (Biological MOTHER)

- Never
- Yes, but quit later,
- Yes, but not frequent
- Frequent
- Don't know

11) Diagnosis of any degenerative neurological disease (Biological MOTHER)

- Multiple sclerosis
- Motor neuron disease
- Dementia

- Parkinson's disease
- Epilepsy
- None of the above
- Don't know

12) Health conditions (Biological MOTHER)

- Activity limitation
- Anaemia
- Arthritis
- Atrial fibrillation
- Cerebrovascular disease
- Chronic kidney disease
- Diabetes
- Dizziness
- Dyspnoea
- Falls
- Foot problems
- Fragility fracture
- Hearing loss

- Heart failure
- Heart valve disease
- Housebound
- Hypertension (high blood pressure)
- Hypotension (low blood pressure)
- Ischemic heart disease
- Memory/cognitive problems
- Mobility problems
- Osteoporosis
- Parkinson's
- Peptic ulcer
- Peripheral vascular disease
- Poly-pharmacy
- Require care
- Respiratory disease
- Skin ulcer
- Sleep disturbance
- Social vulnerability
- Thyroid Disease

- Urinary incontinence
- Urinary system disease
- Visual impairment
- Weight loss/anorexia
- None of the above
- Don't know

13) Current Age of Biological FATHER (if died, please choose the Age of death)

36-40 41-45 46-50 51-55 56-60 61-65 66-70 71-75 76-80 81-85 86-90 91-95

14) If your Biological FATHER died, please provide the reason of death. If he is still alive, please note "alive". _____

15) Previous smoking (Biological FATHER)

- Never
- Yes, but quit later
- Yes, but not frequent
- Frequent
- Don't know

16) Lifelong average alcohol consumption (Biological Never FATHER)

- Yes, but quit later,
- Yes, but not frequent
- Frequent
- Don't know

17) Diagnosis of degenerative neurological disease (Biological FATHER)

- Multiple sclerosis
- Motor neuron disease
- Dementia
- Parkinson's disease
- Epilepsy
- None of the above
- Don't know

18) Health conditions (Biological FATHER)

- Activity limitation
- Anaemia
- Arthritis
- Atrial fibrillation

- Cerebrovascular disease
- Chronic kidney disease
- Diabetes
- Dizziness
- Dyspnoea
- Falls
- Foot problems
- Fragility fracture
- Hearing loss
- Heart failure
- Heart valve disease
- Housebound
- Hypertension (high blood pressure)
- Hypotension (low blood pressure)
- Ischemic heart disease
- Memory/cognitive problems
- Mobility problems
- Osteoporosis
- Parkinson's

- Peptic ulcer
- Peripheral vascular disease
- Poly-pharmacy
- Require care
- Respiratory disease
- Skin ulcer
- Sleep disturbance
- Social vulnerability
- Thyroid Disease
- Urinary incontinence
- Urinary system disease
- Visual impairment
- Weight loss/anorexia
- None of the above
- Don't know

19) Current Age of Biological MATERNAL GRANDMOTHER (if died, please choose the Age of death)

< 50 51-60 61-70 71-80 81-90 91-100

20) If your Biological MATERNAL GRANDMOTHER died, please provide the reason of death. If she is still alive, please note "alive".

_____

21) Previous smoking (Biological MATERNAL GRANDMOTHER)

- Never
- Yes, but quit later
- Yes, but not frequent
- Frequent
- Don't know

22) Lifelong average alcohol consumption (Biological Never MATERNAL GRANDMOTHER)

- Yes, but quit later,
- Yes, but not frequent
- Frequent
- Don't know

23) Diagnosis of degenerative neurological disease (Biological MATERNAL GRANDMOTHER)

- Multiple sclerosis
- Motor neuron disease
- Dementia
- Parkinson's disease
- Epilepsy
- None of the above
- Don't know

24) Health conditions (Biological MATERNAL GRANDMOTHER) Activity limitation

- Anaemia
- Arthritis
- Atrial fibrillation
- Cerebrovascular disease
- Chronic kidney disease
- Diabetes
- Dizziness
- Dyspnoea
- Falls
- Foot problems

- Fragility fracture
- Hearing loss
- Heart failure
- Heart valve disease
- Housebound
- Hypertension (high blood pressure)
- Hypotension (low blood pressure)
- Ischemic heart disease
- Memory/cognitive problems
- Mobility problems
- Osteoporosis
- Parkinson's
- Peptic ulcer
- Peripheral vascular disease
- Poly-pharmacy
- Require care
- Respiratory disease
- Skin ulcer
- Sleep disturbance

- Social vulnerability
- Thyroid Disease
- Urinary incontinence
- Urinary system disease
- Visual impairment
- Weight loss/anorexia
- None of the above
- Don't know

25) Current Age of Biological MATERNAL GRANDFATHER (if died, please choose the Age of death)

< 50 51-60 61-70 71-80 81-90 91-100

26) If your Biological MATERNAL GRANDFATHER died, please provide the reason of death. If he is still alive, please note "alive".

_____

27) Previous smoking (Biological MATERNAL GRANDFATHER)

- Never
- Yes, but quit later

- Yes, but not frequent
- Frequent
- Don't know

28) Lifelong average alcohol consumption (Biological MATERNAL GRANDFATHER)

- Never
- Yes, but quit later,
- Yes, but not frequent
- Frequent
- Don't know

29) Diagnosis of degenerative neurological disease (Biological MATERNAL GRANDFATHER)

- Multiple sclerosis
- Motor neuron disease
- Dementia
- Parkinson's disease
- Epilepsy
- None of the above
- Don't know

30) Health conditions (Biological MATERNAL GRANDFATHER)

- Activity limitation
- Anaemia
- Arthritis
- Atrial fibrillation
- Cerebrovascular disease
- Chronic kidney disease
- Diabetes
- Dizziness
- Dyspnoea
- Falls
- Foot problems
- Fragility fracture
- Hearing loss
- Heart failure
- Heart valve disease
- Housebound
- Hypertension (high blood pressure)
- Hypotension (low blood pressure)

- Ischemic heart disease
- Memory/cognitive problems
- Mobility problems
- Osteoporosis
- Parkinson's
- Peptic ulcer
- Peripheral vascular disease
- Poly-pharmacy
- Require care
- Respiratory disease
- Skin ulcer
- Sleep disturbance
- Social vulnerability
- Thyroid Disease
- Urinary incontinence
- Urinary system disease
- Visual impairment
- Weight loss/anorexia
- None of the above

- Don't know

31) Current Age of Biological PATERNAL GRANDMOTHER (if died, please note the Age of death)

< 50 51-60 61-70 71-80 81-90 91-100

32) If your Biological PATERNAL GRANDMOTHER died, please provide the reason of death. If she is still alive, please note "alive". _____

33) Previous smoking (Biological PATERNAL GRANDMOTHER)

- Never
- Yes, but quit later
- Yes, but not frequent
- Frequent
- Don't know

34) Lifelong average alcohol consumption (Biological Never PATERNAL GRANDMOTHER)

- Yes, but quit later,
- Yes, but not frequent

- Frequent
- Don't know

35) Diagnosis of degenerative neurological disease (Biological PATERNAL GRANDMOTHER)

- Multiple sclerosis
- Motor neuron disease
- Dementia
- Parkinson's disease
- Epilepsy
- None of the above
- Don't know

36) Health conditions (Biological PATERNAL GRANDMOTHER)

- Activity limitation
- Anaemia
- Arthritis
- Atrial fibrillation
- Cerebrovascular disease
- Chronic kidney disease

- Diabetes
- Dizziness
- Dyspnoea
- Falls
- Foot problems
- Fragility fracture
- Hearing loss
- Heart failure
- Heart valve disease
- Housebound
- Hypertension (high blood pressure)
- Hypotension (low blood pressure)
- Ischemic heart disease
- Memory/cognitive problems
- Mobility problems
- Osteoporosis
- Parkinson's
- Peptic ulcer
- Peripheral vascular disease

- Poly-pharmacy
- Require care
- Respiratory disease
- Skin ulcer
- Sleep disturbance
- Social vulnerability
- Thyroid Disease
- Urinary incontinence
- Urinary system disease
- Visual impairment
- Weight loss/anorexia
- None of the above
- Don't know

37) Current Age of Biological PATERNAL GRANDFATHER (if died, please note the Age of death)

< 50 51-60 61-70 71-80 81-90 91-100

38) If your Biological PATERNAL GRANDFATHER died, please provide the reason of death. If he is still alive, please note "alive".

_____

39) Previous smoking (Biological PATERNAL GRANDFATHER)

- Never
- Yes, but quit later
- Yes, but not frequent
- Frequent
- Don't know

40) Lifelong average alcohol consumption (Biological PATERNAL GRANDFATHER)

- Never
- Yes, but quit later,
- Yes, but not frequent
- Frequent
- Don't know

41) Diagnosis of degenerative neurological disease (Biological PATERNAL GRANDFATHER)

- Multiple sclerosis
- Motor neuron disease
- Dementia
- Parkinson's disease
- Epilepsy
- None of the above
- Don't know

42) Health conditions (Biological PATERNAL GRANDFATHER)

- Activity limitation
- Anaemia
- Arthritis
- Atrial fibrillation
- Cerebrovascular disease
- Chronic kidney disease
- Diabetes
- Dizziness
- Dyspnoea
- Falls
- Foot problems

- Fragility fracture
- Hearing loss
- Heart failure
- Heart valve disease
- Housebound
- Hypertension (high blood pressure)
- Hypotension (low blood pressure)
- Ischemic heart disease
- Memory/cognitive problems
- Mobility problems
- Osteoporosis
- Parkinson's
- Peptic ulcer
- Peripheral vascular disease
- Poly-pharmacy
- Require care
- Respiratory disease
- Skin ulcer
- Sleep disturbance

- Social vulnerability
- Thyroid Disease
- Urinary incontinence
- Urinary system disease
- Visual impairment
- Weight loss/anorexia
- None of the above
- Don't know

43) Your weight (please annotate kg/stone/pound)

_____

44) Your height (please annotate cm/foot/m)

_____

45) Your smoking habit (cigarettes/day)? If you smoke in the past and now quit, please note "in the past'' and provide previous smoking habit (cigarettes/day).

_____

46) Your alcohol consumption (units/week)? If you don’t know, please provide how much, what kind of alcohol and how many times you drink per week. E.g., two bottles of beer three times a week

If you drink in the past and now quit, please note

"In the past" and provide previous alcohol consumption

_____

47) Do you still work? How many hours do you work per week?

_____

48) Your exercise (hours/week)?

_____

49) Do you have a mood disorder (depression, anxiety, etc.)?

- Yes
- No

50) Your health conditions

- Activity limitation
- Anaemia

- Arthritis
- Atrial fibrillation
- Cerebrovascular disease
- Chronic kidney disease
- Diabetes
- Dizziness
- Dyspnoea
- Falls
- Foot problems
- Fragility fracture
- Hearing loss
- Heart failure
- Heart valve disease
- Housebound
- Hypertension (high blood pressure)
- Hypotension (low blood pressure)
- Ischemic heart disease
- Memory/cognitive problems
- Mobility problems

- Osteoporosis
- Parkinson's
- Peptic ulcer
- Peripheral vascular disease
- Poly-pharmacy
- Require care
- Respiratory disease
- Skin ulcer
- Sleep disturbance
- Social vulnerability
- Thyroid Disease
- Urinary incontinence
- Urinary system disease
- Visual impairment
- Weight loss/anorexia
- None of the above
- Don't know

51) Do you wish to be informed by letter of any significant findings from this study? We can send you by post/e-mail.

- Yes
- No

Appendix 4: Consent form

CONSENT FORM

Title of Study: The effect of family longevity on the disability progression in patients with Multiple Sclerosis: An Epidemiological Evaluation

Name of Researcher: Dr Nikos Evangelou, Dr Xin Gao

Name of Participant:

1. I confirm that I have read and understood the information sheet version 2.0 25/June/2018 for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. If the information is disclosed during the study that could pose a risk of harm to me or others, the researcher will discuss this with the Chief Investigator and where appropriate, report it accordingly to your general practitioner.

3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorized individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyze and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential.

4. I understand that the information collected about me will be used to support other research in the future and may be shared anonymously with other researchers.

5. I agree to take part in the above study.

_____	_____	_____
Name of Participant	Date	Signature
_____	_____	_____
Name of Person taking consent	Date	Signature

3 copies: 1 for the participant, 1 for the project notes and 1 for the medical notes

Reference

.

ALSALLOM, F., WOODSON, S., BRIGGS, F., SERRA, A. & ABBOUD, H. 2018. Impact of multiple comorbidities on EDSS at presentation in patients with multiple sclerosis: A cross-sectional study. (P2.379). *Neurology,* 90.

AMATO, M. P. & PONZIANI, G. 2000. A prospective study on the prognosis of multiple sclerosis. *Neurol Sci,* 21**,** S831-8.

AMOR, S., PEFEROEN, L. A., VOGEL, D. Y., BREUR, M., VAN DER VALK, P., BAKER, D. & VAN NOORT, J. M. 2014. Inflammation in neurodegenerative diseases--an update. *Immunology,* 142**,** 151-66.

BARANZINI, S. E. & OKSENBERG, J. R. 2017. The Genetics of Multiple Sclerosis: From 0 to 200 in 50 Years. *Trends Genet,* 33**,** 960-970.

BJARTMAR, C. & TRAPP, B. D. 2003. Axonal degeneration and progressive neurologic disability in multiple sclerosis. *Neurotox Res,* 5**,** 157-64.

BOLTON, C. & SMITH, P. A. 2018. The influence and impact of ageing and immunosenescence (ISC) on adaptive immunity during multiple sclerosis (MS) and the animal counterpart experimental autoimmune encephalomyelitis (EAE). *Ageing Research Reviews,* 41**,** 64-81.

BORTOLI, S., REHAULT, V., EVENO, E., AUFFRAY, C., BUTLER-BROWNE, G. & PIETU, G. 2003. Gene expression profiling of human satellite cells during muscular aging using cDNA arrays. *Gene,* 321**,** 145-154.

BOZZINI, S. & FALCONE, C. 2017. Genetic Factors Associated with Longevity in Humans. *Senescence Jolanta Dorszewska.* IntechOpen.

BRADL, M. & LASSMANN, H. 2009. Progressive multiple sclerosis. *Semin Immunopathol,* 31**,** 455-65.

BRAND, F. N., KIELY, D. K., KANNEL, W. B. & MYERS, R. H. 1992. Family patterns of coronary heart disease mortality: the Framingham Longevity Study. *J Clin Epidemiol,* 45**,** 169-74.

BROMAN, T., ANDERSEN, O. & BERGMANN, L. 1981. Clinical studies on multiple sclerosis. I. Presentation of an incidence material from Gothenburg. *Acta Neurol Scand,* 63**,** 6-33.

BROWN, M. G., KIRBY, S., SKEDGEL, C., FISK, J. D., MURRAY, T. J., BHAN, V. & SKETRIS, I. S. 2007. How effective are disease-modifying drugs in delaying progression in relapsing-onset MS? *Neurology,* 69**,** 1498-1507.

BROWNER, W. S., KAHN, A. J., ZIV, E., REINER, A. P., OSHIMA, J., CAWTHON, R. M., HSUEH, W. C. & CUMMINGS, S. R. 2004. The genetics of human longevity. *Am J Med,* 117**,** 851-60.

CASELLI, G., POZZI, L., VAUPEL, J. W., DEIANA, L., PES, G., CARRU, C., FRANCESCHI, C. & BAGGIO, G. 2006. Family clustering in Sardinian longevity: a genealogical approach. *Exp Gerontol,* 41**,** 727-36.

CHARLSON, M., SZATROWSKI, T. P., PETERSON, J. & GOLD, J. 1994. Validation of a combined comorbidity index. *J Clin Epidemiol,* 47**,** 1245-51.

CLEGG, A., BATES, C., YOUNG, J., RYAN, R., NICHOLS, L., ANN TEALE, E., MOHAMMED, M. A., PARRY, J. & MARSHALL, T. 2016. Development and validation of an electronic frailty index using routine primary care electronic health record data. *Age Ageing,* 45**,** 353-60.

COMPSTON, A. & COLES, A. 2008. Multiple sclerosis. *Lancet,* 372**,** 1502-17.

CONFAVREUX, C. & VUKUSIC, S. 2006a. Age at disability milestones in multiple sclerosis. *Brain,* 129**,** 595-605.

CONFAVREUX, C. & VUKUSIC, S. 2006b. [The natural history of multiple sclerosis]. *Rev Prat,* 56**,** 1313-20.

CONFAVREUX, C., VUKUSIC, S. & ADELEINE, P. 2003. Early clinical predictors and progression of irreversible disability in multiple sclerosis: an amnesic process. *Brain,* 126**,** 770-82.

CONFAVREUX, C., VUKUSIC, S., MOREAU, T. & ADELEINE, P. 2000. Relapses and progression of disability in multiple sclerosis. *N Engl J Med,* 343**,** 1430-8.

CONWAY, D. S., THOMPSON, N. R. & COHEN, J. A. 2017. Influence of hypertension, diabetes, hyperlipidemia, and obstructive lung disease on multiple sclerosis disease course. *Mult Scler,* 23**,** 277-285.

CORREALE, J. & FAREZ, M. F. 2015. The Role of Astrocytes in Multiple Sclerosis Progression. *Front Neurol,* 6**,** 180.

D'HOOGHE M, B., HAENTJENS, P., NAGELS, G. & DE KEYSER, J. 2012. Alcohol, coffee, fish, smoking and disease progression in multiple sclerosis. *Eur J Neurol,* 19**,** 616-24.

DE BENEDICTIS, G., TAN, Q., JEUNE, B., CHRISTENSEN, K., UKRAINTSEVA, S. V., BONAFE, M., FRANCESCHI, C., VAUPEL, J. W. & YASHIN, A. I. 2001. Recent advances in human gene-longevity association studies. *Mech Ageing Dev,* 122**,** 909-20.

DECKER, L., PICARD-RIERA, N., LACHAPELLE, F. & BARON-VAN EVERCOOREN, A. 2002. Growth factor treatment promotes mobilization of young but not aged adult subventricular zone precursors in response to demyelination. *J Neurosci Res,* 69**,** 763-71.

DELUTY, J. A., ATZMON, G., CRANDALL, J., BARZILAI, N. & MILMAN, S. 2015. The influence of gender on inheritance of exceptional longevity. *Aging (Albany NY),* 7**,** 412-8.

DENIC, A., JOHNSON, A. J., BIEBER, A. J., WARRINGTON, A. E., RODRIGUEZ, M. & PIRKO, I. 2011. The relevance of animal models in multiple sclerosis research. *Pathophysiology,* 18**,** 21-9.

DUTTA, R. & TRAPP, B. D. 2014. Relapsing and progressive forms of multiple sclerosis: insights from pathology. *Curr Opin Neurol,* 27**,** 271-8.

FILIPPINI, G., MUSICCO, M, FRATIGLIONI, L, BISANTI, L, CITTERIO, A, COSI, V, ZERBI, D, MARFORIO, S, SOLARI, A, BORDO, B, ARTUSO, A, MOLINARI, S, TRONCI, A, ROSSI, G, PERNISI, O, BENEDETTI, D, COCCIA, G, BEVILACQUE, L & MARIANI, C 1989. Migration and Infectious-Diseases in the Etiology of Multiple-Sclerosis - a Case-Control Study. *Multiple Sclerosis Research,* 843**,** 147-158.

FITZNER, D. & SIMONS, M. 2010. Chronic progressive multiple sclerosis - pathogenesis of neurodegeneration and therapeutic strategies. *Curr Neuropharmacol,* 8**,** 305-15.

FRANKLIN, R. J., ZHAO, C. & SIM, F. J. 2002. Ageing and CNS remyelination. *Neuroreport,* 13**,** 923-8.

FRANKLIN, R. J. M. 2002. Why does remyelination fail in multiple sclerosis? *Nature Reviews Neuroscience,* 3**,** 705-714.

FRAUSSEN, J., DE BOCK, L. & SOMERS, V. 2016. B cells and antibodies in progressive multiple sclerosis: Contribution to neurodegeneration and progression. *Autoimmun Rev,* 15**,** 896-9.

GAJOFATTO, A., CALABRESE, M., BENEDETTI, M. D. & MONACO, S. 2013. Clinical, MRI, and CSF markers of disability progression in multiple sclerosis. *Dis Markers,* 35**,** 687-99.

GERALDES, R., ESIRI, M. M., DELUCA, G. C. & PALACE, J. 2017. Age-related small vessel disease: a potential contributor to neurodegeneration in multiple sclerosis. *Brain Pathol,* 27**,** 707-722.

GOODIN, D. S., REDER, A. T., EBERS, G. C., CUTTER, G., KREMENCHUTZKY, M., OGER, J., LANGDON, D., RAMETTA, M., BECKMANN, K., DESIMONE, T. M. & KNAPPERTZ, V. 2012. Survival in MS A randomized cohort study 21 years after the start of the pivotal IFN beta-1b trial. *Neurology,* 78**,** 1315-1322.

GRACA, D. L. & BLAKEMORE, W. F. 1986. Delayed remyelination in rat spinal cord following ethidium bromide injection. *Neuropathol Appl Neurobiol,* 12**,** 593-605.

GUAN, J. Z., GUAN, W. P., MAEDA, T., XIE, G. Q., WAN, G. Z. & MAKINO, N. 2015. Patients with multiple sclerosis show increased oxidative stress markers and somatic telomere length shortening. *Molecular and Cellular Biochemistry,* 400**,** 183-187.

GUBBI, S., SCHWARTZ, E., CRANDALL, J., VERGHESE, J., HOLTZER, R., ATZMON, G., BRAUNSTEIN, R., BARZILAI, N. & MILMAN, S. 2017. Effect of Exceptional Parental Longevity and Lifestyle Factors on Prevalence of Cardiovascular Disease in Offspring. *Am J Cardiol,* 120**,** 2170-2175.

GUDMUNDSSON, H., GUDBJARTSSON, D. F., FRIGGE, M., GULCHER, J. R. & STEFANSSON, K. 2000. Inheritance of human longevity in Iceland. *Eur J Hum Genet,* 8**,** 743-9.

HAMPTON, D. W., INNES, N., MERKLER, D., ZHAO, C., FRANKLIN, R. J. & CHANDRAN, S. 2012. Focal immune-mediated white matter demyelination reveals an age-associated increase in axonal vulnerability and decreased remyelination efficiency. *Am J Pathol,* 180**,** 1897-905.

HANLON, P., NICHOLL, B. I., JANI, B. D., LEE, D., MCQUEENIE, R. & MAIR, F. S. 2018. Frailty and pre-frailty in middle-aged and older adults and its association with multimorbidity and mortality: a prospective analysis of 493 737 UK Biobank participants. *Lancet Public Health,* 3**,** E323-E332.

HAWKES, K. 2004. Human longevity: the grandmother effect. *Nature,* 428**,** 128-9.

HEALY, B. C., ALI, E. N., GUTTMANN, C. R., CHITNIS, T., GLANZ, B. I., BUCKLE, G., HOUTCHENS, M., STAZZONE, L., MOODIE, J., BERGER, A. M., DUAN, Y., BAKSHI, R., KHOURY, S., WEINER, H. & ASCHERIO, A. 2009. Smoking and disease progression in multiple sclerosis. *Arch Neurol,* 66**,** 858-64.

HEDSTROM, A. K., SUNDQVIST, E., BAARNHIELM, M., NORDIN, N., HILLERT, J., KOCKUM, I., OLSSON, T. & ALFREDSSON, L. 2011. Smoking and two human leukocyte antigen genes interact to increase the risk for multiple sclerosis. *Brain,* 134**,** 653-64.

HERNAN, M. A., JICK, S. S., LOGROSCINO, G., OLEK, M. J., ASCHERIO, A. & JICK, H. 2005. Cigarette smoking and the progression of multiple sclerosis. *Brain,* 128**,** 1461-5.

HERNAN, M. A., OLEK, M. J. & ASCHERIO, A. 2001. Cigarette smoking and incidence of multiple sclerosis. *Am J Epidemiol,* 154**,** 69-74.

HORTON, M., RUDICK, R. A., HARA-CLEAVER, C. & MARRIE, R. A. 2010. Validation of a self-report comorbidity questionnaire for multiple sclerosis. *Neuroepidemiology,* 35**,** 83-90.

HUBBARD, R. E., SEARLE, S. D., MITNITSKI, A. & ROCKWOOD, K. 2009. Effect of smoking on the accumulation of deficits, frailty and survival in older adults: a secondary analysis from the Canadian Study of Health and Aging. *J Nutr Health Aging,* 13**,** 468-72.

IMITOLA, J., CHITNIS, T. & KHOURY, S. J. 2006. Insights into the molecular pathogenesis of progression in multiple sclerosis: potential implications for future therapies. *Arch Neurol,* 63**,** 25-33.

INTERNATIONAL MULTIPLE SCLEROSIS GENETICS, C., WELLCOME TRUST CASE CONTROL, C., SAWCER, S., HELLENTHAL, G., PIRINEN, M., SPENCER, C. C., PATSOPOULOS, N. A., MOUTSIANAS, L., DILTHEY, A., SU, Z., FREEMAN, C., HUNT, S. E., EDKINS, S., GRAY, E., BOOTH, D. R., POTTER, S. C., GORIS, A., BAND, G., OTURAI, A. B., STRANGE, A., SAARELA, J., BELLENGUEZ, C., FONTAINE, B., GILLMAN, M., HEMMER, B., GWILLIAM, R., ZIPP, F., JAYAKUMAR, A., MARTIN, R., LESLIE, S., HAWKINS, S., GIANNOULATOU, E., D'ALFONSO, S., BLACKBURN, H., MARTINELLI BONESCHI, F., LIDDLE, J., HARBO, H. F., PEREZ, M. L., SPURKLAND, A., WALLER, M. J., MYCKO, M. P., RICKETTS, M., COMABELLA, M., HAMMOND, N., KOCKUM, I., MCCANN, O. T., BAN, M., WHITTAKER, P., KEMPPINEN, A., WESTON, P., HAWKINS, C., WIDAA, S., ZAJICEK, J., DRONOV, S., ROBERTSON, N., BUMPSTEAD, S. J., BARCELLOS, L. F., RAVINDRARAJAH, R., ABRAHAM, R., ALFREDSSON, L., ARDLIE, K., AUBIN, C., BAKER, A., BAKER, K., BARANZINI, S. E., BERGAMASCHI, L., BERGAMASCHI, R., BERNSTEIN, A., BERTHELE, A., BOGGILD, M., BRADFIELD, J. P., BRASSAT, D., BROADLEY, S. A., BUCK, D., BUTZKUEVEN, H., CAPRA, R., CARROLL, W. M., CAVALLA, P., CELIUS, E. G., CEPOK, S., CHIAVACCI, R., CLERGET-DARPOUX, F., CLYSTERS, K., COMI, G., COSSBURN, M., COURNU-REBEIX, I., COX, M. B., COZEN, W., CREE, B. A., CROSS, A. H., CUSI, D., DALY, M. J., DAVIS, E., DE BAKKER, P. I., DEBOUVERIE, M., D'HOOGHE M, B., DIXON, K., DOBOSI, R., DUBOIS, B., ELLINGHAUS, D., et al. 2011. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature,* 476**,** 214-9.

IRVINE, K. A. & BLAKEMORE, W. F. 2006. Age increases axon loss associated with primary demyelination in cuprizone-induced demyelination in C57BL/6 mice. *Journal of Neuroimmunology,* 175**,** 69-76.

JACOBSEN, M., SCHWEER, D., ZIEGLER, A., GABER, R., SCHOCK, S., SCHWINZER, R., WONIGEIT, K., LINDERT, R. B., KANTARCI, O., SCHAEFER-KLEIN, J., SCHIPPER, H. I., OERTEL, W. H.,

HEIDENREICH, F., WEINSHENKER, B. G., SOMMER, N. & HEMMER, B. 2000. A point mutation in PTPRC is associated with the development of multiple sclerosis. *Nat Genet,* 26**,** 495-9.
JADASZ, J. J., AIGNER, L., RIVERA, F. J. & KURY, P. 2012. The remyelination Philosopher's Stone: stem and progenitor cell therapies for multiple sclerosis. *Cell Tissue Res,* 349**,** 331-47.
KEEGAN, M., PINEDA, A. A., MCCLELLAND, R. L., DARBY, C. H., RODRIGUEZ, M. & WEINSHENKER, B. G. 2002. Plasma exchange for severe attacks of CNS demyelination: predictors of response. *Neurology,* 58**,** 143-6.
KENEALY, S. J., PERICAK-VANCE, M. A. & HAINES, J. L. 2003. The genetic epidemiology of multiple sclerosis. *Journal of Neuroimmunology,* 143**,** 7-12.
KERBER, R. A., O'BRIEN, E., SMITH, K. R. & CAWTHON, R. M. 2001. Familial excess longevity in Utah genealogies. *J Gerontol A Biol Sci Med Sci,* 56**,** B130-9.
KIM, S., WELSH, D. A., CHERRY, K. E., MYERS, L. & JAZWINSKI, S. M. 2013. Association of healthy aging with parental longevity. *Age (Dordr),* 35**,** 1975-82.
KOCH, M., MOSTERT, J., HEERSEMA, D. & DE KEYSER, J. 2007a. Progression in multiple sclerosis: Further evidence of an age dependent process. *Journal of the Neurological Sciences,* 255**,** 35-41.
KOCH, M., VAN HARTEN, A., UYTTENBOOGAART, M. & DE KEYSER, J. 2007b. Cigarette smoking and progression in multiple sclerosis. *Neurology,* 69**,** 1515-20.
KOCH-HENRIKSEN, N., THYGESEN, L. C., STENAGER, E., LAURSEN, B. & MAGYARI, M. 2018. Incidence of MS has increased markedly over six decades in Denmark particularly with late onset and in women. *Neurology,* 90**,** E1954-E1963.
KOJIMA, G., ILIFFE, S. & WALTERS, K. 2015. Smoking as a predictor of frailty: a systematic review. *Bmc Geriatrics,* 15.
KOUDRIAVTSEVA, T. 2014. Thrombotic processes in multiple sclerosis as manifestation of innate immune activation. *Frontiers in Neurology,* 5.
KOUDRIAVTSEVA, T., RENNA, R., PLANTONE, D. & MAINERO, C. 2015. Demyelinating and thrombotic diseases of the central nervous system: common pathogenic and triggering factors. *Front Neurol,* 6**,** 63.
KUMAR, A., LANGSTRAAT, C. L., DEJONG, S. R., MCGREE, M. E., BAKKUM-GAMEZ, J. N., WEAVER, A. L., LEBRASSEUR, N. K. & CLIBY, W. A. 2017. Functional not chronologic age: Frailty index predicts outcomes in advanced ovarian cancer. *Gynecologic Oncology,* 147**,** 104-109.
KURTZKE, J. F. 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology,* 33**,** 1444-52.
LASSMANN, H. & VAN HORSSEN, J. 2011. The molecular basis of neurodegeneration in multiple sclerosis. *FEBS Lett,* 585**,** 3715-23.
LUDWIN, S. K. 1980. Chronic demyelination inhibits remyelination in the central nervous system. An analysis of contributing factors. *Lab Invest,* 43**,** 382-7.
MACKENZIE, I. S., MORANT, S. V., BLOOMFIELD, G. A., MACDONALD, T. M. & O'RIORDAN, J. 2014. Incidence and prevalence of multiple sclerosis in the UK 1990-2010: a descriptive study in the General Practice Research Database. *Journal of Neurology Neurosurgery and Psychiatry,* 85**,** 76-84.
MAHAD, D. H., TRAPP, B. D. & LASSMANN, H. 2015. Pathological mechanisms in progressive multiple sclerosis. *Lancet Neurol,* 14**,** 183-93.
MANOUCHEHRINIA, A., TENCH, C. R., MAXTED, J., BIBANI, R. H., BRITTON, J. & CONSTANTINESCU, C. S. 2013. Tobacco smoking and disability progression in multiple sclerosis: United Kingdom cohort study. *Brain,* 136**,** 2298-304.
MAO, P. Z. & REDDY, P. H. 2010. Is multiple sclerosis a mitochondrial disease? *Biochimica Et Biophysica Acta-Molecular Basis of Disease,* 1802**,** 66-79.
MARRIE, R., HORWITZ, R., CUTTER, G., TYRY, T., CAMPAGNOLO, D. & VOLLMER, T. 2008. Comorbidity, socioeconomic status and multiple sclerosis. *Mult Scler,* 14**,** 1091-8.

MARRIE, R.-A., PATEL, R., MARRIOTT, J., FIGLEY, C., KORNELSEN, J., GRAFF, L., BOLTON, J., BERNSTEIN, C. & FISK, J. 2018. Anxiety and Diabetes Adversely Affect Cognition in Multiple Sclerosis (P1.423). *Neurology,* 90.

MARRIE, R. A., RUDICK, R., HORWITZ, R., CUTTER, G., TYRY, T., CAMPAGNOLO, D. & VOLLMER, T. 2010. Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. *Neurology,* 74**,** 1041-1047.

MARZETTI, E., CALVANI, R., TOSATO, M., CESARI, M., DI BARI, M., CHERUBINI, A., BROCCATELLI, M., SAVERA, G., D'ELIA, M., PAHOR, M., BERNABEI, R., LANDI, F. & CONSORTIUM, S. 2017. Physical activity and exercise as countermeasures to physical frailty and sarcopenia. *Aging Clin Exp Res,* 29**,** 35-42.

MCKAY, K. A., TREMLETT, H., FISK, J. D., ZHANG, T., PATTEN, S. B., KASTRUKOFF, L., CAMPBELL, T., MARRIE, R. A. & SCLEROSIS, C. T. I. T. E. A. I. O. C. O. M. 2018. Psychiatric comorbidity is associated with disability progression in multiple sclerosis. *Neurology,* 90**,** e1316-e1323.

MIKAELOFF, Y., CARIDADE, G., TARDIEU, M. & SUISSA, S. 2007. Parental smoking at home and the risk of childhood-onset multiple sclerosis in children. *Brain,* 130**,** 2589-2595.

MILLER, D., BARKHOF, F., MONTALBAN, X., THOMPSON, A. & FILIPPI, M. 2005. Clinically isolated syndromes suggestive of multiple sclerosis, part I: natural history, pathogenesis, diagnosis, and prognosis. *Lancet Neurol,* 4**,** 281-8.

MILLER, D. H., CHARD, D. T. & CICCARELLI, O. 2012. Clinically isolated syndromes. *Lancet Neurol,* 11**,** 157-69.

MILLER, S. D., KATZ-LEVY, Y., NEVILLE, K. L. & VANDERLUGT, C. L. 2001. Virus-induced autoimmunity: epitope spreading to myelin autoepitopes in Theiler's virus infection of the central nervous system. *Adv Virus Res,* 56**,** 199-217.

MITNITSKI, A. B., GRAHAM, J. E., MOGILNER, A. J. & ROCKWOOD, K. 2002. Frailty, fitness and late-life mortality in relation to chronological and biological age. *BMC Geriatr,* 2**,** 1.

MITNITSKI, A. B., MOGILNER, A. J. & ROCKWOOD, K. 2001. Accumulation of deficits as a proxy measure of aging. *ScientificWorldJournal,* 1**,** 323-36.

MOTL, R. W. & PILUTTI, L. A. 2012. The benefits of exercise training in multiple sclerosis. *Nat Rev Neurol,* 8**,** 487-97.

MULLER, R. 1951. Course and prognosis of disseminated sclerosis in relation to age of onset. *AMA Arch Neurol Psychiatry,* 66**,** 561-70.

NAKAMURA, K. I., TAKUBO, K., IZUMIYAMA-SHIMOMURA, N., SAWABE, M., ARAI, T., KISHIMOTO, H., FUJIWARA, M., KATO, M., OSHIMURA, M., ISHII, A. & ISHIKAWA, N. 2007. Telomeric DNA length in cerebral gray and white matter is associated with longevity in individuals aged 70 years or older. *Experimental Gerontology,* 42**,** 944-950.

NEUMANN, H., KOTTER, M. R. & FRANKLIN, R. J. M. 2009. Debris clearance by microglia: an essential link between degeneration and regeneration. *Brain,* 132**,** 288-295.

NEWBOULD, R. D., NICHOLAS, R., THOMAS, C. L., QUEST, R., LEE, J. S. Z., HONEYFIELD, L., COLASANTI, A., MALIK, O., MATTOSCIO, M., MATTHEWS, P. M., SORMANI, M. P., WALDMAN, A. D. & MURAROC, P. A. 2014. Age independently affects myelin integrity as detected by magnetization transfer magnetic resonance imaging in multiple sclerosis. *Neuroimage-Clinical,* 4**,** 641-648.

OZAKBAS, S., KAYA, D. & IDIMAN, E. 2012. Early onset multiple sclerosis has worse prognosis than adult onset multiple sclerosis based on cognition and magnetic resonance imaging. *Autoimmune Dis,* 2012**,** 563989.

PALACE, J., DUDDY, M., BREGENZER, T., LAWTON, M., ZHU, F., BOGGILD, M., PISKE, B., ROBERTSON, N. P., OGER, J., TREMLETT, H., TILLING, K., BEN-SHLOMO, Y. & DOBSON, C. 2015. Effectiveness and cost-effectiveness of interferon beta and glatiramer acetate in the UK Multiple Sclerosis Risk Sharing Scheme at 6 years: a clinical cohort study with natural history comparator. *Lancet Neurology,* 14**,** 497-505.

PENA, F. G., THEOU, O., WALLACE, L., BROTHERS, T. D., GILL, T. M., GAHBAUER, E. A., KIRKLAND, S., MITNITSKI, A. & ROCKWOOD, K. 2014. Comparison of alternate scoring of variables on the performance of the frailty index. *BMC Geriatr,* 14**,** 25.

PITTAS, F., PONSONBY, A. L., VAN DER MEI, I. A., TAYLOR, B. V., BLIZZARD, L., GROOM, P., UKOUMUNNE, O. C. & DWYER, T. 2009. Smoking is associated with progressive disease course and increased progression in clinical disability in a prospective cohort of people with multiple sclerosis. *J Neurol,* 256**,** 577-85.

PLANTONE, D., DE ANGELIS, F., DOSHI, A. & CHATAWAY, J. 2016. Secondary Progressive Multiple Sclerosis: Definition and Measurement. *CNS Drugs,* 30**,** 517-26.

RAJPATHAK, S. N., LIU, Y., BEN-DAVID, O., REDDY, S., ATZMON, G., CRANDALL, J. & BARZILAI, N. 2011. Lifestyle factors of people with exceptional longevity. *J Am Geriatr Soc,* 59**,** 1509-12.

RAWJI, K. S., MISHRA, M. K., MICHAELS, N. J., RIVEST, S., STYS, P. K. & YONG, V. W. 2016. Immunosenescence of microglia and macrophages: impact on the ageing central nervous system. *Brain,* 139**,** 653-61.

RICHTER, B., MOHNI, J., PANDEY, K., FORD, R., BACON, T. & KISTER, I. 2016. Body Mass Index Is Modestly Correlated with Disease Severity in Multiple Sclerosis. *Neurology,* 86.

RIISE, T., GRONNING, M., FERNANDEZ, O., LAUER, K., MIDGARD, R., MINDERHOUD, J. M., NYLAND, H., PALFFY, G., POSER, S. & AARLI, J. A. 1992. Early prognostic factors for disability in multiple sclerosis, a European multicenter study. *Acta Neurol Scand,* 85**,** 212-8.

RIST, J. M. & FRANKLIN, R. J. M. 2008. Taking ageing into account in remyelination-based therapies for multiple sclerosis. *Journal of the Neurological Sciences,* 274**,** 64-67.

ROCKWOOD, K. & MITNITSKI, A. 2011. Frailty defined by deficit accumulation and geriatric medicine defined by frailty. *Clin Geriatr Med,* 27**,** 17-26.

ROVARIS, M., CONFAVREUX, C., FURLAN, R., KAPPOS, L., COMI, G. & FILIPPI, M. 2006. Secondary progressive multiple sclerosis: current knowledge and future challenges. *Lancet Neurol,* 5**,** 343-54.

ROXBURGH, R. H. S. R., SEAMAN, S. R., MASTERMAN, T., HENSIEK, A. E., SAWCER, S. J., VUKUSIC, S., ACHITI, I., CONFAVREUX, C., COUSTANS, M., LE PAGE, E., EDAN, G., MCDONNELL, G. V., HAWKINS, S., TROJANO, M., LIGUORI, M., COCCO, E., MARROSU, M. G., TESSER, F., LEONE, M. A., WEBER, A., ZIPP, F., MITERSKI, B., EPPLEN, J. T., OTURAI, A., SORENSEN, P. S., CELIUS, E. G., LARA, N. T., MONTALBAN, X., VILLOSLADA, P., SILVA, A. M., MARTA, M., LEITE, I., DUBOIS, B., RUBIO, J., BUTZKUEVEN, H., KILPATRICK, T., MYCKO, M. P., SELMAJ, K. W., RIO, M. E., SA, M., SALEMI, G., SAVETTIERI, G., HILLERT, J. & COMPSTON, D. A. S. 2005. Multiple sclerosis severity score - Using disability and disease duration to rate disease severity. *Neurology,* 64**,** 1144-1151.

RUCKH, J. M., ZHAO, J. W., SHADRACH, J. L., VAN WIJNGAARDEN, P., RAO, T. N., WAGERS, A. J. & FRANKLIN, R. J. 2012. Rejuvenation of regeneration in the aging central nervous system. *Cell Stem Cell,* 10**,** 96-103.

RUNMARKER, B. & ANDERSEN, O. 1993. Prognostic factors in a multiple sclerosis incidence cohort with twenty-five years of follow-up. *Brain,* 116 (Pt 1)**,** 117-34.

SADOVNICK, A. D. & EBERS, G. C. 1993. Epidemiology of multiple sclerosis: a critical overview. *Can J Neurol Sci,* 20**,** 17-29.

SANAI, S. A., SAINI, V., BENEDICT, R. H., ZIVADINOV, R., TETER, B. E., RAMANATHAN, M. & WEINSTOCK-GUTTMAN, B. 2016. Aging and multiple sclerosis. *Mult Scler,* 22**,** 717-25.

SCALFARI, A., NEUHAUS, A., DAUMER, M., DELUCA, G. C., MURARO, P. A. & EBERS, G. C. 2013. Early relapses, onset of progression, and late outcome in multiple sclerosis. *JAMA Neurol,* 70**,** 214-22.

SCALFARI, A., NEUHAUS, A., DAUMER, M., EBERS, G. C. & MURARO, P. A. 2011. Age and disability accumulation in multiple sclerosis. *Neurology,* 77**,** 1246-52.

SCALFARI, A., NEUHAUS, A., DEGENHARDT, A., RICE, G. P., MURARO, P. A., DAUMER, M. & EBERS, G. C. 2010. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. *Brain,* 133**,** 1914-29.

SCHMIERER, K., SCARAVILLI, F., ALTMANN, D. R., BARKER, G. J. & MILLER, D. H. 2004. Magnetization transfer ratio and myelin in postmortem multiple sclerosis brain. *Ann Neurol,* 56**,** 407-15.

SEARLE, S. D., MITNITSKI, A., GAHBAUER, E. A., GILL, T. M. & ROCKWOOD, K. 2008. A standard procedure for creating a frailty index. *BMC Geriatr,* 8**,** 24.

SELLNER, J., KRAUS, J., AWAD, A., MILO, R., HEMMER, B. & STUVE, O. 2011. The increasing incidence and prevalence of female multiple sclerosis--a critical analysis of potential environmental factors. *Autoimmun Rev,* 10**,** 495-502.

SERAFINI, B., ROSICARELLI, B., FRANCIOTTA, D., MAGLIOZZI, R., REYNOLDS, R., CINQUE, P., ANDREONI, L., TRIVEDI, P., SALVETTI, M., FAGGIONI, A. & ALOISI, F. 2007. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. *J Exp Med,* 204**,** 2899-912.

SHIELDS, S. A., GILSON, J. M., BLAKEMORE, W. F. & FRANKLIN, R. J. M. 1999. Remyelination occurs as extensively but more slowly in old rats compared to young rats following gliotoxin-induced CNS demyelination. *Glia,* 28**,** 77-83.

SIM, F. J., ZHAO, C., PENDERIS, J. & FRANKLIN, R. J. 2002. The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. *J Neurosci,* 22**,** 2451-9.

SIMPSON, J. E., FERNANDO, M. S., CLARK, L., INCE, P. G., MATTHEWS, F., FORSTER, G., O'BRIEN, J. T., BARBER, R., KALARIA, R. N., BRAYNE, C., SHAW, P. J., LEWIS, C. E., WHARTON, S. B., FUNCTION, M. R. C. C. & AGEING NEUROPATHOLOGY STUDY, G. 2007. White matter lesions in an unselected cohort of the elderly: astrocytic, microglial and oligodendrocyte precursor cell responses. *Neuropathol Appl Neurobiol,* 33**,** 410-9.

SOTGIU, S., PUGLIATTI, M., FOIS, M. L., ARRU, G., SANNA, A., SOTGIU, M. A. & ROSATI, G. 2004. Genes, environment, and susceptibility to multiple sclerosis. *Neurobiology of Disease,* 17**,** 131-143.

SPANN, S. J. & OTTINGER, M. A. 2018. Longevity, Metabolic Disease, and Community Health. *Prog Mol Biol Transl Sci,* 155**,** 1-9.

STERNE, J. A., WHITE, I. R., CARLIN, J. B., SPRATT, M., ROYSTON, P., KENWARD, M. G., WOOD, A. M. & CARPENTER, J. R. 2009. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ,* 338**,** b2393.

STRASSMANN, B. I. & GARRARD, W. M. 2011. Alternatives to the grandmother hypothesis: a meta-analysis of the association between grandparental and grandchild survival in patrilineal populations. *Hum Nat,* 22**,** 201-22.

SVEJGAARD, A. 2008. The immunogenetics of multiple sclerosis. *Immunogenetics,* 60**,** 275-86.

TRAPP, B. D. & NAVE, K. A. 2008. Multiple sclerosis: an immune or neurodegenerative disorder? *Annu Rev Neurosci,* 31**,** 247-69.

TROJANO, M., AVOLIO, C., MANZARI, C., CALO, A., DE ROBERTIS, F., SERIO, G. & LIVREA, P. 1995. Multivariate analysis of predictive factors of multiple sclerosis course with a validated method to assess clinical events. *J Neurol Neurosurg Psychiatry,* 58**,** 300-6.

TSANG, B. K. & MACDONELL, R. 2011. Multiple sclerosis- diagnosis, management and prognosis. *Aust Fam Physician,* 40**,** 948-55.

VAGERO, D., ARONSSON, V. & MODIN, B. 2018. Why is parental lifespan linked to children's chances of reaching a high age? A transgenerational hypothesis. *SSM Popul Health,* 4**,** 45-54.

WANG, C. X., SONG, X. W., MITNITSKI, A., YU, P. L., FANG, X. H., TANG, Z., SHI, J. & ROCKWOOD, K. 2013. Gender Differences in the Relationship Between Smoking and Frailty: Results From the Beijing Longitudinal Study of Aging. *Journals of Gerontology Series a-Biological Sciences and Medical Sciences,* 68**,** 338-346.

WEINSHENKER, B. G., O'BRIEN, P. C., PETTERSON, T. M., NOSEWORTHY, J. H., LUCCHINETTI, C. F., DODICK, D. W., PINEDA, A. A., STEVENS, L. N. & RODRIGUEZ, M. 1999. A randomized trial of

plasma exchange in acute central nervous system inflammatory demyelinating disease. *Ann Neurol,* 46**,** 878-86.

YIN, J., VALIN, K. L., DIXON, M. L. & LEAVENWORTH, J. W. 2017. The Role of Microglia and Macrophages in CNS Homeostasis, Autoimmunity, and Cancer. *J Immunol Res,* 2017**,** 5150678.

YOU, D. Z., GU, D. N. & YI, Z. 2010. Familial Transmission of Human Longevity Among the Oldest-Old in China. *Journal of Applied Gerontology,* 29**,** 308-332.

YOUNGERMAN, B. E., NEUGUT, A. I., YANG, J., HERSHMAN, D. L., WRIGHT, J. D. & BRUCE, J. N. 2018. The modified frailty index and 30-day adverse events in oncologic neurosurgery. *J Neurooncol,* 136**,** 197-206.