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Late Breaking News Poster Session

LBP - Late Breaking Poster Session

LBP1

Testicular hypofunction and multiple sclerosis risk: a record-linkage study

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Background: The demographic profile of multiple sclerosis (MS) suggests that gender may be an important factor influencing disease risk and clinical course. MS in males typically onsets at a later age compared to females.This coincides with an age-related decline in testosterone levels suggesting that testosterone may be implicated in MS aetio-pathogenesis in males. The influence of gonadal hormones on MS is not well characterised and has thus far been investigated primarily in animal models or as a proposed therapeutic approach.

Objectives: We aimed to investigate a potential association between testicular hypofunction (TH), as a proxy for low testosterone levels, and MS risk.

Methods: A retrospective cohort study was conducted through analysis of linked English national Hospital Episode Statistics and mortality data from 1999 to 2011. A TH cohort of 5049 males was constructed by identifying the first recorded episode of day-case care or hospital admission during the study period in which TH was coded. A reference cohort (3.4 million males) and obesity cohort (given the possibility of obesity being a confounding factor) were constructed in similar ways. We searched for any subsequent day-case or inpatient admission for, or death from, MS in these cohorts. We calculated rates for MS, stratified and then standardized by age, sex, calendar year of first recorded admission, region of residence, and socio-economic status.

Results: The standardised rates of MS were 37.79 per 100,000 person-years in the TH cohort (based on 11 cases observed, and 2.4 expected) and 8.17 per 100,000 years in the reference cohort (based on 1734 cases observed, and 1742.6 expected). The adjusted rate ratio (RR) was 4.62 (95% confidence interval 2.30-8.24, $p< 0.0001$). In the TH cohort, all observed cases of MS occurred more than one year after first admission or day-case care for TH. We found an overall adjusted RR of MS following obesity in males of 1.47 (95% CI 1.20-1.80, $p=0.0001$). Given that the point estimate for MS following TH (4.6) lies outside the confidence interval for MS following obesity (1.20-1.80), the difference is significant and obesity is unlikely to be an important confounder.

Conclusions: We report a strong positive association (a five-fold elevation of rates) between TH and subsequent MS in males. The strengths of this study are the huge size of the database and the person-based cohort design. Future work should aim more directly to elucidate the relationship between testosterone levels and MS in both males and females.

LBP2

Greater spinal cord atrophy predicts disability status five years after a clinically isolated syndrome

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Background: Spinal cord pathology is an important substrate for long term disability in people with multiple sclerosis (MS). The upper cervical cord cross-sectional area (UCCA) is independently associated with disability in established MS. Patients with a clinically isolated syndrome (CIS), the first clinical manifestation of relapse-onset MS, have been shown to have a smaller UCCA than matched controls, suggesting that spinal cord atrophy may begin at the earliest stages of MS. However, the rate of spinal cord atrophy after a CIS and the relationship to disability during follow up is unknown.

Objectives: To investigate the longitudinal change in UCCA over the first five years after CIS and to determine whether spinal cord atrophy predicts disability status in the short to medium term.

Methods: Data were obtained from a prospectively recruited CIS cohort with serial clinical and MRI follow up. Spinal cord imaging was done on a 1.5 T scanner with 60x1mm sagittal slices of the cervical cord. Images were reformatted axially to create five contiguous 3mm slices at the level of C2/C3. An active surface model was used to measure the UCCA and an average obtained over the five slices. The percentage change in the UCCA between baseline and follow up was calculated. MS was diagnosed using the 2010 McDonald criteria and disability measured using the Expanded Disability Status Scale (EDSS). Patients with an EDSS of 3 or more were considered disabled.

Results: 121 patients were seen at baseline (mean age 32.8 years, 60% female) and for follow up after a mean of 5.63 years after CIS. 39 (31%, median EDSS 1) patients remained CIS and 84 developed MS, of whom 69 (57%, median EDSS 1) were classified as non-disabled and 15 (12%, median EDSS 4) as disabled. The baseline UCCA was similar in patients who remained CIS and those who developed MS. At follow up the mean change in UCCA was greater among disabled MS patients compared with

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those who were non-disabled (-7.5% vs -2.2%, p< 0.01) or remained CIS (-7.5% vs -0.6%, p< 0.01).
Conclusions: Over a follow up period of five years, a greater degree of spinal cord atrophy was observed in CIS patients who developed MS with significant neurologic disability. These findings support the use of spinal cord atrophy as an imaging biomarker of disease progression in early MS.

LBP3
Increased coinfection of human herpesviruses 6A (HHV-6A) and HHV-6B in multiple sclerosis patients
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Background: Human herpesvirus 6 (HHV-6) is a ubiquitous DNA virus, with seroprevalence rates exceeding 95% in the adult population. HHV-6 is comprised of two subgroups, HHV-6A and HHV-6B, which were once called variants but increasing evidence of their biological differences had led to their reclassification as distinct viral species.
Objectives: As it is now especially relevant to investigate HHV-6A and HHV-6B separately, our objectives were to design a multiplex HHV-6A and HHV-6B ddPCR assay and subsequently investigate the incidence of HHV-6A and HHV-6B coinfection in samples from healthy donors and patients with MS, a disease in which HHV-6 is thought to play a role.
Methods: The ubiquitous human herpesviruses HHV-6A and HHV-6B have been associated with various neurologic disorders partly due to the detection of elevated viral DNA levels in patients compared to controls. However, the reported frequency of detection varies widely, likely reflecting differences in detection methodologies. Our study employed a novel technology called digital droplet PCR (ddPCR), which is a third generation PCR technology that enables the absolute quantification of target DNA molecules.
Results: In our assessment of healthy donors, we observed a heretofore-underappreciated high frequency of coinfection in PBMC and serum. We observed higher viral loads in the saliva compared to the blood of healthy donors, and therefore we chose this sample type to compare HHV-6 levels between healthy donors and patients with MS. We detected a significantly elevated frequency of coinfection in MS saliva. Such increased detection of HHV-6A in MS patients is consistent with other studies suggesting that this viral species (thought to be more neurotropic and neurovirulent compared to HHV-6B) is more prevalent among MS patients compared to healthy donors.
Conclusions: Our data supports the hypothesis that if HHV-6B is the predominant species of childhood infection, an abundance of detectable HHV-6A may be pathophysiologic. As the biology and disease associations between these two viral species differ, studies of coinfection may prove clinically relevant and enhance our understanding of the roles of each virus in human health and disease.

LBP4
Low-frequency coding variation in PRF1 and GALC mediate multiple sclerosis risk
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Background: Multiple sclerosis (MS) is an autoimmune disease in which the myelin sheath surrounding brain neurons is destroyed by the immune system, leading to progressive physical and cognitive morbidity, brain atrophy and death. Over the last decade we have conducted a series of genome-wide association studies showing that the genetic architecture of MS is complex and likely involves hundreds of risk loci. We have identified almost 100 such loci explaining over 50% of the heritability and our ongoing common variant mapping efforts in ~36,000 cases and ~44,000 controls will substantially increase this number. To complement these efforts we have interrogated 250,000 low-frequency non-synonymous (NS) coding variants across all exons in the genome using Illumina's exome chip in a total of ~40,000 cases and ~60,000 controls of European descent.
Objectives: To assess the contribution of low-frequency non-synonymous coding variants to MS susceptibility risk.
Methods: In our interim analysis, we have analyzed 26,231 cases and 24,031 controls in 14 country-level strata following stringent quality control to eliminate technical artefacts and population stratification. We tested >80,000 NS polymorphic variants for association to MS risk using a per-variant univariate allelic test. Gene burden tests and pathway analyses are ongoing.
Results: We find that two low-frequency variants (MAF < 0.05) show convincing evidence of association ($p < 6.25 \times 10^{-7}$, Bonferroni-adjusted $p < 0.05$ for number of variants tested). These variants are in genes PRF1 (preforin 1, OR = 1.2) and GALC (galactosylceramidase, OR = 0.77). A further variant in HDAC7, a histone deacetylase, has suggestive evidence of association ($p = 1.97 \times 10^{-5}$, OR = 0.77). We are currently analyzing our remaining samples to perform a joint analysis across approximately 100,000 cases and controls.
Conclusions: Overall, our results suggest that low-frequency variation identifies a number of MS risk genes in both known risk loci and elsewhere in the genome. These effects account for a small but significant proportion of disease risk heritability and reveal novel risk genes and aspects of MS susceptibility biology.

LBP5
Quality of life after optic neuritis in MS: new data for the 10-item neuro-ophthalmic supplement to the NEI-VFQ-25
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Background: Patient-reported outcomes have an increasingly important role in MS for demonstrating quality of life (QOL) and showing how clinical measures reflect meaningful changes in structure and function. The 10-Item Neuro-Ophthalmic

Supplement is a new patient-reported outcome measure of vision-specific QOL designed to complement the 25-Item National Eye Institute Visual Functioning Questionnaire (NEI-VFQ-25).

Objectives: We examined the 10-Item Neuro-Ophthalmic Supplement in an MS cohort as a complement to the NEI-VFQ-25. We also determined how well 10-Item Supplement scores may distinguish people with MS with vs. without a history of optic neuritis (ON), and how QOL scores relate to measures of visual pathway structure and function.

Methods: Participants self-administered the NEI-VFQ-25 and 10-Item Neuro-Ophthalmic Supplement. Low-contrast letter acuity (LCLA) and high-contrast visual acuity (VA) were tested. Spectral-domain (SD) OCT was performed to determine peripapillary retinal nerve fiber layer thickness (RNFLT) and macular ganglion cell + inner plexiform layer (GCL+IPL) thickness. The King-Devick (K-D) test of rapid number naming that requires intact fast eye movements (saccades) was administered.

Results: Analyses of data from 265 patients with MS (528 eyes, age 47.1±11.6 years) and 21 disease-free controls (42 eyes, age 31.0±10.9 years) showed significantly worse scores for the 10-Item Supplement (mean=77.7±18.5 out of 100 vs 96.9±4.9 in controls, $p< 0.001$) and for the NEI-VFQ-25 composite score (mean=84.5±16.0 vs 98.1±2.1, $p=0.001$). The 10-Item Supplement was able to distinguish MS patients with history of ON (area under ROC curve=0.65, $p< 0.001$, logistic regression accounting for age). Both the 10-Item Supplement and NEI-VFQ-25 composite were excellent discriminators of MS patients vs. controls; combining them resulted in an ROC area of 0.95 ($p< 0.001$, accounting for age). The Supplement score was likewise a strong predictor of peripapillary RNFLT in MS ($p=0.002$, GEE models accounting for age and within-patient, inter-eye correlations) as was GCL+IPL thickness ($p=0.003$). KD scores were able to predict QOL scores ($p< 0.001$).

Conclusions: The 10-item Neuro-Ophthalmic Supplement is a useful measure of vision-specific QOL that complements the NEI-VFQ-25 in people with MS, and is a predictor of ON history. 10-Item Supplement scores reflect both afferent and efferent visual pathway structure and function, supporting a role for this patient-reported outcome in MS clinical trials that incorporate vision.

LBP6

Impact of siponimod (BAF 312) on CNS remyelination in a transgenic Xenopus model

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Background: In MS complete ablation of immune system may stop the evolution but do not induce repair and clinical improvement.

Objectives: To promote remyelination there is a need to develop new alternative experimental models to investigate remyelination.

Methods: We have developed a transgenic *Xenopus laevis* as a model to study demyelination and remyelination by conditionally triggering apoptotic ablation of myelinating oligodendrocytes. This transgenic line (*pMBP-GFP-NTR*) expresses the green fluorescent protein reporter fused to the bacterial nitroreductase enzyme under the control of the mouse 1.9kb myelin basic protein ((-1907bp/+36bp *MBP*) promoter region). In our transgenic *Xenopus laevis* the *GFP-NTR* transgene is expressed specifically in myelin-forming oligodendrocytes, (Kaya et al., 2012). The *E. Coli* enzyme nitroreductase (NTR) converts the nitro radical of prodrugs such as metronidazole to a hydroxylamine derivative highly cytotoxic.

Results: Transgenic *pMBP-GFP-NTR Xenopus* tadpoles exposed for 3 to 10 days to the metronidazole prodrug demonstrated demyelination, restricted to the central nervous system, without axonal damage. The level of demyelination obtained depends on the concentration and duration of exposure to metronidazole. After cessation of metronidazole treatment, spontaneous remyelination occurs. Due to the transparency of the embryo, demyelination and remyelination can be monitored and quantified *in vivo* by examination under fluorescent macroscope or two-photon microscope (Kaya et al., 2012).

Conclusions: In the present study we show, that using this model, treatment with 13-cis-retinoic acid (100 nM), a factor recently shown to have an effect on maturation of oligodendroglial cells (Huang et al., 2011), resulted in increased remyelination and that BAF 312 (3nM), a next generation dual S1P1/5 modulator, which currently is tested in Phase III for SPMS and which was previously shown to promote myelination *in vitro* (Jackson et al., 2011) is as potent as retinoic acid to promote remyelination.

LBP7

Neuromyelitis optica patients on rituximab: predicting response to therapy

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Background: While the anti-CD20 monoclonal antibody rituximab has been established as an effective medication for relapse prevention in neuromyelitis optica (NMO) and neuromyelitis optica spectrum disorder (NMOSD), little is known about factors that influence individual patient response to rituximab.

Objectives: To correlate demographic factors, disease specific factors, and immunological laboratory parameters with relapse risk in NMO and NMOSD patients on rituximab.

Methods: A single center retrospective chart review was performed. Patients with a diagnosis of probable or definite NMO or NMOSD were included. Demographic information, disease specific information, history of rituximab treatment, relapse history, and lymphocyte subsets counts were documented. ANOVA was performed to correlate demographic factors (age, race) and disease specific factors (Aq-4 antibody status, definite NMO status) with annualized relapse rate (ARR) while on drug. Logistic regression was used to assess whether time from last dose, lymphocyte subset counts (B cell, T cell, Thelper cells, Tsupressor cells, NK cell, absolute lymphocyte count), demographic or disease specific characteristics were predictive of the presence of relapse at a given time point.

Results: 23 patients, all female, met inclusion criteria. Mean age was 38 (range 16-64); 9 patients were African American, 7 Hispanic, and 7 Caucasian. 15 (65%) met Wingerchuk 2006 criteria for definite NMO and 17 (73%) tested positive for the Aq-4 Ab. Patients had a median of 2 infusion cycles (range 1-6) and were followed for a mean of 22 months (range 1-92), with a median of 8 months between infusion cycles. The median ARR was 0.24.

Lower B cell counts (p=0.035), as well as a diagnosis of definite NMO (p=0.012) and presence of Ab (p=0.025) correlated with a lower risk for relapse at a given time point. Aq-4 Ab status was predictive of ARR while on rituximab: those who were Ab positive had lower ARRs (p=0.042). Demographic factors and cell counts other than B cells were unrelated to relapse risk.

Conclusions: In our sample those who had a definite NMO diagnosis and those who were Aq-4 Ab positive had the lowest risk of relapse while on rituximab. We hypothesize that there is pathologic heterogeneity such that some individuals without the classic, Aq-4 mediated form of disease are less responsive to B cell directed therapy. This may have implications for treatment choice as well as for our emerging understanding of the classification of NMO disease spectrum.

LBP8

Increased frequency of IL17-IL22 dual secreting T-cells in relapsing-remitting multiple sclerosis

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Background: Higher Th17 cell frequencies have been reported in many autoimmune diseases along with their correlation with disease activity, as seen in psoriasis and rheumatoid arthritis. Th17 cells are critically involved in experimental autoimmune encephalomyelitis (EAE), and also suggest that they play a major role in multiple sclerosis (MS), but their association with disease activity and severity is less clear. Emerging literature suggests Th17 to be a plastic and heterogeneous cell population, they not only produce IL17 but are capable of expressing/co-expressing other associated cytokines.

Objectives: To investigate whether Th17 lineage cell frequencies are altered in patients with MS compared to healthy controls and if these correlate with disease activity and severity.

Methods: We recruited 100 patients, 50 with relapsing-remitting MS and 50 with secondary progressive MS (SPMS); along with 50 age and gender-matched Healthy Controls (HC) from the Neurology Department of the University Hospital North Staffordshire. Blood samples were collected and peripheral blood mononuclear cells were isolated, stimulated and stained for a T-helper cell panel followed by flow cytometry.

Results: Median age of the patients was 49 years (range19 to 68 years; male=28, female=82). Fifteen patients were in relapse at the time of recruitment. All SPMS patients and 50%of RRMS patients were treatment naïve. The frequencies of CD4+ CD45RA- memory cells were significantly reduced in the RRMS compared to HC ($p < 0.05$). There was no significant difference in total CD4 cells producing IL17 (CD4+IL17+ cells) or memory CD4 cells producing IL-17 (CD4+CD45RA-ve IL17+ cells) frequency in RRMS versus SPMS or HCs. Interestingly, the proportion of CD4 memory cells co-expressing IL17 and IL22 was increased in the RRMS group compared to HC ($P < 0.05$). When gated on CD45RA- cells, these cells represented a small proportion of the CD4 memory compartment (< 0.5%). Increased IL17+IL22+ T cell frequencies observed in the RRMS group showed no significant correlation with disease activity as measured by annualized relapse rate and/or disease severity as measured by the expanded disability status score.

Conclusions: IL17+IL22+ CD4 memory cell frequencies are increased in RRMS. In view of the overall decreased total memory cell frequencies this relative enrichment of dual secreting memory cells may play an important role in the RRMS phase of the disease but not in SPMS.

LBP9

Oligodendroglial cells and signs of remyelination are present in a subset of early active and inactive MS lesions despite pronounced axonal damage

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Background: Multiple sclerosis (MS) is the most frequent inflammatory demyelinating disease of the central nervous system (CNS) in young adults. Axonal injury and loss in the CNS is a main cause of irreversible neurological deficits. In MS lesions, axonal loss can range from mild to severe. Oligodendrocytes, the myelin-forming cells in the CNS, are presumably a target of the immune-mediated demyelinating process in MS. Remyelination as one of the major mechanisms contributing to axonal protection is often incomplete or fails in MS lesions.

Objectives: The aim of this study was to assess oligodendroglial cells and remyelination in acute and inactive MS lesions with pronounced axonal damage.

Methods: Biopsy tissue from patients with early MS was assessed. In most cases, the biopsies had been performed for diagnostic reasons to exclude neoplastic or infectious diseases. All of the lesions fulfilled the accepted criteria for the diagnosis of multiple sclerosis. The CNS tissue of these biopsied patients, all of whom had white matter lesions (WM), was evaluated for the extent of axonal damage. Using silver staining to detect axonal density, we selected WM lesions with pronounced axonal loss for further characterization. We assessed inflammatory cells, acute axonal damage on sections stained for amyloid precursor protein (APP) and for non-phosphorylated neurofilament (SMI32), as well as de- and remyelination on stainings with luxol fast blue, myelin basic protein (MBP), myelin-associated glycoprotein (MAG) and CNPase. The number of oligodendroglial cells was quantified using antibodies against NogoA and MAG.

Results: Inactive lesions without active ongoing myelin degradation as well as active lesions appeared mainly demyelinated. The axonal damage was marked in these MS lesions compared to normal-appearing white matter. Even in inactive lesions acutely

damaged axons staining positively for APP were still observed. Interestingly, these destructive MS lesions displayed many oligodendrocytes and gradual signs of remyelination.

Conclusions: Our study provides evidence that in a subset of early active and inactive MS lesions oligodendroglial cells and signs of remyelination may be present despite pronounced axonal damage.

LBP10

High-mobility group box 1 (HMGB1) expression is increased in the normal-appearing brain tissue of multiple sclerosis (MS) patients vs. controls

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Background: MS is characterised pathologically by focal inflammatory, demyelinating plaques. More recently, normal-appearing white and grey matter (NAWM/NAGM) distal to these lesions have also been shown to be altered in MS, suggesting more widespread abnormality. High-mobility group box 1 (HMGB1) is a damage-associated pattern recognition molecule (DAMP), able to sense sterile/infectious injury and propagate subsequent inflammatory responses following translocation from its normal nuclear position into the cytoplasm/extracellular space. Increased cytoplasmic HMGB1 expression has been shown previously in macrophages within active lesions, but its profile in non-lesional tissue has not been examined.

Objectives: Analysis of HMGB1 expression pattern in both lesional and non-lesional brain tissue in MS patients vs. non-MS controls.

Methods: Immunohistochemical analysis of HMGB1 expression in biopsy (n=1) and post-mortem brain tissue of MS patients (n=15) and non-MS controls (n=6) was performed. MS tissue blocks were categorized using H&E, LFB, MBP and CD68 as NAWM, 'active lesion' and 'chronic active lesion'. Active/chronic active were grouped together when assessing non-lesional tissue away from the lesion border. Images were digitally scanned (Leica SCN400) and different magnifications of the same region-of-interest captured. Immunoreactivity (IR) was scored using x40 images by three blinded observers (MC, SN, AMG). The intra-class coefficient between raters was >0.8.

Results: IR analysis showed increased HMGB1 expression in active WM lesions in MS vs. control WM, affecting macrophages/activated microglial cells. However, IR was also significantly increased in both NAWM/ NAGM in MS patients vs. controls, with the changes consistently marked in oligodendroglial cells in both. In addition, neuronal cells in non-lesional cortical regions demonstrated significantly greater cytoplasmic translocation of HMGB1. These changes were most marked in regions distal to active lesional areas but were also noted in tissue blocks without an active lesion ('NAWM' tissue block). Both chronic WM and GM demyelinating regions showed reduced expression of HMGB1.

Conclusions: Increased expression and subcellular redistribution of HMGB1 in CNS-resident cells in particular supports the notion of pervasive tissue alteration early-on in MS brain tissue. Whether this pattern reflects a pro-inflammatory, destructive process or a protective response to surrounding cellular stress, or a balance between the two is yet to be determined.

LBP11

A 1-year smartphone data collection study for MS: feasibility and utility of frequent sampling of patient performance in a natural setting

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Background: There is a need for sensitive surrogate endpoints for clinical trials in chronic neurologic disorders such as multiple sclerosis (MS).

Objectives: We piloted a multi-dimensional patient-friendly smartphone (SP) longitudinal MS data collection platform.

Methods: 37 pairs (1 MS patient and 1 cohabitant (CH)) were recruited at the Partners MS Center. Patients were aged 18-55 years and met McDonald criteria. The Android HTC Sensation 4G smartphone was used to deploy a custom application suite consisting of 20 tests capturing subject performance (color vision, attention, dexterity and cognition) and patient-reported outcomes (PROs). During the 1-year study, subjects were prompted to complete a quasi-randomly assigned test every day.

Results: A total of 16 out of 37 pairs completed the entire study. Dropout was highest in the first 19 weeks, during which 15 pairs dropped out. Importantly, low initial scores on the SF-36 Mental Component Scale (P=0.04) and the Impact of Visual Impairment Scale (P=0.007), were associated with dropout in MS subjects.

Performance on individual measures fluctuated over time. However, the mean of a performance measure over time provides a much more robust assessment on an individual's performance than any single cross-sectional measure. This is illustrated by the increased significance in correlation between pairs of measures: for example, we see a strong and expected correlation between SF-36 Physical Component Scale and MOS Pain Effects Scale scores at baseline ($p=4 \times 10^{-3}$) and on average ($p=7 \times 10^{-6}$).

A learning curve was identified for most performance scores: after initial trials, scores improved and variance narrowed. Using spline models, scores for most tests stabilized after 10 trials in MS subjects and 5 trials in the cohabitants. Some MS subjects had a learning curve similar to CH subjects but the mean learning period was longer in MS subjects than in CHs in all performance tests.

Conclusions: 43% of MS subjects tolerated daily tasks for 1 year and performed a variety of tasks on a smartphone. The smartphone platform was effective in gathering data at high frequency in the field. Novel phenotypes such as the inflection point in the practice effect were derived from these data. Clinical validation of

these tests is necessary to establish clinical relevance. Future directions include enhanced and more frequent individual estimates of disease course to monitor disease course in large-scale studies.

LBP12

Excitatory post-synaptic injury in experimental autoimmune encephalomyelitis gray matter

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Background: Gray matter degeneration contributes to progressive disability and cognitive impairment in patients with multiple sclerosis (MS). Though axon injury is widely thought to trigger much neurodegeneration in MS, we hypothesized that neurons in the presence of pro-inflammatory molecules and oxidative stress are vulnerable to excitotoxic post-synaptic injury as well.
Objectives: We tested whether neurons in the setting of microglial activation and oxidative stress undergo excitatory post-synaptic injury independent of axotomy or demyelination, using an experimental autoimmune encephalomyelitis (EAE) mouse model of MS gray matter injury in which gray matter axons and myelin are relatively preserved.
Methods: We used measured loss of excitatory pre- and post-synaptic structures via immunostaining for VGLUT1 and PSD95-positive puncta in the hippocampi of C57/BL6 mice with EAE induced by direct immunization with MOG35-55 peptide, which recapitulates much of MS gray matter pathology, compared to sham-immunized controls. We studied their association with Iba1-positive microglia and oxidative stress via EO6 staining for oxidized phospholipids, as well as myelin (MBP) and axon integrity (SMI32).
Results: In EAE hippocampi, microglial activation and oxidative stress was associated with a 30% loss of PSD95-positive excitatory post-synaptic puncta compared to controls, independent of changes to VGLUT1-positive puncta, myelination, or axon integrity. Excitatory post-synaptic loss correlated with increased PSD95 inclusions within nearby activated microglia in EAE mice, and was independent of the severity of EAE clinical score.
Conclusions: Neurons in EAE hippocampi undergo excitatory synaptic injury in the setting of microglial activation and oxidative stress that appears to preferentially affect the post-synaptic structures, and occurs independent of myelin loss or axon injury. This suggests that direct post-synaptic injury may contribute to gray matter dysfunction in MS and may be an important target for neuroprotective interventions.

LBP13

Profiling the autoantibody repertoire on ultra-high-density peptide microarrays reveals novel potential autoimmune targets in multiple sclerosis

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Background: Profiling the autoantibody repertoires for the discovery of novel autoimmune targets requires representations of a significant part of the human proteome. We have previously utilized both planar and bead based protein arrays containing large sets of protein fragments for the profiling of plasma and CSF samples within multiple sclerosis (Ayoglu; *Molecular Cellular Proteomics* 2013. Autoantibody profiling in multiple sclerosis using arrays of human protein fragments. Häggmark; *Proteomics* 2013. Antibody-based profiling of cerebrospinal fluid within multiple sclerosis).
Objectives: Here we utilize an approach where the whole proteome is being represented in the form of high-density peptide arrays and used to profile the autoantibody repertoire in plasma with the aim to identify novel autoimmune candidate targets within multiple sclerosis.
Methods: Ultra-high-density peptide microarrays made with an *in situ* photolithographic synthesis were designed with 2.1 million peptides with whole proteome coverage with 6 out of the 12 amino acids overlap or 175.000 peptides representing a selected set of targets and then also enabling a higher resolution with 11 amino acids in overlap. The whole proteome arrays were used for initial screening and identification of interesting targets. The dedicated arrays were used to profile 24 plasma samples (8 SPMS, 8 CIS non-converters and 8 controls).
Results: The initial screening on the whole proteome arrays revealed a large number of peptide reactivity within each samples, but also a very significant individual heterogeneity. A frequently repeated and specific part of a low-density lipoprotein receptor domain (LDLRA2) was observed as a potential novel autoimmunity target in multiple sclerosis. The dedicated arrays containing targets from the initial screening as well as from literature identified several autoantibody targets with a defined epitope. One of them was the MAP3K7 (mitogen-activated protein kinase kinase kinase 7) with eight consecutive peptides providing signals in three out of the eight SPMS-samples and in two out of the eight CIS non-converters and in none of the eight controls.
Conclusions: A screen for novel autoantigens in the context of multiple sclerosis by utilizing ultra-high density peptide microarrays reveals several potential autoimmune targets with specific epitopes mapped. The most significant findings will be followed up on bead arrays with designed peptides and protein fragments, enabling the analysis of large number of samples.

LBP14

DMF protects neural stem/progenitor cells and differentiated neurons from oxidative damage through regulating anti-oxidative stress genes

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Background: Dimethyl fumarate (DMF, BG12) was recently approved by FDA for the treatment of relapsing forms of Multiple sclerosis (MS), however its mechanism of action is not yet well understood.
Objectives: We aim to study the potential neuroprotective effects of DMF on neural progenitor cells and neurons.
Methods: We investigated the effects of DMF on rodents' NPC neurosphere formation, survival, apoptosis, and proliferation in

vitro. In addition, cortical neuron survival was analyzed by a custom-designed automated longitudinal fluorescence microscopy. We utilized anti-oxidative stress PCR microarray (SuperArray) to screen for genes involved in the DMF mediated effects on NPC.
Results: Using the neurosphere formation assay, we found that DMF increases the frequency of the multipotent neurospheres in culture and increases the survival of rat NPCs following oxidative stress treatment. In addition, utilizing reactive oxygen species (ROS) assays, we showed that DMF reduces ROS production induced by hydrogen peroxide (H_2O_2). We also found that DMF decreased oxidative stress induced apoptosis in NPCs. Using real-time fluorescence microscopy survival analysis we found that DMF significantly decreased the risk of oxidative stress-induced rat cortical neuron death and enhanced neurite outgrowth. Similar effects were seen on motor neurons.

We further analyzed the expression of oxidative stress-induced genes in the NPC cultures. We demonstrated that DMF increases the expression of Nrf2 (transcription factor nuclear factor-erythroid 2-related factor 2). Furthermore, we utilized SuperArray gene screen technology to identify additional anti-oxidative stress genes. Our results showed that under oxidative stress, DMF significantly up-regulates gene expression of Gstp1 (Glutathione S-transferase pi 1), Nqo1 (NAD(P)H dehydrogenase, quinone 1), Sod2 (Superoxide dismutase 2, mitochondrial), Srxn1(Sulfiredoxin1 homolog), and Fth1(Ferritin)—which all play a role in reducing oxidative stress by breaking down ROS. Intriguingly, in NPC culture, DMF also down-regulates Ccl5, a ligand of CCR1, CCR3, and CCR5; potentially reducing the recruitment of leukocytes into inflammatory sites.
Conclusions: DMF protects neural stem/progenitor cells and differentiated neurons from oxidative damage through regulation of Nrf2 depedent and indepedent pathways by reducing ROS. Analysis of oxidative stress mechanisms may yield new insights into the dysregulation of this biological process during MS disease processes.

LBP15

HERV-W envelope protein inhibits oligodendroglial cell differentiation which can be abrogated by the neutralizing antibody GNbAC1

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Background: Myelin repair in the diseased or injured central nervous system (CNS) is mainly mediated via recruitment, activation and differentiation of oligodendroglial precursor cells (OPCs). This endogenous repair process is influenced by multiple extrinsic and intrinsic factors. The human endogenous retrovirus type W (HERV-W) and in particular its envelope protein (ENV) have been shown to be implicated in inflammatory reactions leading to demyelination in multiple sclerosis (MS). In order to prevent ENV-dependent activation of immune cells which fuel and enhance inflammatory and autoimmune reactions, neutralizing antibodies have been developed as a novel therapeutic approach. One of these antibodies, GNbAC1, has been successfully tested in phaseI and IIa clinical trials. Recently, we could demonstrate that HERV-W ENV also interferes with myelin repair processes by inducing nitrosative stress in OPCs (Kremer et al., Ann Neurol 2013). As a consequence of enhanced NO production OPCs form nitrotyrosine groups and, subsequently, reduced myelin protein expression and decreased cellular maturation can be observed.
Objectives: We tested the efficiency of the GNbAC1 anti-ENV antibody in preventing the induction of oligodendroglial stress reactions.
Methods: Primary OPCs were stimulated with recombinant ENV in absence and presence of neutralizing GNbAC1 and assessed for inducible nitric oxide synthase gene expression levels as well as for the generation of nitrotyrosine groups and the expression of myelin basic protein.
Results: Our findings demonstrate that upon pre-incubation with GNbAC1 the ability of the viral envelope protein to induce stress reaction in OPCs is significantly reduced and that the antibody can rescue myelin expression.
Conclusions: These observations indicate that, beyond immune cell modulation, this monoclonal antibody is also able to prevent HERV-W ENV-mediated inhibition of oligodendroglial differentiation which holds significant promise for future remyelination therapies for MS.

LBP16

Follow-up of MS patients from phase IIa clinical study of GNbAC1 reveals unexpected decrease of HERV-W endogenous retrovirus genes expression

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Background: GNbAC1 is an IgG4 humanized monoclonal antibody targeting and neutralizing pathogenic effects of MSRV-Env, a potent pro-inflammatory protein that also blocks oligodendrocyte precursor cell differentiation. This therapeutic target is an endogenous retroviral envelope from HERV-W family, which is abundantly expressed in microglia within active MS lesions and at the rim of chronic progressive plaques. HERV-W *gag*-encoded capsid protein is co-detected in MS lesions, which indicates HERV-W expression beyond the *env*-encoded pathogenic target.
Objectives: Follow-up mRNA expression in circulating blood cells of MS patients treated with GNbAC1 over the first six months of Phase IIa study with quantitative PCR targeting two different HERV-W genes, *env* and *pol*.
Methods: Peripheral blood mononuclear cells (PBMC) of patients collected during the first 6 months of Phase IIa (ClinicalTrials.gov Identifier: NCT01639300) were assessed by real time quantitative polymerase chain reaction (RT-qPCR). Expression levels of

messenger RNA (mRNA) were assessed by different primers/probe sets specific for retroviral *env* and *pol* HERV-W genes.
Results: At inclusion, no difference in HERV-W transcript levels was observed between patients being enrolled in both cohorts (2 and 6mg/kg) and between recruiting centres. Nonetheless, the different qPCR protocols showed the highest HERV-W mRNA levels in Primary Progressive MS (PPMS) patients. Despite low numbers, statistically significant correlations were found between HERV-W *env* and *pol* mRNA levels and disease duration and/or progression index.

A decrease of all HERV-W mRNA levels was observed at the 6th GNbAC1 administration, when compared to basal values at inclusion in both cohorts. Statistical distribution of values measured before the first, third and sixth GNbAC1 injection in all patients showed unexpected decrease for both *env* and *pol* mRNA
Conclusions: Specifically binding to HERV-W envelope protein and neutralizing its pathogenic activity, GNbAC1 is not expected to impact HERV-W gene expression. A significant decrease of mRNAs encoding retroviral proteins from different HERV-W structural genes therefore appears unique for an anti-envelope antibody and evidences an effect on HERV-W expression itself.

This study showed

i) a global anti-retroviral effect on HERV-W expression over six months of GNbAC1 treatment,
ii) evidence, beyond MSRV-Env endogenous protein itself, of an association between HERV-W expression and MS and
iii) PCR assays for potential companion diagnostic tests.

LBP17

Clinical outcomes in patients with CIS treated with interferon beta-1b: 11-year follow-up of BENEFIT

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Background: Findings from the BENEFIT trial have shown that initiating treatment with interferon beta-1b immediately after the first clinical event indicative of MS (CIS) improved clinical and MRI outcomes after up to 8 years vs patients who delayed treatment initiation up to 2 years.
Objectives: To study the long-term impact of early or delayed treatment with interferon beta-1b on the disease course after CIS.
Methods: Patients with CIS and >=2 MRI lesions suggestive of MS were randomly assigned to receive interferon beta-1b 250 µg (early treatment) or placebo (delayed treatment) subcutaneously every other day. After 2 years or conversion to clinically definite MS (CDMS), patients receiving placebo were offered treatment with interferon beta-1b. Eleven years after the initial randomization, all patients from participating study centers were approached to complete a comprehensive reassessment. We report initial clinical outcomes and employment status.
Results: 278 of the original 468 patients were enrolled (167 from the early and 111 from the delayed treatment arms). Including 3 deceased patients, 60.0% of the cohort originally randomized in the BENEFIT core study was identified, corresponding to 72.6% of BENEFIT patients originally randomized in centers that agreed to participate in the BENEFIT 11 study. Baseline characteristics of the original and the BENEFIT 11 participants were similar. By 11 years post-randomization, differences in time to first relapse (HR 0.655 [95% CI 0.517-0.830], p=0.0005), annualized relapse rate (RR 0.7879 [95% CI 0.6896-0.9003], p=0.0005) and time to CDMS (HR 0.670 [95% CI 0.526-0.854], p=0.0012) still favored the early treatment group. Kaplan Meier estimates of risk of CDMS at 11 years were 65.2% in the early and 75.0% in the delayed treatment groups. EDSS remained low and stable with a median of 2.0 and median change from baseline of 0.5 in both groups. Risk of SPMS at 11 years was 4.5% in the early and 8.3% in the delayed treatment groups. Employment data showed that 73.0% of BENEFIT 11 patients continued to be employed.
Conclusions: BENEFIT 11 provides evidence that even 11 years after randomization early treatment of patients with CIS had a positive impact on clinical outcomes and supports the importance of starting therapy with interferon beta-1b early. Both treatment groups had low and stable EDSS scores indicative of little or no disease progression and the majority of patients remained employed.

LBP18

Exome sequencing unravels novel candidate genes in familial multiple sclerosis

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Background: Multiple Sclerosis (MS) is an inflammatory central nervous system autoimmune disease that affects 2 million people worldwide with increasing incidence. Most MS cases are sporadic but there is a higher incidence of MS in close relatives. Thus, the cause of MS is likely multifactorial, with contribution from both environmental and genetic factors. Although familial MS is rare, families with MS offer a unique opportunity to elucidate genetics of MS.
Objectives: We sought to use cutting-edge technology of exome sequencing coupled with bioinformatic tools to identify mutations in small family pedigrees.
Methods: To find rare variants contributing to familial MS susceptibility, we performed exome sequencing on 21 individuals from 4 unrelated families with MS. We also evaluated epidemiologic data pertaining to these individuals´ smoking, birth place and subsequent residence latitude.
Results: A total of 133,064 variants were found to be shared among 8 unaffected and 13 affected family members of our 4 MS

families. To narrow the list of candidates, we excluded variants with low quality scores, those commonly found in 1000G database, and variants with tolerable mutations. To refine the search criteria for candidate variants, we relied on pedigree inheritance information and literature searches for genes with association to MS, other inflammatory/autoimmune disorders, and genes involved in inflammatory pathways. Using this approach, we identified 12 novel candidate variants within the 4 families. Of affected individuals, most were born and resided at higher latitudes and most were either smokers or exposed to second hand smoke.

Conclusions: Exome sequencing and bioinformatics tools allow for a faster and cheaper opportunity to discover new variants in small family pedigrees. Using this technology, we identified 12 new candidate variants in Familial MS. Further studies on

1) a larger cohort of family members,
2) in a separate larger MS dataset of Familial and sporadic MS, and
3) downstream experimental approaches, will help confirm candidates as causative MS variants.

The discovery of new MS genes and understanding how genes interact with environmental factors will enhance the field of MS and allow for new discoveries of gene-based guided MS therapies.

LBP19

Differential impact of pediatric monophasic demyelinating disorders and multiple sclerosis on brain growth

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Background: Reduced brain volume has been reported in patients with pediatric-onset multiple sclerosis (MS) compared to normal controls (NC).

Objectives: To evaluate how total brain, grey matter (GM) and white matter (WM) growth differ between pediatric NC and children with MS, acute disseminated encephalomyelitis (ADEM), and non-ADEM monophasic acquired demyelinating syndromes (monoADS) with and without T2 brain lesions at onset (monoADSlesion and monoADSnolesion, respectively).

Methods: 114 children (27 MS, 32 monoADSlesion, 35 monoADSnolesion and 20 ADEM) were identified from the prospective Canadian Pediatric Demyelinating Disease Study. 613 serial MRI were acquired on a single 1.5T MRI scanner. The first scan was acquired 6 months on average after the first attack, the age at onset was 10.6±3.6y, the follow-up time was 3.7±2.2y and the median number of visits was 5. 876 1.5T MRI scans from 340 subjects from the NIH-funded MRI study of normal brain development (NIHPD) were used as controls (follow-up: 3.21±0.98y, 2 or 3 visits per subject).

A 4D EM classification algorithm was used to segment the GM and WM. The total brain volume was the sum of the GM and WM volumes.

Mixed effect models were built to estimate the GM, WM and brain growth trajectories of all groups using fixed effects for age (intercept, linear, quadratic and cubic terms) and the interactions of age with sex and age with group, and using intercept and slope terms as random effects.

Results: In the brain growth model, significant group and age interactions ($p < 0.01$) indicate that MS, monoADSlesion, monoADSnolesion and ADEM have different brain growth curves compared to NC. Whereas the NC brain growth plateaus, the curves of all patient groups decline [highest rate for the pMS group (-7.1cc/y) > monoADSlesion (-5.5 cc/y) > ADEM (-4.4 cc/y) > monoADSnolesion(-3.0 cc/y)].

As expected, GM decreases over time in NC. Only the MS and MonoADSlesion differ significantly ($p < 0.01$) from the NC (difference of -3.5cc/y for MS and -2.4cc/y for MonoADSlesion).

In contrast, WM volume increases with age in all groups; however the rate of increase is significantly smaller ($p < 0.01$) for the children with MS, ADEM and monoADSlesion compared to NC.

Conclusions: MS in children shows a clear impact on overall brain growth, resulting in smaller volumes of GM and WM than in NC, while ADEM impacts predominantly WM growth. In children with monoADS the presence of brain lesions is a critical factor for failure of WM growth.

LBP20

A multi-SNP signature predicts high response to Copaxone (Glatiramer Acetate) in RRMS patients

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Background: Copaxone (glatiramer acetate) consistently demonstrated annual relapse rate (ARR) reduction of ~33% compared with placebo, and a favorable safety profile, in clinical trials. On an individual level, patients show variable response levels to Copaxone, some achieving significantly higher response levels. Involvement of proteins with high inter-individual genetic variability in Copaxone mechanism of action (MOA), e.g. *HLA-DRB1*1501*, as well as previous studies, support potential contribution of genetics to level of response.

Objectives: To identify a genetic signature associated with response rate to Copaxone, defined by ARR reduction.

Methods: DNA from consenting RRMS patients in the FORTE study [n= 604, 52% of study population, treated with Copaxone 20mg (n=308) or 40 mg (n=296) daily] and GALA study [n=1158, 82% of study population, treated with 40mg TIW (n=777), or placebo (n=381)] was collected. Analyses to date focused on ~1200 patients classified as "high responder" (reduction of one or more relapses during study compared to prior two years) or "non-responder" (a similar or larger number of relapses during study compared to prior two years), for active and placebo arms. Patients

were genotyped (Illumina HumanOmni5-Quad array) to identify response-associated single-nucleotide polymorphisms (SNPs). The GALA cohort served as a discovery set, with the placebo arm used to assess prognostic (reflecting disease course) versus predictive (reflecting treatment response) SNP association. Predictive modeling was used to develop a SNP-based signature for Copaxone response, and the FORTE cohort was used as validation cohort.

Results: Comparison of Copaxone-treated high responders and non-responders in GALA identified several SNPs that are associated with Copaxone response, none of which were associated with placebo response. The association of these SNPs with response was confirmed in FORTE. Most SNPs are associated with genes related to Copaxone MOA. Based on these findings, a multi-SNP signature was developed and is currently being confirmed in further studies, to be presented at the conference.

Conclusions: Genetic association with response to Copaxone was identified. In Copaxone naïve RRMS patients, the multi-SNP signature may be able to predict high Copaxone responders who will, on average, exhibit ARR reductions significantly higher than the average response reported in Copaxone clinical trials. Confirmation in an independent cohort is ongoing.

LBP21

Cerebrospinal fluid aquaporin-4 antibody levels in neuromyelitis optica attacks

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Background: Neuromyelitis optica spectrum disorders (NMOSD) are characterized by severe attacks of optic neuritis and/or longitudinally extensive transverse myelitis. A significant proportion of NMOSD patients are seropositive for aquaporin-4 antibodies, but serum antibody titers do not correlate well with the amount of inflammation in the central nervous system and astrocyte damage.

Objectives: To elucidate immunopathogenetic roles of aquaporin-4 antibodies in the cerebrospinal fluid (CSF) of NMOSD patients, we analyzed the relationship between aquaporin-4 antibody titers, cellular and inflammatory markers in the CSF.

Methods: We enrolled eleven NMOSD consecutive patients (10 females; 1 male) with a median age of 50 years (range 24 - 71) seen at Hospital das Clinicas, Faculty of Medicine, University of Sao Paulo with detectable aquaporin-4 antibody in sera and CSF at Tohoku University. Astrocyte damage was evaluated measuring glial fibrillary acidic protein (GFAP) levels and cytokines were measured using a multiplexed fluorescent magnetic bead-based immunoassay in the CSF.

Results: The serum aquaporin-4 antibody titers were found at high-levels in all patients during attacks and remission. In contrast, aquaporin-4 antibody titers in the CSF were remarkably higher only in samples collected during attacks. Consequently, the CSF:serum ratio found during remission (1:2,048 ± 1:1,448) was higher than during NMO attacks (1:204 ± 1:175), $p = 0.0030$. The CSF aquaporin-4 antibody levels during attacks (but not in sera) closely correlated with pleocytosis ($r = 0.7679$, $p = 0.0058$), inflammatory cytokines including interleukin-6 ($r = 0.8091$, $p = 0.0026$) that can regulate antibody-producing plasmablasts, interleukin-1 beta ($r = 0.7517$, $p = 0.0076$) that can disrupt the blood-brain barrier, and GFAP levels ($r = 0.9439$, $p < 0.0001$) in the CSF.

Conclusions: The amount of aquaporin-4 antibodies present in the central nervous system may have therapeutic implications, as it is associated with astrocyte injury and inflammatory responses during NMOSD attacks.

LBP22

Identification of pathogenic mutations and risk alleles in familial multiple sclerosis

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Background: Although risk alleles for multiple sclerosis (MS) have already been identified though association studies, most associated variants have a minor effect, typically with odds ratio (OR) below 1.3, and cannot account for the clustering of MS patients observed within families.

Objectives: The goal of this study is to identify pathogenic mutations responsible for Mendelian forms of MS and rare variants of major effect on disease risk.

Methods: Exome sequencing analysis was applied to patients from two multi-incident families. Findings were validated in a case-control series consisting of 2,119 patients and 844 healthy controls collected through the longitudinal Canadian Collaborative Project on the Genetic Susceptibility to Multiple Sclerosis.

Results: A p.R415Q mutation was identified in five patients within the first family. Genotyping patients and controls found one additional familial patient with the p.R415Q mutation. Within this kindred, there was another affected family member and one unaffected sibling who carried the mutation. Both families present the same disease haplotype; and all patients were diagnosed with primary progressive or secondary progressive MS within few years from onset. The amino acid position is highly conserved and p.R415Q is predicted to disrupt the heterodimerization of this transcripton factor, resulting in dysregulation of many genes including myelin protein zero (MPZ) and peripheral myelin protein-22 (PMP22).

The second family resulted in the identification of a p.G420D mutation segregating with disease in six family members. Genotyping patients and controls identified the mutation in one control and 11 MS patients resulting in an OR of 4.8. Nine of the newly identified patients presented family history of disease, genotyping of family members identified the p.G420D mutation in 24 out of 28 MS patients (86%) and 11 out of 28 healthy individuals (39%). Despite the presence of p.G420D in healthy individuals, linkage analysis supports co-segregation with disease (LOD=4.6). Interestingly; the mutation appears to be more penetrant in females with a 1:3.4 unaffected to affected ratio, versus a 1:1.2 in males. The implicated gene activates inflammatory cells, production of cytokines and increases blood-brain barrier permeability allowing for the infiltration of leukocytes.

Conclusions: Application of next generation sequencing technologies to Mendelian forms of MS can be successfully used for the identification of pathogenic mutations and risk variants of major effect.