

## Background:

Multiple Sclerosis (MS) is the most common neurological autoimmune disease in young adults, affecting millions worldwide. The cause of disease itself is not known yet, but multiple risk factors have been identified including genetic risk, such as Human Leukocyte Antigen (HLA) alleles, environmental risk (Vitamin D, smoking) and Epstein-Barr virus (EBV) infection. Current theories on etiological pathology of MS include cross-reactive immune responses between EBV and host CNS proteins, mistaken self and triggering of EBV infected autoreactive B lymphocytes.

## Objectives:

We have taken an integrated approach to investigating the relationship between genetic, environment and EBV infection and their contribution to MS risk utilizing the MS Perth Demyelinating Disease Database (n=426) and the Busselton healthy control cohort (n=186).

## Methods:

To review individual and combined effects on disease risk comparing MS patients and healthy controls from WA, host genetic profiles were determined using Sanger sequence based Human Leukocyte Antigen (HLA) typing. Immune responses to EBV infection (IgG antibodies) directed against viral capsid antigen (VCA), Epstein-Barr nuclear antigen-1 (EBNA-1) and EBNA-1<sub>(short)</sub> (peptide within EBNA-1 [aa 401-411]) were detected by using commercial and in-house ELISAs (Figure 1b). EBV sequence variation was assessed in MS patients (n=54) using semi-nested in-house PCRs, Sanger sequencing and for a subset with 454-FLX technology (Figure 1c).

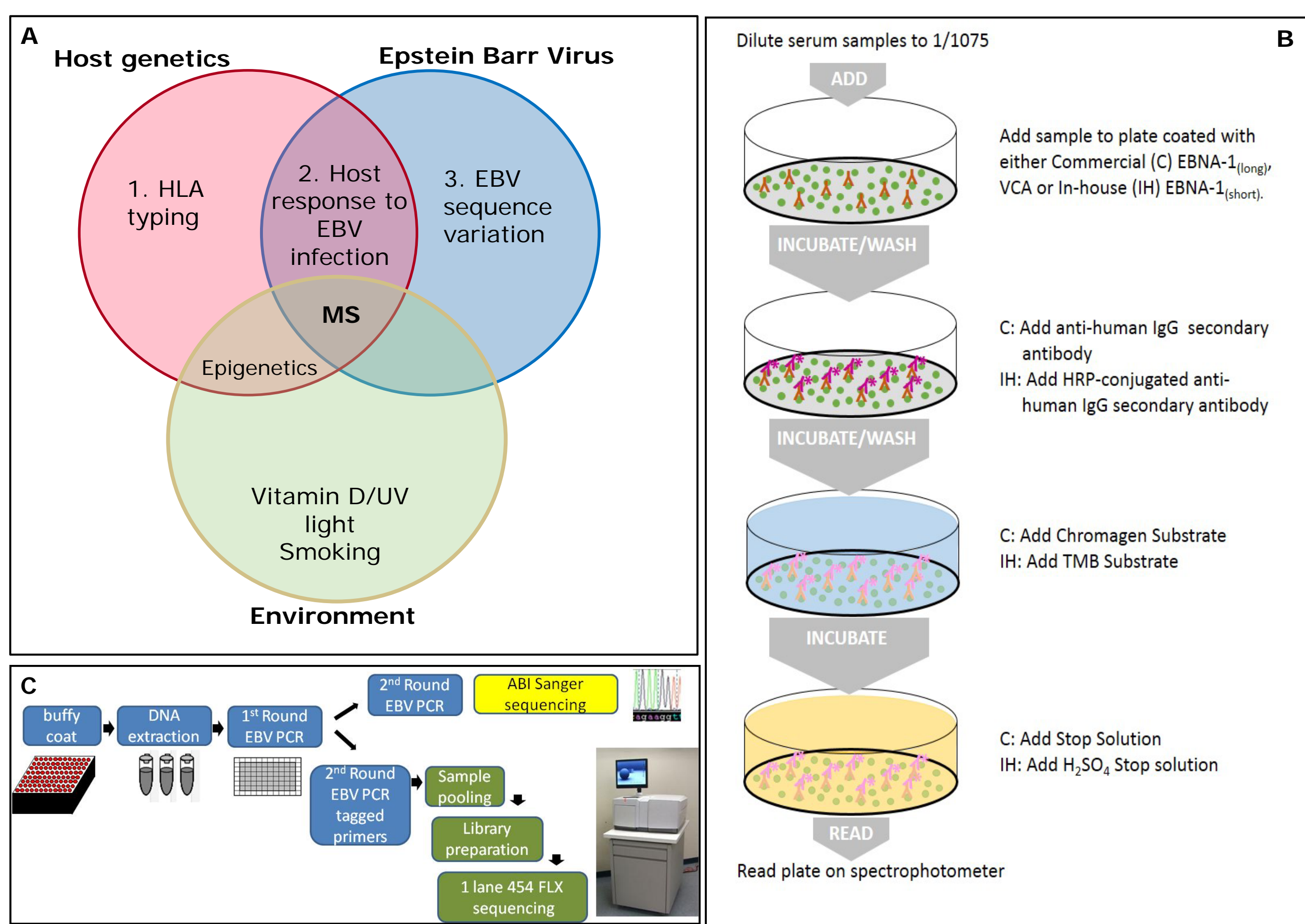
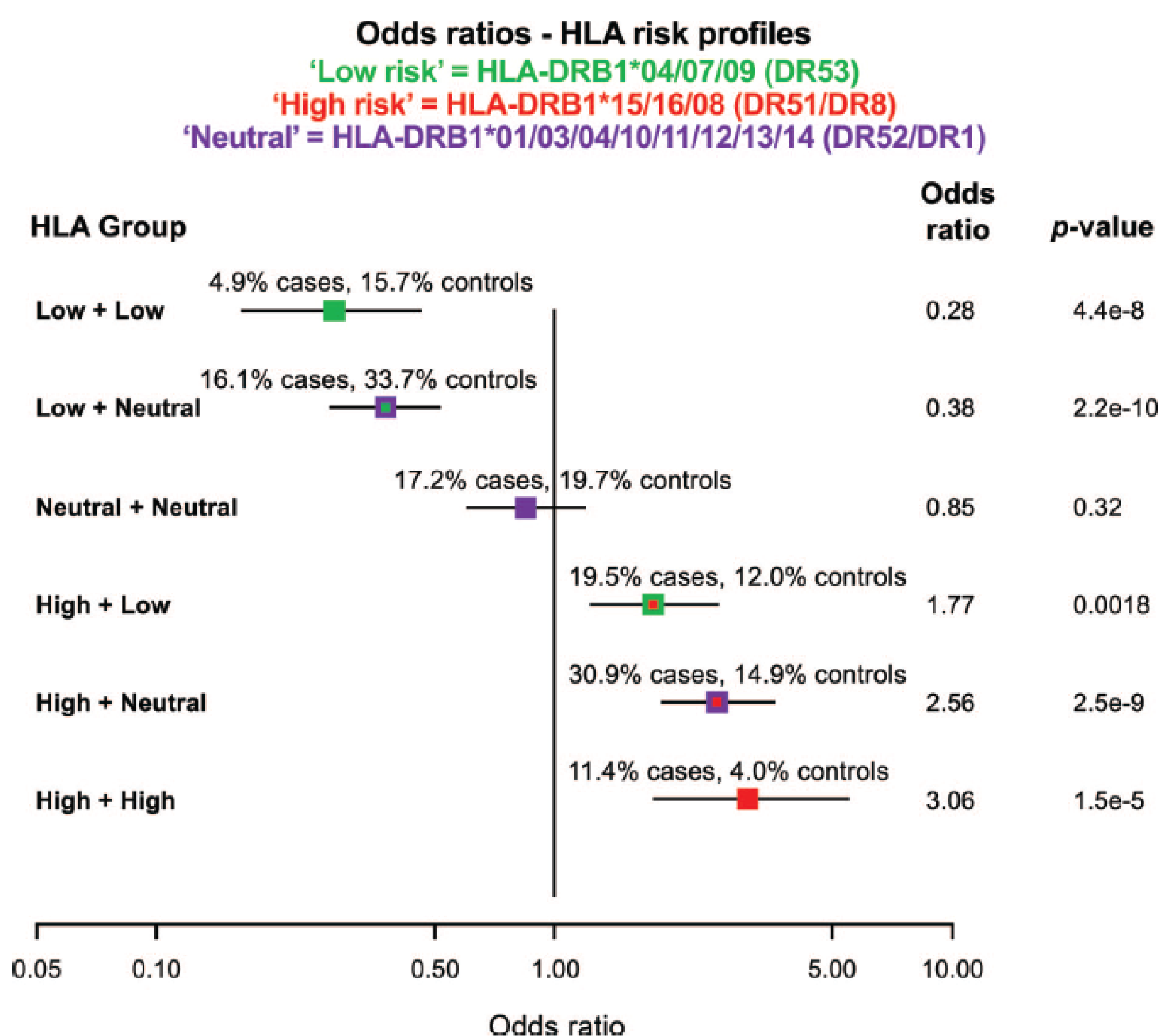


Figure 1(A) Multifaceted approach to MS risk factors. (B) Overview of the ELISA method. Each of the steps illustrated was automated using the Biomek FX robot. (C) Overview of EBV sequencing method for viral sequence variation

## Results:

### 1. Assessment of genetic MS risk (HLA typing)

Comparing class II HLA-DRB allele prevalence in patients and controls, we were able to identify a combined contribution of low risk alleles (HLA-DRB1\*04, \*07, \*09), a group of neutral HLA risk and a group of high MS risk alleles (HLA DRB1\*15, \*16 and \*08) (Figure 2).



Stratification of multiple sclerosis risk according to combined HLA allele profiles, including prevalence rates in cases (n = 466) and controls (n = 498): odds ratios and 95% confidence intervals.

Figure 2. Combined effects of HLA-DR profiles on multiple sclerosis risk (Nolan et al 2012)

## Results:

### 2. Assessment of Immune response to EBV (IgG antibodies)

All MS patients demonstrated immune responses to EBV compared to only 93.5% (EBNA-1) and 90.3% (VCA) of healthy controls. Individuals with high risk HLA-DRB alleles (DRB1\*08, \*15, \*16) had significantly higher antibody titres against EBNA-1<sub>(long)</sub> and EBNA-1<sub>(short)</sub> in comparison to low-risk carriers (p<0.0001; Figure 3A & B), but not significantly different anti-VCA titres (Figure 3C). Incorporating HLA risk allele presence, anti-EBNA-1, anti-EBNA-1<sub>(short)</sub> and anti-VCA antibody levels in a gender adjusted model (Figure 4), achieved a diagnostic sensitivity of 92% and specificity of 64% for our cohorts. Subclassing of anti-EBNA1 antibodies revealed a prevalence of IgG<sub>1</sub> antibodies, although their additional incorporation did not improve the classification model.

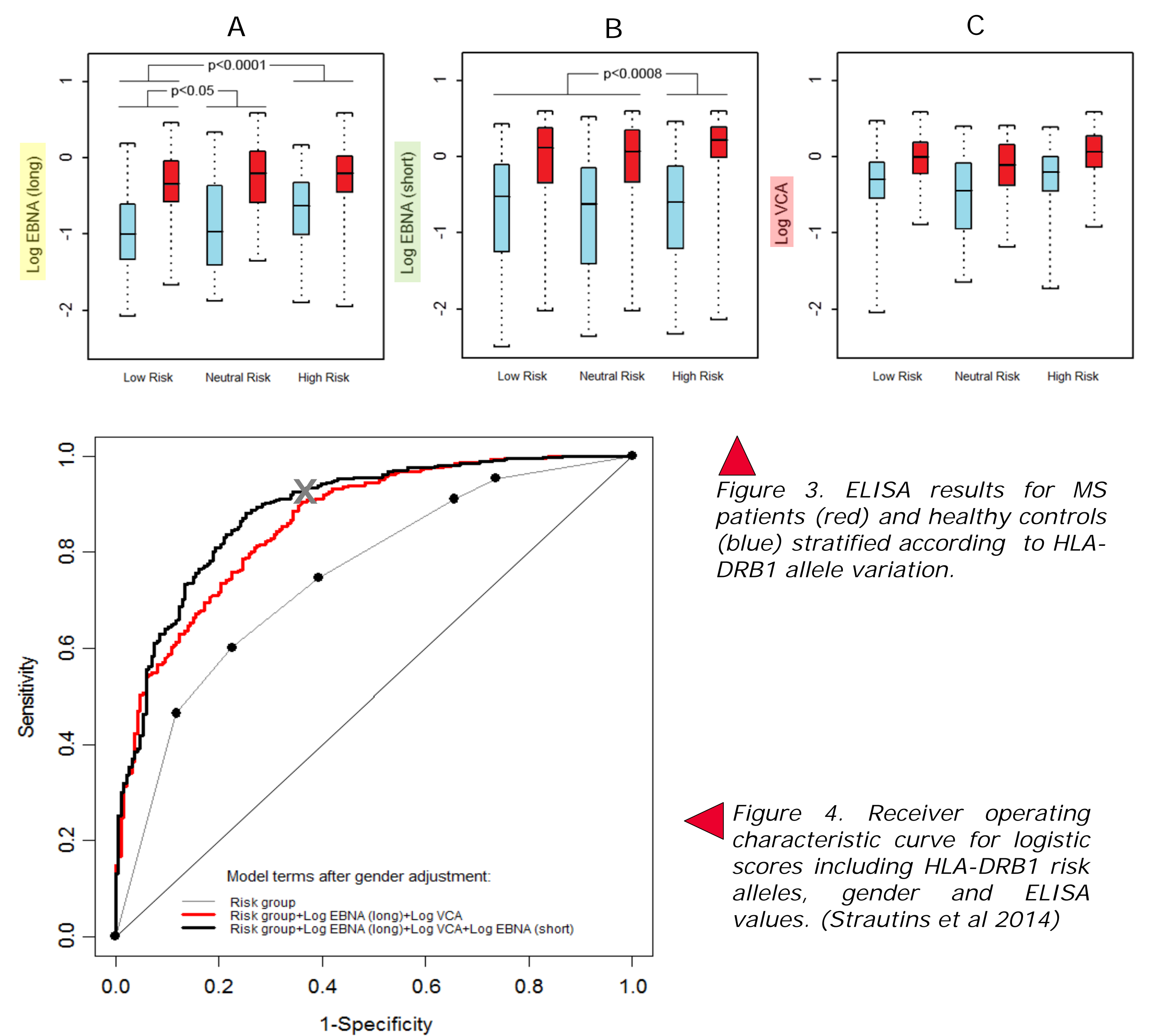


Figure 3. ELISA results for MS patients (red) and healthy controls (blue) stratified according to HLA-DRB1 allele variation.

Figure 4. Receiver operating characteristic curve for logistic scores including HLA-DRB1 risk alleles, gender and ELISA values. (Strautins et al 2014)

### 3. EBV Sequence variation in MS patients

EBNA-1 sequencing including highly sensitive 454-FLX technology did identify low EBV sequence variation and did not reveal MS-specific strains. It did however highlight HLA-DRB1\*1501-restricted T cell epitopes within EBNA-1 that could induce cross-reactive T cell responses against homologous targets within central nervous system proteins (Figure 5).

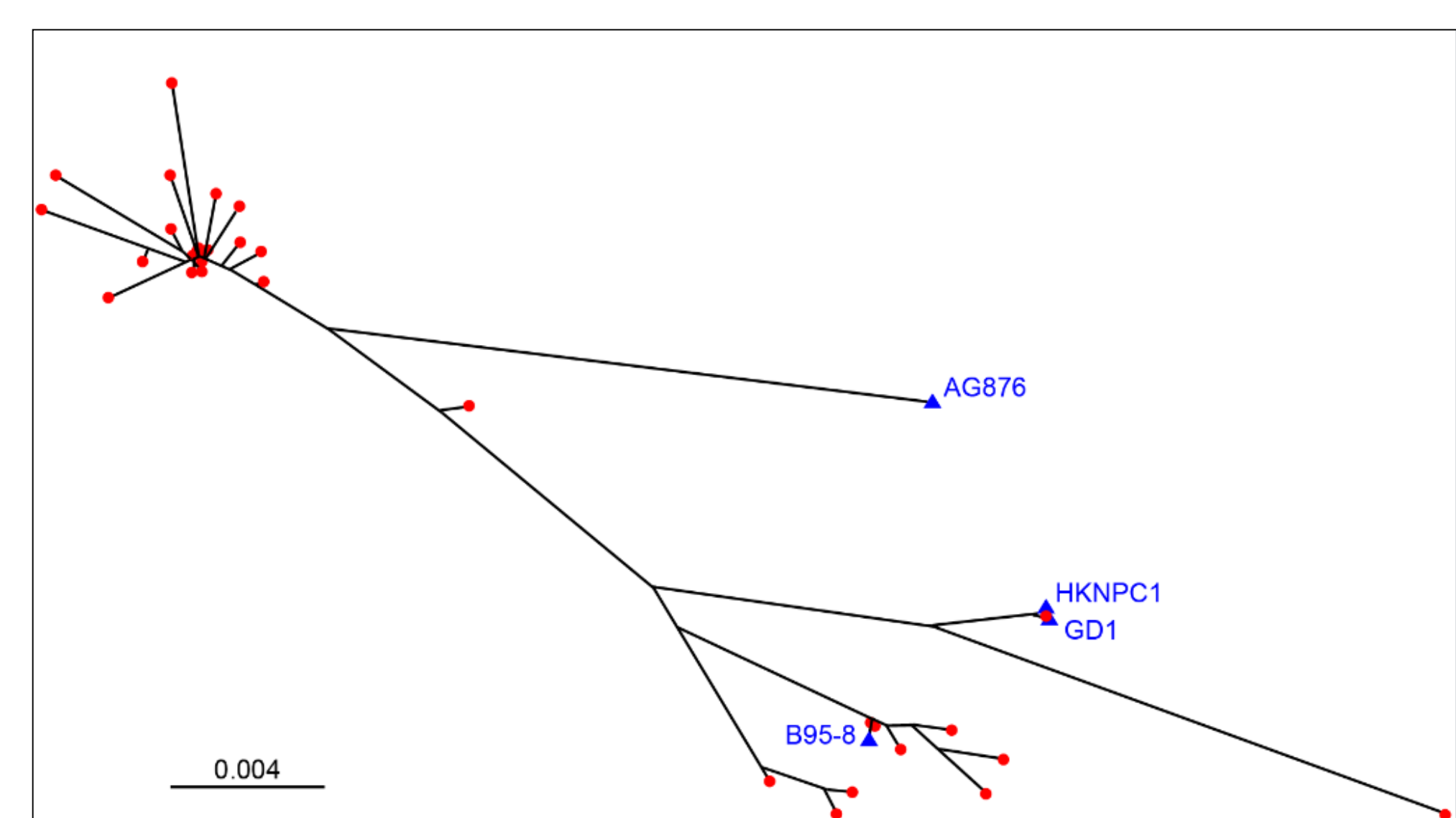


Figure 5. Phylogenetic tree of C-terminal EBNA-1 Sanger sequences including the reference strains B95-8, AG876, GD1 and HKNPC1 (blue triangles) and 53 MS cases (red dots).

## Discussion, Conclusions, Future Aims:

- Including serological anti-EBV Ig levels can improve a purely genetic risk model, where anti-EBNA-1<sub>(long)</sub> IgG can abrogate "high risk" HLA-DRB1 alleles, suggesting common pathways. This risk model should be applied to another independent MS cohort, but supports the important role of EBV in MS development.
- Although EBV sequence variation was observed, no MS-specific EBV strain was identified. Computational analysis further identified potential cross-reactive epitopes, and we now aim to investigate these functionally.
- We are currently investigating the role of B cells in MS (Immunophenotyping) and by identifying latently infected B cells to further investigate pathogenesis, as well as B cell-T cell interactions that may be central disease mediators and possible targets for monitoring and treatment strategies.

## Acknowledgments:

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