

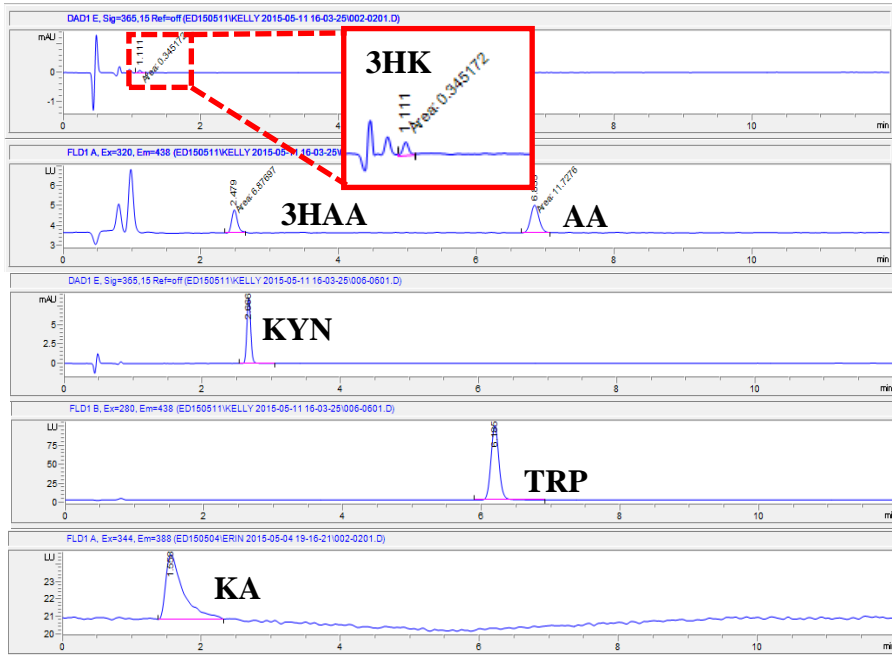
# **Kynurenine pathway metabolomics predicts and provide mechanistic insight into multiple sclerosis progression**

## **Supplementary Information**

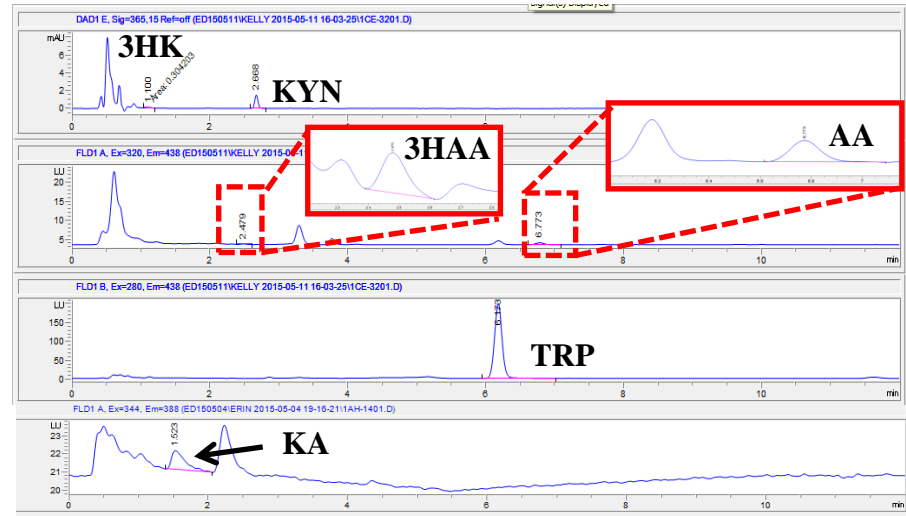
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### SUPPLEMENTARY FIGURE S1A

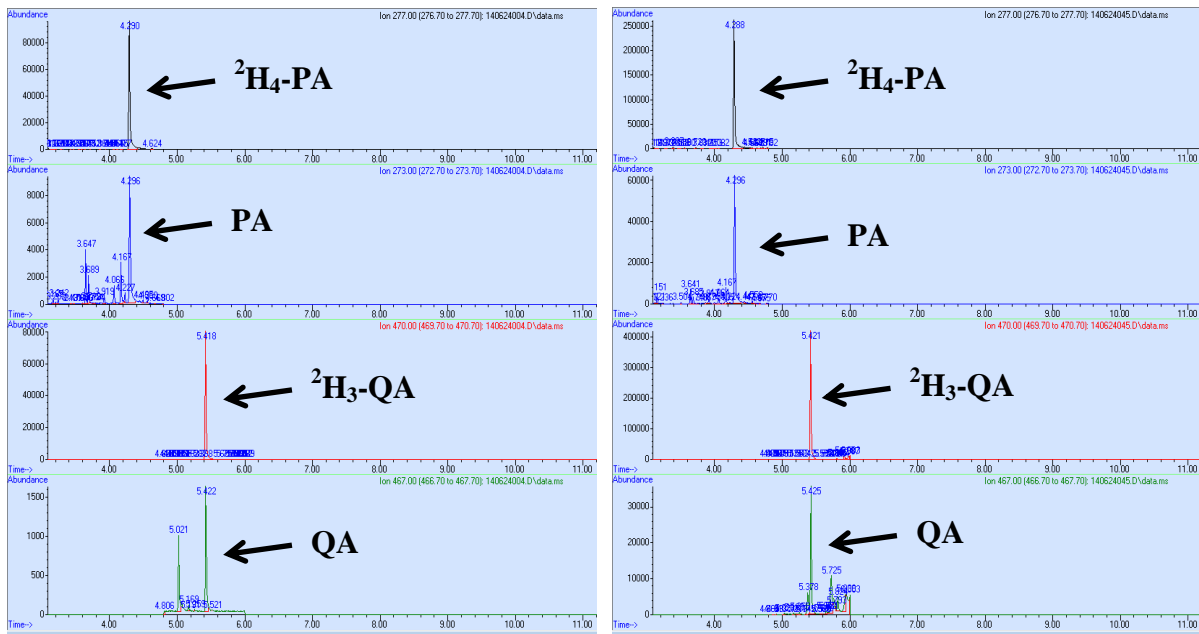


### SUPPLEMENTARY FIGURE S1B



**SUPPLEMENTARY FIGURE S1C**

**SUPPLEMENTARY FIGURE S1D**



**Supplementary Figure 1.** Snapshot of chromatograms of various KP metabolites standards (A) and serum sample (B) by UHPLC. Chromatogram of PA and QA standards (C) and serum samples (D) by GCMS. Although 3HAA was measured in our serum samples, the level was abnormally low. 3HAA is a relatively unstable metabolites having limited application as a biomarker and was not shown in our data.

**SUPPLEMENTARY TABLES**

**Supplementary Table S2.** Cohort 1 KP Metabolic Profiles

| Metabolite                    | Mean or Median | Standard deviation or IQR | MS versus control Differences | P value              |
|-------------------------------|----------------|---------------------------|-------------------------------|----------------------|
| <b>TRP[<math>\mu</math>M]</b> |                |                           |                               |                      |
| Control (n=49)                | 71.11          | $\pm$ 10.20               |                               |                      |
| RRMS (n=50)                   | 57.99          | $\pm$ 12.83               | (D) -13.12                    | <0.0001 <sup>A</sup> |
| SPMS (n=20)                   | 50.44          | $\pm$ 11.63               | (D) -20.67                    | <0.0001 <sup>A</sup> |
| PPMS (n=17)                   | 47.08          | $\pm$ 8.75                | (D) -24.02                    | <0.0001 <sup>A</sup> |
| <b>KYN[<math>\mu</math>M]</b> |                |                           |                               |                      |
| Control (n=49)                | 1.81           | $\pm$ 0.41                |                               |                      |
| RRMS (n=50)                   | 2.05           | $\pm$ 0.51                | (U) 0.25                      | 0.03 <sup>A</sup>    |
| SPMS (n=20)                   | 1.95           | $\pm$ 0.24                |                               | N.S <sup>A</sup>     |
| PPMS (n=17)                   | 1.91           | $\pm$ 0.50                |                               | N.S <sup>A</sup>     |
| <b>K/T Ratio</b>              |                |                           |                               |                      |
| Control (n=49)                | 26.02          | $\pm$ 7.29                |                               |                      |
| RRMS (n=50)                   | 36.78          | $\pm$ 11.04               | (U) 10.75                     | <0.0001 <sup>A</sup> |
| SPMS (n=20)                   | 40.12          | $\pm$ 9.09                | (U) 14.09                     | <0.0001 <sup>A</sup> |
| PPMS (n=17)                   | 41.15          | $\pm$ 10.27               | (U) 15.13                     | <0.0001 <sup>A</sup> |
| <b>KA[nM]</b>                 |                |                           |                               |                      |
| Control (n=49)                | 52.77          | 46.16 – 60.65             |                               |                      |
| RRMS (n=50)                   | 73.84          | 67.48 – 87.42             | (U) 22.64                     | <0.0001 <sup>B</sup> |
| SPMS (n=20)                   | 40.46          | 36.52 – 46.25             | (D) -11.33                    | 0.01 <sup>B</sup>    |
| PPMS (n=17)                   | 40.01          | 34.17 – 49.19             | (D) -12.05                    | <0.01 <sup>B</sup>   |
| <b>3-HK[nM]</b>               |                |                           |                               |                      |
| Control (n=49)                | 49.15          | 37.84 – 54.46             |                               |                      |
| RRMS (n=50)                   | 63.67          | 48.87 – 81.68             | (U) 25.06                     | <0.0001 <sup>B</sup> |
| SPMS (n=20)                   | 83.01          | 51.40 – 95.62             | (U) 32.13                     | <0.0001 <sup>B</sup> |
| PPMS (n=17)                   | 83.61          | 69.46 – 88.92             | (U) 33.74                     | <0.0001 <sup>B</sup> |
| <b>AA[nM]</b>                 |                |                           |                               |                      |
| Control (n=49)                | 130.02         | $\pm$ 80.83               |                               |                      |
| RRMS (n=50)                   | 116.73         | $\pm$ 54.35               |                               | N.S <sup>A</sup>     |
| SPMS (n=20)                   | 134.42         | $\pm$ 46.57               |                               | N.S <sup>A</sup>     |
| PPMS (n=17)                   | 145.54         | $\pm$ 55.36               |                               | N.S <sup>A</sup>     |
| <b>PA[nM]</b>                 |                |                           |                               |                      |
| Control (n=49)                | 392.96         | $\pm$ 77.11               |                               |                      |
| RRMS (n=50)                   | 459.13         | $\pm$ 91.74               | (U) 66.17                     | <0.001 <sup>A</sup>  |
| SPMS (n=20)                   | 388.10         | $\pm$ 66.94               |                               | N.S <sup>A</sup>     |
| PPMS (n=17)                   | 246.90         | $\pm$ 51.53               | (D) -146.07                   | <0.0001 <sup>A</sup> |
| <b>QA[nM]</b>                 |                |                           |                               |                      |
| Control (n=49)                | 322.13         | 262.26 – 377.54           |                               |                      |
| RRMS (n=50)                   | 448.97         | 388.76 – 490.58           | (U) 119.97                    | <0.0001 <sup>B</sup> |
| SPMS (n=20)                   | 552.94         | 511.34 – 617.71           | (U) 240.07                    | <0.0001 <sup>B</sup> |
| PPMS (n=17)                   | 754.62         | 629.20 – 803.55           | (U) 388.33                    | <0.0001 <sup>B</sup> |
| <b>QA/KA Ratio</b>            |                |                           |                               |                      |
| Control (n=49)                | 5.94           | 4.88 – 7.15               |                               |                      |
| RRMS (n=50)                   | 5.88           | 4.91 – 7.08               |                               | N.S <sup>B</sup>     |
| SPMS (n=20)                   | 13.63          | 11.78 – 14.12             | (U) 7.28                      | <0.0001 <sup>B</sup> |
| PPMS (n=17)                   | 17.23          | 14.95 – 20.56             | (U) 11.41                     | <0.0001 <sup>B</sup> |
| <b>NAD<sup>+</sup></b>        |                |                           |                               |                      |
| Control (n=49)                | 33.54          | 27.28 – 39.92             |                               |                      |

|             |       |               |            |                      |
|-------------|-------|---------------|------------|----------------------|
| RRMS (n=50) | 16.81 | 12.36 – 21.83 | (D) -14.87 | <0.0001 <sup>B</sup> |
| SPMS (n=20) | 14.20 | 11.83 – 16.61 | (D) -18.32 | <0.0001 <sup>B</sup> |
| PPMS (n=17) | 13.49 | 8.63 – 17.13  | (D) -20.30 | <0.0001 <sup>B</sup> |

Mean concentration of tryptophan (TRP), kynurenine (KYN), kynurenine/tryptophan (K/T) ratio, kynurenic acid (KA), 3-hydroxykynurenine (3-HK), picolinic acid (PA), quinolinic acid (QA) and QA/KA ratio in stratified MS groups and healthy controls. Metabolites that pass the Shapiro-Wilk normality test ( $p \geq 0.05$ ; data not shown), ANOVA (denoted with A) was used for comparison and reported as mean with standard deviation, otherwise, Kruskal Wallis (denoted as B) was applied and reported as median with interquartile range (IQR) provided. A p value of  $\leq 0.05$  is considered significant for mean difference between the control and stratified MS subtypes with N.S. denotes as no significant. Further illustration of the mean differences is denote by up (U), and down (D).

**Supplementary Table S3.** Cohort 1 27-Plex Cytokines, Chemokine and Growth Factor Profiles

| Cytokines,<br>Chemokines and<br>Growth Factors |        |              | MS versus<br>control<br>Differences <sup>‡</sup> | P value |
|------------------------------------------------|--------|--------------|--------------------------------------------------|---------|
|                                                | Median | IQR          |                                                  |         |
| <b>IL-1<math>\beta</math></b>                  |        |              |                                                  |         |
| Control (n=49)                                 | 1.42   | 1.12-1.74    |                                                  |         |
| RRMS (n=50)                                    | 1.15   | 0.92-1.59    |                                                  | N.S     |
| SPMS (n=20)                                    | 1.34   | 0.95-1.53    |                                                  | N.S     |
| PPMS (n=17)                                    | 1.29   | 1.01-1.69    |                                                  | N.S     |
| <b>IL-1ra</b>                                  |        |              |                                                  |         |
| Control (n=49)                                 | 69.53  | 49.65-107.31 |                                                  |         |
| RRMS (n=50)                                    | 55.49  | 37.23-87.09  | (D)                                              | N.S     |
| SPMS (n=20)                                    | 77.93  | 49.16-96.47  | (U)                                              | N.S     |
| PPMS (n=17)                                    | 57.35  | 48.24-110.38 | (D)                                              | N.S     |
| <b>IL-2</b>                                    |        |              |                                                  |         |
| Control (n=49)                                 | 8.29   | 5.69-12.02   |                                                  |         |
| RRMS (n=50)                                    | 5.54   | 3.64-9.94    | (D)                                              | N.S     |
| SPMS (n=20)                                    | 10.21  | 5.71-12.11   | (U)                                              | N.S     |
| PPMS (n=17)                                    | 8.99   | 5.96-12.65   |                                                  | N.S     |
| <b>IL-4</b>                                    |        |              |                                                  |         |
| Control (n=49)                                 | 1.27   | 0.97-1.52    |                                                  |         |
| RRMS (n=50)                                    | 1.16   | 0.92-1.37    |                                                  | N.S     |
| SPMS (n=20)                                    | 1.17   | 1.09-1.46    |                                                  | N.S     |
| PPMS (n=17)                                    | 1.25   | 1.04-1.51    |                                                  | N.S     |
| <b>IL-5</b>                                    |        |              |                                                  |         |
| Control (n=49)                                 | 2.58   | 1.97-3.08    |                                                  |         |
| RRMS (n=50)                                    | 2.14   | 1.68-2.84    |                                                  | N.S     |
| SPMS (n=20)                                    | 2.34   | 1.90-3.16    |                                                  | N.S     |
| PPMS (n=17)                                    | 2.30   | 2.06-3.12    |                                                  | N.S     |
| <b>IL-6</b>                                    |        |              |                                                  |         |
| Control (n=49)                                 | 4.68   | 3.33-6.21    |                                                  |         |
| RRMS (n=50)                                    | 3.44   | 2.48-5.33    | (D)                                              | N.S     |
| SPMS (n=20)                                    | 5.29   | 3.22-6.98    | (U)                                              | N.S     |
| PPMS (n=17)                                    | 4.50   | 4.17-5.40    |                                                  | N.S     |
| <b>IL-7</b>                                    |        |              |                                                  |         |
| Control (n=49)                                 | 5.88   | 3.95-7.92    |                                                  |         |
| RRMS (n=50)                                    | 5.26   | 4.27-8.85    |                                                  | N.S     |
| SPMS (n=20)                                    | 6.80   | 5.57-7.75    | (U)                                              | N.S     |

|                    |        |              |     |     |
|--------------------|--------|--------------|-----|-----|
| PPMS (n=17)        | 6.78   | 5.65-7.98    | (U) | N.S |
| <b>IL-8</b>        |        |              |     |     |
| Control (n=49)     | 13.13  | 8.73-14.72   |     |     |
| RRMS (n=50)        | 9.95   | 8.26-13.06   | (D) | N.S |
| SPMS (n=20)        | 12.57  | 10.76-14.36  |     | N.S |
| PPMS (n=17)        | 12.98  | 9.90-13.25   |     | N.S |
| <b>IL-9</b>        |        |              |     |     |
| Control (n=49)     | 9.63   | 7.10-13.26   |     |     |
| RRMS (n=50)        | 7.82   | 6.01-9.78    | (D) | N.S |
| SPMS (n=20)        | 8.48   | 6.71-10.84   | (D) | N.S |
| PPMS (n=17)        | 8.49   | 7.36-8.49    | (D) | N.S |
| <b>IL-10</b>       |        |              |     |     |
| Control (n=49)     | 9.71   | 6.75-14.76   |     |     |
| RRMS (n=50)        | 6.49   | 4.95-10.26   | (D) | N.S |
| SPMS (n=20)        | 8.09   | 6.54-12.22   | (D) | N.S |
| PPMS (n=17)        | 8.79   | 6.34-12.78   | (D) | N.S |
| <b>IL-12 (p70)</b> |        |              |     |     |
| Control (n=49)     | 20.76  | 12.13-29.81  |     |     |
| RRMS (n=50)        | 14.73  | 10.51-26.75  | (D) | N.S |
| SPMS (n=20)        | 17.39  | 11.17-28.48  | (D) | N.S |
| PPMS (n=17)        | 25.47  | 13.47-43.14  | (U) | N.S |
| <b>IL-13</b>       |        |              |     |     |
| Control (n=49)     | 7.50   | 5.67-12.71   |     |     |
| RRMS (n=50)        | 5.55   | 4.07-10.72   | (D) | N.S |
| SPMS (n=20)        | 8.68   | 5.95-12.23   | (U) | N.S |
| PPMS (n=17)        | 12.02  | 8.32-15.51   | (U) | N.S |
| <b>IL-15</b>       |        |              |     |     |
| Control (n=49)     | 9.77   | 7.05-16.48   |     |     |
| RRMS (n=50)        | 9.54   | 6.84-12.96   |     | N.S |
| SPMS (n=20)        | 9.60   | 7.98-12.30   |     | N.S |
| PPMS (n=17)        | 7.83   | 6.49-10.49   | (D) | N.S |
| <b>IL-17</b>       |        |              |     |     |
| Control (n=49)     | 35.34  | 26.19-45.91  |     |     |
| RRMS (n=50)        | 28.34  | 22.72-35.88  | (D) | N.S |
| SPMS (n=20)        | 32.37  | 25.60-37.97  | (D) | N.S |
| PPMS (n=17)        | 30.49  | 23.52-36.96  | (D) | N.S |
| <b>Eotaxin</b>     |        |              |     |     |
| Control (n=49)     | 69.92  | 42.24-105.27 |     |     |
| RRMS (n=50)        | 58.19  | 42.52-89.70  | (D) | N.S |
| SPMS (n=20)        | 88.98  | 69.05-137.97 | (U) | N.S |
| PPMS (n=17)        | 62.06  | 46.94-95.00  | (D) | N.S |
| <b>FGF-basic</b>   |        |              |     |     |
| Control (n=49)     | 14.65  | 12.34-18.50  |     |     |
| RRMS (n=50)        | 12.24  | 9.53-15.33   | (D) | N.S |
| SPMS (n=20)        | 13.55  | 9.53-17.89   | (D) | N.S |
| PPMS (n=17)        | 13.85  | 10.23-16.96  | (D) | N.S |
| <b>G-CSF</b>       |        |              |     |     |
| Control (n=49)     | 101.74 | 81.24-122.01 |     |     |
| RRMS (n=50)        | 83.17  | 70.24-103.33 | (D) | N.S |
| SPMS (n=20)        | 97.59  | 80.27-128.77 | (D) | N.S |
| PPMS (n=17)        | 97.91  | 85.10-116.96 | (D) | N.S |
| <b>GM-CSF</b>      |        |              |     |     |
| Control (n=49)     | 7.64   | 5.87-12.21   |     |     |

|                                 |         |                 |     |     |
|---------------------------------|---------|-----------------|-----|-----|
| RRMS (n=50)                     | 5.23    | 4.03-7.05       | (D) | N.S |
| SPMS (n=20)                     | 4.80    | 2.34-9.21       | (D) | N.S |
| PPMS (n=17)                     | 6.70    | 2.03-9.30       | (D) | N.S |
| <b>IFN-<math>\gamma</math></b>  |         |                 |     |     |
| Control (n=49)                  | 31.30   | 24.39-39.38     |     |     |
| RRMS (n=50)                     | 24.83   | 17.90-32.90     | (D) | N.S |
| SPMS (n=20)                     | 30.12   | 25.58-37.85     | (D) | N.S |
| PPMS (n=17)                     | 28.72   | 20.22-33.44     | (D) | N.S |
| <b>IP-10</b>                    |         |                 |     |     |
| Control (n=49)                  | 269.58  | 224.74-465.89   |     |     |
| RRMS (n=50)                     | 296.87  | 232.79-434.15   | (U) | N.S |
| SPMS (n=20)                     | 379.88  | 218.55-468.55   | (U) | N.S |
| PPMS (n=17)                     | 324.31  | 250.42-433.99   | (U) | N.S |
| <b>MCP-1</b>                    |         |                 |     |     |
| Control (n=49)                  | 45.35   | 36.44-65.58     |     |     |
| RRMS (n=50)                     | 33.39   | 22.46-57.41     | (D) | N.S |
| SPMS (n=20)                     | 47.23   | 24.62-62.18     | (U) | N.S |
| PPMS (n=17)                     | 45.95   | 28.91-60.56     |     | N.S |
| <b>MIP-1<math>\alpha</math></b> |         |                 |     |     |
| Control (n=49)                  | 1.75    | 1.37-2.70       |     |     |
| RRMS (n=50)                     | 1.37    | 1.00-1.78       |     | N.S |
| SPMS (n=20)                     | 1.71    | 1.27-2.03       |     | N.S |
| PPMS (n=17)                     | 1.69    | 1.41-2.84       |     | N.S |
| <b>MIP-1<math>\beta</math></b>  |         |                 |     |     |
| Control (n=49)                  | 34.47   | 26.16-47.07     |     |     |
| RRMS (n=50)                     | 28.01   | 20.76-41.40     | (D) | N.S |
| SPMS (n=20)                     | 32.17   | 26.59-39.78     | (D) | N.S |
| PPMS (n=17)                     | 40.23   | 30.76-54.01     | (U) | N.S |
| <b>PDGF-bb</b>                  |         |                 |     |     |
| Control (n=49)                  | 1207.17 | 966.12-1577.31  |     |     |
| RRMS (n=50)                     | 1193.95 | 919.28-1611.26  |     | N.S |
| SPMS (n=20)                     | 1203.98 | 977.71-1642.65  |     | N.S |
| PPMS (n=17)                     | 1201.54 | 1038.91-1380.43 |     | N.S |
| <b>RANTES</b>                   |         |                 |     |     |
| Control (n=49)                  | 1484.55 | 1269.85-2120.25 |     |     |
| RRMS (n=50)                     | 1469.61 | 1158.78-1746.00 |     | N.S |
| SPMS (n=20)                     | 1602.06 | 1217.05-1602.06 | (U) | N.S |
| PPMS (n=17)                     | 1835.80 | 1620.58-2440.05 | (U) | N.S |
| <b>TNF-<math>\alpha</math></b>  |         |                 |     |     |
| Control (n=49)                  | 19.68   | 14.68-25.83     |     |     |
| RRMS (n=50)                     | 15.79   | 12.56-21.68     | (D) | N.S |
| SPMS (n=20)                     | 17.18   | 12.01-22.47     | (D) | N.S |
| PPMS (n=17)                     | 17.45   | 13.20-25.64     | (D) | N.S |
| <b>VEGF</b>                     |         |                 |     |     |
| Control (n=49)                  | 47.58   | 24.57-70.41     |     |     |
| RRMS (n=50)                     | 33.27   | 20.52-53.41     | (D) | N.S |
| SPMS (n=20)                     | 32.43   | 21.74-47.87     | (D) | N.S |
| PPMS (n=17)                     | 44.53   | 35.57-83.25     | (D) | N.S |

Median concentration of interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-1 receptor antagonist (IL-1ra), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-12 (p70) [IL-12 (p70)], interleukin-13 (IL-13), interleukin-15 (IL-15), interleukin-17 (IL-17), Eotaxin, Fibroblast growth factor-basic (FGF-basic), granulocyte

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colony-stimulating factor (G-CSF), granulocyte-macrophages colony-stimulating factor (GM-CSF), interferon gamma (IFN- $\gamma$ ), IFN- $\gamma$ -inducible protein 10 (IP-10), monocyte chemotactic protein-1 (MCP-1), macrophages inflammatory protein-1 $\alpha$  (MIP-1  $\alpha$ ), platelet-derived growth factor (PDGF-bb), macrophages inflammatory protein-1 $\beta$  (MIP-1 $\beta$ ), regulated upon activation normal T-cell expressed and secreted (RANTES), tumour necrosis factor-alpha (TNF- $\alpha$ ) and vascular endothelial growth factor (VEGF) in stratified MS groups and healthy controls. Kruskal Wallis nonparametric test was used and reported with interquartile range (IQR). We did not see any significance in mean difference between the control and stratified MS subtypes with N.S. denotes as no significant. Further illustration of noticeable mean differences is denote by up (U), and down (D). Concentrations of all the inflammatory mediators are expressed in picogram per mililiter (pg/ml).