**eTable 1.** Total Study population by study and by outcome

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  **Outcome** | **Statistical modeling strategy**  | **JHU** | **CombiRx** | **IMID** | **Total N** |
| **Laboratory** |  |  |  |  |  |
| 25(OH)D level, ng/mL | Linear regression | 935 | - | - | 935 |
| **Clinical outcome** |  |  |  |  |  |
| Timed 25-foot walk  | Linear mixed effects model | 144 | 575 | 197 | 916 |
| Nine-hole peg test  | Linear mixed effects model | 144 | 575 | 197 | 916 |
| PASAT-3 | Linear mixed effects model | 144 | 575 | - | 719 |
| EDSS progression (primary) | Linear mixed effects model | 294 | 575 | 197 | 1066 |
| EDSS progression (secondary) | Cox proportional hazards model | 294 | 575 | 197 | 1066 |
| Relapse  | Andersen Gill model for recurrent events  | - | 575 | - | 575 |
| **Brain Imaging Outcome** |  |  |  |  |  |
| Normalized gray matter volume  | Linear mixed effects model | 437 | 575 | - | 1012 |
| Normalized white matter volume | Linear mixed effects model | 437 | 575 | - | 1012 |
| Normalized brain volume | Linear mixed effects model | 437 | 575 | - | 1012 |
| T2 lesion volume | Linear mixed effects model | 437 | 575 | - | 1012 |
| New lesions  | Negative binomial model | 266 | 575 | - | 841 |
| **Retinal Imaging Outcome** |  |  |  |  |  |
| GCIPL | Linear mixed effects model | 1105 | - | - | 1105 |

**eTable 2.** Characteristics of the sub-cohort of JHU participants with measured 25(OH)D levels.

|  |  |
| --- | --- |
| **Characteristics** | **JHU cohort with 25(OH)D levels** |
|
| **N** | 935 |
| Age, years, mean (SD)  | 43.11 (11.92) |
| Male sex, % | 241 (25.8) |
| BMI, kg/m2, mean (SD) | 28.08 (6.51) |
| Disease duration, years, mean (SD) | 6.88 (7.63) |
| RRMS Subtype, n (%) | 780 (83.4) |
| MS DMTs, n (%) |  |
|  Glatiramer acetate | 193 (20.6) |
|  Interferon beta | 144 (15.4) |
|  Glatiramer acetate+Interferon beta | 0 (0.0) |
|  Dimethyl Fumarate | 97 (10.4) |
|  Teriflunomide | 14 (1.5) |
|  Fingolimod | 30 (3.2) |
|  Natalizumab | 117 (12.5) |
|  Anti-CD20 | 32 (3.4) |
|  No therapy | 301 (32.2) |
|  Other | 7 (0.7) |
| Number of relapses in previous 3 years (CombiRx, JHU) or 1 year (IMID), mean (SD)2 | 0.74 (0.89) |
| Use of vitamin D supplement any time during follow-up, n (%) | 542 (58.0) |
| Use of other medication potentially affecting vitamin D levels1, n (%) | 572 (61.2) |

**eTable 3.** Pooled results for models additionally adjusting for vitamin D supplement use and medications potentially impacting vitamin D status

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Quartile of 25(OH)D PGS** | **P for trend1** | **P for het2** | **I2** |
| **Q1** | **Q2** | **Q3** | **Q4** |  |  |  |
| **Clinical outcome, Parameter, 95% CI** |  |  |  |  |  |  |  |
| **%**Change in T25FW, Rate, 95% CI | 0.00 [ref] | -0.14 (-1.37, 1.12) | -1.01 (-2.20, 0.19) | 0.53 (-0.71, 1.80) | 0.76 | 0.42 | 0.00% |
| **%**Change in 9HPT, Rate, 95% CI | 0.00 [ref] | -0.01 (-0.71, 0.70) | -0.22 (-0.93, 0.50) | 0.50 (-0.22, 1.23) | 0.30 | 0.36 | 0.00% |
| **%**Change in PASAT-3, Rate, 95% CI | 0.00 [ref] | 0.28 (-0.39, 0.95) | 0.32 (-0.28, 0.93) | 0.09 (-0.53, 0.72) | 0.70 | 0.57 | 0.00% |
| Rate of change in EDSS, Rate, 95% CI | 0.00 [ref] | 0.046 (-0.004, 0.096) | 0.023 (-0.097, 0.143) | 0.037 (-0.012, 0.086) | 0.40 | 0.25 | 24.09% |
| Relapse rate, HR, 95% CI | 1.00 [ref] | 1.19 (0.86, 1.65) | 1.21 (0.89, 1.64) | 1.07 (0.77, 1.50) | 0.68 | - | - |
| **MRI outcome, Parameter, 95% CI** |  |  |  |  |  |  |  |
| **%**Change in BPF, Rate, 95% CI | 0.00 [ref] | -0.01 (-0.05, 0.04) | -0.01 (-0.06, 0.04) | -0.01 (-0.06, 0.04) | 0.9 | 0.005 | 87.38% |
| **%**Change in GMF, Rate, 95% CI | 0.00 [ref] | 0.01 (-0.02, 0.04) | 0.01 (-0.02, 0.04) | 0.01 (-0.01, 0.04) | 0.11 | 0.41 | 0.00% |
| **%**Change in WMF, Rate, 95% CI | 0.00 [ref] | -0.02 (-0.04, 0.00) | -0.02 (-0.04, 0.00) | -0.01 (-0.04, 0.01) | 0.17 | 0.72 | 0.00% |
| **%**Change in T2 lesion volume, Rate, 95% CI | 0.00 [ref] | -0.14 (-0.74, 0.46) | -0.14 (-0.73, 0.46) | -0.18 (-0.78, 0.43) | 0.39 | 0.83 | 0.00% |
| Rate of new lesions, RR, 95% CI | 1.00 [ref] | 1.48 (1.02, 2.14) | 1.31 (0.90, 1.91) | 1.02 (0.70, 1.49) | 0.92 | 0.4 | 0.00% |
| **Retinal imaging outcome, Parameter, 95% CI** |  |  |  |  |  |  |  |
| **%**Change in GCIP, Rate, 95% CI | 0.00 [ref] | 0.02% (-0.08, 0.13) | 0.00% (-0.10, 0.11) | -0.04% (-0.15, 0.06) | 0.34 | - | - |

**eTable 4.** Mendelian randomization (MR) estimates for 1 SD unit increase in genetically predicted log(25[OH]D) levels and selected MS outcomes (relapse rate1 and rate of new lesions2).

|  |  |  |  |
| --- | --- | --- | --- |
| **Outcome** | **N SNPs** | **Effect Estimate** | **P value**  |
| **Relapse rate** (n exposure=485,762; n outcome=575) |  |  |  |
| IVW  | 81 | 0.50 (0.19, 1.33) | 0.17 |
|  MR Egger | 81 | 0.29 (0.05, 1.89) | 0.20 |
|  Weighted median | 81 | 0.38 (0.08, 1.76) | 0.22 |
|  IVW Radial | 81 | 0.50 (0.21, 1.19) | 0.12 |
| Test for heterogeneity  | - | - | 0.93 |
| MR Egger regression intercept (pleiotropy) | - | 0.01 (-0.03, 0.05) | 0.51 |
| **Rate of new lesions** (n exposure=485,762; n outcome=841) |  |  |  |
| IVW  | 79 | 0.95 (0.26, 3.38) | 0.93 |
|  MR Egger | 79 | 0.78 (0.07, 9.05) | 0.84 |
|  Weighted median | 79 | 1.16 (0.17, 7.86) | 0.88 |
|  IVW Radial | 79 | 0.95 (0.26, 3.38) | 0.93 |
| Test for heterogeneity  | - | - | 0.10 |
| MR Egger regression intercept (pleiotropy) | - | 0.005 (-0.05, 0.06) | 0.86 |

1Relapse IVs were derived from participants in CombiRx (n=575). Initial analyses indicated potential heterogeneity (Q statistic: 111.6; p=0.05). Results displayed exclude potential outlying SNPs (n=10 SNPs). 2Rate of new lesions were derived from pooled estimates derived from JHU and CombiRx participants (n=841). No heterogeneity was observed.

**eFigure 1.** Results for clinical outcomes and continuous 25(OH)D PGS (not adjusted for BMI) for individual studies and the pooled estimate1 across studies.



1Effect estimates displayed are for a 1 SD increase in 25(OH)D PGS. They are adjusted for age, 5 ancestry PCs, MS DMT, disease duration and number of relapses in previous 3 years. The pooled effect estimate is results from a random effects meta-analysis. **A.** Results for rate of EDSS progression (Heterogeneity I2=0.00%; p het=0.53). **B.** Results for annualized percent change T25FW (I2=0.0%; p het=0.85). **C.** Results for annualized percent change 9HPT (I2=12.38%; p het=0.42).

**eFigure 2.** Results for MRI outcomes and continuous 25(OH)D PGS (not adjusted for BMI) for individual studies and the pooled estimate1 across studies.



1Effect estimates displayed are for a 1 SD increase in 25(OH)D PGS. They are adjusted for age, 5 ancestry PCs, MS DMT, disease duration and number of relapses in previous 3 years. The pooled effect estimate is results from a random effects meta-analysis. **A.** Results for annualized percent change in BPF (Heterogeneity I2=71.7%; p het=0.06). **B.** Results for annualized percent change in lesion volume (I2=0.0%; p het=0.74). **C.** Relative rate for new lesions (I2=41.2%; p het=0.19).

**eFigure 3.** Results for clinical outcomes and continuous 25(OH)D PGS for individual studies and the pooled estimate1 across studies using PGS p-value threshold of 5e-5.



1Effect estimates displayed are for a 1 SD increase in 25(OH)D PGS. They are adjusted for age, 5 ancestry PCs, MS DMT, disease duration and number of relapses in previous 3 years. The pooled effect estimate is results from a random effects meta-analysis. **A.** Results for rate of change in EDSS (Heterogeneity I2=0.0%; p het=0.48). **B.** Results for annualized percent change T25FW (I2=0.0%; p het=0.91). **C.** Results for annualized percent change 9HPT (I2=0.0%; p het=0.59).

**eFigure 4.** Results for MRI outcomes and continuous 25(OH)D PGS for individual studies and the pooled estimate1 across studies using PGS p-value threshold of 5e-5.



1Effect estimates displayed are for a 1 SD increase in 25(OH)D PGS. They are adjusted for age, 5 ancestry PCs, MS DMT, disease duration and number of relapses in previous 3 years. The pooled effect estimate is results from a random effects meta-analysis. **A.** Results for annualized percent change in BPF (Heterogeneity I2=74.1%; p het=0.05). **B.** Results for annualized percent change in lesion volume (I2=0.0%; p het=0.66). **C.** Relative rate for new lesions (I2= 4.1%; p het=0.31).

**eFigure 5. Scatterplots for MR of 25(OH)D with relapse and rate of new lesions.** Scatterplots showing the genetic estimates for the outcome along the y axis against the estimates for the exposure along the x axis (log rate ratio with 95% CI). **A**. Effect of genetically determined 25(OH)D levels on log(rate) of relapse. **B**. Effect of genetically determined 25(OH)D levels on log(rate) of new lesions. The slope of each line represents the MR estimate for the corresponding method.

****