Genetically determined serum serine level has a novel causal effect on multiple sclerosis risk and predicts disability progression

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Abstract

Background

There are currently no specific biomarkers for multiple sclerosis (MS). Identifying robust biomarkers for MS is crucial to improve disease diagnosis and management.

Methods

This study first used six Mendelian randomisation methods to assess causal relationship of 174 metabolites with MS, incorporating data from European-ancestry metabolomics (n=8,569-86,507) and MS (n=14,802 MS cases, 26,703 controls) genome-wide association studies. Genetic scores for identified causal metabolite(s) were then computed to predict MS disability progression in an independent longitudinal cohort (AusLong study) of 203 MS cases with up to 15-year follow-up.

Results

We found a novel genetic causal effect of serine on MS onset (OR= 1.67, 95%CI= 1.51-1.84, P= 1.73×10^{-20}), such that individuals whose serine level are one standard deviation above the population mean will have 1.67 times the risk of developing MS. This is robust across all sensitivity methods (OR ranges from 1.49 to 1.67). In an independent longitudinal MS cohort, we then constructed time-dynamic and time-fixed genetic scores based on serine genetic instrument SNPs, where higher scores for raised serum serine level were associated with increased risk of disability worsening, especially in the time-dynamic model (RR=1.25, 95%CI= 1.10-1.42, P=7.52×10^{-4}).

Conclusions

These findings support investigating serine as an important candidate biomarker for MS onset and disability progression.

Key Messages

What is already known on this topic

Several metabolites have been investigated in multiple sclerosis (MS) with mixed findings, and the causality of metabolites with MS remain unclear.

What this study adds

This study used a suite of Mendelian randomisation methods to assess genetic causality of 174 metabolites with MS onset, and subsequently identified serine as a novel candidate biomarker for MS. Serine-related genetic scores were then developed to predict risk of MS disability progression in a longitudinal cohort with long-term follow-up.

How this study might affect research, practice, or policy

Findings from this study support investigating serine in fundamental and mechanistic research as a next step, and if validated, earlier interventions based on serum serine level or genetic scoring could allow personalised MS disease prognoses and optimisation of treatment plans prior to irreversible disability development.

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory/neurodegenerative disease of the central nervous system[1]. The continuing search of a cure for MS is impeded by an incomplete understanding of the causes of MS, which is further compounded by the scarcity of a routinely accessible and sensitive biomarker of MS onset and progression[2]. In addition, there is also uncertainty as to whether MS disease modifying therapy can yield long-term benefits at the individual-level[2]. Consequently, the discovery and validation of MS biomarkers are an important step towards increased treatment precision and enabling assessments of treatment efficacy before the occurrence of irreversible disease events and accumulation of disability.

Metabolites are small molecules reflecting a wide spectrum of biological processes. To date, changes in serum levels of metabolites such as kynurenine have been associated with MS[3], though the causal relationships between the candidate metabolites and MS remain unclear. In addition, there is growing interest in establishing predictive models capable of characterising and monitoring MS disease progression based on molecular biomarkers[4]. Understanding the causality between specific metabolites and MS risk can help identify diagnostic and prognostic biomarkers for the purposes of developing new treatments and building more accurate disease prediction models.

Genetic data is increasingly used to strengthen causal inference in observational research, including the assessments of candidate disease biomarkers[5]. Genome-wide association studies (GWAS) are widely used to detect associations between genetic variants and phenotypic outcomes. Utilising the fact that alleles segregate randomly at meiosis, Mendelian randomisation (MR) simulates a “natural” randomised control trial to test the genetic causality between two traits by comparing the outcome in groups with and without the effect

allele associated with a risk factor of interest. In the last decade, several MR methods[6-11] have been developed to analyse summary-level data generated from GWAS, integrating the effects of multiple genetic variants as instrumental variables (termed "genetic instrument SNPs" that are associated with the risk factor of interest) to assess genetic causal relationship between the risk factor and outcome. A recent metabolomics GWAS by Lotta et al.[12] identified genetic loci regulating serum levels of 174 human metabolites, from which the GWAS summary results can be used to perform MR and assess genetic causality of the metabolites in disease traits. Based on the hypothesis that genetically determined serum metabolite levels may affect MS risk, we leveraged six different MR methods to assess the genetic causal relationships of metabolite levels with MS risk.

After using MR to identify metabolites with consistent genetic causal effects in MS, we hypothesised that genetic instrument SNPs that influence candidate metabolite levels may also influence MS disease progression and thus could be used to model and predict disease worsening. Established methods such as genetic prognostic indexes (GPI)[13] and polygenic risk scores (PRS)[14] can estimate the combined effects of genetic variants identified in population-scale GWAS, with applications in predicting clinical outcomes[13 15]. Thus, we constructed a time-dynamic GPI as well as a PRS based on genetic instrument SNPs for the candidate metabolites and assessed whether these genetic scores could predict disability progression in an independent cohort of people with clinically definite MS with up to 15 years follow-up.

Methods

Metabolomics GWAS Data

We obtained full summary statistics from a European-ancestry metabolomics GWAS for the effects of genetic variants on serum levels of 174 metabolites belonging to different biochemical classes (amino acids, acylcarnitines, phosphatidylcholines, hexoses, biogenic amines, lysophosphatidylcholines, and sphingomyelins)[12]. This was obtained from an open access repository[16]. In brief, Lotta et al.[12] conducted a meta-analysis of GWAS results from the Fenland[17], EPIC-Norfolk[18] and INTERVAL[19] studies, where the metabolites were captured by AbsoluteIDQ Biocrates p180 kit (Biocrates Life Sciences) and measured using mass spectrometry. The sample size for each metabolite varied from 8,569 to 86,507 individuals, depending on the contributing studies. For each metabolite, we excluded multiallelic and ambiguous SNPs, and those with minor allele frequency <0.05. No further quality control procedures were performed on the summary statistics.

MS GWAS Data

MS GWAS summary data on the effects of genetic variants on MS risk was from a meta-analysis of 15 MS GWAS cohorts of European ancestry (n= 14,802 MS cases and 26,703 controls)[20]. This data was obtained via application online[21]. Allele frequencies were annotated using the 1000 Genome Project phase 3 EUR population reference[22]. We excluded SNPs that were either multiallelic, ambiguous, with minor allele frequency <0.05, or not present in all 15 GWAS cohorts. After these steps, a total of 5,543,512 SNPs were included in our analysis.

AusLong Data

The Ausimmune Longitudinal (AusLong) study has prospectively followed an Australian cohort of clinically isolated syndrome cases for up to 15 years following referral after a first clinical episode of CNS demyelination[23]. AusLong cases were genotyped for 665,608

variants using the Infinium Global Screening Array-24 Kit v2.0. These data were imputed to ~9.7 million SNPs using the IMPUTE4 program[24]. After excluding those who had not been diagnosed with clinically definite MS at their last review, 203 MS cases with 1,267 repeated disability observations were included in our survival analysis.

Disability assessments utilising the Expanded Disability Status Scale (EDSS)[25] were undertaken at baseline, 2-3, 5, 10, and 15 years post first clinical episode of CNS demyelination by an accredited study neurologist, as well as annually after the 5th year review by computer-assisted telephone interviews using a validated telephone EDSS[23]. As in previous work[13], a mixed-effect proportional model was used to impute missing EDSS scores (15.4%) wherein we adjusted for the effects of clinical and environmental risk factors including age at first demyelinating event, sex, relapse rate, anti-Epstein-Barr virus (EBV) nuclear antigen IgG titres, body mass index (BMI), smoking status, Hospital Anxiety and Depression Scores (HADS), previous EDSS scores, and auxiliary variables (latitude, vitamin D and MS type) after having demonstrated their significant and independent associations with EDSS. Key cohort characteristics have been summarised in Supplementary Table S1.

Mendelian Randomisation Analysis

We first used generalised-summary-data-based Mendelian randomisation (GSMR) to assess whether metabolite levels had genetic causal effects on MS onset. GSMR is a method that incorporates independent genome-wide significant variants for the exposure as genetic instrument SNPs[6], which has a built-in "HEIDI-outlier" method to remove genetic instrument SNPs with apparent pleiotropic effects on both exposure and outcome. To perform GSMR analysis, we screened 174 available metabolites and retained those with at least ten valid

genetic instrument SNPs ($P < 5x10^{-8}$ and $P_{HEIDI} > 0.01$). Bonferroni correction method was applied to account for multiple testing.

Further, we performed sensitivity analysis using five different two-sample MR methods, including causal analysis using summary effect estimates method (CAUSE)[7], inverse variance weighted method (IVW)[8], Mendelian randomisation Egger (MR-Egger)[9], weighted median[10] and weighted mode[11]. Each of these models estimates the causal effect based on different assumptions and weighting of pleiotropic effects that arise from shared genetic factors between traits. In the sensitivity analysis, we applied $P < 5x10^{-8}$ as the threshold for selecting genetic instrument SNPs across the methods except for CAUSE, where a threshold of $P< 1x10^{-5}$ was applied. Different to many existing MR methods that focus on the variants most strongly associated with the risk factor of interest, CAUSE can improve power by incorporating information from more variants[7]. Detailed description of the MR analyses is available in Supplementary Methods. To assess instrument strength for instrument SNPs in the IVW models, F-statistic was calculated using the formula established by Burgess et al.[26]:

$$F = (\frac{n-k-1}{k})(\frac{R^2}{1-R^2})$$

where R^2 is the proportion of variance in the phenotype explained by the genetic variants, n is the sample size, and k is the number of instruments. A commonly accepted threshold for sufficient instrument strength is F >10. Other validity tests including Cochran's Q-test[27], leave-one-out analysis[28] and Egger intercept test[29] were also conducted to support validity of identified instrument SNPs.

Metabolite-related Genetic Scores and Survival Analysis

For the candidate metabolite(s) that had consistent association signals across the six MR methods, we performed survival analyses to examine the prospective associations of the

identified metabolite-associated genetic instrument SNPs in predicting worsening of disability in the AusLong study. We computed two models to evaluate this, one of which was a time-dynamic GPI based on the identified genetic instrument SNPs. Briefly, a univariate Cox analysis was initially performed to assess prognostic effects of each genetic instrument SNP, followed by a multivariate Cox model with backward selection (5% false discovery rate; including identified genetic instrument SNPs for metabolite, previous EDSS states, and their interactions) to identify SNPs associated with the time-to-worsening of disability. A time-dynamic cross-validation based GPI was then constructed to combine the effects of the selected SNP alleles[13 30]. Next, we also computed PRS using the genetic instrument SNPs identified for the candidate metabolite(s). Different to the GPI model, the PRS model was intrinsically independent of time points at which the survival predictions were made.

Detailed description of the genetic scores and subsequent survival analysis is available in Supplementary Methods. Briefly, the survival endpoint in our survival analysis was time elapsed since first demyelinating event to disability worsening outcome, where an increase in EDSS was defined as a "worsening" event (y= 1) and a decrease or no change in EDSS was an "improved/stable" event (y= 0). Separate mixed-effects Cox models were fitted to examine associations of GPI and PRS with EDSS worsening outcome, adjusting for age, sex, anti-EBV nuclear antigen IgG titres, relapse rate, BMI, smoking status, HADS, and vitamin D supplementation. We further assessed and compared the models based on the Akaike information criterion (AIC) and estimated the intra-class correlation due to individual-level and phenotype-level differences in EDSS progression rates.

Statistical Analysis

Data analyses were performed using the following software: R version 3.6.1 (R package 'gsmr' version 1.0.9, 'TwoSampleMR' version 0.4.26, 'cause' version 1.0.0, 'JointAI' version 1.0.2, 'coxme' version 2.2-16, 'mstate' version 0.3.2, 'msm' version 1.6.9, 'mfp' version1.5.2.2, 'survival' version 3.3-1, 'dynpred' version 0.1.2, 'JM' version 1.5-1), PLINK version 1.90, and IMPUTE4.

Results

Novel Genetic Causal Effect of Serine on MS risk

We found that 41 of 174 metabolites had ten or more valid genetic instrument SNPs that could be taken forward into MR analysis to investigate the effects of the metabolites on MS risk (Figure 1A; Supplementary Table S2). Thus, we used a Bonferroni-corrected p-value threshold of $0.05/41=1.22\text{x}10^{-3}$. Summarised in Figure 1, serine and kynurenine were the two metabolites that passed this threshold in the GSMR analysis, where genetically determined higher levels of both metabolites increased genetic susceptibility for MS. Upon sensitivity analysis using other MR methods, we observed a consistent pattern of positive association with MS onset for genetic serine level (Figure 1B; Supplementary Table S3; Supplementary Figure S1), including the CAUSE method ($p = 0.03$ for the causal model being a better fit than the model without causal effect). In the GSMR analysis, the built-in HEIDI test was able to remove two outliers and retain 18 of 20 instrument SNPs identified for serine (Supplementary Table S4). We then conducted additional tests to examine validity of these 18 SNPs. Firstly, we performed Cochran's Q test and found no evidence of heterogeneity for the 18 SNPs ($Q = 26.7$, $DF = 17$, $P = 0.06$). Secondly, we calculated the F-statistic of instrument strength for the 18 SNPs ($F = 69.0$), which satisfied the accepted threshold of $F>10$. Thirdly, we also performed leave-one-out analysis by leaving out each instrument SNP in turn and found that the association was unlikely to be driven by a single instrument SNP

based on the consistent effect estimates (Supplementary Table S6). Fourthly, Egger intercept test for these 18 SNPs (Supplementary Table S7) suggested no evidence of directional horizontal pleiotropy driving the results (Egger intercept=-5.2×10^{-3}, P = 0.71). Based on these, the 18 instrument SNPs passed all validity tests and we found consistent association patterns to GSMR when using these SNPs in sensitivity MR methods (Supplementary Table S3). Therefore, these 18 SNPs were used to generate serine-based genetic scores in subsequent analyses. Additionally, we repeated the same validity tests for the full set of 20 serine instrument SNPs and summarised in Supplementary Table S5-7. Furthermore, we assessed potential reverse causality by examining MS onset as the risk factor and genetically determined serum serine level as the outcome, and we found no evidence supporting genetic causality in this direction (Figure 1C; Supplementary Table S3).

For kynurenine, we noted a pattern of positive associations with similar effect sizes in sensitivity analysis (Figure 1B; Supplementary Table S3), though not reaching statistical significance. Additionally, we also found evidence supporting genetic causality in the reverse direction (Figure 1C; Supplementary Table S3), which suggested that the relationship of genetically determined serum kynurenine level with MS risk may arise from shared genetic factors rather than causal effects.

Serine-related Genetic Scores Predicted Worsening of Disability in MS

Based on the 18 genetic instrument SNPs for serum serine level (Supplementary Table S4), we found in univariable Cox mixed-effect models that two variants had suggestive association with survival time to EDSS worsening (Supplementary Table S8). Using backward elimination in the multivariable model including identified serine genetic instrument SNPs, previous EDSS states, and their interactions, we retained a model with 17

genetic effects (Supplementary Table S9) that were predictive of survival probability for disability worsening outcome, which were subsequently used to construct the serine GPI. In line with findings from the MR analysis, we found a one unit increase in serine GPI was associated with increased risk of transitioning into higher EDSS states, after adjusting for covariates in the GPI model (relative risk (RR) =1.25, 95%CI= 1.10-1.42, $P=7.52x10^{-4}$). When splitting into quartiles, we observed statistically significant differences between the quartiles of serine GPI (Figure 2, $P=7.8x10^{-35}$ for survival curves' comparisons using log-rank test). Consistent associations were also seen in the quartile GPI model after adjustments (Supplementary Table S10).

The PRS approach showed a similar association pattern after adjusting for covariates in the model (RR = 1.12, 95%CI= 1.00-1.27, P=0.06). When splitting into quartiles, we did not observe clear differences between quartiles in log-rank test (P = 0.10; Supplementary Figure S2), though there was a significant difference (P = 0.02) in survival curves for those with serine PRS in the lowest quartile compared to those in the combined upper three quartiles (Supplementary Figure S3). Supplementary Table S10 summarises associations from the quartile PRS model after adjustments, which had consistent direction of effect compared to the GPI model.

In the longitudinal MS cohort, the GPI model (AIC=2182.07) performed better than the PRS model (AIC=2189.37). The GPI scores alone explained a greater percentage of between individual worsening rates (r^2=78%) compared to the PRS scores alone (r^2=72%), where the PRS model may have assigned smaller risk scores to individuals with worse prognosis over time and underestimated the risk of worsening of disability.

Discussion

In this study, we analysed the genetic determinants of 174 metabolites from a recent large metabolomics GWAS study[12], and found consistent strong evidence supporting genetic causality of raised serine levels with MS risk. We then hypothesised that these genetic determinants of raised serine may also influenced worsening of disability among people with MS. We examined the combined effects of genetic instrument SNPs for serine levels on the rate of EDSS progression in the AusLong prospective cohort of early MS based on two different models: a time-dynamic risk prediction model (GPI) and a time-independent risk prediction model (PRS). In these models, the serine-related genetic scores showed consistent associations with worsening disability as measured by EDSS transitions to a higher (worse) disability state. Based on the GPI model, there was a significant five-year difference between the highest and lowest quartiles of the serine GPI in median survival time to worsening of disability. Together, these findings support further investigations of serine as an important candidate biomarker for MS onset and disability progression.

The MR analysis revealed consistent positive association signals for genetically determined serum serine level across different models, with similar effect sizes, suggesting that genetically determined higher serine levels are associated with increased risk of MS. Further, despite modest contributions of individual serine-related SNPs to time-to-worsening of disability outcome, genetic scores combining their effects were consistently predictive of the time to EDSS worsening. Based on previous research[13], MS disability prognoses at the individual- and group-level can change over time. Utilising this information in a time-dynamic GPI model, we demonstrated that a serine-related GPI was significantly predictive of disability worsening over time.

A previous small study (n= 33 MS cases and 17 controls)[31] found increased plasma concentrations of L-serine-O-phosphate (the immediate precursor to L-serine in the serine synthesis pathway) in MS cases compared to controls, though could not detect any significant differences in plasma concentrations of serine likely due to limited statistical power. Further, there is significant evidence that oligodendrocytes are involved in MS pathophysiology[32-34], while recent studies have found that increased ceramide abundance was associated with oligodendrocyte apoptosis[35] and EDSS progression[36] in MS. The ceramide synthesis pathway begins with the condensation of palmitoyl-CoA and serine[37], and targeting the rate-limiting enzyme (serine palmitoyltransferase) in this step has been proposed as a potential therapeutic approach for MS[37]. Furthermore, serine is a key source of one-carbon units involving in one-carbon metabolism, and T cells are known to depend on serine for proliferation and effector function[38 39]. In the one-carbon metabolism pathway, MTHFD2 is an enzyme highly expressed in inflammatory diseases, and a recent study by Sugiura et al.[39] showed that pharmacological inhibition of MTHFD2 *in vivo* decreased pathology in mouse models of MS. Additionally, serine synthesis pathway inhibition has been reported to augment the therapeutic efficacy of dietary serine limitation as a treatment for cancer[40], although similar studies have not been conducted in MS. If our results are confirmed in future functional studies, modulation of serine synthesis pathways combined with serine-restricted diet interventions may be fruitful areas of research to prevent or treat MS particularly in those with a higher serine GPI.

In our study, we integrated large genetic datasets to first identify serine as a high-confidence candidate in MS risk, followed by analysis in an independent clinical dataset to demonstrate the use of serine-associated genetic instrument SNPs in predicting worsening of MS disability outcome. If serine is validated as a MS biomarker, serine-related measurements

may be useful for personalised disease prognoses, and potentially increase precision in disease management strategies such as treatment selection and serine levels could be modulated by dietary interventions. It is currently necessary to wait for further disability to develop to know that treatment is not working[2], and earlier interventions based on biomarkers (serum serine levels) or genetic scoring could allow optimisation of treatment plans prior to irreversible disability development.

Limitations and Strengths

Our analysis is not without its limitations. Firstly, the MR methods were not able to fully distinguish pleiotropic effects from causal effects. That said, the HEIDI-outlier method implemented in the GSMR analysis was used to remove a proportion of genetic instrument SNPs with pleiotropic effects, while the CAUSE model could account for the effects of correlated pleiotropy. Secondly, the sample size for our longitudinal cohort is modest compared to cross-sectional GWAS studies, which may partly explain why the PRS failed to clearly discriminate risk of worsening between individuals as observed in Figure 2. Nevertheless, the AusLong dataset has a long follow-up time up to 15 years, measuring a wide range of clinical, environmental, and genetic information that we were able to incorporate in our survival analysis to strengthen our findings. Thirdly, despite the capabilities of GPI-based prediction models in capturing dynamic predictive effects over time while accounting for previous disability measurements, these prognostic models are not as useful for making individual predictions of survival endpoints[13]. However, dynamic risk predictions using the GPI is an efficient way of aggregating evidence from genetic markers when predicting disability in MS, with the goal of assigning higher risk scores to individuals with worst prognosis and vice-versa[13]. Fourthly, we analysed EDSS using a continuous-time evolution of the disability progression process, which is a powerful way of modelling

disability progression given the long-term follow-up in AusLong cohort. However, the model as per all measures of EDSS could not account for potential misclassifications (e.g., test-retest variability, and inter- and intra-rater variability) in longitudinal EDSS assessments. Lastly, we identified a causal effect of genetically determined serum serine level on MS onset, though we could not ascertain the causality of serine with MS disability worsening. It is possible that the SNPs associated with higher serum serine levels may affect MS disability worsening through mechanistic pathways unrelated to serine. Similarly, serum serine level itself may not be a robust biomarker in the absence of knowledge about an individual's genetic makeup. A future step for mechanistic research could be a longitudinal multi-omics study that integrates multiple layers of high-throughput omics data (e.g., genomics, epigenomics, metabolomics, and lipidomics) and phenotype data to further understand serine and relevant metabolites in MS progression.

Conclusions

In summary, our findings provide the first evidence for causality of genetically determined serum serine level with MS risk, which was robust across six different MR methods. The MR results also pointed to a relationship between kynurenine and MS that may arise from shared genetic factors. In an independent longitudinal cohort of people with clinically definite MS, we showed that serine-related genetic scores could predict survival probability for disability worsening. Thus, findings from this study warrant further fundamental and mechanistic research as a next step to investigate serine in MS onset and disability progression, which yield opportunities for renewing mechanistic understanding of MS or even novel therapeutic approaches.

Data Availability

Summary statistics for MS GWAS are available through application from: https://imsgc.net/?page_id=31 Summary statistics for metabolites are available through an interactive web server (https://omicscience.org/apps/crossplatform/) as well as the GWAS Catalog database (https://www.ebi.ac.uk/gwas/, accession numbers GCST90010722–GCST90010862). The AusLong dataset is available from the authors upon reasonable request but is not publicly available due to ethical requirements.

Code Availability

All codes used for the analyses are available upon request.

Contributors

XL, BVT and YZ contributed to the conception and design of the study.
XL, YY, VFN and YZ contributed to the acquisition and analysis of data.
AusLong Investigator Group contributed to the design and data collection of AusLong study.
BVT and YZ supervised the study.
XL, YY, VFN, XY, SSY, IvdM, SAB, ALP, AusLong Investigator Group, KB, BVT and YZ contributed to the interpretations of the findings and the critical revision of the manuscript.

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committees. Authors listed below belong to the AusLong/Ausimmune Investigators Group, which contributed to the design and data collection of AusLong study:
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Conflicts of Interest and Financial Disclosures

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Figure 1. Causality of genetically determined metabolite levels with MS risk.

A. In total, 41 metabolites of different biochemical classes (coloured) were included in GSMR main analysis, two of which passed the adjusted p-value threshold; **B.** Genetic causality of genetically determined serum serine and kynurenine levels (exposures) with MS onset (outcome) based on six Mendelian randomisation methods; **C.** Genetic causality of MS onset (exposure) with serine and kynurenine metabolites (outcomes) based on the six methods. "-log10(P)" is based on p-values for GSMR estimates. "N" refers to the number of SNPs included in the different models as genetic instrument SNPs. Error bars in forest plots represent the 95% confidence intervals for GSMR estimates. Abbreviations: CAUSE = causal analysis using summary effect estimates; GSMR = generalised-summary-data-based Mendelian randomisation; IVW = inverse variance weighted; MR-Egger = Mendelian randomisation-Egger; MS = multiple sclerosis.

Figure 2. Survival curve comparison between quartiles of a time-dynamic serine genetic prognostic index. This figure visualises and compares the survival curves for 1267 repeated observations (n = 203 individuals with clinically definite multiple sclerosis) separated into four quartiles based on unadjusted serine genetic prognostic index (GPI). The serine GPI was calculated based on serine-associated genetic instrument SNPs and their interaction effects with previous disability measurements that passed the backward elimination procedure in a multivariable Cox mixed-effect model. For the disability worsening outcome, we defined having an increase in the Expanded Disability Status Scale (EDSS) as a "worsening" event (y= 1) and having a decrease or no change in EDSS as an "improved/stable" event (y= 0). The time-to-worsening of disability (observation time for EDSS) was the cumulative sum of time intervals between EDSS visits, adjusting for disease duration at the time of EDSS measurement. Top right panel illustrates the distribution of the serine GPI. Bottom table summarised the risk sets for disability worsening over time. Coloured area around the survival curves indicates the corresponding 95% confidence intervals.