nature portfolio

Peer Review File

Elucidating genetic backgrounds of myasthenia gravis in Japanese by genome-wide association studies and multi-omics analyses of thymoma

Corresponding Author: Professor Yukinori Okada

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
This is a well-crafted, thorough analysis of myasthenia gravis within a Japanese population. In addition to studying an Asian cohort, the manuscript's notable strengths include its emphasis on disease subtypes, the large control sample size, supplementary downstream analyses like PheWas, and the examination of the TERT variant's impact.

I have only minor comments, none of which will detract from the paper's main messages.
• Add the Lambda1000, which is a metric often used when the case and control groups are unbalanced.
• Include the ICD9/10 codes used to select healthy individuals as controls from the Japanese biobank. If ICD codes were not used, describe in more detail how the control cohort was selected from the large biobank collection.
• Add the effect allele (the allele that increases the OR above 1) to Tables 1 and 2; although it may seem trivial, it greatly aids comparisons with other datasets.
• There is limited evidence linking TERT to European-ancestry populations. It may be worthwhile to speculate in the Discussion on why the genetic architecture of myasthenia differs across populations. For example, the incidence of myasthenia gravis is lower in Japan than elsewhere. Do current genetic findings account for this difference? This is not a limitation of the paper.
• It would be helpful to state in the Methods that the whole genome sequence data were generated using blood-derived DNA, as telomere length measurements are more susceptible to noise in this tissue type.
• Although it is described in the Methods, I had to search for it; add a sentence to the Results section describing the model used to analyze the data.

Reviewer #2

(Remarks to the Author)
This is a very interesting GWAS into Myasthenia Gravis in patients with a Japanese background. Indeed, it is to be expected that the genetic architecture between geographical regions varies, and that is indeed what is shown here, comparing the current results with previous, mostly EU based GWAS-es.

The study is strong in explicitly analysing clinical subtypes of MG, which usually is a major challenge in GWAS where usually minimal clinical data are available. Moreover, some interesting, subtype dependent results were obtained.
Main results are two loci, TERT and HLA where TERT seems mostly associated with generalized/ thymoma dependent MG, and HLA with non-thymoma associated MG. Results are plausible, in the sense that TERT is most abundantly expressed in the thymus.

I do have some comments:

1. The authors do present the qqplots and lambda GC, but I would like to also see an LDSC based analysis of intercept and slope, to explore if inflation is mainly polygenic or still based on residual confounding. I realise that sample size might be a

bit tricky, but could the authors explore what the SNP based heritability is according to LDSC (and/ or LDAK)?

2. I applaud the authors that they also analyse the EU data on MG, and they do present a combined Manhattan plot in the supplement. Main question I have there is: do all signals from the EU GWAS go down, do the Japanese hits also go down, with two new hits? Signals are so significant in the original GWASes that even with "negative" replication, they can remain significant. The EU GWAS used Manhattan plots where "towers" are depicted with arrows, with arrows point up showing loci that improve, and arrows going down signals that get less significant after combined analysis. Could the authors present the combined results like that? Also, in the tables where EU and Japanese SNPs are listed, some loci have "NA" in the Japanese study, what does that mean?

3. Lastly, and perhaps related to number 2: do the authors think that the TERT result is solely driven bij thymoma associated MG, or is there still a group of gMG that is also TERT driven? They could explore this by doing some more subanalyses, exploring what happens if subcohorts are removed from the gMG total group.

I especially like figure 1 to show subtype results.

Reviewer #3

(Remarks to the Author)
The authors present important findings from a highly valuable and well characterized genetic cohort of myasthenia gravis patients in Japan. This study represents the first-ever GWAS of East Asian ancestry in this disease and includes a cross-ancestry meta-analysis, as well as comprehensive follow-up analyses focusing on the HLA region and the TERT locus. While the study is both timely and relevant, I have several suggestions for improving the manuscript:

1) Although the authors discuss the novel association at the TERT locus in detail, the newly identified loci from the meta-analysis with European cohorts (EPS15L1 and RALGAPA2) are barely addressed. The authors should expand on these meta-analysis findings and provide further interpretation. Supplementary Table 3 could also be added to the main text.
2) In Supplementary Table 2+3, there are missing values (NAs) that require explanation. Are these due to all SNPs not being present in the current cohort? If so, the authors could consider using proxy SNPs in LD, unless all SNPs in LD are infrequent in the current cohort, which should be noted in the legend. Additionally, sign tests and heterogeneity tests should be included to complement the results.
3) An OR of 1.92 for the lead variant rs2736099 near the TERT locus is unusually high for a GWAS hit. However, it does not replicate strongly in previous studies. The authors should either seek an additional (East Asian) cohort for replication or discuss this in more detail (e.g. it could be driven by thymoma?).
4) It is unclear whether the raw genotypes of case and control samples were merged and subsequently jointly QCed and imputed. This should be described explicitly in the methods.
5) The number of SNPs and MAF cutoff used to calculate genomic inflation (Lambda) and generate the QQ plots should be reported in the supplementary figure legends. Ideally, this analysis should be repeated using a MAF cutoff of 0.02.

Minor:
1) In general, all tables and figures require more detailed legends to ensure they are fully interpretable. If possible, provide Excel/CSV supplementary tables instead of PDF tables
2) Table 1 appears redundant, as the lead SNPs are in moderate LD and yield nearly identical results. Furthermore, the conditional analyses demonstrate that these effects are not independent. The same applies to Figure 2b.
3) The PCA plot for PC3 appears to contain a nested or improperly formatted sub-plot. Please provide a proper legend and clarify the visualization. In addition, the PC4 plots suggest that MHC may not have been sufficiently clumped.
4) Figures 1a and 1d could be combined into a single graphic for conciseness.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
I would like to thank the authors for all modifications and answers, also to other reviewer's comments. I do have one tiny comment still: could the authors also try and present the SNP based heritability on the liability scale? Now they present the observed scale, for liability one needs some assumptions on life time risk etc, but the liability scale number translates much better to daily practice.

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REVIEWER COMMENTS
Reviewer #1 (Remarks to the Author):

This is a well-crafted, thorough analysis of myasthenia gravis within a Japanese population. In addition to studying an Asian cohort, the manuscript's notable strengths include its emphasis on disease subtypes, the large control sample size, supplementary downstream analyses like PheWas, and the examination of the TERT variant's impact.

Response:
We sincerely appreciate the reviewer's thoughtful and constructive feedback. Thank you for the time and effort you devoted to evaluating our manuscript. Your comments have been valuable in improving the clarity and quality of the work.

I have only minor comments, none of which will detract from the paper's main messages.
• Add the Lambda1000, which is a metric often used when the case and control groups are unbalanced.

Response:
We appreciate your insightful comment on this important point. In accordance with your suggestion, we have added the λ_{GC1000} value to the manuscript. Following a comment from Reviewer 3, we recalculated both λ_{GC} and λ_{GC1000} using 6,889,985 variants with MAF ≥ 0.02. The updated λ_{GC1000} is 1.002, so we consider that no inflation of the test statistics were observed.

Modification:
To reflect the reviewer's comments, we added the descriptions as;
"No inflation of test statistics were observed with the genomic control factor (λ_{GC}) = 1.005 and (λ_{GC1000}) = 1.002 in the quantile-quantile (QQ) plots based on 6,889,985 SNPs with MAF ≥ 0.02. (**Supplementary Fig. 2**)."
in **Results** (page 6, lines 167-169).

• Include the ICD9/10 codes used to select healthy individuals as controls from the Japanese biobank. If ICD codes were not used, describe in more detail how the control cohort was selected from the large biobank collection.

Response:
Thank you for pointing out an important point. In this study, we used BioBank Japan (BBJ) as the control cohort. BBJ is a hospital-based biobank that targets specific diseases. The medical history information extracted from electronic medical records was all based on diagnoses made by professional physicians. Therefore, ICD-9 or ICD-10 were not used in the selection of the control cohort (Sakaue S et al., ***Nat Genet*** 2021 and Yata T et al., ***Cell Genom*** 2025).

Modification:

To reflect the reviewer's comments, we added the descriptions as;
"As for control dataset, among the target diseases of BBJ, cases of rheumatoid arthritis, atopic dermatitis, Graves' disease, liver cirrhosis, chronic hepatitis B, chronic hepatitis C, nephrotic syndrome, bronchial asthma, cervical cancer, and hepatocellular carcinoma were excluded as the MHC-related diseases. In addition, cases of autoimmune diseases (listed in Supplementary Table 8), MG, and thymoma were further excluded by referring to medical history information extracted from electronic medical records. BBJ is a hospital-based biobank that targets specific diseases. The medical history information extracted from electronic medical records was all based on diagnoses made by professional physicians. Therefore, ICD-9 or ICD-10 were not used in the selection of the control cohort.[23,59]"
in **Methods** (page 27, lines 562-566).

Modified references are listed below.
23. Sakaue, S. *et al*. A cross-population atlas of genetic associations for 220 human phenotypes. ***Nat Genet*** 53, 1415–1424 (2021).
59. Yata, T. *et al*. Contribution of germline and somatic mutations to risk of neuromyelitis optica spectrum disorder. ***Cell Genomics*** 5, 100776 (2025).

• Add the effect allele (the allele that increases the OR above 1) to Tables 1 and 2; although it may seem trivial, it greatly aids comparisons with other datasets.

Response:
Thank you for pointing out an important point. In **Table 1**, we have added information on the effect and other alleles. **Table 3** (**Table 2** was now moved to **(new) Table3** to reflect a comment from reviewer 2) shows the odds ratios for the presence of specific HLA variants compared to their absence.

Modification:
Table1. Associations of the lead variant at the *TERT* locus with the MG subtypes.

SNP (rsID)	Position (GRCh37)	Reference/ Effect allele	Subgroup	Case		Control		OR (95% CI) of Effect allele	*P* value
				n	Effect allele frequency	n	Effect allele frequency		
rs2736099	5:1287340	G/A	all cases	1,434	0.350	42,913	0.295	1.30 (1.19-1.41)	3.2×10^{-10}
			OMG	300	0.294			1.00 (0.83-1.19)	0.98
			GMG	1,134	0.364			1.38 (1.26-1.52)	1.6×10^{-12}
			AChR-Ab(+)MG	934	0.382			1.49 (1.35-1.65)	2.1×10^{-15}
			AChR-Ab(-)MG	200	0.283			0.95 (0.76-1.18)	0.63
			TAMG	335	0.442			1.92 (1.64-2.26)	1.1×10^{-15}
			TA(-)MG	599	0.348			1.29 (1.13-1.46)	4.5×10^{-5}
			g-EOMG	293	0.370			1.43 (1.19-1.72)	7.6×10^{-5}
			g-LOMG	306	0.328			1.16 (0.97-1.38)	0.10

OR: odds ratio, CI: confidence interval

To reflect the reviewer's comments, we added the descriptions as;
"Odds ratios compare the presence versus absence of each HLA variant."

in the **legend of Table 3.**

• There is limited evidence linking TERT to European-ancestry populations. It may be worthwhile to speculate in the Discussion on why the genetic architecture of myasthenia differs across populations. For example, the incidence of myasthenia gravis is lower in Japan than elsewhere. Do current genetic findings account for this difference? This is not a limitation of the paper.

Response:
We thank you for highlighting this important point. The lower incidence of myasthenia gravis in Japan and other population differences are indeed interesting. Population- or environment-specific factors may help explain the differences in genetic architecture. *TERT* has shown ancestry-specific associations in cancer (El Zarif T et al., ***Oncologist*** 2024), which may also be relevant to its association with thymoma-associated MG in our study.

Modification:
To reflect the reviewer's comments, we added the descriptions as;
"These emphasize the value of considering disease subtypes and clinical endotypes across MG risk loci, as well as biological involvement of *TERT* in MG pathogenesis. Although *TERT* has not been identified as a significant locus in previous GWAS of MG in European populations, its association with MG in our study may reflect population-specific genetic or environmental factors, given that *TERT* has shown ancestry-specific associations in cancer[51]."
in **Discussion** (page 24, lines 477-480).

The modified reference is listed below.
51. Zarif, T. El *et al*. TERT Promoter Mutations Frequency Across Race, Sex, and Cancer Type. ***Oncologist*** 29, 8–14 (2024).

• It would be helpful to state in the Methods that the whole genome sequence data were generated using blood-derived DNA, as telomere length measurements are more susceptible to noise in this tissue type.

Response:
We sincerely appreciate your insightful comment on this important point. As you rightly noted, the whole genome sequencing data was generated from blood-derived DNA.

Modification:
To reflect the reviewer's comments, we added the descriptions as;
"For association analysis with telomere length, we estimated telomere length using Telseq for blood-derived DNA WGS samples in TMM cohort using Illumina Novaseq 6000 (Illumina, CA, USA)."
in **Methods** (page 29, line 649).

• Although it is described in the Methods, I had to search for it; add a sentence to the Results section describing the model used to analyze the data.

Response:

Thank you for pointing out an important point. We have added descriptions of the models and software used for following analyses; GWAS, GWAS meta-analysis, risk variant fine-mapping within the MHC region, PheWAS, colocalization analysis, allele-specific expression (ASE) analysis, and transcription factor (TF) motif binding analysis.

Modification:

To reflect the reviewer's comments, we added the descriptions as;

"We performed GWAS of imputed dosages of each variant by single-variant association test of SAIGE software version 1.2.0, which is based on a generalized mixed model and assumes an additive effect model. In the GWAS of all the MG cases, the *TERT* gene locus at 5p15 satisfied genome-wide significance threshold with MG ($P < 5.0\times10^{-8}$; Fig. 1c)."

in **Results** (page 6, lines 176-178),

"Next, we performed cross-population GWAS meta-analysis by integrating the Japanese GWAS (all MG cases) with those of the Europeans (7,142 MG cases and 474,941 controls in total), using the inverse-variance method under a fixed-effects model for the effect of each variant."

in **Results** (page 11, lines 254-255),

"We imputed genotypes of 4-digit alleles and amino acid polymorphisms of the class I and II HLA genes in the MHC region using DEEP*HLA with the Japanese population-specific reference panel and conducted the association study by using logistic regression model that assumed additive effects of allele dosages on a log-odds scale."

in **Results** (page 13, lines 278-279),

"We utilized BBJ genotype and phenotype data (n_{Sample} = 232,060), which consisted of 125 diseases as defined by the 10th revision of the International Classification of Diseases (ICD-10) and 75 quantitative traits consisting of physical measurements, blood tests (biochemistry, serum, blood cell count), blood pressure, echocardiography, and lifestyle questionnaires (diet, exercise, alcohol consumption, smoking). Binary traits were tested by logistic regression analysis, continuous traits by linear regression analysis."

in **Results** (page 18, lines 364-365),

"We evaluated phenome-wide colocalization of the *TERT* regional associations with MG by calculating the posterior probability (PP) using the approximate bayes factor method in the Coloc R package (version 5.2.2)"

in **Results** (page 18, lines 387-388),

"We utilized PWMScan based on position weight matrix of 746 TFs registered in JASPAR CORE 2020. *P* values are calculated based on the null hypothesis that the target sequence is a random sequence."

in **Results** (pages 22, lines 444-446),

"d) Results of ASE quantification of rs2736099-A/G by full-length RNA-seq (RamDA-seq). Each value indicates the expression fraction and the numbers in brackets indicate the number of read counts. *P* value was calculated by two-tailed binomial test."

in **the legend of Figure 6**.

Reviewer #2 (Remarks to the Author):

This is a very interesting GWAS into Myasthenia Gravis in patients with a Japanese background. Indeed, it is to be expected that the genetic architecture between geographical regions varies, and that is indeed what is shown here, comparing the current results with previous, mostly EU based GWAS-es.

The study is strong in explicitly analysing clinical subtypes of MG, which usually is a major challenge in GWAS where usually minimal clinical data are available. Moreover, some interesting, subtype dependent results were obtained.
Main results are two loci, TERT and HLA where TERT seems mostly associated with generalized/ thymoma dependent MG, and HLA with non-thymoma associated MG. Results are plausible, in the sense that TERT is most abundantly expressed in the thymus.

Response:
We sincerely thank the reviewer for the positive and thoughtful comments. We appreciate your recognition of the value of analyzing clinical subtypes and the relevance of population-specific genetic findings. We are also grateful for your insightful suggestions, which helped to deepen the significance and impact of our study.

I do have some comments:
1. The authors do present the qqplots and lambda GC, but I would like to also see an LDSC based analysis of intercept and slope, to explore if inflation is mainly polygenic or still based on residual confounding. I realise that sample size might be a bit tricky, but could the authors explore what the SNP based heritability is according to LDSC (and/ or LDAK)?

Response:
Thank you for your suggestion regarding an LD Score Regression (LDSC) and LD Adjusted Kinships (LDAK) analysis. In response, we performed SNP-based heritability estimation using LDSC, based on LD scores calculated from East Asian populations of the 1000 Genomes Project and restricted to HapMap3 SNPs. The estimated SNP heritability on the observed scale was 0.020 (SE = 0.011). The LDSC intercept was 0.994 (SE = 0.0064), and the mean χ^2 was 1.012, resulting in a lambda GC of 0.996. These results indicate that test statistic inflation is minimal and mainly reflects polygenic signal, with the intercept near 1 suggesting little impact from population stratification or other biases. We also estimated SNP-based heritability using LDAK 6 with pre-computed BLD-LDAK taggings for East Asian populations (HapMap3 SNPs). The estimated SNP heritability on the observed scale was 0.016 (SE = 0.0080), which is slightly lower than the LDSC estimate but generally consistent, suggesting that the LDAK-based results are robust and supportive of the LDSC findings.

Modification:
To reflect the reviewer's comments, we added the descriptions as;
"We estimated SNP-based heritability using Linkage Disequilibrium Score Regression (LDSC)[24] and Linkage Disequilibrium Adjusted Kinships (LDAK) SumHer[25]. LDSC showed an observed-scale SNP heritability of 0.020 (SE = 0.011), with an intercept of 0.994 (SE =

0.0064), a mean χ^2 of 1.012, and a λ_{GC} of 0.996, while LDAK showed an observed-scale SNP heritability of 0.016 (SE = 0.0080). These findings show that the inflation of test statistics is minimal and mainly reflects polygenicity rather than confounding, and the consistency across methods supports the robustness of the estimates."
in **Results** (page 6, lines 169-175),
"**SNP-based heritability estimation**
We estimated SNP-based heritability using LD Score Regression (LDSC)[24]. We calculated LD scores from East Asian populations of the 1000 Genomes Project[64] and restricted the analysis to HapMap3[65] SNPs. LDSC estimates both SNP heritability and intercept, which reflects the contribution of residual confounding such as population stratification. We also estimated SNP-based heritability using LDAK 6 (SumHer)[25] with pre-computed BLD-LDAK taggings for East Asian populations (HapMap3 SNPs). LDAK uses a weighted linear mixed model to account for linkage disequilibrium and minor allele frequency, providing a robust estimate of SNP heritability even in modest sample sizes."
in **Methods** (page 28, lines 596-604).

Modified References are listed below.
24. Bulik-Sullivan, B. *et al.* LD score regression distinguishes confounding from polygenicity in genome-wide association studies. ***Nat Genet*** 47, 291–295 (2015).
25. Speed, D. & Balding, D. J. SumHer better estimates the SNP heritability of complex traits from summary statistics. ***Nat Genet*** 51, 277–284 (2019).
64. Auton, A. *et al*. A global reference for human genetic variation. ***Nature*** 526, 68–74 (2015).
65. Altshuler, D. M. *et al*. Integrating common and rare genetic variation in diverse human populations. ***Nature*** 467, 52–58 (2010).

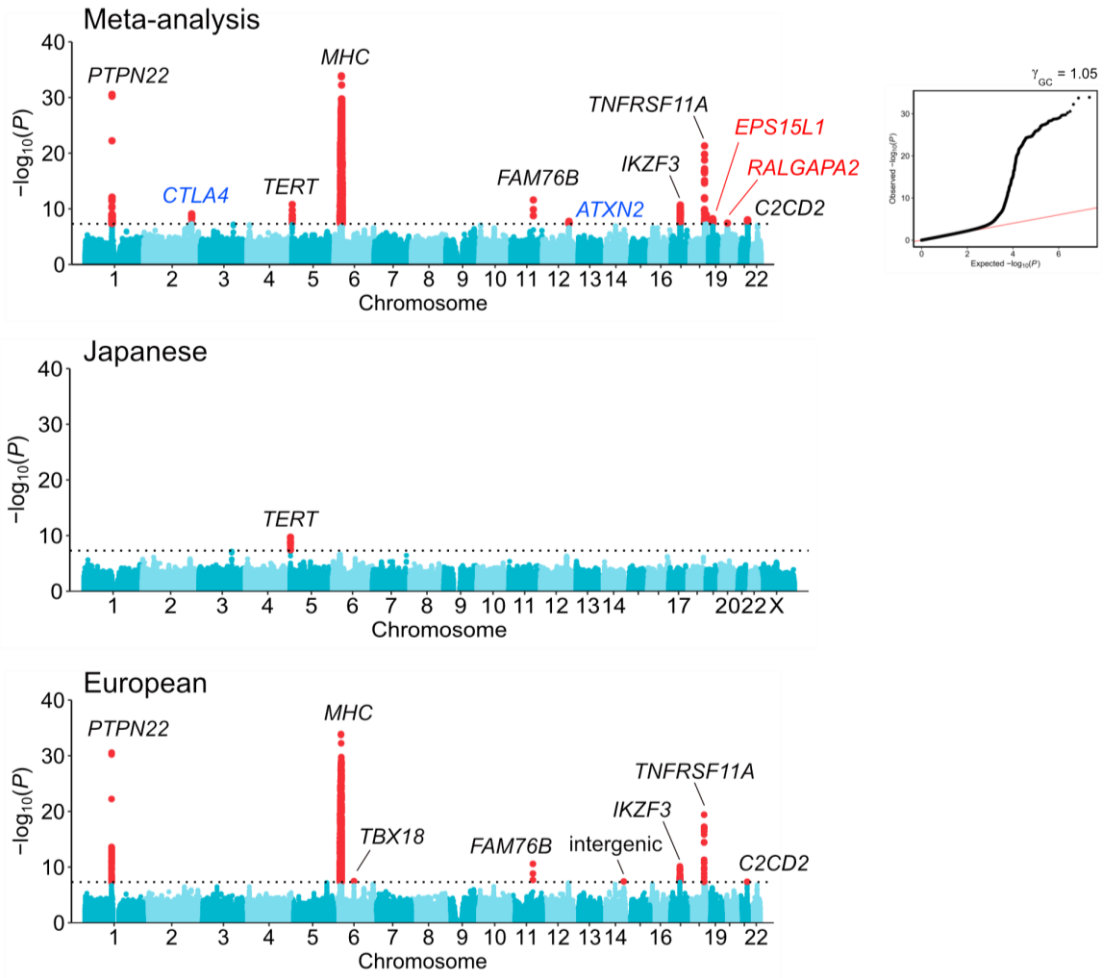
2. I applaud the authors that they also analyse the EU data on MG, and they do present a combined Manhattan plot in the supplement. Main question I have there is: do all signals from the EU GWAS go down, do the Japanese hits also go down, with two new hits? Signals are so significant in the original GWASes that even with "negative" replication, they can remain significant. The EU GWAS used Manhattan plots where "towers" are depicted with arrows, with arrows point up showing loci that improve, and arrows going down signals that get less significant after combined analysis. Could the authors present the combined results like that?

Response:
Thank you for pointing out this important point. In the meta-analysis, the Japanese hit at the *TERT* locus and European significant signals at *CTLA4*, *IKZF3*, *TNFRSF11A*, and *C2CD2* showed stronger associations. In contrast, the signal for *FAM76B*, previously identified in the European GWAS, was weakened. For *PTPN22* and the MHC region, the lead SNPs identified in European cohorts were neither genotyped nor imputed in the Japanese study, as they were very rare in the Japanese population. Detailed results are summarized in **Table 2** and we have added separate Manhattan plots for the Japanese and European GWAS results as **Supplementary Figure 4**.

Modification:
To reflect the reviewer's comments, we added the descriptions as;

“The Japanese significant signal at the *TERT* locus and European significant signals at *CTLA4*, *IKZF3*, *TNFRSF11A*, and *C2CD2* showed stronger associations. In contrast, the signal for *FAM76B*, previously identified in the European GWAS, was weakened. For *PTPN22* and the MHC region, the lead SNPs identified in European cohorts were neither genotyped nor imputed in the Japanese study, as they were very rare in the Japanese population.”
in **Results** (page 11, lines 260-265),

Supplementary Figure 4. Cross-population GWAS meta-analysis by integrating the Japanese GWAS with those of the Europeans



A Manhattan plot and a quantile-quantile plot show the result of cross-population GWAS meta-analysis by integrating the Japanese GWAS with those of the Europeans. Manhattan plots of the Japanese and European GWAS are shown below the meta-analysis plot for comparison. The dotted horizontal black line indicates the genome-wide significance level ($P = 5.0\times10^{-8}$), and red circles indicate SNPs that satisfied genome-wide significance level. Newly identified loci are labeled in red, and previously reported loci that also reached genome-wide significance by meta-analysis are shown in blue.

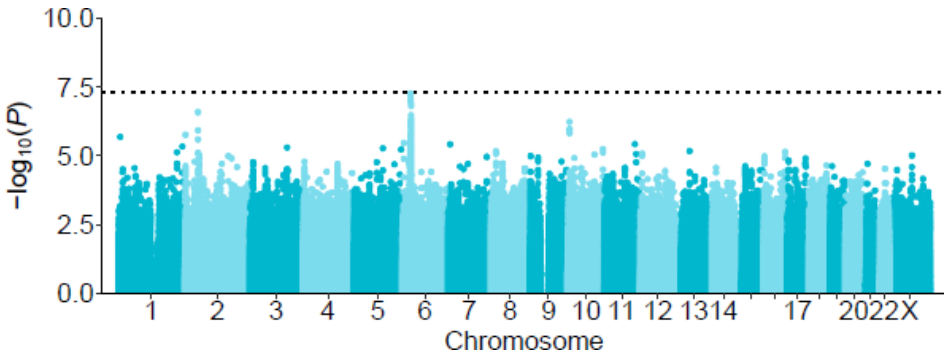
Also, in the tables where EU and Japanese SNPs are listed, some loci have "NA" in the Japanese study, what does that mean?

Response:
Thank you for pointing out an important point. “NA” denotes variants that were neither genotyped in the current SNP array nor imputed, as they were not present in the reference panel. For rs3131618 in at MHC region (**Table2**), rs2476601 at *PTPN22* locus, rs1264706 at MHC region, and rs4766578 at *ATXN2* locus (**Supplementary Table 2**), this is presumably due to their extremely low frequency in the Japanese population. On the other hand, although rs2358816 at *PTPN22* locus (**Table 2**) had a minor allele frequency of approximately 0.2 in the Japanese population, it was not imputed in our dataset. We also investigated tag SNPs with strong linkage disequilibrium ($r^2 > 0.8$) with rs2358816 in East Asians, but none showed significant associations. Furthermore, no association signals were detected across the *PTPN22* locus.

Modification:
To reflect the reviewer’s comments, we added the descriptions as;
“'NA' indicates that the SNPs were neither genotyped nor imputed in the JAMG-R dataset.“
in the legend of **Table 2** and **Supplementary Table 2**.
(**Supplementary Table 3** was moved to **(new)Table 2** to reflect a comment of reviewer 3.)

3. Lastly, and perhaps related to number 2: do the authors think that the TERT result is solely driven bij thymoma associated MG, or is there still a group of gMG that is also TERT driven? They could explore this by doing some more subanalyses, exploring what happens if subcohorts are removed from the gMG total group.

Response:
We appreciate your insightful and valuable comment. Please note that all thymoma-associated MG cases were included in the seropositive generalized MG (gMG) group, therefore we consider that stratification by thymoma status has already been addressed in our analysis. Furthermore, we performed GWAS in the gMG without thymoma and confirmed that no significant association signals were detected (**Response figure 1**). So *TERT* signal could solely be driven by thymoma associated MG. However, as we also noted in the **Discussion** of the main manuscript, our data showed that treatment response in EOMG is associated with rs2736099-A in the *TERT*, indicating that the risk allele might underlie the pathological mechanism of MG.



Response figure 1. The Manhattan plot shows GWAS result for gMG without thymoma

Modification:

To reflect the reviewer's comments, we added the descriptions as;

"In the stratified GWAS, we again identified genome-wide significant associations at the *TERT* region for gMG, g-AChR-Ab(+)MG, and g-TAMG (**Fig. 1c, d**). We also performed GWAS in the gMG without thymoma and confirmed that no significant association signals were detected (data not shown). "

in **Results** (page 9, lines 213-214).

I especially like figure 1 to show subtype results.

Response:

We really appreciate your thoughtful comments and support.

Reviewer #3 (Remarks to the Author):

The authors present important findings from a highly valuable and well characterized genetic cohort of myasthenia gravis patients in Japan. This study represents the first-ever GWAS of East Asian ancestry in this disease and includes a cross-ancestry meta-analysis, as well as comprehensive follow-up analyses focusing on the HLA region and the TERT locus.

Response:
We sincerely thank the reviewer for the positive and encouraging comments. We also appreciate your acknowledgment of the comprehensive follow-up analyses, including the cross-ancestry meta-analysis and evaluations of the HLA region and *TERT* locus.

While the study is both timely and relevant, I have several suggestions for improving the manuscript:
1) Although the authors discuss the novel association at the TERT locus in detail, the newly identified loci from the meta-analysis with European cohorts (EPS15L1 and RALGAPA2) are barely addressed. The authors should expand on these meta-analysis findings and provide further interpretation.

Response:
We thank the reviewer for the helpful comment. We have expanded the discussion of *EPS15L1* and *RALGAPA2*, newly identified loci in the meta-analysis. *EPS15L1*, which is involved in intracellular trafficking, was also implicated in the TWAS analysis[17], supporting its potential relevance to MG. *RALGAPA2*, a member of the *RALGAP* family, regulates Ral GTPase signaling[56] and may influence immune function. While their roles in MG remain unclear, these findings merit further investigation.

Modification:
To reflect the reviewer's comments, we added the descriptions as;
"In cross populational GWAS meta-analysis, we identified novel loci of *EPS15L1* and *RALGAPA2*. *EPS15L1* was highlighted in a recent transcriptome-wide association study as significantly associated with MG susceptibility[17], supporting its potential involvement in disease pathogenesis. *RALGAPA2* is a *RALGAP* family gene that inactivates RalA and RalB, involved in intracellular signaling[57]. Although their role in autoimmunity is unclear, altered Ral signaling may affect immune regulation and warrants further study in MG."
in **Discussion** (page 25, lines 509-514).

Modified references are listed below.
17. Braun, A. *et al*. Genome-wide meta-analysis of myasthenia gravis uncovers new loci and provides insights into polygenic prediction. ***Nature Communications*** 15, 9839 (2024).
57. Shirakawa, R. & Horiuchi, H. Ral GTPases: Crucial mediators of exocytosis and tumourigenesis. ***J Biochem*** 157, 285–299 (2015).

Supplementary Table 3 could also be added to the main text.

Response: Thank you for your helpful suggestion.

Modification:
We have moved **Supplementary Table 3** to **(new) Table 2** in the main manuscript.

2) In Supplementary Table 2+3, there are missing values (NAs) that require explanation. Are these due to all SNPs not being present in the current cohort? If so, the authors could consider using proxy SNPs in LD, unless all SNPs in LD are infrequent in the current cohort, which should be noted in the legend.

Response:
Thank you for pointing out an important point. “NA” denotes variants that were neither genotyped in the current SNP array nor imputed, as they were not present in the reference panel. For rs3131618 in at MHC region (**Table2**), rs2476601 at *PTPN22* locus, rs1264706 at MHC region, and rs4766578 at *ATXN2* locus (**Supplementary Table 2**), this is presumably due to their extremely low frequency in the Japanese population. On the other hand, although rs2358816 at *PTPN22* locus (**Table 2**) had a minor allele frequency of approximately 0.2 in the Japanese population, it was not imputed in our dataset. We also investigated tag SNPs with strong linkage disequilibrium ($r^2 > 0.8$) with rs2358816 in East Asians, but none showed significant associations. Furthermore, no association signals were detected across the *PTPN22* locus.

Modification:
To reflect the reviewer’s comments, we added the descriptions as;
“'NA' indicates that the SNPs were neither genotyped nor imputed in the JAMG-R dataset.“
in the legend of **Table 2** and **Supplementary Table 2**.

Additionally, sign tests and heterogeneity tests should be included to complement the results.

Response:
Thank you for your valuable suggestion. We performed a sign test. We conducted a sign test for the 9 SNPs (excluding ‘NA’ SNPs) presented in **Table 2**, and the result was statistically significant with a *P* value of 0.002. We agree that heterogeneity tests are important to assess the consistency of effect sizes across studies. However, since our meta-analysis includes only two studies, we did not perform heterogeneity tests.

Modification:
To reflect the reviewer’s comments, we added the descriptions as;
“We identified novel loci of *EPS15L1* at 19p13 (rs10404046, OR = 1.13, 95%CI = 1.08–1.18, $P = 5.9\times10^{-9}$) and *RALGAPA2* at 20p11 (rs6075661, OR = 1.13, 95%CI = 1.08–1.18, $P = 3.7\times10^{-8}$; **Supplementary Fig.4** and **Table 2**) with genome-wide significance. We conducted

a sign test for the 9 SNPs presented in **Table 2** (excluding 'NA' SNPs), and confirmed concordant effect directions (P = 0.002)"
in **Results** (page 11, lines 258-260).

3) An OR of 1.92 for the lead variant rs2736099 near the TERT locus is unusually high for a GWAS hit. However, it does not replicate strongly in previous studies. The authors should either seek an additional (East Asian) cohort for replication or discuss this in more detail (e.g. it could be driven by thymoma?).

Response:
We appreciate your thoughtful comment on this important point. All previous studies have been conducted in European populations, and to our knowledge, this is the first GWAS of MG conducted in an East Asian population. Moreover, previous studies did not stratify between thymoma-associated and non-thymoma-associated cases. Therefore, at this time, it is difficult to obtain a dataset suitable for the analysis you suggested. However, we fully agree that similar investigations across different countries and ethnic groups are of great importance, and we hope to pursue such studies in the future.

Modification:
To reflect the reviewer's comments, we added the descriptions as;
"*TERT* is genetically, clinically, and biologically involved in MG, where the risk allele downregulates the *TERT* expression in thymoma. Further investigations in other East Asian cohorts and stratified analyses by clinical subtypes will be important to validate and extend our findings."
in **Discussion** (page 25, lines 517-519).

4) It is unclear whether the raw genotypes of case and control samples were merged and subsequently jointly QCed and imputed. This should be described explicitly in the methods.

Response:
Thank you for pointing out this important issue. As you correctly noted, quality control and imputation were performed after merging the case and control samples.

Modification:
To reflect the reviewer's comments, we added the descriptions as;
"Genotype dosages were imputed by using Minimac4. In-house whole genome sequencing data of Japanese (n = 11,754) were used for the reference panel. Variant QC and imputation were performed after merging the case and control samples."
in Methods (page 27, line 579).

5) The number of SNPs and MAF cutoff used to calculate genomic inflation (Lambda) and generate the QQ plots should be reported in the supplementary figure legends. Ideally, this analysis should be repeated using a MAF cutoff of 0.02.

Response:

Thank you for pointing out this important issue. We appreciate your insightful comment on this important point. In the original analysis, we created the QQ plot using 9,034,566 SNPs with a MAF of ≥ 0.005, in line with the GWAS threshold. Following your suggestion, we have re-generated the QQ plot using 6,889,985 SNPs with a MAF ≥ 0.02.

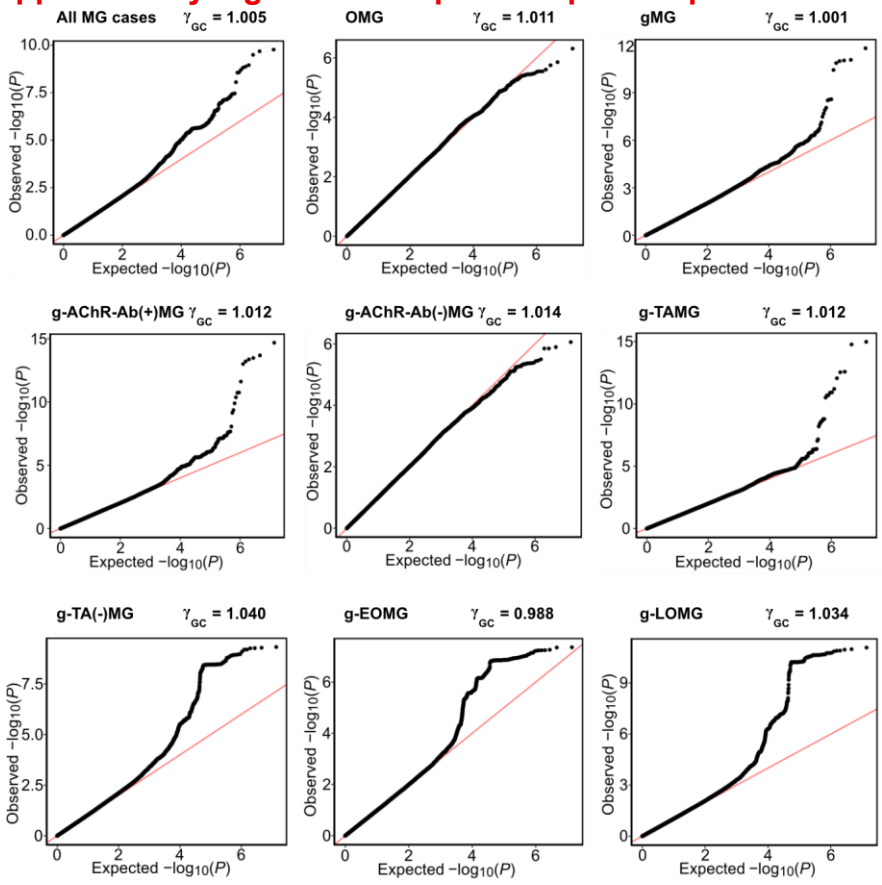
Modification:

To reflect the reviewer's comments, we added the descriptions as;

"No inflation of test statistics were observed with the genomic control factor (λ_{GC}) = 1.005 and (λ_{GC}1000) = 1.002 in the quantile-quantile (QQ) plots based on 6,889,985 SNPs with MAF ≥ 0.02. (**Supplementary Fig. 2**)."

in Results (page 6, lines 167-169),

Supplementary Figure 2. The quantile-quantile plots of GWAS of each MG subtype



The horizontal and vertical axes indicate expected and observed -$\log_{10}$(P values) for the association tests. 6,889,985 variants with minor allele frequency (MAF) ≥ 0.02 which passed post imputation QC of Rsq ≥ 0.5 were tested including both autosomal and X chromosomes.

Minor:

1) In general, all tables and figures require more detailed legends to ensure they are fully interpretable. If possible, provide Excel/CSV supplementary tables instead of PDF tables

Response:

Thank you for pointing out this important issue. We have revised the legends of each figure and table to provide more detailed explanations. We will also submit the supplementary tables as Excel files to facilitate accessibility and review.

2) Table 1 appears redundant, as the lead SNPs are in moderate LD and yield nearly identical results. Furthermore, the conditional analyses demonstrate that these effects are not independent. The same applies to Figure 2b.

Response:

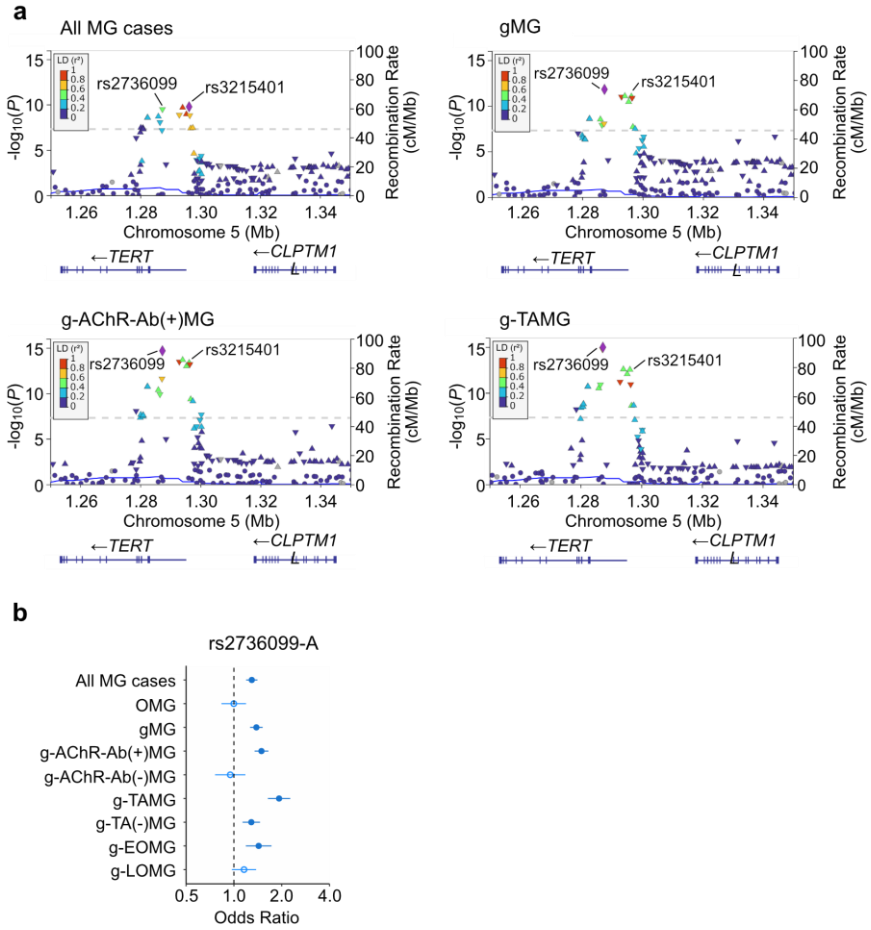
Thank you for pointing out this important issue. In accordance with your suggestion, we removed the results of rs3215401 in **Table 1** and forest plot of rs3215401 in **Figure 2**.

Modification:

Table 1. Associations of the lead variant at the *TERT* locus with the MG subtypes.

SNP (rsID)	Position (GRCh37)	Reference/ Effect allele	Subgroup	Case		Control		OR (95% CI) of Effect allele	*P* value
				n	Effect allele frequency	n	Effect allele frequency		
rs2736099	5:1287340	G/A	all cases	1,434	0.350	42,913	0.295	1.30 (1.19-1.41)	3.2×10^{-10}
			OMG	300	0.294			1.00 (0.83-1.19)	0.98
			GMG	1,134	0.364			1.38 (1.26-1.52)	1.6×10^{-12}
			AChR-Ab(+)MG	934	0.382			1.49 (1.35-1.65)	2.1×10^{-15}
			AChR-Ab(-)MG	200	0.283			0.95 (0.76-1.18)	0.63
			TAMG	335	0.442			1.92 (1.64-2.26)	1.1×10^{-15}
			TA(-)MG	599	0.348			1.29 (1.13-1.46)	4.5×10^{-5}
			g-EOMG	293	0.370			1.43 (1.19-1.72)	7.6×10^{-5}
			g-LOMG	306	0.328			1.16 (0.97-1.38)	0.10
~~rs3215401~~	~~5:1296255~~	~~A/AG~~	~~All MG cases~~	~~1,434~~	~~0.306~~	~~42,913~~	~~0.253~~	~~1.32 (1.21-1.43)~~	~~1.7×10^{-10}~~
			~~OMG~~	~~300~~	~~0.264~~			~~1.06 (0.88-1.28)~~	~~0.51~~
			~~gMG~~	~~1,134~~	~~0.317~~			~~1.39 (1.26-1.53)~~	~~9.3×10^{-12}~~
			~~g-AChR-Ab(+)MG~~	~~934~~	~~0.332~~			~~1.49 (1.34-1.65)~~	~~4.3×10^{-14}~~
			~~g-AChR-Ab(-)MG~~	~~200~~	~~0.245~~			~~0.97 (0.77-1.22)~~	~~0.81~~
			~~g-TAMG~~	~~335~~	~~0.382~~			~~1.86 (1.57-2.20)~~	~~2.9×10^{-13}~~
			~~g-TA(-)MG~~	~~599~~	~~0.305~~			~~1.31 (1.15-1.49)~~	~~3.4×10^{-5}~~
			~~g-EOMG~~	~~293~~	~~0.312~~			~~1.38 (1.14-1.68)~~	~~6.4×10^{-4}~~
			~~g-LOMG~~	~~306~~	~~0.297~~			~~1.26 (1.02-1.55)~~	~~0.016~~

Figure 2. Associations of the *TERT* region with MG disease subtypes.



(We removed the forest plot of rs3215401 from **Figure 2b**)

3) The PCA plot for PC3 appears to contain a nested or improperly formatted sub-plot. Please provide a proper legend and clarify the visualization. In addition, the PC4 plots suggest that MHC may not have been sufficiently clumped.

Response:

We thank the reviewer for pointing this out. We have reformatted **Supplementary Figure 1** to correct the issue. We also thank you for pointing out this important issue regarding the PCA plots. We note that we had performed principal component analysis (PCA) after excluding the MHC region defined as Chr6:20–40 Mb. The long-range haplotype structure in the Japanese population has been reported to span up to 24–35 Mb (Hirata J et al., ***Nat Genet*** 2019), and we considered that the excluded region sufficiently covers this range. In addition, we conducted GWAS using PC1 to PC5 as phenotypes, but no significant signals were observed on Chr6.

Modification:

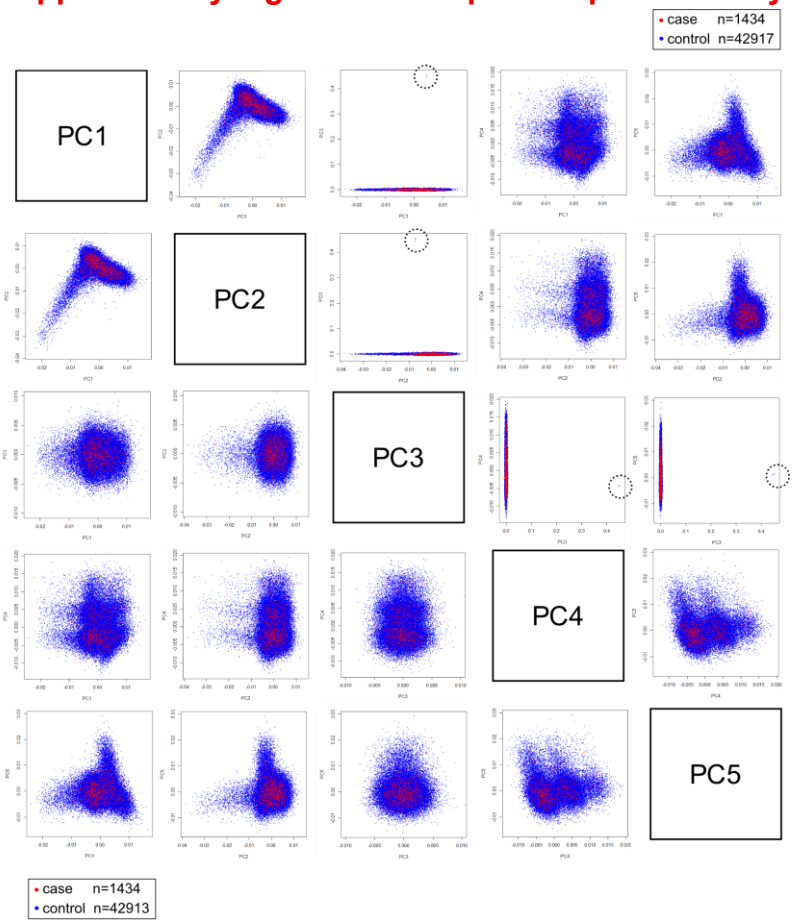
To reflect the reviewer's comments, we added the descriptions as;

"Principal component (PC) analysis was conducted using QC-passed samples after widely excluding the MHC region (chromosome 6: 20–40 Mb) to reduce population stratification

effects. The long-range haplotype structure in the Japanese population has been reported to span up to 24–35 Mb[58]. The analysis showed that there were 4 samples in the control group with outliers in the third PC, which were excluded (**Supplementary Fig. 1**)."
in Methods (page 26, lines 539-541).

The modified reference is listed below.
58. Hirata, J. et al. Genetic and phenotypic landscape of the major histocompatibilty complex region in the Japanese population. Nat Genet 51, 470–480 (2019).

Supplementary Figure 1. Principal component analysis of GWAS subject datasets



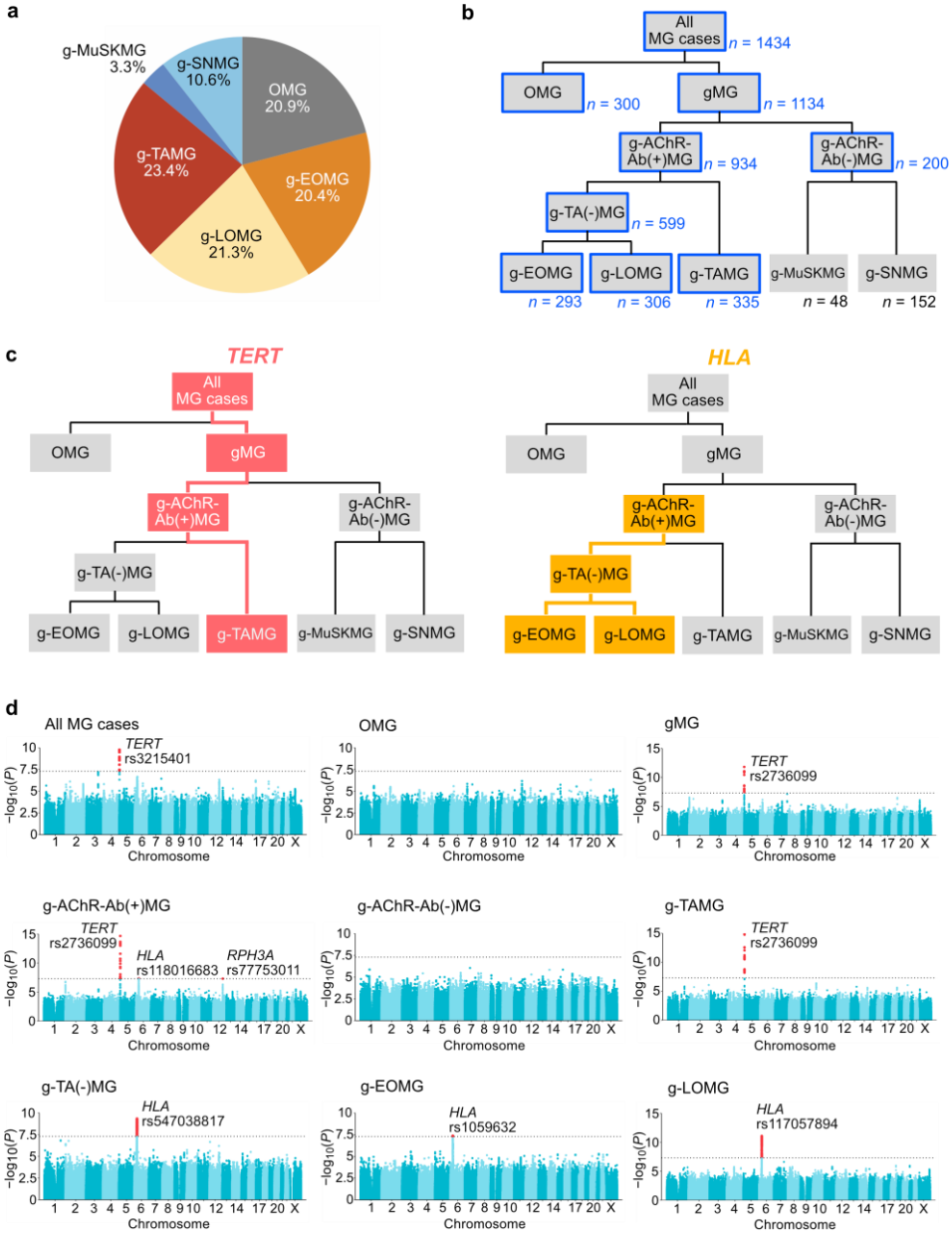
Scatter plots of principal components (PCs) 1 through 5. Each point represents a sample: red for MG cases (n = 1,434), blue for controls (n = 42,917). The upper right panels show the distribution before outlier exclusion. Dotted circles indicate four control samples identified as PC outliers, which were excluded from subsequent GWAS analysis. The lower left panels show the distribution after exclusion of these outliers.

4) Figures 1a and 1d could be combined into a single graphic for conciseness.

Response:
Thank you for pointing out this important issue. In accordance with your suggestion, we moved **Figure 1d** to **Figure 1c** to improve graphical conciseness.

Modification:

Figure 1. The genome-wide association studies of MG and its clinical subtypes



a) Proportion of each disease type in the current analysis. **b**) Disease subtype classification and analysis according to the O/g-ELTMuN classification, as well as the number of the subtyped samples. **c**) Disease classifications with significant associations with *TERT* and *HLA* are shown in red and blue, respectively. **d**) Manhattan plots show GWAS results for all the MG cases and subtypes. The dotted horizontal black line indicates the genome-wide significance level ($P = 5.0\times10^{-8}$), and red circles indicate SNPs that satisfied genome-wide significance level.

Responses to the editorial requests

We ask you to revise your paper to address our editorial requests (in the attachedAuthor Checklist) and any remaining comments from reviewers (included at the end of thisemail, if applicable).

Thank you for indicating the key points of modification. We have fully addressed all items in the Author Checklist and all remaining reviewer comments. Our detailed responses to the Author Checklist and reviewer comments are provided below.

AUTHOR CHECKLIST

Please check the items below carefully and add a response in each row of the table to indicate the changes that you have made. Please also check through any additional marked-up edits we may have provided within the manuscript file.

We sincerely thank the editors for their careful review and guidance. We have provided detailed responses in the attached Author Checklist and revised both the main manuscript and Supplementary Information accordingly.

REVIEWER COMMENTS

Reviewer #2 (Remarks to the Author):

I would like to thank the authors for all modifications and answers, also to other reviewer's comments. I do have one tiny comment still: could the authors also try and present theSNP based heritability on the liability scale? Now they present the observed scale, for liability one needs some assumptions on life time risk etc, but the liability scale number translates much better to daily practice.

Response:

We sincerely thank the reviewer for the positive and thoughtful comment. We have now also assumed the liability scale SNP heritability using LD Score Regression (LDSC). The liability-scale estimate was calculated assuming a population prevalence of 23.1 per 100,000 (0.000231) for myasthenia gravis in Japan[66] and a sample prevalence of 0.0323 (n_{case} = 1,434, $n_{control}$ = 42,913). LDSC showed a liability-scale SNP heritability of 0.045 (SE = 0.025), with an intercept of 0.994 (SE = 0.0064), a mean χ^2 of 1.012, and a λ_{GC} of 0.996.

Modification:

To reflect the reviewer's comments, we added the descriptions as;

"LDSC showed a observed-scale SNP heritability of 0.020 (SE = 0.011), with an intercept of 0.994 (SE = 0.0064), a mean χ^2 of 1.012, and a λ_{GC} of 0.996, and a liability-scale SNP heritability of 0.045 (SE = 0.025), with an intercept of 0.994 (SE = 0.0064), a mean χ^2 of 1.012, and a λ_{GC} of 0.996, respectively."

in **Results** (page 7, lines 187-189),

"For this case–control study, SNP-based heritability was estimated on the observed scale and subsequently transformed to the liability scale assuming a population prevalence of 23.1 per 100,000 in Japan[66] and a sample prevalence of 0.0323 (n_{case} = 1,434, $n_{control}$ = 42,913)."

in **Methods** (page 19, lines 524-527).

Modified references are listed below.

66. Yoshikawa, H. et al. Two-step nationwide epidemiological survey of myasthenia gravis in Japan 2018. ***PLoS One*** 17, (2022).