Single variant rankings - Loci

Study selection. The Gene Verification Committee (GVC) performs literature reviews of large-scale genome-wide association studies (GWAS) of Alzheimer's disease (AD) and closely related phenotypes, including studies of array genotypes, whole exome sequencing (WES) and whole genome sequencing (WGS) data. We also review studies identified by expert investigators (GVC group) and review loci listed in the Alzheimer's Disease Variant Portal (ADVP) and the National Human Genome Research Institute/European Bioinformatics Institute (EBI) GWAS catalogue. Reports from BioRxiv or MEDRxiv are not considered. See Table 3 for full selection criteria. The following types of publications are examined but not formally reviewed:

1. Publications with sample sizes less than 1,000 subjects.
2. Publications where novel untested methods were used.

Evaluation. Association signals from these studies are classified into seven tiers, based on the evidence supporting them. Each manuscript with at least one locus having suggestive or genome-wide significant evidence is then reviewed by the GVC to evaluate the evidence that the particular signal is associated with AD. Evidence from the latest/largest study may supersede evidence from previous studies when samples overlap, and analyses are comparable. Adjudication will determine which study will be given top priority.

Phenotypes. The GVC considers multiple phenotypes closely related to AD. We consider each phenotype independently and rank each separately. Under this system, a given locus will have multiple rankings depending on the phenotypes reviewed. Current phenotypes under consideration are:

- **AD** defined either clinically (possible or probable AD) or by autopsy (confirmed AD)
- **Dementia** is less-precisely defined as evidence of cognitive impairment, and includes information collected from self-report or by proxy.

The most specific phenotype considered is clinically/autopsy-defined AD, whereas the family history of AD and dementia class will include AD as well as other causes of dementia. In some studies, subjects diagnosed with AD are mixed with dementia cases in a way where the two subject classes cannot be distinguished; in these cases, we consider them as "Dementia" studies. Future phenotypes may include AD endophenotypes (*e.g*. CSF biomarkers), AD related disorders (ADRD) and stratified analyses (*i.e. APOE*, sex) of AD phenotypes.

Sources of evidence. To assign a locus to a tier, the GVC gathers evidence for the strength of association between the variant or another in strong linkage disequilibrium (LD), the consistency of the signal within the study, the robustness of the results, and the robustness of the analysis.

Specifically:

- **Significance:** Evidence for association between the AD-related phenotype and a variant or an LD proxy is measured by the p-value (*P*).
  - **Genome-wide significance** is defined using standard criteria ($P < 5 \times 10^{-8}$). We recognize that this value may be too high when populations other than non-Hispanic Whites (non-Spanish European origin subjects) are considered or for rare-variant analyses.
  - **Genome-wide suggestive evidence** is defined using standard criteria ($P < 1 \times 10^{-6}$).
  - **Nominal evidence** is defined as $P < 0.05$.

- **Consistent association** within a meta-analysis.
  - **Within the final meta-analysis**, the direction of effect is mostly the same for the different cohorts/datasets included within the meta-analysis, and results are not driven by a single data set among data sets representing similar populations (AD clinical/neuropath and Dementia are not considered similar populations.
  - Forest plots, effect size statistics (odds ratios, beta coefficients, etc.) and i^2 heterogeneity values (or equivalent data) should be presented so that heterogeneity of individual results within the meta-analysis can be evaluated.
- **Robust results.**
  - Conditional analysis for adjacent signals are presented when applicable.
  - Support from adjacent SNVs near the lead SNV (common SNVs only). LocusZoom plots showing multiple SNVs for a signal (or equivalent data) should be provided for novel signals**.** "Equivalent data" are association results in tabular form from multiple adjacent SNVs (~ +/- 1 Mb)
- **Robust analysis.** Example signatures of solid associations include:
  - Evidence for genomic inflation is evaluated and reported with a genomic inflation factor near 1. Q-Q plots should be provided but are not required.
  - Population stratification is evaluated and accounted for in the analysis model.
  - Standard quality control methods are applied (e.g. relatedness, call rate, imputation quality, and DNA sample quality filtering)
  - Batch effects such as coverage harmonization are considered and accounted for including quality control (to assess the presence of batch effects and other confounders) and model specification (inclusion of batch effects and other confounders when appropriate). Example strategies include:
    - Depth and exome capture kits are the same across cases and controls or are controlled/modeled for in the association tests
    - Accounting for sequencing site and/or technology in the analysis model
    - Quality control filtering including standard methods such as sites not equally covered between cases and controls are excluded from analysis
  - Proper choice of association test including accounting for imbalance in cases and controls if applicable.
  - Significance thresholds are appropriate (genome-wide, acknowledges multiple testing)
- **Additional criteria for consideration:**
  - Sub-threshold evidence of association in an independent dataset ($P < 0.05$), such as one from a different ancestry, should be recorded in the comment field of the locus report.
  - Note: Disagreement in results from another ancestry is not a reason for exclusion from a specific tier.

Tier System. To begin, we evaluate each analysis within the publication. If it is a meta-analysis, we only evaluate the final meta-analysis results and not each stage contributing to the meta-analysis. Each locus reported within the main text of the publication is ranked based on the strength of evidence for association. See **Table 1** for a summary of each tier requirements. Once tiers have been assigned for all analyses and publications, shared loci are defined using FUMA or a similar program. If the tier ranking of a locus is not the same across different publications or analyses, the GVC adjudicates the tier assignments based on sample size and overlap, phenotypes used, ethnic composition of the studies, quality of the analyses, consistence of effect direction, and support from adjacent SNVs.

Below and in table 1, “meta-analysis” refers to evaluation of multiple cohorts/data sets. “Analysis” implies that only one data set/cohort is considered in the evaluation. The definition of a “cohort” or “data set” is made by the authors of the manuscript under review.

Table 1. Summary of criteria for tier system.

Tier 1: Sufficient evidence of an association	**Tier 2 Sufficient evidence of an association but some sources of evidence are missing, or evidence is not optimal.**	**Tier 3: Suggestive evidence of an association**	**Tier 4: Suggestive evidence of an association but some sources of evidence are missing, or some evidence is suboptimal.**	**Tier 5: Suggestive evidence of an association from a single data set**	**Tier 6: Limited evidence for an association**	**Tier 7: Insufficient evidence of association**
A meta or joint analysis provides: • Genome-wide significant evidence for association (Col U) • Robust analysis (Col AO decision) • Consistent association (Col AA decision) • Robust association (Col AE decision) • All required information (see table 2) necessary to evaluate the analysis and association signals is provided and is fully supportive of a robust/consistent evaluation. (i.e. all option "1"s in cols AO, AA and AE) • All data/analysis is provided to evaluate effect direction and support from adjacent SNVs for a signal (cols AB, AC and AD, and/or AE),	A meta or joint analysis provides: • Genome-wide significant evidence for association (Col U) • Not all required information (see table 2) necessary to evaluate the analysis and associations is provided and/or some of the information is suboptimal. • However, the strength of the association and methods used are such that there is strong support for a locus. (col U and AF to AL? (judgement calls for these methods whether they are good or not) OR • In an AD + Dementia study; most of the statistical evidence comes from either Dementia cohorts (e.g. UKBB or 23&Me) or AD clinic/path cohorts.	A meta or joint analysis provides: • Genome-wide suggestive evidence for association • Robust signal • Consistent association • Robust association • All data/analysis is provided to evaluate effect direction and support from adjacent SNVs for a signal. . **OR** Analysis of a single cohort/dataset provides: • Genome-wide significant evidence for association • Robust analysis • Robust association • All required sources of evidence are provided, and the evidence is optimal	A meta-analysis or joint analysis provides: • Genome-wide suggestive evidence for association Not all required information (see table 2) necessary to evaluate the analysis and associations is provided and/or some of the information is suboptimal **OR** Analysis of a single cohort/dataset* provides: • Genome-wide significant evidence for association • Not all required information (see table 2) necessary to evaluate the analysis and associations is provided and/or some of the information is suboptimal	Analysis of a single cohort/dataset* provides: • Genome-wide suggestive evidence for association • Robust analysis • Robust association • All required sources of evidence are provided, and the evidence is optimal	A meta or joint analysis provides: • Genome-wide suggestive or significant evidence for association There are moderate limitations present in the evidence for a robust signal, and/or a consistent association or robust association. • The strength of the association and/or methods used are such that there is only weak support for a locus.	A meta-analysis or analysis provides: • Genome-wide suggestive or significant evidence for association • Severe limitations, such as: ○ Evidence the association is a false positive. ○ Concerns about technical/analytic issues ○ Data necessary to evaluate the robustness and consistence of the analysis are missing such that there is only weak or no support for a locus. **OR** • No suggestive or genome-wide significant evidence is provided

Tier 1: Sufficient evidence of an association. Evidence is sufficient to conclude that there is an association between this locus and the phenotype based on the following:

- There is a large-scale meta-analysis providing genome-wide significant statistical evidence of a genetic association between a variant and the trait of interest.
- Association results are consistent across the cohorts used in the final meta-analysis presented in the main body of the paper.
- All information is provided to support a robust association as defined below.
- All information is provided to support a robust analysis
- Analysis methods are well-documented and are supported by other work in the literature.
- All data/analysis is provided to evaluate effect direction
- Support from adjacent SNVs (up to +/- 1 Mb) support the signal from the peak SNV (either Locus Zoom plot or tabular data).

Tier 2: Evidence of an association but some information missing or not optimal.

- A large-scale meta-analysis providing genome-wide significant statistical evidence of a genetic association between a variant and the trait of interest. At least one variant meeting the inclusion criteria, or its LD proxy, has genome-wide significant evidence for association with the AD-related trait.
- Not all required information necessary to evaluate the analysis and associations is provided and/or is not fully supportive of robust/consistent evaluation.
- The reviewer will note what information is missing and/or other supportive evidence.
- Analysis methods are well-documented and are supported by other work in the literature.
- The strength of the association and methods used are such that there is strong support for a locus.

OR

- In a study that includes both cohorts where the primary phenotype is **AD** defined by clinical and/or neuropathologic data and where the primary phenotype is **Dementia** (e.g. UKBB or 23&Me or other dementia cohorts), most of the statistical evidence comes from either the Dementia cohorts or from AD clinical/pathology cohorts.

Tier 3: Suggestive evidence of an association. Evidence suggests that there is an association between this locus and the phenotype.

Tier 3 has:

- A large-scale meta-analysis provides suggestive evidence of a genetic association variant and the trait of interest
- Results are consistent across the cohorts used in the final meta-analysis presented in the main body of the paper.
- All information is provided to support a robust association.
- All information is provided to support a robust analysis defined below
- Analysis methods are well-documented and are supported by other work in the literature.
- All data/analysis is provided to evaluate effect direction and support from adjacent SNVs for a signal.

OR

- Genome-wide significant association at a locus in a single cohort
- Robust analysis
- Robust association
- All required sources of evidence are provided, and the evidence is optimal

- All data/analysis is provided to evaluate effect direction and support from adjacent SNVs for a signal.

Tier 4: Suggestive evidence of an association but some information is missing or suboptimal.

- A large-scale meta-analysis providing suggestive evidence of a genetic association between a variant and the trait of interest.
- Not all the required information is provided to conclude that the analysis is robust.
- Not all the required information is provided to determine that the association is robust.
- Analysis methods are well-documented and are supported by other work in the literature.
- The strength of the association and methods used are such that there is support for a locus.

OR

- Analysis of a single cohort that yields genome-wide evidence for an association.
- Not all required information (see table 2) necessary to evaluate the analysis and associations is provided and/or some of the information is suboptimal.

Tier 5: Suggestive evidence of an association from a single data set

- Analysis of a single cohort/dataset provides genome-wide suggestive evidence for association
- All information is provided to support a robust analysis as defined below.
- All information is available to support a robust association as defined below.
- Analysis methods are well-documented and are supported by other work in the literature.
- All required sources of evidence are provided, and the evidence is optimal

Tier 6: Limited evidence of an association. There is some evidence of an association between this locus and AD or closely related phenotypes, but additional supporting evidence is needed to be confident the results are not due to chance, confounding factors, or bias. Criteria for this tier are:

- Genome-wide suggestive or significant evidence for association
- Moderate limitations present in the evidence for a robust signal
- Moderate limitations present in the evidence for a robust association
- Moderate limitations present in the evidence for a consistent association.
- The strength of the association and/or methods used are such that there is only weak support for a locus.

Tier 7: Insufficient evidence to determine whether an association exists. While there is evidence of an association between this locus and AD or a closely related trait in at least one analysis, additional supporting evidence is needed to be confident the results are not a false-positive association due to genotyping/sequencing error, chance, confounding factors, or bias. There is reason to believe the result may not be a true association. Examples of associations that may fall in this tier include:

- The variant may have evidence of genotyping or sequencing error
- The analysis model may not have adequately controlled for confounding factors, bias, *etc*.
- The association was identified in a study with a small sample size (ex., N < 1,000).
- Severe limitations, such as:
  - Evidence the association is a false positive.
  - Concerns about technical/analytic issues
  - Data necessary to evaluate the robustness and consistence of the analysis are missing such that there is only weak or no support for a locus.

OR

- No suggestive or genome-wide significant evidence for an association is provided.

The elements in Table 2 are used to define:

- Variant definition
- Significance
- Consistent results
- Robust results
- Robust analysis

Table 2.

		Tier requirements	Notes
Variant information	Reference genome	Must have	
	Chr:Pos:Ref	Must have	chr:pos with ref allele
	Lead SNP identifier	Must have	rsID
	Lead SNP position	Must have	
	Lead SNP risk allele	Must have	Reported by the publication
	Can you identify the elevated or protective allele?	Must have	Reported by the publication
	Lead SNP risk allele frequency	Extra	Overall AF on risk allele reported by publication
	Lead SNP risk allele frequency – cases	Extra	
	Lead SNP risk allele frequency – controls	Extra	
	Lead SNP beta	Must have one of these	
	Lead SNP beta std error	Must have one of these	
	Lead SNP Z-score	Must have one of these	
	Lead SNP OR + C.I.	Must have one of these	

	lead SNP Bayes' Factor	Must have one of these	
	Lead SNP p-value	Must have	
Significance	Genome-wide significance with $P < 5 \times 10^{-08}$ or Genome-wide suggestive with $P < 1 \times 10^{-06}$	Must have	The minimum is "suggestive" for the data we are collecting
Consistent results	Forest plots	Must have one of these	
	Signed statistics for each separate cohort (OR, beta, *etc.*). Cohort is defined by authors.	Must have one of these	
	line plot (+/-)	Must have one of these	
	heterogeneity statistic	Must have one of these	
	phenotype match (AD, Dementia, or AD + Dementia). AD clin/path cohorts and Dementia cohorts are not considered “similar” cohorts for considering heterogeneity.	Must have an AD-related outcome.	AD-related outcome as defined in the inclusion criteria
	Conclusion ranking for consistent associations	Must have	The overall rank for whether a result is consistent
Robust results	Was conditional analysis of close SNVs performed?	Desirable but not required – note in comments	If not present grade each 'close locus' separately but note no conditional analysis to determine independent loci
	If performed, does conditional analysis concluded the locus is independent?	Desirable but not required – note in comments	If not present grade each 'close locus' separately but note no conditional analysis to determine independent loci
	Locus Zoom or information from supporting nearby SNVs (required for novel common variant loci)	Must have for novel loci - supporting suggestive or significant SNVs in the region	
	Conclusion ranking for robust results	Must have	The overall rank for whether a result is robust
Robust analysis	Q-Q plot	Not required	
	inflation statistics	Must have	
	methodology (Novelty)	Must have	As part of the literature review, we removed papers that only used novel methods; if the paper has a novel method and an established method, we just review the established method
	methods - QC appropriate, not worried about batch effects, etc.	Must have	

	methods - model appropriate	Must have	
	significance thresholds are appropriate (genome-wide, acknowledges multiple testing)	Must have	Using fixed significance threshold of 5e-08 and suggestive threshold of 1e-06
	Population stratification corrected for (PCs, *etc*.)	Must have	
	Analysis reviewed	Must have	If a meta-analysis, focus on the meta-analysis results not individual stages. For reports where multiple models are tested, we will consider the primary analysis (if stated). If not stated, we will consider each model separately and note whether the significance was corrected for multiple testing.
	Analysis type	Must have	Is the analysis of a single dataset or multiple datasets/meta-analysis
	Conclusion ranking for robust analysis	Must have	The overall rank for whether an analysis is robust

Table 3. Complete Criteria for selecting manuscripts to review