**The variant call format provides efficient and robust storage of GWAS summary statistics**

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**Genome-wide association study (GWAS) summary statistics are a fundamental resource for a variety of research applications 1–6. Yet despite their widespread utility, no common storage format has been widely adopted, hindering tool development and data sharing, analysis and integration. Existing tabular formats 7,8 often ambiguously or incompletely store information about genetic variants and their associations, and also lack essential metadata increasing the possibility of errors in data interpretation and post-GWAS analyses. Additionally, data in these formats are typically not indexed, requiring the whole file to be read which is computationally inefficient. To address these issues, we propose an adaptation of the variant call format** 9 **(GWAS-VCF) and have produced a suite of open-source tools for using this format in downstream analyses. Simulation studies determine GWAS-VCF is 9-46x faster than tabular alternatives when extracting variant(s) by genomic position. Our results demonstrate the GWAS-VCF provides a robust and performant solution for sharing, analysis and integration of GWAS data. We provide open access to over 10,000 complete GWAS summary datasets converted to this format (available from:** [**https://gwas.mrcieu.ac.uk**](https://gwas.mrcieu.ac.uk)**).**

**Main**

The GWAS is a powerful tool for identifying genetic loci associated with any trait, including diseases and clinical biomarkers, as well as non-clinical and molecular phenotypes such as height and gene expression 3 (eQTLs). Sharing of GWAS results as summary statistics (i.e. variant, effect size, standard error, p-value etc.) has enabled a range of important secondary research applications including: causal gene and functional variant prioritisation 1, causal cell/tissue type nomination 2, pathway analysis 3, causal inference (Mendelian randomization; MR) 4, risk prediction 3, genetic correlation 5 and heritability estimation 6. However, the utility of GWAS summary statistics is hampered by the absence of a universally adopted storage format and associated tools.

Historic lack of a common standard has resulted in GWAS analysis tools outputting summary statistics in different tabular formats (e.g. plink 10, GCTA 11, BOLT-LMM 12, GEMMA 13, Matrix eQTL 14 and meta-analysis tools e.g. METAL 15). As a consequence, various processing issues are typically encountered during secondary analysis. First, there is often inconsistency and ambiguity of which allele relates to the effect size estimate (the “effect” allele). Confusion over the effect allele can have disastrous consequences on the interpretation of GWAS findings and the validity of post-GWAS analyses. For example MR studies may provide causal estimates with incorrect effect directionality 16. Likewise, prediction models based on polygenic risk scores might predict disease wrongly or suffer reduced power if some of the effect directionalities are incorrect. Second, the schema (i.e. which columns/fields are included and how they are named) of these tabular formats varies greatly. Absent fields can limit analyses and although approaches exist to estimate the values of some of these missing columns (e.g. standard error from P value) imprecision is introduced reducing subsequent test power. Varying field names are easily addressed in principle but can be cumbersome and error prone. Third, data are frequently distributed with no or insufficient metadata describing the study, trait(s), and variants (e.g., trait measurement units, variant id/annotation sources, etc.) which can lead to errors, impede integration of results from different studies and hamper reproducibility. Fourth, querying unindexed text files is slow and memory inefficient, making some potential applications computationally infeasible (e.g. systematic hypothesis-free analyses).

Some proposals for a standard tabular format have been made. The EBI-NHGRI GWAS catalog ([www.ebi.ac.uk/gwas](http://www.ebi.ac.uk/gwas)) developed a tab-separated values (TSV) text format with a minimal set of required (and optional) columns along with standardised headings 7. The SMR tool 8 introduced a binary format for rapid querying of quantitative trait loci. These approaches are adequate for storing variant level summary statistics but do not enforce allele consistency or support embedding of essential metadata. Learning from these examples and our experiences performing high-throughput analyses across two research centres, we developed a set of requirements for a suitable universal format (Table 1). These features place emphasis on consistency and robustness, capacity for metadata to provide a full audit trail, efficient querying and file storage, ensuring data integrity, interoperability with existing open-source tools and across multiple datasets to support data sharing and integration. We determined that adapting the variant call format (VCF) 9 was a convenient and constructive solution to address these issues. We provide evidence demonstrating how the VCF meets our requirements and showcase the capabilities of this medium (Table 1).

The VCF is organised into three components: a flexible file header containing metadata (lines beginning with ‘#’), and a file body containing variant- (one locus per row with one or more alternative alleles/variants) and sample-level information (one sample per column). We adapt this format to include GWAS-specific metadata and utilise the sample column to store variant-trait association data (Figure 1; Supplementary Table 1).

According to the VCF specification, the file header consists of metadata lines containing 1) the specification version number, 2) information about the reference genome assembly and contigs, and 3) information (ID, number, type, description, source and version) about the fields used to describe variants and samples (or variant-trait associations in the case of GWAS-VCF) in the file body. We take advantage of the VCF file header to store additional information about the GWAS including 1) source and version of summary statistics, 2) study IDs (e.g., PMID/DOI of publication describing the study, or accession number and repository of individual-level data), 3) description of the trait(s) studied (e.g., type, association test used, sample size, ancestry and measurement unit) as well as the source and version of trait IDs (e.g., Experimental Factor Ontology 17, Human Phenotyping Ontology 18 or Medical Subject Headings 19 IDs for clinical and other traits, or Ensembl Gene IDs for eQTL datasets).

Unlike VCF where a row can contain information about multiple alternative alleles observed at the same site/locus (and thus may store more than one variant), the GWAS-VCF specification requires that each variant is stored in a separate row of the file body. Each row contains eight mandatory fields: chromosome name (CHROM), base-pair position (POS), unique variant identifier (ID), reference/non-effect allele (REF), alternative/effect allele (ALT), quality (QUAL), filter (FILTER) and variant information (INFO). The ID, QUAL and FILTER fields can contain a null value represented by a dot. Importantly, the ID value (unless null) should not be present in more than one row. [SHOULD WE USE QUAL FOR IMPUTATION QUALITY/INFO SCORE?] The FILTER field may be used to flag poor quality variants for exclusion in downstream analyses. The INFO column is a flexible data store for additional variant-level key-value pairs (fields) and may be used to store for example: population frequency (AF), allele count in called genotypes (AC), total number of alleles in called genotypes (AN), number of samples/individuals with called genotypes (NS), genomic annotations and variant functional effects. We also use the INFO field to store the dbSNP locus identifier (rsid) for the site at which the variant resides. This is because (despite their common usage as variant identifiers) rsids uniquely identify loci (not variants!) and thus cannot be used in the ID field, as we will discuss further at the end of this manuscript. Following the INFO column is a format field (FORMAT) and one or more sample columns which we use to store variant-trait association data, with values for the fields listed in the FORMAT column for example: effect size (ES), standard error (SE) and -log10 P-value (LP).

This format has a number of advantages over existing solutions. First, the VCF provides consistent and robust approaches to storing genetic variants, annotations and metadata. Furthermore, defined variable type requirements reduce parsing errors and prevent unexpected program operation. Second, the VCF is well established and supported by existing tools providing a range of functions for querying, annotating, transforming and analysing genetic data. Third, the GWAS-VCF file header stores comprehensive metadata about the GWAS. Fourth, a GWAS-VCF file can store individual or multiple traits (in one or more sample columns) in a single file which is beneficial for the distribution of GWAS datasets where genotypes of each sample/individual have been tested for association with multiple traits (e.g., eQTL datasets).

Simulations of query performance demonstrate compressed GWAS-VCF is substantially quicker than unindexed and uncompressed TSV format for querying by genomic position. On average GWAS-VCF was 16x faster to extract a single variant using chromosome position (mean query duration in GWAS-VCF 0.08 seconds [95% CI 0.08, 0.08]) vs mean query duration in TSV 1.29 seconds [95% CI 1.29, 1.30]) and 9x quicker using the rsid (0.09 seconds [95% CI 0.09, 0.09] vs 0.81 seconds [95% 0.80, 0.82]). Using a 1Mb window of variants GWAS-VCF was 46x quicker (0.11 seconds [95% CI 0.11, 0.11] vs 5.02 seconds [95% CI 4.99, 5.04]). Although querying on association P value was faster using TSV (mean query duration in TSV 7.18 seconds [95% CI 7.09, 7.26] vs mean query duration in GWAS-VCF 18.04 seconds [95% CI 17.92, 18.16]) GWAS-VCF could be improved by using variant flags (i.e. in the INFO field) to highlight records below prespecified thresholds if the exact value is unimportant. For example, all variants below genome-wide significance (P < 5e-8) or a more relaxed threshold (e.g. P < 5e-5).

To automate the conversion of existing summary statistics files to the GWAS-VCF format, we developed open-source Python3 software (Gwas2VCF; Table 2). The application reads in metadata and variant-trait association data using a user-defined schema. During processing, variants are harmonised using a supplied reference genome file to ensure the non-effect allele matches the reference sequence enabling consistent directionality of allelic effects across studies. Insertion-deletion variants are left-aligned and trimmed for consistent representation. Finally, the VCF is indexed using tabix 20 and rsidx 22 which enable rapid queries by genomic position and rsid , respectively. We have developed a freely available web application providing a user-friendly interface for this implementation and encourage other centres to deploy their own instance (Table 2).

Once stored in a GWAS-VCF file, summary statistics can be read and queried using R or Python programming languages with our open-source libraries (Table 2) or from the command line using for example: bcftools 20, GATK 21 or bedtools 23. Alternatively, GWAS-VCF may be converted to NHGRI-EBI format or any other tabular format to support incompatible tools. Further, gwasglue provides convenient R programming functions to automate preparation of genetic association data for downstream analysis (Table 2). Currently, methods exist for streamlining variant fine-mapping 24–28, colocalization 29, MR 30 and data visualisation 31. New methods are being actively added and users may request new features via the repository issues page.

To encourage adoption, we made openly available over 10,000 complete GWAS summary statistics in GWAS-VCF format as part of the IEU GWAS database. These studies include a broad range of traits, diseases and molecular phenotypes initially collected for the MR Base platform 32.

A limitation of current summary statistics formats, including GWAS-VCF, is the lack of a widely adopted and stable representation of sequence variants that can be used as universal unique identifier for said variants. Published summary statistics often use rsids 33 to identify variants but this practice is inappropriate because rsids are locus identifiers and do not distinguish between multiple alternative alleles observed at the same site. Moreover, rsids are not stable as they can be merged and retired over time. The reason this is a problem is that in GWAS summary statistics every record represents the effect of a specific allele on one or more traits, and if a record identifier is used that is not unique for each allele it cannot technically be considered an identifier. An alternative approach is to concatenate chromosome, base-position, reference and alternative allele field values into a single string, but this is non-standardised, and genome build specific. Worst still is the common approach of mixing these types of identifiers within a single file. In version 1.0.0 of the GWAS-VCF specification we suggest querying variants by chromosome and base-position and filtering the output to retain the target substitution (implemented in our parsers), but we acknowledge that this approach can be cumbersome and difficult to interoperate with other software. The ideal solution would be to populate the ID column of a GWAS-VCF file using universally accepted and unique variant identifiers. We have reviewed several existing variant identifier formats as candidates for the variant identifier field, to be implemented in the next version of the specification (Supplementary Table 2). However, we refrain from making a unilateral choice at this juncture because successful implementation will require consultation from a range of stakeholders. The genetics community uses different approaches already to deal with the problem of sequence variant representation and there is a need to coalesce upon a single format.

Here we present an adaptation of the VCF specification for GWAS summary statistics storage that is amenable to high-throughput analyses and robust data sharing and integration. We implement open-source tools to convert existing summary statistics formats to GWAS-VCF, and libraries for reading or querying this format and integrating with existing analysis tools. Finally, we provide complete GWAS summary statistics for over 10,000 traits in GWAS-VCF. These resources enable convenient and efficient secondary analyses of GWAS summary statistics and support future tool development.

**Code availability**

Query performance evaluation source code available from GitHub (https://github.com/MRCIEU/gwas-vcf-performance) or pre-built image available from DockerHub (mrcieu/gwas-vcf-performance)

**Data availability**

Version 1.0.0 of the GWAS -VCF format specification is available from: <https://github.com/MRCIEU/gwas-vcf-spec/releases/tag/1.0.0>

Full summary statistics for over 10,000 GWAS in VCF format are available from the IEU GWAS Database (<https://gwas.mrcieu.ac.uk>)

**Method**

**Specification**

The specification was developed through experience of collecting and harmonising GWAS summary data across two research centres at scale 32 and performing a range of representative high throughput analyses on these data (for example LD score regression 34, MR 35, genetic colocalisation analysis 36 and polygenic risk scores 37).

**Query performance simulation**

Densely imputed summary statistics (13,791,467 variants) for a large GWAS of body mass index data were obtained from Neale et al 38. The data were mapped to VCF using Gwas2VCF v1.1.1 and processed using bcftools v1.10 20 to remove multiallelic variants or records with missing dbSNP identifiers. A tabular (unindexed) file was prepared from the VCF to replicate a typical storage medium currently used for distributing summary statistics. Query runtime performance was compared between tabix and standard UNIX commands under the following conditions: single variant selection using dbSNP identifier or chromosome position, multi-variant selection by association P value (thresholds: P < 5e-8, 0.2, 0.4, 0.6, 0.8) or 1 Mb genomic interval. Tests were undertaken with 100 repetitions using VCF or unindexed text formats with and without GZIP compression on an Ubuntu v18.04 server with Intel Xeon(R) 2.0 Ghz processor. All comparisons were performed using singled thread operations and therefore differences in runtime performance were due to tool and/or file index usage.

**References**

1. Hou, L. & Zhao, H. A review of post-GWAS prioritization approaches. *Front. Genet.* **4**, 280 (2013).

2. Finucane, H. K. *et al.* Partitioning heritability by functional annotation using genome-wide association summary statistics. *Nat. Genet.* **47**, 1228–1235 (2015).

3. Visscher, P. M. *et al.* 10 Years of GWAS Discovery: Biology, Function, and Translation. *American Journal of Human Genetics* **101**, 5–22 (2017).

4. Smith, G. D. & Ebrahim, S. ‘Mendelian randomization’: Can genetic epidemiology contribute to understanding environmental determinants of disease? *International Journal of Epidemiology* (2003). doi:10.1093/ije/dyg070

5. Bulik-Sullivan, B. *et al.* LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat. Genet.* (2015). doi:10.1038/ng.3211

6. Yang, J., Zeng, J., Goddard, M. E., Wray, N. R. & Visscher, P. M. Concepts, estimation and interpretation of SNP-based heritability. *Nature Genetics* **49**, 1304–1310 (2017).

7. Buniello, A. *et al.* The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Res.* **47**, D1005–D1012 (2019).

8. Zhu, Z. *et al.* Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. *Nat. Genet.* **48**, 481–487 (2016).

9. Danecek, P. *et al.* The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158 (2011).

10. Purcell, S. *et al.* PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* (2007). doi:10.1086/519795

11. Yang, J., Lee, S. H., Goddard, M. E. & Visscher, P. M. GCTA: A tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* **88**, 76–82 (2011).

12. Loh, P. R. *et al.* Efficient Bayesian mixed-model analysis increases association power in large cohorts. *Nat. Genet.* **47**, 284–290 (2015).

13. Zhou, X. & Stephens, M. Genome-wide efficient mixed-model analysis for association studies. *Nat. Genet.* **44**, 821–824 (2012).

14. Shabalin, A. A. Gene expression Matrix eQTL: ultra fast eQTL analysis via large matrix operations. **28**, 1353–1358 (2012).

15. Willer, C. J., Li, Y. & Abecasis, G. R. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinforma. Appl. NOTE* **26**, 2190–2191 (2010).

16. Hartwig, F. P., Davies, N. M., Hemani, G. & Smith, G. D. Two-sample Mendelian randomization: avoiding the downsides of a powerful, widely applicable but potentially fallible technique. *Int. J. Epidemiol.* 1717–1726 (2016). doi:10.1093/ije/dyx028

17. Malone, J. *et al.* Databases and ontologies Modeling sample variables with an Experimental Factor Ontology. **26**, 1112–1118 (2010).

18. Carmody, L. *et al.* Expansion of the Human Phenotype Ontology (HPO) knowledge base and resources. *Hanns LochmüllerLochm¨Lochmüller* **47**, (2019).

19. Medical Subject Headings - Home Page. Available at: https://www.nlm.nih.gov/mesh/meshhome.html. (Accessed: 16th April 2020)

20. Li, H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. *Bioinformatics* **27**, 2987–93 (2011).

21. McKenna, A. *et al.* The genome analysis toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* (2010). doi:10.1101/gr.107524.110

22. bioforensics/rsidx: Library for indexing VCF files for random access searches by rsID. Available at: https://github.com/bioforensics/rsidx. (Accessed: 5th March 2020)

23. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinforma. Appl. NOTE* **26**, 841–842 (2010).

24. Benner, C. *et al.* Genetics and population analysis FINEMAP: efficient variable selection using summary data from genome-wide association studies. doi:10.1093/bioinformatics/btw018

25. Kichaev, G. *et al.* Integrating Functional Data to Prioritize Causal Variants in Statistical Fine-Mapping Studies. *PLoS Genet.* **10**, e1004722 (2014).

26. Kichaev, G. & Pasaniuc, B. Leveraging Functional-Annotation Data in Trans-ethnic Fine-Mapping Studies. *Am. J. Hum. Genet.* **97**, 260–271 (2015).

27. Kichaev, G. *et al.* Improved methods for multi-trait fine mapping of pleiotropic risk loci. *Bioinformatics* **33**, 248–255 (2017).

28. Hormozdiari, F., Kostem, E., Kang, E. Y., Pasaniuc, B. & Eskin, E. Identifying causal variants at loci with multiple signals of association. *Genetics* **198**, 497–508 (2014).

29. Wallace, C. Statistical Testing of Shared Genetic Control for Potentially Related Traits. *Genet. Epidemiol.* **37**, 802–813 (2013).

30. hemani, gibran *et al.* MRCIEU/TwoSampleMR: WellcomeOpen. (2019). doi:10.5281/ZENODO.3298001

31. jrs95/gassocplot: Regional association plotter for genetic and epigenetic data. Available at: https://github.com/jrs95/gassocplot. (Accessed: 21st April 2020)

32. Hemani, G. *et al.* The MR-base platform supports systematic causal inference across the human phenome. *Elife* **7**, (2018).

33. Home - SNP - NCBI. Available at: https://www.ncbi.nlm.nih.gov/snp/. (Accessed: 16th March 2020)

34. Zheng, J. *et al.* Databases and ontologies LD Hub: a centralized database and web interface to perform LD score regression that maximizes the potential of summary level GWAS data for SNP heritability and genetic correlation analysis. *Bioinformatics* **33**, 272–279 (2017).

35. Hemani, G. *et al.* Automating Mendelian randomization through machine learning to construct a putative causal map of the human phenome. *bioRxiv* 173682. (2017). doi:10.1101/173682

36. Richardson, T. G., Hemani, G., Gaunt, T. R., Relton, C. L. & Davey Smith, G. A transcriptome-wide Mendelian randomization study to uncover tissue-dependent regulatory mechanisms across the human phenome. *Nat. Commun.* **11**, 1–11 (2020).

37. Richardson, T. G., Harrison, S., Hemani, G. & Smith, G. D. An atlas of polygenic risk score associations to highlight putative causal relationships across the human phenome. *Elife* **8**, (2019).

38. UK Biobank — Neale lab. Available at: http://www.nealelab.is/uk-biobank/. (Accessed: 25th February 2020)

39. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinforma. Appl. NOTE* **25**, 2078–2079 (2009).

40. Obenchain, V. *et al.* Sequence analysis VariantAnnotation: a Bioconductor package for exploration and annotation of genetic variants. **30**, 2076–2078 (2014).

41. Gentleman, R. C. *et al.* *Open Access Bioconductor: open software development for computational biology and bioinformatics*. *Genome Biology* **5**, (2004).

42. Huber, W. *et al.* Orchestrating high-throughput genomic analysis with Bioconductor. *Nat. Methods* **12**, 115–121 (2015).

43. Bioconductor - Home. Available at: https://www.bioconductor.org/. (Accessed: 27th March 2020)

44. pysam-developers/pysam: Pysam is a Python module for reading and manipulating SAM/BAM/VCF/BCF files. It’s a lightweight wrapper of the htslib C-API, the same one that powers samtools, bcftools, and tabix. Available at: https://github.com/pysam-developers/pysam. (Accessed: 10th March 2020)

45. broadinstitute/picard: A set of command line tools (in Java) for manipulating high-throughput sequencing (HTS) data and formats such as SAM/BAM/CRAM and VCF. Available at: https://github.com/broadinstitute/picard. (Accessed: 25th February 2020)

46. GenomicsDB/GenomicsDB: Highly performant data storage in C++ for importing, querying and transforming variant data with Java/Spark. Used in gatk4. Available at: https://github.com/GenomicsDB/GenomicsDB. (Accessed: 25th February 2020)

47. Voss, K., Gentry, J. & Auwera, G. Van Der. GATK4 + WDL + Cromwell. *F1000Research* **6**, 4 (2017).

48. Morales, J. *et al.* A standardized framework for representation of ancestry data in genomics studies, with application to the NHGRI-EBI GWAS Catalog. *Genome Biol.* **19**, 21 (2018).

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**Author contributions**

All authors contributed the manuscript and storage format specification. G.H. and E.M. designed the research. M.L. and G.H. wrote software packages and performed query performance simulations. B.E. and G.H. prepared the GWAS data.

**Competing interest**

None.

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Table 1. Requirements for a summary statistics storage format and solutions offered by the VCF

|  |  |
| --- | --- |
| Requirement | Solution using the variant call format |
| Human readable and easy to parse | Easily read with any text viewer. Mature open-source parsing libraries are available (HTSLIB 39 and HTSJDK 39) and implemented in most modern programming languages, for example: VariantAnnotation 40 R-package is available from Bioconductor 41–43 and python package pysam 44. Bcftools 20, GATK 21, bedtools 23 and others provides user-friendly functionality from the command line. |
| Unambiguous interpretation of the data | Data field descriptions, value types and number of values are required and defined in the file header. File validity is enforced during each read/write. |
| Unambiguous representation of bi-allelic, multi-allelic and insertion-deletion variants | Every variant substitution is represented by reference and alternative allele haplotypes defining the exact base change on the forward strand. The reference allele is required to match genome sequences defined in the file header. The alternative allele is always the effect allele allowing consistency between studies for ease of comparison. |
| Genomic information can be validated | The file header contains information about reference genome assembly and contigs. Reference alleles must match the sequence in the referenced genome build (in FASTA format). GATK 21 ValidateVariants can be used to verify file format validity and compare reference allele information against the corresponding genome reference sequence. |
| Flexibility on which GWAS fields are recorded and enforcement of essential fields | All fields are defined in the file header and can be set optional or required as desired. The supplement contains essential fields and reserved keys. |
| Capacity to store metadata about the study and trait(s) | The file header contains information about the source and version of summary statistics, study IDs (e.g., PMID/DOI of publication describing the study, or accession number and repository of individual-level data), description of the trait(s) studied (e.g., type, association test used, and measurement unit) as well as the source and version of trait IDs (e.g., Experimental Factor Ontology 17, Human Phenotyping Ontology 18 or Medical Subject Headings 19 IDs for clinical and other traits, or Ensembl Gene IDs for eQTL datasets). |
| Allows multiple traits to be stored together | The SAMPLE column was chosen to store variant-trait association data to allow for storage of multiple traits in a single VCF file, or as individual files if desired. |
| Rapid querying by variant identifier, genomic position interval or GWAS summary statistics value (range or exact value) | The file is sorted karyotypically and indexed by chromosome position using tabix 20 to enable fast queries by genomic position. Secondary indexing on dbSNP identifier is also provided using rsidx 22. Refer to performance comparisons of indexed VCF files and standard UNIX tools. |
| File compression | VCF files may be compressed with block GZIP 20 or converted to a binary call file which is a binary VCF companion format 20. |
| Readable by existing open-source tools | A large number of tools support VCF files including: GATK 21, Picard 45, bcftools 20, bedtools 23, vcftools 9 and plink 10. Bcftools 20 can also provide a tabular extract for use with non-compatible tools. |
| Amenable to cloud-based streaming and database storage | Genomic intervals may be extracted over a network using a range-request which extracts file segments without transferring the whole file. This enables rapid streaming of queries over the internet. For high-throughput and distributed storage and querying, VCF files can be easily imported into GenomicsDB 46. |

GWAS, genome-wide association study. dbSNP, database of single-nucleotide polymorphisms. HTSLIB, high-throughput sequencing data library. HTSJDK, high-throughput sequencing data java development kit. GATK, genome-analysis toolkit. dbSNP, single nucleotide polymorphism database. eQTL, expression quantitative trait loci.

Table 2. Open-source tools for working with GWAS-VCF

|  |  |  |  |
| --- | --- | --- | --- |
| Program | Purpose | Implementation | Source code link |
| gwas2vcf | Mapping tabular GWAS summary statistics and NHGRI-EBI format to VCF | Python3 (Docker) | <https://github.com/mrcieu/gwas2vcf> |
| gwas2vcfweb [http://vcf.mrcieu.ac.uk](http://vcf.mrcieu.ac.uk/) | Front-end and queue schedular for gwas2vcf | Python3, Cromwell47  (Docker) | <https://github.com/mrcieu/gwas2vcfweb> |
| R/gwasvcf | Library for querying and reading GWAS-VCF files | R | <https://github.com/mrcieu/gwasvcf> |
| pygwasvcf | Library for querying and reading GWAS-VCF files | Python3 | <https://github.com/mrcieu/pygwasvcf> |
| R/gwasglue | Library for processing GWAS summary statistics ready for secondary analysis | R | <https://github.com/mrcieu/gwasglue> |
| LD Score Regression 5 (patch) | Estimating genetic correlation and heritability | Python | <http://github.com/explodecomputer/ldsc> |

GWAS, genome-wide association study. LD, linkage disequilibrium. VCF, variant call format. NHGRI-EBI, National Human Genome Research Institute and European Bioinformatics Institute.

Figure 1. VCF format adapted to store GWAS summary statistics (GWAS-VCF)



The VCF file contains study and trait(s) metadata, variant-level data, and variant-trait association summary statistics. Each field is defined in the file header including variable type and number of values. The format can store the results of a GWAS with one or more traits in a single file.

Figure 2. Performance comparison for querying summary statistics in plain text and VCF format



Mean query time (log milliseconds [lower is quicker]; repetitions n=100) to extract either: a single variant using the chromosome position or dbSNP identifier or multiple variants using a 1 Mb interval or association P value. AWK, grep, bcftools 20 and rsidx 22 were evaluated using uncompressed and GZIP/BGZIP 20 compressed unindexed text and VCF. Error bars represent the 95% confidence interval.

Supplementary Table 1. Reserved fields for GWAS-VCF file metadata

|  |  |  |
| --- | --- | --- |
| Field | Location | Description |
| Trait fields | | |
| Trait | Header | Phenotype description |
| Ontology | Header | Ontology used to describe phenotype (or null if ontology not used) |
| Unit | Header | Phenotype units |
| Article | Header | Reference to publication in uniform resource identifier format (scheme:path) i.e. doi:10.1000/xyz123 or pubmed:12345678 |
| Population | Header | Participant ancestry (or mixed ancestry) using the standardised framework 48 |
| TotalControls\* | Header | Total number of controls in the association study (or total sample size if continuous outcome) |
| TotalCases | Header | Total number of cases in the association study (or null if continuous trait) |
| TraitType\* | Header | Type of GWAS outcome [Continuous or Binary] |
| TotalVariants\* | Header | Total number of variants in input |
| VariantsNotRead\* | Header | Number of variants that could not be read |
| HarmonisedVariants\* | Header | Total number of harmonised variants |
| VariantsNotHarmonised\* | Header | Total number of variants that could not be harmonised |
| SwitchedAlleles\* | Header | Total number of variants strand switched |
| Variant fields | | |
| RSID | Info | dbSNP identifier for variant locus |
| AF | Info or Sample¥ | Alternate allele frequency in the association study |
| AC | Info or Sample¥ | Allele count in called genotypes |
| AN | Info or Sample¥ | Total number of alleles in called genotypes |
| NS | Info or Sample¥ | Number of samples/individuals with called genotypes |
| SS | Info or Sample¥ | Sample size used to estimate genetic effect |
| EZ | Sample | Z-score provided if it was used to derive the ES and SE fields |
| SI | Sample | Accuracy score of summary association statistics imputation |
| NC | Info or Sample¥ | Number of cases used to estimate genetic effect |
| ID | Info or Sample¥ | Variant identifier provided in the summary statistics (i.e. marker identifier) |
| ES\* | Sample | Effect size estimate relative to the alternative allele |
| SE\* | Sample | Standard error of effect size estimate |
| LP\* | Sample | -log10 P-value for effect estimate |

Header, VCF header. Info, VCF variant-level information field. Sample, VCF trait-level information. \* Required fields. ¥ Variable is placed in the INFO field if the value is the same across all traits or the SAMPLE field if different for each trait

Supplementary Table 2. Proposed variant identifier schemes for the ID column in the VCF file body and file configuration

|  |  |  |
| --- | --- | --- |
| VCF row identifier (ID column) | Advantages | Disadvantages |
| dbSNP rsID with multiallelic variants on a single row  Example:  rs376272854 | * No duplication of information already in the row * Rsidx provides fast dbSNP ID queries * Widely used * Short length * Compatibility with existing tools (rsid is encouraged by VCF v4.2 specification) | * Refers to a position rather than a substitution * Complexity and ambiguity of manipulating multiallelic rows * Does not distinguish between multiple alternative alleles and therefore a positional identifier * Multiple rsids can point to the same position (e.g. new dbSNP entries awaiting merge with existing records) |
| No value in ID column with multiallelic variants on separate rows | * No duplication of information already in the row * Avoids the complexities of a variant identifier | * Variant queries include multiple fields (chromosome, position, reference and alternative allele) * No guarantees of row uniqueness * Difficult to operate with other software that requires a unique substitution identifier |
| HGVS DNA nomenclature with multiallelic variants on separate rows  Example:  chr2:g.84918761\_84918811del | * Unique identifier for every substitution * Supports one substitution per row in the VCF which is easier to parse * Short insertion-deletion encoding * Known format | * Duplicates information already stored in the row * Not stable between genome builds * Comparing between builds is difficult * Not widely used for GWAS |
| Concatenation of chromosome, position and alleles with multiallelic variants on separate rows  Example:  chr2:84918760:  CCCAACCCTGCTGTCAT  AATGCATAAGCAGCCAC  AGACAGTAAGTGAATGAA:C | * Unique identifier for every substitution * Supports one substitution per row in the VCF which is easier to parse * Known format | * Duplicates information already stored in the row * Comparing between builds is difficult * Not stable between genome builds * Long insertion-deletion coding |
| SPDI (Sequence-id, Position, Deleted Sequence, Insertion Sequence separated by a colon) with multiallelic variants on separate rows  Example:  NC\_000002.12: 84918760: CCCAACCCTGCTGTCAT  AATGCATAAGCAGCCAC  AGACAGTAAGTGAATGAA:C | * Unique identifier for every substitution * Supports one substitution per row in the VCF which is easier to parse   Known format | * Duplicates information already stored in the row * Comparing between builds is difficult * Not stable between genome builds * Long insertion-deletion coding |
| Concatenation of chromosome, position and alleles using MD5 hash to shorten long alleles with multiallelic variants on separate rows  Example:  chr2:84918760-7c43e7284b58ba06e  7438bff62376edf:C | * Unique (almost) identifier for every substitution * Supports one substitution per row in the VCF which is easier to parse * Short insertion-deletion coding | * Duplicates information already stored in the row * Not stable between genome builds * Comparing between builds is difficult * Cannot reverse hash without database * Not widely used * Very tiny chance of a hash collision |
| GA4GH Variation Representation (SHA-512 message digest of the chromosome position and alternative allele with multiallelic variants on separate rows  Example:  ga4gh:VA.yOoxi7-uUnJyn4QkQ23h6RJuT4Zqarow | * Unique (almost) identifier for every substitution * Supports one substitution per row in the VCF which is easier to parse   Short insertion-deletion coding | * Duplicates information already stored in the row * Not stable between genome builds * Comparing between builds is difficult * Cannot reverse hash without database * Not widely used * Very tiny chance of a hash collision |

GWAS, genome-wide association study. VCF, variant call format. Rsidx, file index using the dbSNP identifier. MD5, message-digest algorithm. HGVS, Human Genome Variation Society. GA4GH, Global Alliance for Genomics and Health. SHA, Secure Hash Algorithm.