Supplemental Information

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The version of the Genome Aggregation Database (gnomAD) v3 presented in this manuscript is a catalog containing 759,302,267 short nuclear variants (644,267,978 passing stringent variant quality control [QC]) based on whole-genome sequencing of 76,156 samples (passing QC from an initial collection of 153,030 samples) mapped to the GRCh38 build of the human reference genome. In this release, we have included more than 3,000 new samples specifically chosen to increase the ancestral diversity of the resource, and for the first time, we provide individual genotypes in addition to variant calls for a subset of gnomAD, which includes new data from >60 distinct populations from Africa, Europe, the Middle East, South and Central Asia, East Asia, Oceania, and the Americas. Many of the processing, quality control, and analysis procedures closely resemble those from the 15,748 genomes from the gnomAD v2 manuscript (Karczewski et al. 2020). In this supplement, we highlight the differences where applicable.

Data processing and variant calling

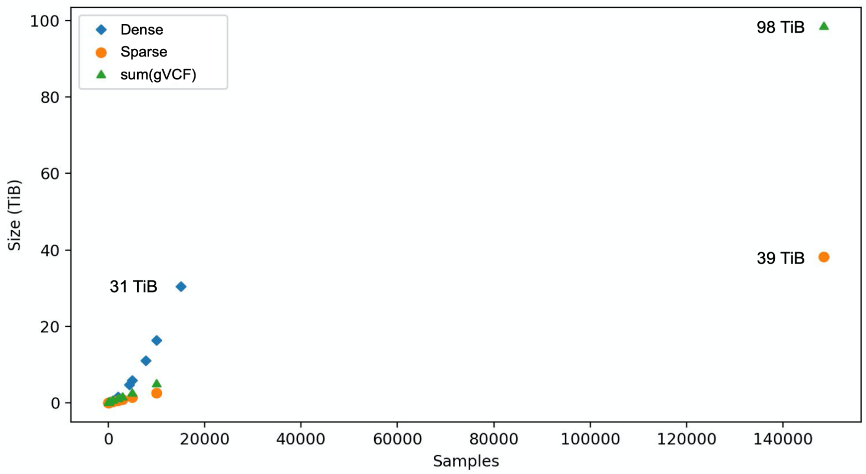
Whole genome sequences were mapped using bwa mem 0.7.15.r1140 against the GRCh38 version hs38DH, which includes decoy contigs and HLA genes (FASTA located at https://console.cloud.google.com/storage/browser/gcp-public-data--broad-references/hg38/v0/). Reads were then processed using the GATK best practices using GATK4 for BQSR and GATK3.5 for HaplotypeCaller to produce gVCFs.

Previous approaches (e.g. gnomAD v2) have typically involved the joint calling of the full cohort using GATK to produce a VCF file with a genotype for each sample at every position where at least one sample contains a non-reference allele. However, this approach would not scale to 150,000 samples, due to time and memory limitations, as well as storage: the output would require about 900TB and be prohibitively expensive to store and compute over.

Instead, we implemented and used a novel combiner within Hail (described in detail in (Karczewski et al. 2021)), which combines gVCFs into a sparse MatrixTable (MT). gVCFs are single-sample files, which contain one row for each genomic position where a non-reference allele is found in the sample and, unlike VCFs, a row for each reference block start. Reference blocks are contiguous bases where the sample is homozygous reference within certain confidence boundaries. In gnomAD v3, we used the following three confidence bins: No coverage / evidence; Genotype quality < Q20; and Genotype quality >= Q20. For each of these bins, the reference block stores the minimum and median coverage, and the minimum genotype quality for the bases residing in the block.

Using this new sparse data format, the full gnomAD v3 MT only requires 20TB of storage (Supplementary Figure 1). This new format scales linearly with the number of samples and is lossless with respect to the input sample gVCFs. Importantly, much more granular QC metrics, previously collapsed into the INFO field across samples, are preserved at each non-reference genotype in the data, such as strand balance, read position metrics (ReadPosRankSum), etc. Thus, new data can be appended to existing data without re-processing of the previously processed samples. We demonstrated the power of this data format by adding 4,598 genomes to our original gnomAD v3 release of 71,702 genomes. Finally, while not currently implemented, it is possible to re-export a gVCF from this format, removing the need for storing the gVCFs.

Supplementary Figure 1 | Growth of data size with number of samples. The dense representation (VCF) grows super-linearly, while the aggregate gVCF size and the SparseMT representation grow linearly. The



final gnomAD v3 sparse dataset was smaller still (20TiB) due to increased compression from broader reference block confidence bins.

Sample QC

The sample QC process was similar to that of gnomAD v2 (Karczewski et al. 2020). Briefly, hard filters were applied to remove samples of poor quality as well as samples that did not have permissions for public release of aggregate data. Next, we inferred sex for each sample and removed samples with sex chromosome aneuploidies or ambiguous sex assignment: here, we modified the original pipeline by using normalized coverage on both X and Y in order to infer sample sex. We defined and used a set of high quality sites to infer relatedness between samples, allowing us to filter to a set of unrelated individuals, and assign ancestry to each sample. Finally, we filtered samples that were determined to be outliers based on sample QC metrics, using a novel regression-based method. All quality control and processing steps were performed using Hail 0.2.62 (Hail Team. Hail 0.2.62-84fa81b9ea3d. https://github.com/hail-is/hail/commit/84fa81b9ea3d.).

Hard filtering

We computed sample QC metrics using the Hail ‘sample_qc’ module on all autosomal bi-allelic single nucleotide variants (SNVs). We removed samples that were clear outliers for the number of SNVs (< 2.4 million or > 3.75 million), number of singletons (> 100,000), ratio of heterozygous to homozygous variants > 3.3, and a mean coverage on chromosome 20 of < 15X. Additionally, for 87,756 of the 92,306 releasable samples where BAM-level metrics were available, we removed samples that were outliers for percent contamination (> 5%), percent chimeras: (> 5%), and median insert size (< 250bp).

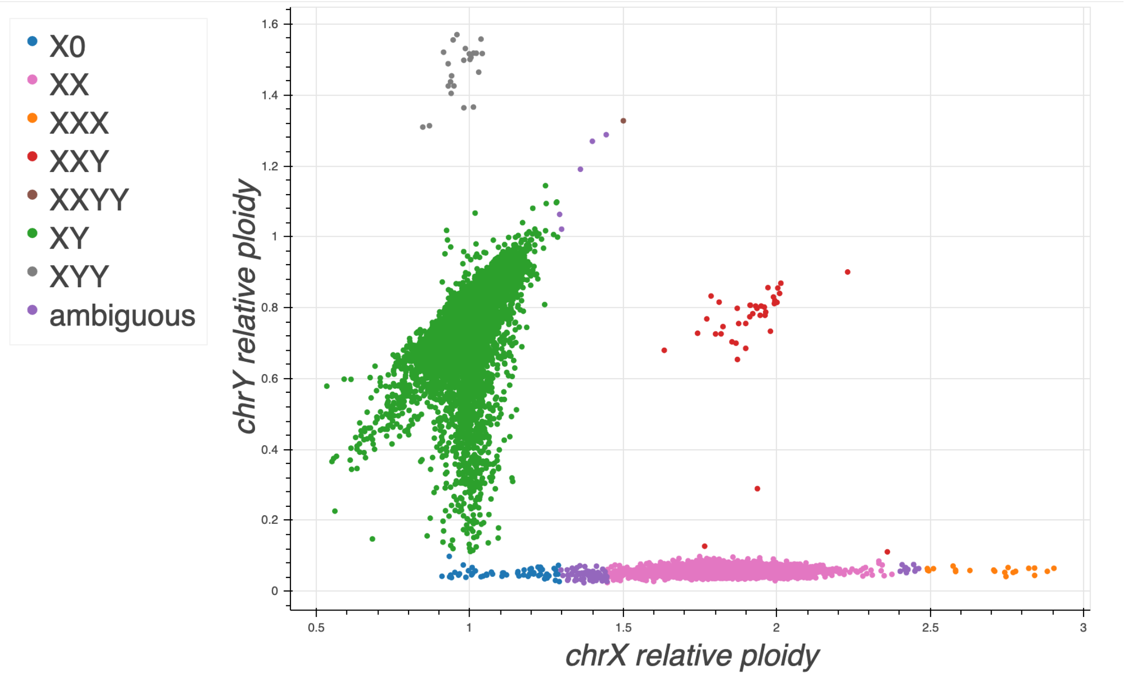
Sex inference

To infer sex, we computed the mean coverage on non-pseudoautosomal (non-PAR) regions of chromosome X and Y and normalized these values using the mean coverage on chromosome 20. In addition, we ran the Hail ‘impute_sex’ function on the non-PAR regions of chromosome X to compute the inbreeding coefficient F-stat. Based on these three metrics, we assigned a number of X chromosomes based on normalized X coverage 0-1.2913 (1 X), 1.4477-2.3961 (2 X), or 2.4909+ (3 X), and a number of Y chromosomes based on normalized Y coverage 0-0.1 (no Y), 0.1-1.1645 (1 Y), 1.2381+ (2 Y). The final assignments are shown in Supplementary Table 1 and Supplementary Figure 2.

Supplementary Table 1 | Sex chromosome inference. The coverages for normalized X and Y coverages are shown for each sex chromosome inference assignment, alongside total number of releasable samples that pass QC to this point.

		X chromosome coverage		Y chromosome coverage	
Inferred chromosomes	**sex Total**	**Lower cutoff**	**Upper cutoff**	**Lower cutoff**	**Upper cutoff**
XX	46,361	1.4477	2.3961	0	0.1
XY	45,129	0	1.2913	0.1	1.1645
XO	425	0	1.2913	0	0.1
XXY	107	1.4477	2.3961	1.2381	1.1645
XXX	34	2.4909	-	0	0.1
XYY	31	0	1.2913	1.2381	-
XXXY	4	2.4909	-	0.1	1.1645
XXYY	3	1.4477	2.3961	1.2381	-
ambiguous	212	All others		All others	

Supplementary Figure 2 | Sex inference. A scatter plot of ploidy on chromosomes X and Y is shown for each individual in the dataset. Points are colored by inferred sex haplotype.



Defining a high quality set of sites for QC

In order to perform relatedness and ancestry inference, we first selected a set of high quality QC sites as follows:

1. We took all sites that were used for gnomAD v2.1 and lifted them over to GRCh38
2. We added ~5k sites widely used for quality control of GWAS data (Purcell et al. 2014) and lifted these sites over to GRCh38
3. From these two sets of sites, we then selected all bi-allelic SNVs with an Inbreeding coefficient > -0.25 (no excess of heterozygotes)

In total, we ended up with 76,419 high quality variants for relatedness and ancestry inference.

Relatedness inference

We used PC-Relate (implemented in Hail 'pc_relate') (Conomos et al. 2016) to compute relatedness, followed by Hail's 'maximal_independent_set' in order to select as many samples as possible, while asserting that the final dataset includes no pairs of first and second degree relatives. When multiple samples could be selected, we kept the sample with the highest coverage as a tie-breaker.

Ancestry assignment

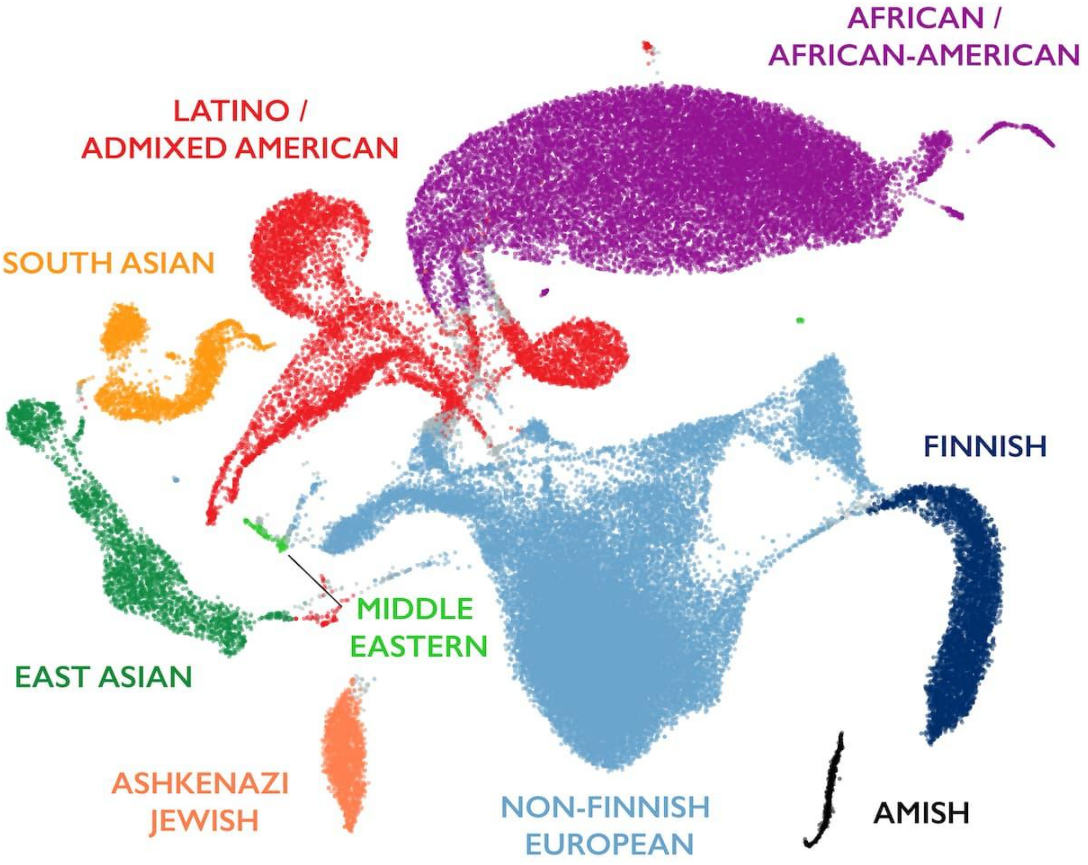
We used principal component analysis (PCA; using the 'hwe_normalized_pca' function in Hail) on the set of high quality variants in our unrelated samples, and selected the first 16 PCs to assign ancestry. We then trained a random forest classifier using 22,054 samples with known ancestry and 14,828 samples for which we had a population label from gnomAD v2 as training samples and using the PCs as features. We assigned ancestry to all samples for which the probability of that ancestry was > 75% according to the random forest model. All other samples were unassigned (labeled oth). A UMAP embedding of the first

PCs is shown in Supplementary Figure 3 (Diaz-Papkovich, Anderson-Trocme, and Gravel 2018; McInnes et al. 2018).

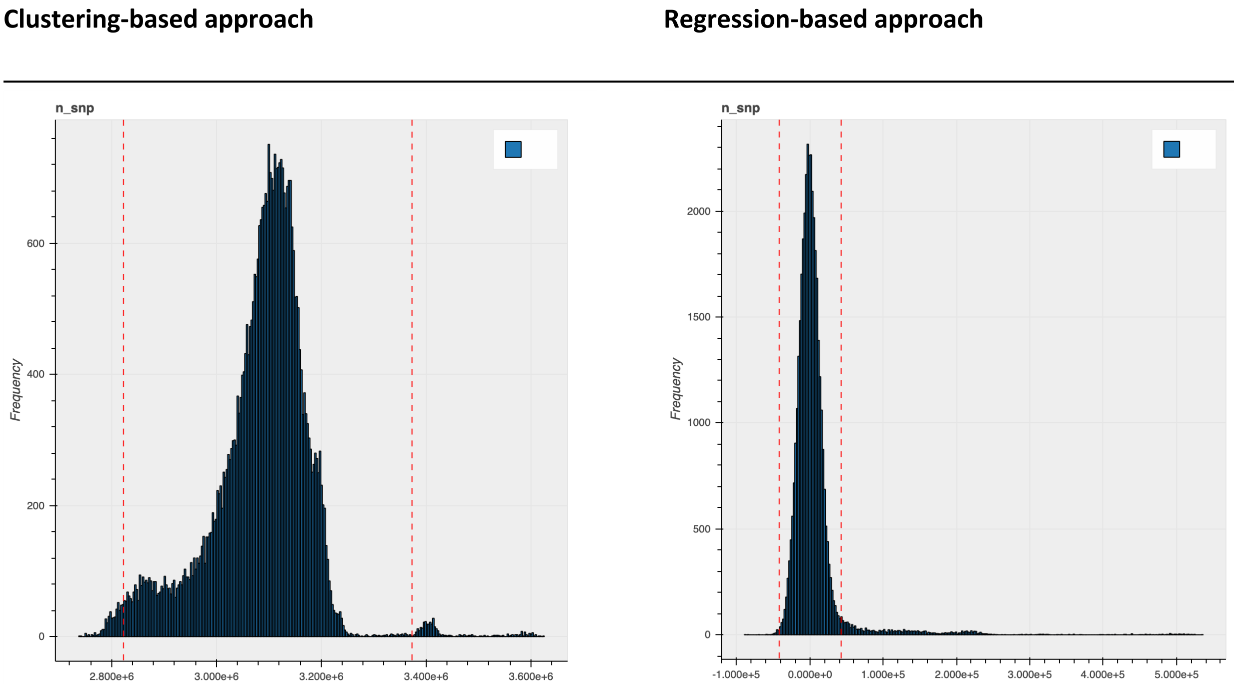
Supplementary Figure 3 | UMAP embedding of PCA results (PCs 1-6 and 8-16) of all individuals in the gnomAD release. This embedding is an illustration of the structure present in the dataset: note that long-range distances in this projection do not reflect genetic distance between populations.

Filtering based on QC metrics

In gnomAD v2, we grouped samples based on their ancestry assignments and filtered outliers within each ancestry based on various quality metrics such as number of SNPs. Here, we built a single model for each metric across all individuals, regressing out the PCs computed during the ancestry assignment, and filtered samples based on the residuals for each of the QC metrics. This strategy allowed us to consider the samples' ancestry as a continuous spectrum and was particularly beneficial for admixed samples and samples that did not get an ancestry assignment (assigned as oth; previously, we lumped all



these samples together even though they were drawn from multiple populations). An illustration of the improvement from this new method is shown in Supplementary Figure 4.



Clustering-based approach **Regression-based approach**

Supplementary Figure 4 | The distribution of the number of SNPs for the African ancestry samples using the clustering-based and regression-based approach.

We used the sample QC metrics computed using the Hail ‘sample_qc’ module on all autosomal bi-allelic SNVs and filtered samples that were 4 median absolute deviations (MADs) from the median for the following metrics: n_snp, r_ti_tv, r_insertion_deletion, n_insertion, n_deletion, r_het_hom_var, n_het, n_hom_var, n_transition and n_transversion. In addition, we filtered samples that fell outside 8 MADs above the median n_singleton metric and over 4 MADs above the median r_het_hom_var metric. The final set of samples is shown in Supplementary Table 2.

After some downstream analysis, we noted that this regression approach removes samples from populations with extreme diversity, such as individuals from the San, Papuan, and Pygmy populations in HGDP. We have adjusted this filter for future releases and provide the data for all these individuals in a joint-called subset of gnomAD (see Code and Data Availability section below, as well as accompanying manuscript) under release v3.1.2. However, we note that this current dataset excludes these individuals, as newly computing the frequency metrics was prohibitively expensive.

Supplementary Table 2 | Final number of individuals for each population

Population code	Description	Number of Genomes
afr	African/African American	20,744
ami	Amish	456
amr	Latino/Admixed American	7,647
asj	Ashkenazi Jewish	1,736
eas	East Asian	2,604
fin	Finnish	5,316
nfe	Non-Finnish European	34,029
mid	Middle Eastern	158
sas	South Asian	2,419
oth	Other (population not assigned)	1,047
Total		76,156

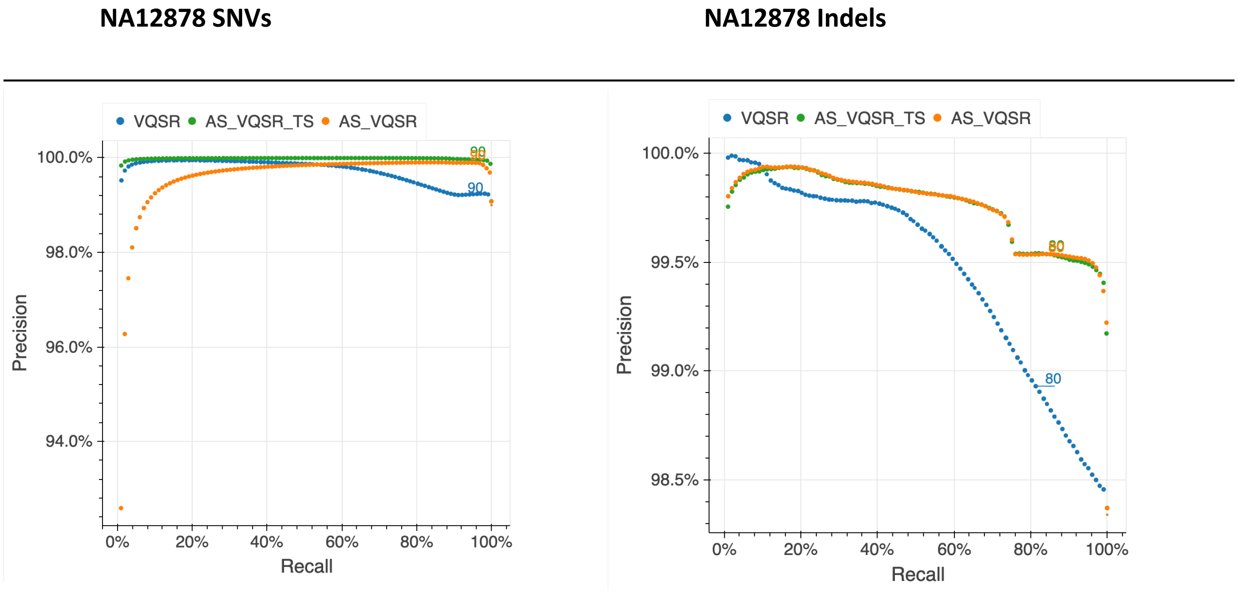
Variant QC and annotation

Because the new sparse MatrixTable format contains all the information encoded in the gVCFs, we computed all variant QC metrics within Hail, which enabled the separate computation for each allele (rather than site-level as was typically done previously). The code to compute these metrics is available at https://github.com/broadinstitute/gnomad_methods/blob/master/gnomad/utils/sparse_mt.py. We then used the allele-specific version of GATK Variant Quality Score Recalibration (VQSR) to compute a confidence score for each allele in the dataset. We used the following allele-specific features: FS, SOR, ReadPosRankSum, MQRankSum, and QD for SNPs and indels, as well as MQ for SNPs.

In addition to the GATK bundle training resources (HapMap, Omni, 1000 Genomes, and Mills indels), we also used a set of ~19M transmitted singletons (alleles observed exactly twice in the dataset, only in a parent/child duo) from 6,743 trios present in our raw data.

We assessed the results of the filtering by plotting, as a function of quality tranche, the number of potential *de novo* mutations in the 6,743 trios, the Ti/Tv ratio, proportion singletons, proportion bi-allelic variants, and variants in ClinVar, as well as precision and recall in two truth samples present in our data: NA12878 and a pseudo-diploid sample (A mixture of DNA [est. 50.7% / 49.3%] from two haploid CHM cell lines).

In gnomAD v3 (prior to the addition of 4,598 new samples), we assessed the performance of the classic site-level VQSR, to a new algorithm, the allele-specific VQSR (AS_VQSR), as well as one with transmitted singletons included (AS_VQSR_TS). Supplementary Figure 5 illustrates the superior performance of the allele-specific approach by precision-recall curves of gold standard SNVs and indels from one sample, NA12878.



NA12878 SNVs

NA12878 Indels

Supplementary Figure 5 | Precision-recall curves for the previous site-level (VQSR), allele-specific (AS_VQSR) and allele-specific with transmitted singletons (AS_VQSR_TS) approaches. Both allele-specific approaches outperform VQSR, while AS_VQSR_TS shows a slight improvement for SNVs.

In addition to VQSR, we also applied the following hard filters:

- AC0: No sample had a high quality genotype at this variant site (GQ>=20, DP>=10 and allele balance > 0.2 for heterozygotes)
- InbreedingCoeff: there was an excess of heterozygotes at the site compared to Hardy-Weinberg expectations using a threshold of -0.3 on the InbreedingCoefficient metric.

In total, 12.2% of SNVs and 32.5% of indels were filtered, resulting in 569,860,911 SNVs and 74,407,067 indels that passed all filters in our release.

Functional annotation

All variants are annotated using version 101 of the Variant Effect Predictor (VEP) based on the gene models from Gencode v35, with the LOFTEE plugin as described previously (Karczewski et al. 2020). For GRCh38, LOFTEE is similar to the previous implementation, without the extended splice predictions.

Code and data availability

Release files

We release the aggregated allele frequency dataset at https://gnomad.broadinstitute.org, in a browser and bulk downloads for VCFs and Hail Tables, as well as all constraint statistics described in this manuscript. Additionally, we provide a subset of the dataset that includes individual level data for the HGDP (Bergström et al. 2020) and the 1000 Genomes projects (1000 Genomes Project Consortium et al. 2015): the generation and use of this dataset is described in a companion manuscript.

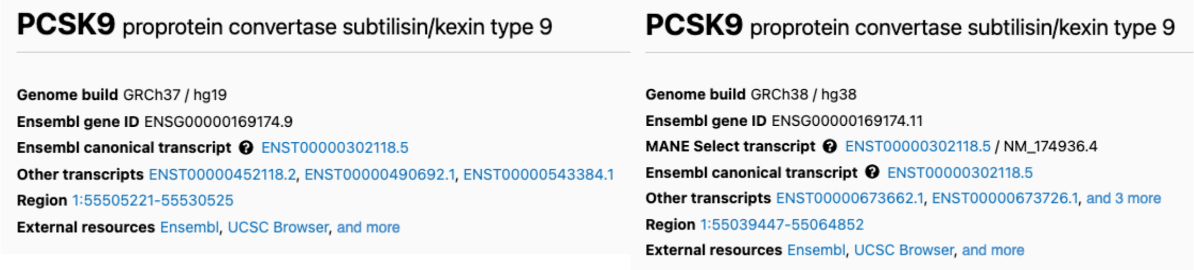
Code availability

All code to perform quality control of the resource is publicly available at https://github.com/broadinstitute/gnomad_qc, and many of the functions are documented in a Python package (gnomad) at https://broadinstitute.github.io/gnomad_methods/index.html. The code to compute the constraint statistics is available at https://github.com/atgu/gnomad_nc_constraint.

The gnomAD browser

Support for multiple reference genomes

Alongside the release of gnomAD v3, we wanted to retain information from previous releases (gnomAD v2) in the browser for reproducibility. To do so, we added support for multiple reference genomes to the browser (Supplementary Figure 6).



PCSK9 proprotein convertase subtilisin/kexin type 9

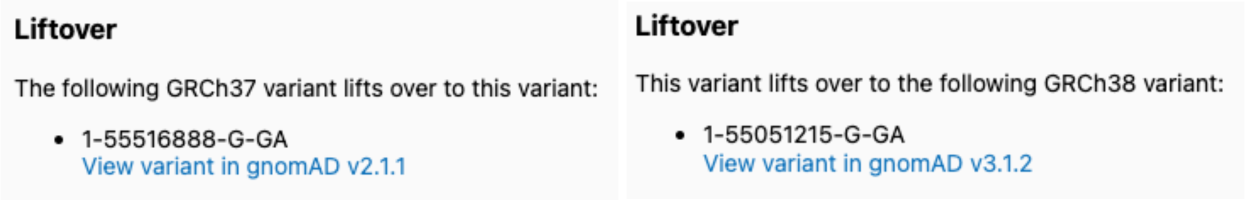
Genome build GRCh37 / hg19
Ensembl gene ID ENSG00000169174.9
Ensembl canonical transcript ❓ ENST00000302118.5
Other transcripts ENST00000452118.2, ENST00000490692.1, ENST00000543384.1
Region 1:55505221-55530525
External resources Ensembl, UCSC Browser, and more

PCSK9 proprotein convertase subtilisin/kexin type 9

Genome build GRCh38 / hg38
Ensembl gene ID ENSG00000169174.11
MANE Select transcript ❓ ENST00000302118.5 / NM_174936.4
Ensembl canonical transcript ❓ ENST00000302118.5
Other transcripts ENST00000673662.1, ENST00000673726.1, and 3 more
Region 1:55039447-55064852
External resources Ensembl, UCSC Browser, and more

Supplementary Figure 6 | The same gene viewed in gnomAD v2 and v3.

Additionally, to make it easier to transition between reference builds and gnomAD versions, we added a liftover function for all variants in gnomAD v2 (Supplementary Figure 7).



Liftover

The following GRCh37 variant lifts over to this variant:

- 1-55516888-G-GA
  View variant in gnomAD v2.1.1

Liftover

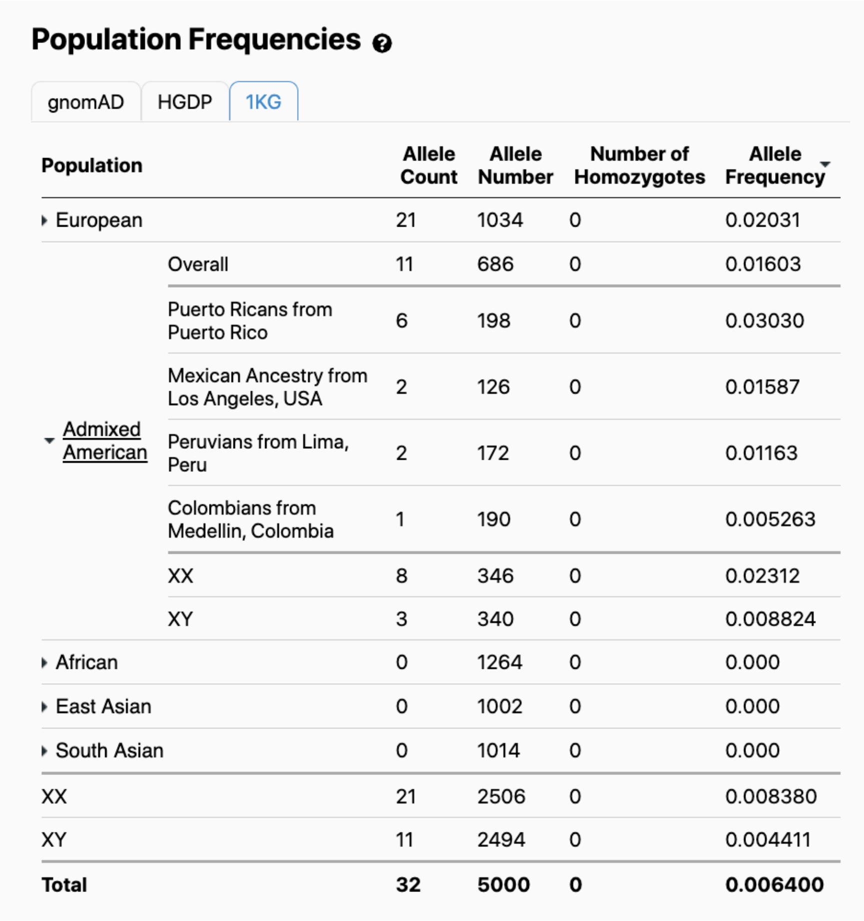
This variant lifts over to the following GRCh38 variant:

- 1-55051215-G-GA
  View variant in gnomAD v3.1.2

Supplementary Figure 7 | Liftover section on a gnomAD v2 and v3 variant page

HGDP and 1000 Genomes population frequencies

For variants found in the HGDP / 1000 Genomes subset, the browser now includes population frequencies based on known populations from the HGDP / 1000 Genomes sample metadata (Supplementary Figure 8).



Population Frequencies

Population		Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
European		21	1034	0	0.02031
Admixed American	Overall	11	686	0	0.01603
	Puerto Ricans from Puerto Rico	6	198	0	0.03030
	Mexican Ancestry from Los Angeles, USA	2	126	0	0.01587
	Peruvians from Lima, Peru	2	172	0	0.01163
	Colombians from Medellin, Colombia	1	190	0	0.005263
	XX	8	346	0	0.02312
	XY	3	340	0	0.008824
African		0	1264	0	0.000
East Asian		0	1002	0	0.000
South Asian		0	1014	0	0.000
XX		21	2506	0	0.008380
XY		11	2494	0	0.004411
Total		**32**	**5000**	**0**	**0.006400**

Supplementary Figure 8 | Detailed population frequencies. Here, we show the frequency table for the 1000 Genomes project.

Read data in non-coding regions

In previous releases, we provide short read data for exonic variants at the bottom of the variant page to enable detailed quality assessment. In this release, we provide short read data for all variants, including those in non-coding regions.

Supplementary Datasets

Supplementary Dataset 1 | Variant counts in gnomAD genomes and mutation rates. The number of possible and observed rare (MAF1%) SNVs in the gnomAD genomes, along with the estimated mutation rate (`fitted_proportion_observed`) for each context, reference, and alternate allele, stratified by methylation levels for CpG transitions.

Supplementary Dataset 2 | Genome-wide constraint Z scores at 1kb scale. A .bed file containing constraint Z scores for 1,797,153 1kb genomic windows (passing final quality controls). Coordinates are on GRCh38.

Supplementary Dataset 3 | Constraint Z scores of enhancers linked to specific genes. Enhancer-gene links were obtained from the Roadmap Epigenomics Enhancer-Gene Linking database. For each gene, the enhancer with the highest Z score was selected for analysis, and the membership of each gene in gene lists analyzed in Fig. 5b is annotated.

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