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The ExAC browser: displaying reference data information from over 60 000 exomes

Konrad J. Karczewski[1,2,*], Ben Weisburd[1,2], Brett Thomas[1,2], Matthew Solomonson[1,2], Douglas M. Ruderfer[3], David Kavanagh[3], Tymor Hamamsy[3], Monkol Lek[1,2], Kaitlin E. Samocha[1,2], Beryl B. Cummings[1,2], Daniel Birnbaum[1,2], The Exome Aggregation Consortium, Mark J. Daly[1,2] and Daniel G. MacArthur[1,2]

[1]Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA, 02114, USA, [2]Medical and Population Genetics, Broad Institute of Harvard and MIT, Cambridge, MA, 02142 USA and [3]Division of Psychiatric Genomics, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, 10029 USA

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ABSTRACT

Worldwide, hundreds of thousands of humans have had their genomes or exomes sequenced, and access to the resulting data sets can provide valuable information for variant interpretation and understanding gene function. Here, we present a lightweight, flexible browser framework to display large population datasets of genetic variation. We demonstrate its use for exome sequence data from 60 706 individuals in the Exome Aggregation Consortium (ExAC). The ExAC browser provides gene- and transcript-centric displays of variation, a critical view for clinical applications. Additionally, we provide a variant display, which includes population frequency and functional annotation data as well as short read support for the called variant. This browser is open-source, freely available at http://exac.broadinstitute.org, and has already been used extensively by clinical laboratories worldwide.

INTRODUCTION

Recently, large reference datasets, such as those from the 1000 Genomes Project Consortium (1), Exome Sequencing Project (ESP) (2) and Exome Aggregation Consortium (ExAC) (3), have become publicly available for the benefit of the biomedical community. These datasets are beneficial for many applications, including clinical as well as basic research. In particular, as a variant's frequency is among the best predictor of its deleteriousness, clinical geneticists use reference datasets to discern pathogenic mutations from benign polymorphisms. Additionally, genetics researchers rely on variant and allele frequency data to infer gene and variant function (e.g. whether a gene is essential) as well as for population genetics analyses. These large-scale projects release raw data in the form of variant call format (VCF) files, but these files require bioinformatics expertise to parse and synthesize. Genome browsers, such as the UCSC genome browser (4), have become a popular method for non-technical audiences to visualize large genome-scale datasets.

Additionally, browsers of variation data, including the Exome Variant Server (EVS) from ESP and the 1000 Genomes Browser, have been developed to present population data, but these are limited in the data they display. For instance, deviations in coverage, which affect one's confidence of the absence of variation, are not natively shown: EVS contains a link to a coverage track on UCSC, but coverage is not visualized on the page itself.

There are a number of practical considerations for the optimal display of reference data. Specifically, as one primary use case for genome browsers involves gene-level analyses, the display of gene summary information is a central view for a genome browser, including integration of summary statistics as well as data for individual single nucleotide variants (SNV), insertions and deletions (indel) and copy number variants (CNV). Of course, detailed information on each variant, including annotations and quality metrics, is of paramount importance. However, an equally important display is that of the absence of variation: whether a missing variant implies a lack of observed variation, low or no coverage in the genomic region, or variation filtered due to poor quality.

The Exome Aggregation Consortium (ExAC) has collected, harmonized, and released exome sequence data from 60 706 individuals (3). Already, these data have proven useful in filtering variants for identifying causal variants for rare disease (5,6,7). Here, we present a visual browser of the ExAC dataset. The browser is intended for use by clinical geneticists researching variants of interest for patients as well as biologists exploring variation in specific genes.

[*]To whom correspondence should be addressed. Tel: +1 617 714 7220; Email: konradjkarczewski@gmail.com

ExAC BROWSER

We designed the ExAC browser as an intuitive interface to enable clinical geneticists and biologists to explore variants and genes of interest. We built a scalable browser framework to display qualitative and quantitative information for genes and variants in the ExAC dataset (see Methods), including both quality control information as well as summary statistics. The front page of the browser includes a search bar, which is seeded with autocomplete suggestions based on gene symbols and aliases, as well as sample queries. From here, there are two central units of the ExAC browser: the gene (or transcript) page and the variant page.

Gene/transcript page

The ExAC browser gene page is an overview page for gene-level information, including summary statistics, coverage, and variants. The page begins with gene metadata and external references, along with constraint information, which summarizes the gene's intolerance to variation for multiple functional classes (3,8) (Figure 1A). Next, we present single base-resolution coverage information for each exon for a number of metrics including mean, median, and proportion of individuals covered at a number of depth cutoffs (Figure 1B). Immediately below, an exon summary plot displays the position and frequency of each SNV and indel, as well as CNV count information broken down by population (Figure 1C). All individual CNV calls are provided in the form of UCSC tracks, which are linked at the top of the page and above the CNV display. Finally, the browser provides a comprehensive table for variant information, which includes the worst functional annotation across transcripts for each variant, as well as frequency information. The table is sortable and can be exported to a CSV format (Figure 1D).

By default, the gene summary page presents a table of all variants in the gene, annotated with the worst consequence across all transcripts, as well as coverage information for the canonical transcript: we also present a transcript page that includes annotation and coverage information specific to that transcript.

Variant page

The variant page includes a diverse set of annotations for the given variant. First, a site overview (Figure 2A) and site- and genotype-level quality metrics are provided (Figure 2B). The user is notified whether any individual has another variant in the same codon (suggesting a multi-nucleotide variant, or MNV), whether the site is multi-allelic, or if a low number of individuals is covered at this locus. Functional annotations of the variant against each transcript including PolyPhen2 (9), SIFT (10), and LOFTEE annotations, as well as a sortable table of population frequencies are provided (Figure 2C). Finally, for users that wish to evaluate the validity of specific variants, raw short-read data from a subset of individuals is available for each variant. We provide an IGVweb visualization of the read pileup of a 125 bp window around the variant (Figure 2D) for a random sample of individuals with each variant, as well as a sampling of homozygous individuals, if available. For the first time among genome browsers, we provide users with a mechanism to efficiently visualize the raw read support for a variant and make assessments of its quality that may not have been detected by variant calling algorithms.

Non-variant information

One important consideration for displaying genetic data includes the display of non-variant sites. In particular, if a variant or region is queried, we display metadata about the locus, whether or not variants are present in the dataset. When a user searches for variants or regions that are not covered in the ExAC dataset, the user is shown a page with coverage information for the general region for the variant.

Additional considerations

As current web browsers and connections benefit from smaller data transfers and footprints, we have developed a number of optimizations to the browser, including compression and caching of data for large genes (Methods). Finally, the browser is optimized for mobile browsing, where extraneous information is hidden when browsing from a mobile device.

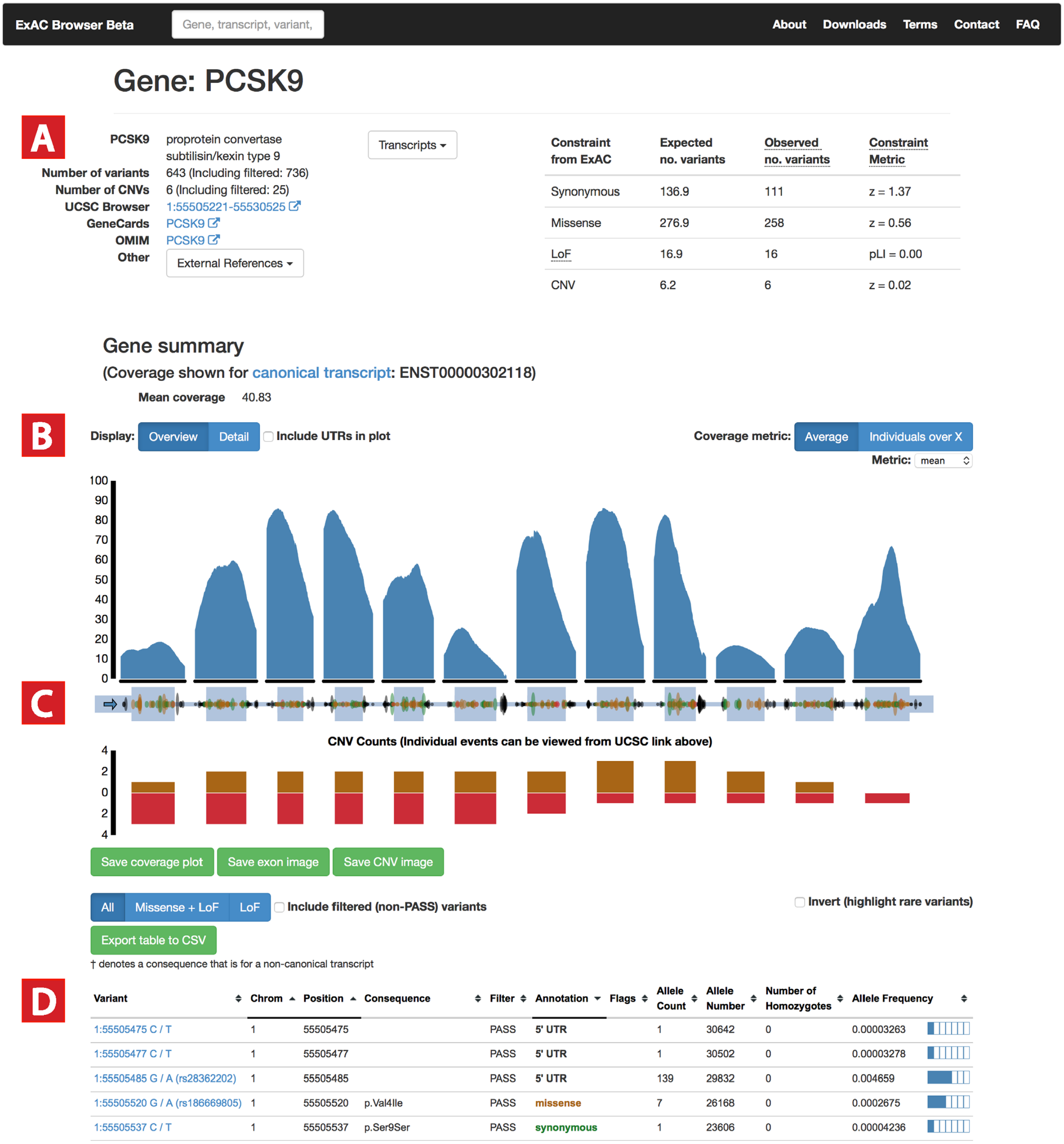
DISCUSSION

Here, we have described a browser for reference variation data, whose use has become widespread in clinical genetics laboratories across the world. As of this writing (8/1/2016), the browser has had over 5 million pageviews and 250 000 users spanning over 188 countries.

The top 10 genes and top three variants visited by users are shown in Table 1.

There are a number of limitations to the ExAC browser. First, the browser only displays data from the exome, or the coding regions of the genome. While these are typically of highest relevance for clinical genetics, there are many non-coding regions that are known to be important for human disease and studied by researchers. An updated version of the browser to allow whole-genome data will be useful for these applications, but additional scalability considerations will be required to display these expanded datasets. Accordingly, queries are limited to 100 kb to ensure a timely return of results. The addition of an optimized API would provide additional flexibility for the browser, as well as serve the needs of researchers needing to do bulk queries for larger-scale analyses. Finally, the quality of the database relies on the quality of the variant calls and annotations contained within ExAC: as new callsets become available, the browser will be updated with new versions that adopt new computational methods and sequencing technologies.

The code is open-source and available at http://github.com/konradjk/exac_browser. The browser framework established can be privately cloned and used for internal sequencing projects, as well as extended to a number of applications, such as a browser for results from genome-wide association studies (GWAS).



Gene: PCSK9

PCSK9	proprotein convertase subtilisin/kexin type 9
Number of variants	643 (Including filtered: 736)
Number of CNVs	6 (Including filtered: 25)
UCSC Browser	1:55505221-55530525
GeneCards	PCSK9
OMIM	PCSK9
Other	External References

Constraint from ExAC	Expected no. variants	Observed no. variants	Constraint Metric
Synonymous	136.9	111	z = 1.37
Missense	276.9	258	z = 0.56
LoF	16.9	16	pLI = 0.00
CNV	6.2	6	z = 0.02

Gene summary

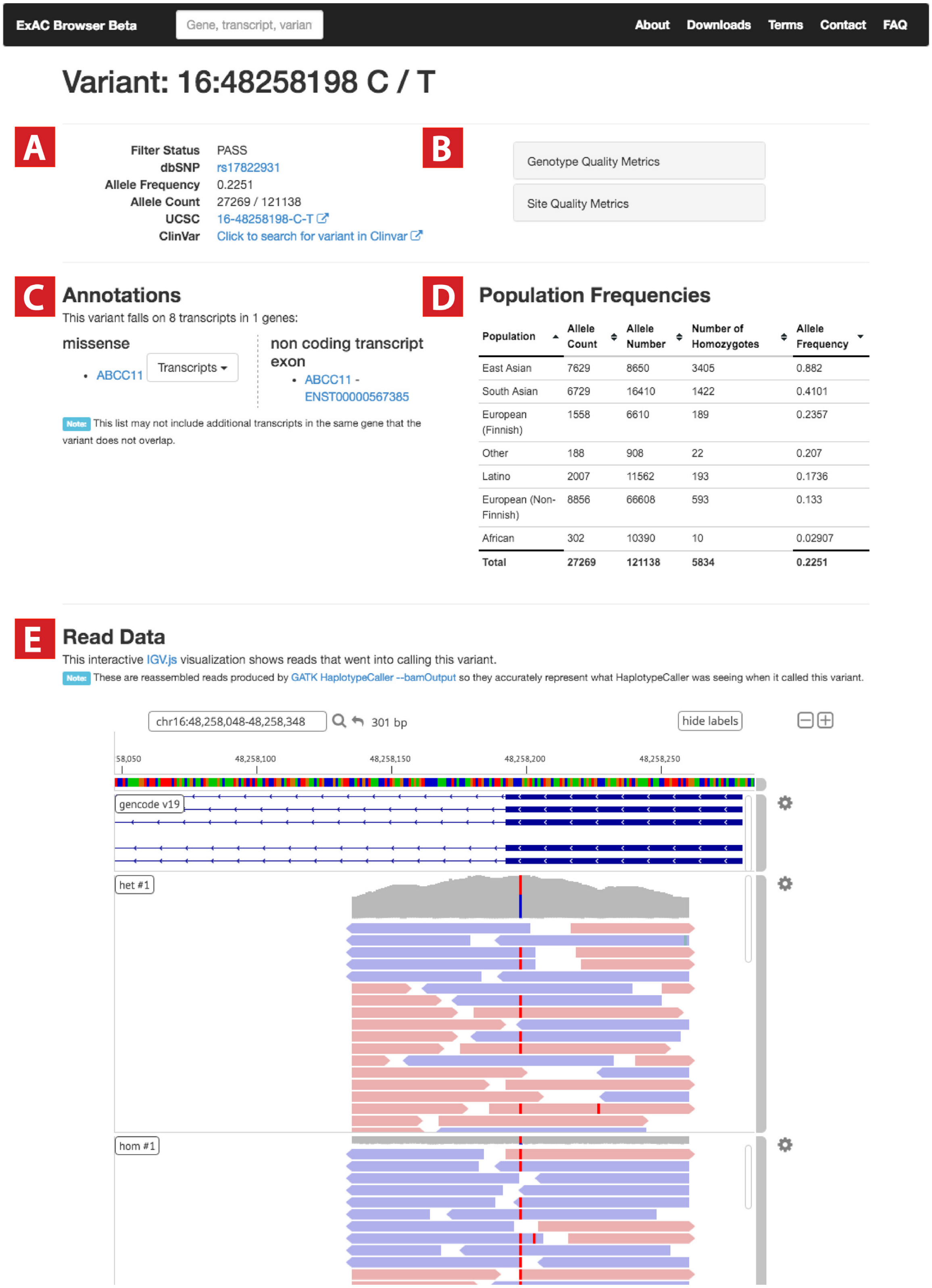
(Coverage shown for canonical transcript: ENST00000302118)

Mean coverage 40.83

† denotes a consequence that is for a non-canonical transcript

Variant	Chrom	Position	Consequence	Filter	Annotation	Flags	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
1:55505475 C / T	1	55505475		PASS	5′ UTR		1	30642	0	0.00003263
1:55505477 C / T	1	55505477		PASS	5′ UTR		1	30502	0	0.00003278
1:55505485 G / A (rs28362202)	1	55505485		PASS	5′ UTR		139	29832	0	0.004659
1:55505520 G / A (rs186669805)	1	55505520	p.Val4Ile	PASS	missense		7	26168	0	0.0002675
1:55505537 C / T	1	55505537	p.Ser9Ser	PASS	synonymous		1	23606	0	0.00004236

Figure 1. Gene page. (**A**) Gene information is summarized, including links to various external resources, as well as constraint information as described in (3). For all exons in the canonical transcript, we display (**B**) base-level coverage for a number of metrics (mean coverage by default), as well as (**C**) position and frequency information for all variants, including CNVs. (**D**) A table of all variants is provided with additional annotation information and links to variant pages.



Variant: 16:48258198 C / T

Filter Status	PASS
dbSNP	rs17822931
Allele Frequency	0.2251
Allele Count	27269 / 121138
UCSC	16-48258198-C-T
ClinVar	Click to search for variant in Clinvar

Genotype Quality Metrics

Site Quality Metrics

Annotations

This variant falls on 8 transcripts in 1 genes:

missense

- ABCC11 Transcripts ▾

non coding transcript exon

- ABCC11 - ENST00000567385

Note: This list may not include additional transcripts in the same gene that the variant does not overlap.

Population Frequencies

Population ▲	Allele Count ⇕	Allele Number ⇕	Number of Homozygotes ⇕	Allele Frequency ▾
East Asian	7629	8650	3405	0.882
South Asian	6729	16410	1422	0.4101
European (Finnish)	1558	6610	189	0.2357
Other	188	908	22	0.207
Latino	2007	11562	193	0.1736
European (Non-Finnish)	8856	66608	593	0.133
African	302	10390	10	0.02907
Total	**27269**	**121138**	**5834**	**0.2251**

Read Data

This interactive IGV.js visualization shows reads that went into calling this variant.

Note: These are reassembled reads produced by GATK HaplotypeCaller --bamOutput so they accurately represent what HaplotypeCaller was seeing when it called this variant.

Figure 2. Variant page. (**A**) Variant metadata is displayed, including links to dbSNP, UCSC and Clinvar. (**B**) Users can browse quality metrics based on genotypes (genotype quality and depth) as well as site-level quality metrics from GATK. (**C**) Annotations for each transcript are provided—if a variant overlaps multiple transcripts with the same functional annotation, a dropdown box provides additional details for the annotations. (**D**) Allele frequency information is displayed for each continental group. (**E**) Short read data is provided for more technical users to assess validity of the variant call.

Table 1. Top genes and variants viewed in the ExAC browser

Gene/Variant	Associated syndromes	Pageviews
PCSK9	(Linked on front page of browser) Hypercholesterolemia	13 540
BRCA1	Breast cancer susceptibility	8251
BRCA2	Breast cancer susceptibility	7408
CFTR	Cystic Fibrosis	5179
FBN1	Marfan Syndrome	4886
TP53	Cancer susceptibility	3712
TTN	Gene that encodes for the largest protein, cardiomyopathy	3528
MYH7	Cardiomyopathy	3497
MYBPC3	Cardiomyopathy	3398
SCN5A	Brugada syndrome, long QT, cardiomyopathy	3175
rs113993960	Cystic fibrosis (CFTR deltaF508)	203
rs1799966	Breast cancer (BRCA1 missense variant)	157
rs11571833	Breast cancer (BRCA2 stop-gained variant)	120

METHODS

Data sources

As of this writing (8/1/2016), version 0.3.1 of ExAC dataset, as described in (3), was used for the ExAC Browser. Variants were annotated using the Variant Effect Predictor (VEP) version 81 (11,12) against the Gencode v19 transcript set. RSIDs were obtained from dbSNP version 142 and gene names and aliases were extracted from dbNSFP (13,14). Histograms for various genotype-specific quality metrics, such as per-sample genotype quality and depth, are pre-computed using a custom python script (https://github.com/macarthur-lab/exac_2015/blob/master/src/prepare_exac_sites_vcf.py). MNVs and constraint metrics are pre-calculated as described in (3).

Reassembled read data was generated for each of the 9.8 million variants in ExAC v0.3.1 by running GATK HaplotypeCaller 3.1 (full version: v3.1-1-ga70dc6e) with the -*bamout* flag on each sample containing the particular variant (up to a limit of five homozygous and five heterozygous samples). Only samples with a read depth (DP) ≥10 and genotype quality (GQ) ≥20 were included. When a variant was present in more than five such samples, the five samples with the highest GQ were selected. Overall, HaplotypeCaller was run 22.3 million times to produce over 5 Tb of small BAM files—with each BAM file storing reassembled reads for a several-hundred base pair window around the variant. Batches of several thousand of these small BAM files were then combined into larger BAM files to improve compression ratios, while using read groups to keep track of the original source of each read. The final dataset comprised ∼23 000 BAMs and spanned 540 Gb. These BAM files were made directly available over the web and visualized in the ExAC browser using IGV.js.

Besides the -*bamout* flag, these additional flags were passed to HaplotypeCaller to ensure that gVCF genotype calls matched the original ExAC gVCF genotypes, which are reproduced here to facilitate reproducibility:

```
-ERC GVCF
 - -paddingAroundSNPs 300
 - -paddingAroundIndels 300
 - -max_alternate_alleles 3
-A DepthPerSampleHC
-A StrandBiasBySample
 - -maxNumHaplotypesInPopulation 200
-stand_call_conf 30.0
-stand_emit_conf 30.0
 - -disable_auto_index_creation_and_locking_when_reading_rods
 - -minPruning 3
 - -variant_index_type LINEAR
 - -variant_index_parameter 128000
```

This data processing was managed by a python-based pipeline available here: https://github.com/macarthur-lab/exac_readviz_scripts.

CNVs were generated using XHMM (15) and based on GENCODE v19 coding regions: all details of CNV calling and quality control have been published previously (16). Gene summary CNV counts and related constraint scores are presented based on likelihoods of the CNV occurring within the genomic range of the gene, as described (16). Exon CNV counts and CNVs presented in the UCSC browser are based on all confidently called CNVs (XHMM SQ > 60) across the genome. All overlapping CNVs, regardless of amount of overlap, are included in Exon CNV counts.

Website design

The ExAC browser was built primarily on open-source tools. On the server, a lightweight Flask framework serves content built on Python scripts available at http://github.com/konradjk/exac_browser. All variants and metadata are loaded into MongoDB (version 2.4.14). The major components loaded include the variant data (directly from the VCF format), coverage data (generated by a modified version of samtools, as described in (3)), MNV and constraint information, as well as gene models from Gencode and RSID information from dbSNP.

The HTML backbone was created based on Bootstrap version 3.1.1 (https://github.com/twbs/bootstrap) and JQuery version 1.11.1 (http://jquery.org). Plotting was performed using d3 version 3 (17). Read visualization is powered by IGVweb version 0.9.3 (https://github.com/igvteam/igv.js/releases/tag/0.9.3).

The entire system runs on a Linux virtual machine with eight cores, 32 GB RAM, and 2T of disk space using Apache 2.4.12. Page tracking is provided by Google Analytics (http://www.google.com/analytics/).

Optimizations

Bootstrap is a mobile-first web framework, which enables the ExAC browser's optimizations for mobile browsing: specifically, much extraneous information (such as the coverage information or additional variant annotations) is hidden when the browser is used on a smaller screen. Additionally, the pages for large genes are pre-computed, allowing for faster load times for these genes. Finally, user search is optimized using typeahead version 0.10.2, with most search terms, including gene names and all aliases, populating the search bar. The single search bar is used to search for variants (formatted as RSIDs or in a chromosome and position format), genes and transcripts (symbols, aliases or Ensembl identifiers) and regions.

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