SOFTWARE TOOL ARTICLE

SNPnotes: high-throughput tissue-specific functional annotation of single nucleotide variants [version 1; peer review: 1 approved with reservations]

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Abstract
A key challenge in the application of whole-genome sequencing (WGS) for clinical diagnostic and research is the high-throughput prioritization of functional variants in the non-coding genome. This challenge is compounded by context-specific genetic modulation of gene expression, and variant-gene mapping depends on the tissues and organ systems affected in a given disease; for instance, a disease affecting the gastrointestinal system would use maps specific to genome regulation in gut-related tissues. While there are large-scale atlases of genome regulation, such as GTEx and NIH Roadmap Epigenomics, the clinical genetics community lacks publicly-available stand-alone software for high-throughput annotation of custom variant data with user-defined tissue-specific epigenetic maps and clinical genetic databases, to prioritize variants for a specific biomedical application. In this work, we provide a simple software pipeline, called SNPnotes, which takes as input variant calls for a patient and prioritizes those using information on clinical relevance from ClinVar, tissue-specific gene regulation from GTEx and disease associations from the NHGRI-EBI GWAS catalogue. This pipeline was developed as part of SVAI Research's "Undiagnosed-1" event for collaborative patient diagnosis. We applied this pipeline to WGS-based variant calls for an individual with a history of gastrointestinal symptoms, using 12 gut-specific eQTL maps and GWAS associations for metabolic diseases, for variant-gene mapping. Out of 6,248,584 SNPs, the pipeline identified 151 high-priority variants, overlapping 129 genes. These top SNPs all have known clinical pathogenicity, modulate gene expression in gut tissues and have genetic associations with metabolic disorders, and serve as starting points for hypotheses about mechanisms driving clinical symptoms. Simple software changes can be made to customize the
pipeline for other tissue-specific applications. Future extensions could integrate maps of tissue-specific regulatory elements, higher-order chromatin loops, and mutations affecting splice variants.

**Keywords**

bioinformatics, genomics, genetics, GWAS, variant annotation, SNPs, software, clinical genetics, epigenetics, epigenomics

This article is included in the Research to the People collection.

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Introduction

Genome sequencing has become an invaluable tool for clinical diagnostics. Several methodologies exist to look at the human genome, each with own benefits and pitfalls. Whole exome sequencing has been a cost-effective technique to identify structural and nucleotide variants in protein-coding regions of the genome, and has identified variants associated with a number of diseases, including psoriasis\(^1\), Factor V Leiden thrombophilia\(^2\), and Miller Syndrome\(^3\). Genome-wide associations studies, originally based on SNP microarrays, have found that roughly half of genetic associations with disease are located outside gene bodies; this fraction approaches 90% with the inclusion of intronic regions\(^4\). With dropping costs for DNA sequencing, whole genome sequencing (WGS) promises to extend the ability to identify disease-associated single and structural nucleotide variants in the clinic, to non-coding regions of the genome, including gene and chromatin regulatory sequences. However, the increased size and complexity of the data creates a parallel challenge of annotating non-coding variants, as it requires knowledge of tissue-specific gene regulation (or epigenetics), including regulatory elements such as promoters and enhancers, and features of higher order chromatin organization such as Topological Associated Domains.

Purpose and approach

Several large-scale epigenomics projects have catalogued tissue-specific regulatory elements. This includes genetic modulation of gene expression (GTEx project)\(^5\), chromatin state (Roadmap Epigenomics)\(^6\), and enhancer-promoter loops for mapping of distal regulatory elements to genes (FANTOM and individual studies)\(^7,8\). A computational workflow that integrated these functional annotation maps to annotate variants from WGS assays would be a valuable resource to prioritize variants with potential functional impact in tissues of interest. Popular high-throughput variant annotation tools, such as BioMart and Variant Effect Predictor\(^9,10\), do not provide tissue-specific annotation. While FUMA\(^11\) integrates comprehensive epigenetic annotation, it is a web-based service used to annotate top-ranking variants from GWAS studies, rather than being a standalone tool for variant annotation.

In this work, we describe and provide variant annotation software that starts with output from a WGS assay and prioritizes variants based on epigenomic resources described above, as well as clinical genetic and GWAS catalogs of variant-disease association. This tool will allow users to capture functional and clinical information and to analyze variants simply by providing the commonly-used VCF file format. We demonstrate the software’s functionality by prioritizing variants from WGS data for a single patient.

Methods

This work was undertaken as part of SVAI Undiagnosed-1 (https://sv.ai/undiagnosed-1), which was a collaborative event with the goal of diagnosing a patient with an unknown genetic condition. As data, participant groups were provided with detailed medical history, genotyping and metabolic data from a 33-year old Caucasian male patient, JCM. The event was hosted in June 2019 by the not-for-profit organization SVAI (http://sv.ai), with participants located in the San Francisco Bay Area (USA) as well as in Toronto, Canada.

Pipeline

Figure 1 shows the workflow for the pipeline. Patient genotypes are provided in Variant Call Format as input. Conceptually, the tool compiles prior knowledge about the functional significance of variants from the perspective of tissue-specific regulatory information, known genetic disease associations in the literature, tissue-specific genetic modulation of gene expression, and clinical pathogenicity. The annotation sources are integrated with the genotype calls in the VCF file, and this integration results in a single output table with available annotation for the variant.
The user can then prioritize variants based on the combination of known or predicted functional consequences.

**Tissue-specific regulatory regions**

The NIH Roadmap Epigenomics project performed comprehensive mapping of noncoding DNA in 111 epigenomes to identify putative regulatory elements in diverse human tissue and cell types (http://www.roadmapepigenomics.org/). The presence of a variant overlapping a tissue-specific promoter or enhancer element signifies that variant alteration could change the regulation of a tissue-specific gene, i.e. that it has regulatory impact on gene expression. Tissue-specific 15-state chromatin state models were downloaded from the Roadmap Epigenomics Portal (downloaded from https://egg2.wustl.edu/roadmap/data/byFileType/chromhmmSegmentations/ChmmModels/coreMarks/jointModel/final/all.mmoments.bedFiles.tar.gz). As the symptoms for John M, hereafter referred to as “the Patient”, included impaired function of the gastrointestinal tract, we limited our annotation to tissues and organs of the digestive system (e.g. esophagus, stomach, intestine). Chromatin states for digestive system tissues were used, including fetal stomach, small and large intestine, sigmoid colon, colonic mucosa, mucosa from stomach, duodenum and rectum, esophagus, rectal mucosa, and stomach mucosa. Regions with open chromatin were included for variant annotation (Table 1; states ≤ 1–7).

**Genetic modulation of gene expression in the gut**

The GTEx project identified variants that significantly modulate gene expression in each of 44 human tissues. Variants that modulate transcription in gut tissues (gut eQTLs) were included for variant annotation (from ClinVar, associated GWAS trait and p-value, coordinates positionally-overlapping genes, associated clinical significance from ClinVar, associated GWAS trait and p-value, coordinates and state name of overlapping open chromatin states in gut tissues, and name of tissue and genes for significant eQTL associations. The pipeline then filters this file to report only those SNPs with GWAS hits that achieve genome-wide significance (p < 5×10^-8); this file is titled “GWASsignificant.txt”, and creates a third file with the list of unique genes that meet this criterion (“GWASsignificant_genes.unique.txt”). The user may filter this data still further to identify SNPs with known clinical pathogenicity, as well as SNPs in functionally annotated non-coding regions.

**Variant disease associations**

Genome-wide SNP-disease associations were downloaded from the NHGRI-EBI GWAS catalog, using the TargetValidation.org API; only those associations mapped to metabolic disorders were included (EFO:0000589). In addition, information on known clinical pathogenicity was downloaded from the ClinVar database (downloaded from ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/tab_delimited/variant_summary.txt.gz).

**Annotation file preparation**

The pipeline software was implemented in bash and R 3.4.4. VCFtools v0.1.15 was used to filter SNPs from the VCF input file. SNP locations were downloaded from the dbSNP 151 database ((downloaded from ftp://ftp.ncbi.nih.gov.snp/organisms/human_9606_b151_GRCh37p13/VCF/common_all_20180423.vcf.gz)). All coordinates are in GRCh37/hg19 build. SNP coordinates were converted to bed format using awk. dbSNP identifiers were converted to genomic coordinates using dbSNP 151 reference (see above). Bedtools v2.28.0 was used to identify overlap of SNP coordinates with individual annotation sources. Finally, R merge was used to join tables by variant location.

**Output format**

The output file (“final_table.txt”) contains SNP coordinates, positionally-overlapping genes, associated clinical significance from ClinVar, associated GWAS trait and p-value, coordinates and state name of overlapping open chromatin states in gut tissues, and name of tissue and genes for significant eQTL associations. The pipeline then filters this file to report only those SNPs with GWAS hits that achieve genome-wide significance (p < 5×10^-8); this file is titled “GWASsignificant.txt”, and creates a third file with the list of unique genes that meet this criterion (“GWASsignificant_genes.unique.txt”). The user may filter this data still further to identify SNPs with known clinical pathogenicity, as well as SNPs in functionally annotated non-coding regions.

**Results**

For our test case, we used the SVAI Undiagnosed-1 Patient whole genome sequencing data provided for the Undisclosed-1 hackathon event, hosted by SVAI/Research to the People. We applied our pipeline to chromosomes 1 to 22 and X, Y chromosomes. Out of 6,248,584 SNPs, we identified 151 high-priority variants, overlapping 129 genes, which demonstrate strong evidence for functional significance (Table 3). Therefore,

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### Table 1. Samples from the NIH Roadmap Epigenomics project used for gut-specific regulatory region annotation.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Tissue name</th>
</tr>
</thead>
<tbody>
<tr>
<td>E075</td>
<td>Colonic mucosa</td>
</tr>
<tr>
<td>E077</td>
<td>Duodenum Mucosa</td>
</tr>
<tr>
<td>E079</td>
<td>Esophagus</td>
</tr>
<tr>
<td>E084</td>
<td>Fetal large intestine</td>
</tr>
<tr>
<td>E085</td>
<td>Fetal small intestine</td>
</tr>
<tr>
<td>E092</td>
<td>Fetal stomach</td>
</tr>
<tr>
<td>E094</td>
<td>Gastric</td>
</tr>
<tr>
<td>E101</td>
<td>Rectal Mucosa Donor 29</td>
</tr>
<tr>
<td>E102</td>
<td>Rectal Mucosa Donor 31</td>
</tr>
<tr>
<td>E106</td>
<td>Sigmoid colon</td>
</tr>
<tr>
<td>E109</td>
<td>Small intestine</td>
</tr>
<tr>
<td>E110</td>
<td>Stomach Mucosa</td>
</tr>
</tbody>
</table>

### Table 2. Samples from the GTEx dataset used to obtain significant gut expression QTLs (eQTLs).

<table>
<thead>
<tr>
<th>File names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon_Sigmoid.v7.signif_variant_gene_pairs.txt.gz</td>
</tr>
<tr>
<td>Colon_Transverse.v7.signif_variant_gene_pairs.txt.gz</td>
</tr>
<tr>
<td>Esophagus_Muscularis.v7.signif_variant_gene_pairs.txt.gz</td>
</tr>
<tr>
<td>Esophagus_Mucosa.v7.signif_variant_gene_pairs.txt.gz</td>
</tr>
<tr>
<td>Small_Intestine_Terminal_Lleum.v7.signif_variant_gene_pairs.txt.gz</td>
</tr>
<tr>
<td>Stomach.v7.signif_variant_gene_pairs.txt.gz</td>
</tr>
</tbody>
</table>
this pipeline allows the user to prioritize certain variants for further downstream analyses, or clinical follow up.

Conclusions and next steps
This pipeline will allow easy integration of several epigenomic functional annotation maps that could assist in SNV prioritization for clinical and basic research applications. Our provided software can be customized by someone with basic bioinformatics or scripting expertise to generalize to other tissues profiled in the GTEx and NIH Roadmap Epigenomics project.

One beneficial extension would be the identification of variants predicted to affect gene splicing. Such predictions are available in databases of splicing variants, such as dbscSNV, or simple splice site prediction algorithms such as MaxEntScan; the latter uses sequence motifs and maximum entropy calculations for its predictions. This approach is limited by the quality of variant databases, and by models that are limited to predicting only in instances that follow canonical rules for splice site regulation. Another promising avenue to predict aberrant splicing is SpliceAI, a deep learning-based model that predicts splice junctions from an arbitrary pre-mRNA transcript sequence. Another valuable addition would be that of using higher-order chromatin interaction maps, which allow the mapping of distal regulatory elements, such as enhancers, to genes (e.g. 8).

Ethical statement and consent
This article is based on research that occurred at the Undisclosed-1 hackathon event, hosted by SVAI/Research to the People.

The patient provided written informed consent for data release of their medical records, including genetic results, blood work and clinical laboratory reports, to the organisers of the event (SVAI/Research to the People). This consent included data release to participants of Undisclosed-1 during the event, and subsequently for this data to be hosted by SVAI/Research to the People in an online data repository with restricted access (see details in “Data Availability”).

The patient provided written informed consent for the publication of all articles based on the research that was carried out at Undisclosed-1 and any accompanying images.

Since the medical records are the patient’s property, the patient was fully informed of what data release would entail and written informed consent was obtained for the release of the medical records. No ethical approval was sought for the Undisclosed-1 event or publication of articles relating to this event.

Data availability

License information: Since the data contains detailed medical records, access is restricted in order to protect the identity of the patient. Intermediary data is provided throughout the article. In order to access the data, applicants must be registered users of synapse.org and must provide a proposal detailing what the data will be used for. Applicants will also be required to sign a statement that ensures that the data are not shared with others who have not applied to use the data from the SVAI. Please submit applications for data access to hello@sv.ai.

Software availability
Software for this pipeline is available at: https://github.com/shraddhapai/SNPNotes

Archived release at time of publication is located at: http://doi.org/10.5281/zenodo.3352276.

License: MIT

Data in this project contains:
- data/final_table.txt.informative.txt contains the list of informative genes obtained by running this pipeline on WGS data from the patient at the hackathon.
- data/NHGRI_GWAS contains precompiled GWAS associations by disease category.

Table 3. Examples of variants prioritized by SNPNotes. Information includes mapped genes, disease associations from genetic studies, eQTL associations from GTEx, and overlap with open chromatin regions in gut tissues.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Gene</th>
<th>GWAS (p &lt; 5 ×10^-8)</th>
<th>eQTL</th>
<th>Open chromatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs964184</td>
<td>ZPR1</td>
<td>Diabetes mellitus (p &lt; 10^-20)</td>
<td>Esophagus</td>
<td>Transcribed</td>
</tr>
<tr>
<td>rs9268645</td>
<td>HLA-DRA</td>
<td>Type I diabetes mellitus (p &lt;10^-100)</td>
<td>Esophagus muscularis and mucosa</td>
<td>Weak transcription</td>
</tr>
<tr>
<td>rs4148325</td>
<td>UGT1A8, UGT1A9, UGT1A5, UGT1A4, UGT1A6, UGT1A3</td>
<td>Obesity (p&lt; 5×10^-10)</td>
<td>Esophagus mucosa</td>
<td>Enhancer</td>
</tr>
<tr>
<td>rs964184</td>
<td>ZPR1</td>
<td>Metabolic syndrome (3×10^-11)</td>
<td>Esophagus muscularis</td>
<td>Transcribed</td>
</tr>
<tr>
<td>rs2292239</td>
<td>ERBB3</td>
<td>Type I diabetes (p &lt; 3×10^-7)</td>
<td>Sigmoid colon, Transverse colon, Small intestine terminal ileum, Stomach</td>
<td>Transcribed</td>
</tr>
</tbody>
</table>
References


Open Peer Review

Current Peer Review Status:  

Version 1

Reviewer Report 27 November 2019

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Deepti Jain
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The authors report the workflow and pipeline code they developed to annotate variants. The authors were motivated to develop this pipeline specifically so that a user could use tissue- and disease-specific resources of annotation and annotate a large set of variants, such as from a whole genome sequencing (WGS) analysis, provided in a VCF format. As a use case, their provided code is supposed to download annotations linked with gut related tissues and metabolic disorders, format them and use them to annotate and filter variants provided in a VCF format. The authors applied the provided code to annotate 6,248,584 variants from a patient with history of gastrointestinal symptoms and generated a filtered set of 151 variants using the annotations.

The manuscript reports the pipeline-code for a workflow which is very important and useful for generating a filtered set of variants that likely have biological function and which could be followed-up in more detail after a WGS study. However I have major concerns about the practical feasibility and interests of others using this code because it is tailored for a very specific use case and lacks documentation. For these reasons I hesitate to endorse its acceptance at the present stage.

Major concerns:

- The features for which the authors developed the pipeline i.e ability to use tissue- and disease-specific annotations, and to provide VCF as an input is already available in the Whole Genome Sequence Annotator (WGSA, https://sites.google.com/site/jpopgen/wgsa)\(^1\). In addition, WGSA has a large selection of a recently updated annotation resources that a user can choose to annotate the single nucleotide variants as well as indels.

- The code provided is very specific to a use case. A user wanting another set of annotations or more than one set of annotations might end up having multiple versions of the code. In general, such an approach in not considered a good practice as it leads to duplication of lot of code. This can be avoided if the code is updated to handle user specifications provided through a config file.

- A user's ability to use tissue specific annotations in a format different than the currently used resources (example annotations from long range chromatin interactions experiments) will be
limited.

- I did not come across any documentation associated with the code. A documentation and vignette will be very helpful so that user can use the code as is, as well as modify it if they desire to use other resources.

- It would be helpful to add a description about how the code handles and reports multiple annotations for a given variant from a given resource. For example, if a variant was found as an eQTL for two different tissues is that variant reported twice or the information from the two tissues combined and reported in a specific format

**Minor suggestion:**
- Annotation of a large set of variants can have a huge computational burden. A user would find it useful if the authors could provided software performance benchmarks.

References

**Is the rationale for developing the new software tool clearly explained?**
Yes

**Is the description of the software tool technically sound?**
Partly

**Are sufficient details of the code, methods and analysis (if applicable) provided to allow replication of the software development and its use by others?**
No

**Is sufficient information provided to allow interpretation of the expected output datasets and any results generated using the tool?**
Partly

**Are the conclusions about the tool and its performance adequately supported by the findings presented in the article?**
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Molecular biology and Human genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.
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