ASHG Interactive Workshop: Overview and Interpretation of GTEx Resources: eQTLs and Gene Expression

No Relevant Conflicts to Disclose:
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Overview and Interpretation of GTEx Resources: eQTLs and Gene Expression

ASHG 2017 Annual Meeting
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GTEx Workshop Agenda

- Overview of study and data
- Portal demonstration
- Jupyter notebook
- GWAS-eQTL challenges
Association of common DNA variants with diseases and traits

Genome-wide association studies (GWAS) led to discovery of >10,000 common DNA variants associated with >600 diseases/traits.

~95% GWAS SNPs located in non-coding regions

https://www.ebi.ac.uk/gwas
Hypothesis: the functional effect of most (non-coding) GWAS variants is modification of gene expression

Regulatory variation is measured as expression quantitative trait loci (eQTLs)

Measured in a population:

Regulatory variation is measured as expression quantitative trait loci (eQTLs)
Regulation of gene expression: multi-tissue and multi-individual

Across a population (e.g., eQTL studies in blood)

Assessing role of genetic variation on gene function requires **both dimensions**

Across tissues or cell types
Functional genomic maps (e.g., ENCODE, Roadmap Epigenomics)
The Genotype Tissue-Expression project

Atlas of gene expression and eQTLs in non-diseased human tissues from up to 960 recently deceased donors

- 53 tissue sites
- 11 distinct brain regions
- 2 cell lines

This workshop

- Core molecular assays:
  - WGS/WES (primarily whole blood)
  - RNA-seq
  - Small RNA-seq (future)
eGTEx: the Enhancing GTEx project

**eGTEx data types**

- Protein quantifications (x2)
- Methylation (WGBS)
- Histone modifications (ChIP-seq)
- Dnase-seq
- mmPCR-seq (deep ASE)
- Somatic DNA-seq (deep exome seq)
- Analysis of telomere structure

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**Table 1**

<table>
<thead>
<tr>
<th>Data Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein quantification</td>
<td>Analysis of protein abundance across tissues.</td>
</tr>
<tr>
<td>Methylation</td>
<td>Quantification of DNA methylation at a high resolution.</td>
</tr>
<tr>
<td>Histone modifications</td>
<td>Examination of histone modifications.</td>
</tr>
<tr>
<td>Dnase-seq</td>
<td>Assay for DNA accessibility.</td>
</tr>
<tr>
<td>mmPCR-seq (deep ASE)</td>
<td>Single-nucleotide resolution of allele-specific expression.</td>
</tr>
<tr>
<td>Somatic DNA-seq (deep exome seq)</td>
<td>Detailed analysis of somatic mutations.</td>
</tr>
<tr>
<td>Analysis of telomere structure</td>
<td>Study of telomere length and function.</td>
</tr>
</tbody>
</table>

---

**Figure 1**

- Telomere length
- DNA accessibility
- Histone modifications
- DNA methylation
- Somatic mutation
- Protein quantification
- Translation
- RNA methylation
- Allele-specific expression
- Transcription

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eGTEx Project, *Nat. Genet.*, 2017
Sample and data processing overview

**DNA Analysis**
- OMNI 2.5M/5M: 450 donors
- WES (100x)
- WGS (30x): HiSeq 2000, HiSeq X

**RNA sequencing**
- QC: RIN $\geq$ 5.5
- polyA+ (Illumina TruSeq)
- 2x76bp, $\geq$ 50M reads

**Quality control**
Data processing and quality control pipelines

**Genotype QC: samples & variants**
- Sample QC
  - Sample QC
  - Sample metrics
  - GATK, haplotype caller
- Variant QC
  - VCF
  - Quality control
  - Technical & batch effects
  - QC'd VCF
  - Phased VCF

**RNA-seq alignment, quantification & QC**
- Genome BAM
- Picard (Aligned reads)
- STAR
- Gene expression
- Isoform expression
- QC metrics

**eQTL mapping**
- VCF
- Read counts
- TPM
- Covariates
- Normalized expression
- PEER
- Combined covariates
- (e)Gene-level statistics
- Variant-gene pairs

**Expression tables, Covariates**
Genotype QC pipeline

Sample QC
- Sample QC
  - Sample metrics
    - GATK HaplotypeCaller
      - VCF
        - Variant metrics
          - Sample QC'd VCF

Variant QC
- Sample QC'd VCF
- GenomeSTRiP LCNV
  - Genotype posteriors
    - 1KG ref. panel

VCF
- Call rate
  - Technical
    - Batch effects
  - QC'd VCF
    - Phasing
      - Phased VCF
RNA-seq pipeline: alignment, quantification, QC

Raw reads FASTQ → STAR → STAR index → Genome BAM → RNA-SeQC → Collapsed GTF → QC metrics

Raw reads FASTQ → Picard (SamToFastq) → Genome BAM → Transcriptome BAM → RSEM → Isoform expression

[Diagram of the RNA-seq pipeline with various steps and tools mentioned]
RNA-seq and eQTL pipeline details

<table>
<thead>
<tr>
<th>Release</th>
<th>V6p</th>
<th>V7</th>
<th>V8</th>
<th>V9</th>
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</thead>
<tbody>
<tr>
<td>Genome build</td>
<td>GRCh37</td>
<td>GRCh37</td>
<td>GRCh38</td>
<td>GRCh38</td>
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<tr>
<td>GENCODE annotation</td>
<td>v19</td>
<td>v19</td>
<td>v26</td>
<td>v26</td>
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<tr>
<td>Aligner</td>
<td>TopHat 1.4.1</td>
<td>STAR 2.4.2a</td>
<td>STAR 2.5.3a</td>
<td>STAR 2.5.3a</td>
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<td>Gene expression</td>
<td>RNA-SeQC 1.1.8</td>
<td>RNA-SeQC 1.1.9</td>
<td>RNA-SeQC 1.1.9</td>
<td>RNA-SeQC 1.1.9</td>
</tr>
<tr>
<td>Transcript expression</td>
<td>FluxCapacitor 1.6</td>
<td>RSEM 1.2.22</td>
<td>RSEM 1.3.0</td>
<td>RSEM 1.3.0</td>
</tr>
<tr>
<td>Quality control metrics</td>
<td>RNA-SeQC 1.1.8</td>
<td>RNA-SeQC 1.1.9</td>
<td>RNA-SeQC 1.1.9</td>
<td>RNA-SeQC 1.1.9</td>
</tr>
<tr>
<td>QTL mapper</td>
<td></td>
<td>FastQTL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Pipeline components selected and updated based on internal and published benchmarks (e.g., Teng et al., Genome Biology, 2016).
Overview of GTEx resources: open-access data

- Expression
  - Gene-level expression (TPM, counts)
  - Transcript-level expression (TPM, counts, isoform proportions)
  - Exon read counts
- QTLs
  - Single-tissue eQTLs (cis- and trans-)
  - Multi-tissue eQTLs
  - Future: splicing QTLs
- Histology images
- De-identified public access sample and subject metadata

All open-access data is available at gtexportal.org
Overview of GTEx resources: protected data

- Sequence data:
  - RNA-seq (2x76 bp, unstranded, >50M reads/sample)
  - WGS (30x coverage) and WES (100x coverage)
  - Illumina Omni2.5/5 microarray genotypes (subset of 450 donors)
- Allele-specific expression (ASE)
- Full sample and subject metadata
- Future: eGTEx sequence data
  - ChIP-seq
  - WGBS-seq

All protected-access data is available at dbGaP, under accession phs000424
### GTEx data releases

<table>
<thead>
<tr>
<th>Release</th>
<th>V6/V6p</th>
<th>V7</th>
<th>V8</th>
<th>V9</th>
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</thead>
<tbody>
<tr>
<td>RNA-seq</td>
<td>8,555</td>
<td>11,688</td>
<td>17,382</td>
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<tr>
<td>WGS</td>
<td>148</td>
<td>635</td>
<td>838</td>
<td>~960</td>
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<tr>
<td>WES</td>
<td>520</td>
<td>603</td>
<td>~960</td>
<td></td>
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<tr>
<td>OMNI</td>
<td>450</td>
<td>450</td>
<td>450</td>
<td>450</td>
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<tr>
<td>RNA-seq w/ GT</td>
<td>7333</td>
<td>10361</td>
<td>15253</td>
<td>~20,000</td>
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<tr>
<td>eQTL tissues</td>
<td>44</td>
<td>48</td>
<td>49</td>
<td>49</td>
</tr>
</tbody>
</table>

**Current public release**

**Analysis freezes**

Midpoint publications: V6p
- Full list available at [https://gtexportal.org/home/publicationPage](https://gtexportal.org/home/publicationPage)
- Data remains available on GTEx Portal

No publication embargo on V7
GTEx data production: samples per donor
Expression data on GTEx Portal

**Gene-level expression**
- Based on collapsed GENCODE annotation
- Quantified with RNA-SeQC
- TPM
- Read counts
- No covariate correction

**Transcript-level expression**
- Based on full GENCODE annotation
- Quantified with RSEM
- TPM
- Expected read counts
- No covariate correction

**eQTL inputs**
- Based on gene-level quantifications
- Additional normalization: TMM of read counts; inverse normal transform
- Covariates (hidden + known) in separate file
Annotation used for gene-level expression quantification

• RNA-seq protocol:
  • polyA+
  • Unstranded

• Ambiguity in quantifying exon domains shared between sense and anti-sense transcripts

• Collapsing procedure:
  • Masks overlapping intervals
  • Mask ‘readthrough’ and ‘retained intron’ transcripts
Definition of *cis*-eQTLs in GTEx

• *cis*-eQTL: genome-wide significant association between ≥ 1 eVariant and eGene, with associations tested within ±1Mb *cis*-window around TSS. Does not imply evidence of allelic effects at each locus.

• eGene: gene with at least one significant eQTL (at 5% FDR).

• eVariant: variant with a significant association to ≥1 eGene.

• Effect allele: ALT allele (not necessarily the minor allele).
Data normalization for eQTL analyses

• Expression thresholds:
  • \( \geq 6 \) counts in \( \geq 20\% \) of samples AND
  • \( \geq 0.1 \) TPM in \( \geq 20\% \) of samples

• Normalization:
  • Between sample normalization: TMM (from edgeR)
    • Corrects for library size differences and expression outlier effects
  • Within-gene normalization: inverse normal transform
    • Attenuates outliers
Covariate correction in eQTL analyses

- Genotype: top 3 PCs, sex, sequencing platform (HiSeq 2000, HiSeq X)
- Expression: significant technical confounders may be unknown; estimation of hidden confounders is key (e.g., through PEER factors)
eQTL mapping and eGene discovery

• Variants in cis-window (±1Mb from TSS) may be correlated due to linkage disequilibrium (LD)
• LD must be incorporated in multiple hypothesis testing correction when establishing genome-wide significance
  • Empirical p-values from permutation of genotypes
Multiple hypothesis correction for eGene detection

Gene A

Gene B

Gene C

Empirical p-value distribution

q-values (Storey)
eGenes at ≤ 0.05 FDR

Delaneau et al., Bioinformatics, 2016
Storey & Tibshirani, PNAS, 2003
Threshold for significant variant-gene pairs

Nominal p-value threshold for each gene $g$:

$$F_g^{-1}(p_t)$$ where $F_g^{-1}$ is the inverse cumulative Beta distribution of the gene.

$p_t$: empirical p-value of gene closest to 0.05 FDR threshold
Example for portal demonstration

**GTex Consortium, Science, 2015**

*GTex Consortium, Science, 2015*
The interactive parts of the workshop will be conducted using a Jupyter notebook, GTEx_ASHG17_workshop.ipynb.

On the GTEx Portal, go to https://gtexportal.org/workshop.html

Click on “Start the notebook” to begin. This will launch a cloud-based instance of the notebook, with access to all data examples. Please note that the notebook is read-only.

The notebook is also available for download at https://github.com/broadinstitute/gtex-ashg2017-workshop
Organization of GTEx data: common identifiers

- All sample attributes are indexed by Sample ID
- All donor attributes are indexed by Donor ID
- The donor-specific tissue collection ID is not a proxy for tissue type

![Sample ID Diagram]

Sample ID: GTEX-1117F-0226-SM-5GZZ7

Donor ID  Aliquot ID

Donor-specific
tissue collection ID
Implications of GTEx for interpreting GWAS signals
Many genes in the same region have eQTLs
...with different effects across tissues
...with different effects across tissues

Which one(s) explain the disease risk?
Lots of eQTL data means that seemingly significant associations are the norm
eQTL/GWAS interpretation needs to be examined more cautiously.

The possibility of a central, tissue-specific effect of the ESR1 variant rs67229052 is supported by its demonstration as an eQTL for ESR1 in only one of ~50 Genotype-Tissue Expression (GTEx) tissues (brain_caudate_basal_ganglia; using the proxy SNP, rs4305732, with $r^2 = 0.98$); the allele associated with higher ESR1 expression ($P = 0.0004$)

~1/3 of all variants could meet this criterion.
Co-localization approaches combine eQTL and GWAS signals

iPython notebook task

- Correlation of GWAS and eQTL summary statistics over an associated hit for BMI.
Co-localization of eQTLs and GWAS in GTEx

Graph showing the number of GWAS loci for various traits, with bars indicating the presence of co-localization with eQTLs. The traits are ordered from top to bottom, with the number of loci decreasing as you move down the list.
• Correlation of GWAS and eQTL summary statistics for two separate genes over an associated hit for BMI

Toy example
Not necessarily the causal gene or tissue
Bimodal distribution of tissue-specificity of *cis-eQTLs*

Multi-tissue eQTL meta-analysis: Metasoft (Han, B and Eskin, E, AJHG 2011)
Number of tissues per eQTL in LD with GWAS variants increases with increased power (multi-tissue analysis)

Single-tissue analysis
Median = 5 tissues

Multi-tissue analysis
Median = 31 tissues

# tissues per GWAS variant

eQTL detected only with multi-tissue analysis

Multi-tissue eQTL posterior probability
Co-localization of eQTLs and GWAS in GTEx
• Correlation of GWAS and eQTL summary statistics for two separate tissues over an associated hit for BMI

Toy example
Not necessarily the causal gene or tissue
Detecting GWAS/eQTL overlap is easy in principle

GWAS

eQTL

Same signal in GWAS and eQTL: colocalization!
Difficult in practice

GWAS

eQTL

Unclear if same signal in both

significance

position

GWAS significance

eQTL significance
## Methods to detect colocalization

<table>
<thead>
<tr>
<th>Method</th>
<th>method archetype</th>
<th>identifies causal variants?</th>
<th>multiple causal variants?</th>
</tr>
</thead>
<tbody>
<tr>
<td>COLOC</td>
<td>Bayesian</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Sherlock</td>
<td>Bayesian</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>eCAVIAR</td>
<td>complete likelihood (exhaustive search)</td>
<td>Yes</td>
<td>Yes, but intractable</td>
</tr>
<tr>
<td>FINEMAP</td>
<td>complete likelihood (stochastic search)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>SMR</td>
<td>Mendelian randomization</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>TWAS</td>
<td>TWAS</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>MetaXcan</td>
<td>TWAS</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Further caveats: Some genes have multiple independent eQTLs

• Could explain complexity of GWAS/eQTL signals
• Conditional eQTLs not yet tested for colocalization with GWAS

Another challenge: Identifying causal variants in eQTL regions

Fine-mapping methods propose credible sets of causal variants for an eQTL

- CAVIAR (Hormozdiari et al. Genetics 2014) results will be on GTEx Portal soon!
eQTL limited in capturing rare variant effects

Gene expression outliers can point to rare variants with large effects
Interpreting personal variants using genetic and functional genomics data

Li, Kim, Tsang, Davis, Nature, 2017
Using GTEx to help solve rare disease cases.

Patient muscle (n=63) ↔ GTEx control muscle (n=184)

Aberrant splicing

Allele imbalance

Variant Calling

See Talk by Beryl Cummings Friday 10:45 AM

Cummings et al, Science Trans Med, 2017
Interpreting genetic variants in disease

Genetic variation influence gene expression of ~90% of all known protein-coding genes

Abundance of eQTL data requires care when conducting GWAS follow-up
  - Multiple testing can lead to false discoveries
  - Co-localization methods required
  - 40% of all variants do not co-localize with their nearest gene

Gene expression outliers can identify large-effect rare variants
- Can be used to interpret individual risk factors and identify rare disease genes and variants
GTEx pipelines

- Source code is available at https://github.com/broadinstitute/gtex-pipeline
  - Includes wrapper scripts, Dockerfiles

- Pipelines are available on FireCloud (http://firecloud.org)
  - Namespace: broadinstitute_gtex
The biobank from the GTEx project is hosted at the Broad Institute.

Samples can be searched and requested at https://gtexportal.org/home/samplesPage.

Sample requests for research complementing the primary project are welcome.
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