Integrating genetic and gene expression evidence into genome-wide association analysis of gene sets

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Integration of both approaches for complementary evidence.

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(4) Missing heritability: genetic variation explains 5% of hight variation.

(5) Very weak predictive power.

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(5) Can we find evidence that expression variation predictive of trait variation is genetic.

Expression quantitative trait loci eQTL

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(3) Can help with causal versus reactive.
(4) Need expression data and SNP data from same individuals.
(5) Missing heritability still a problem.

Pathway based analysis

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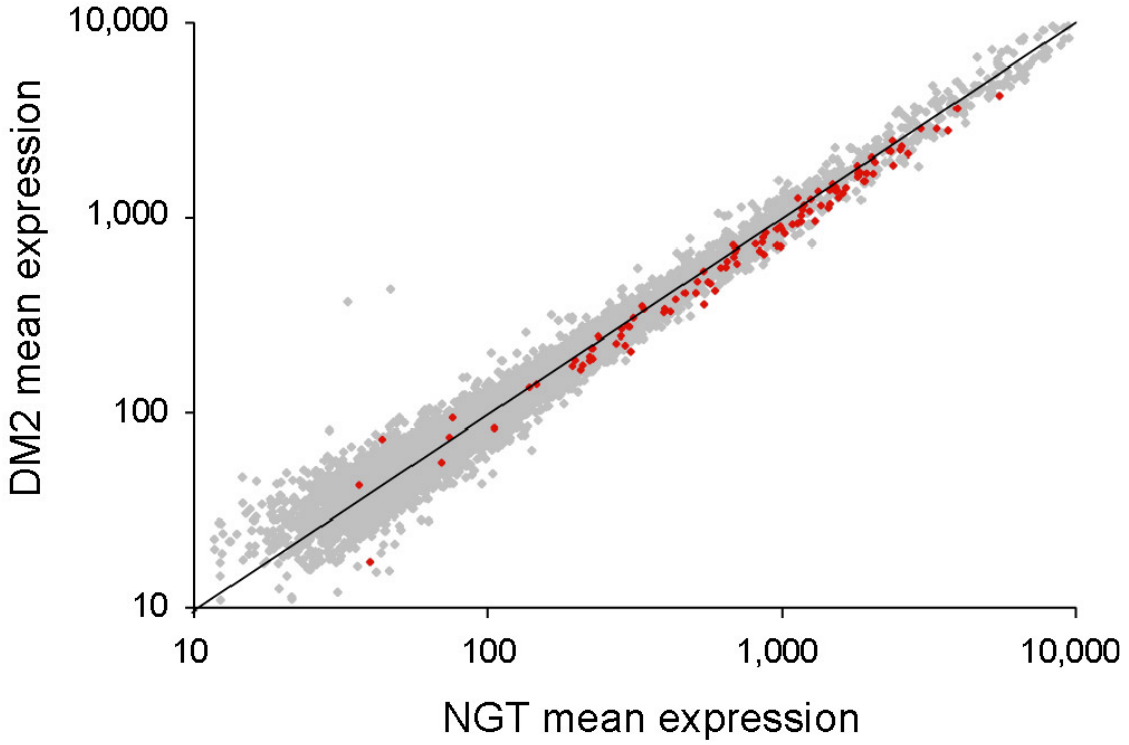

Mootha et al 2005

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(4) Facilitates interpretation of the results from association studies.

Gene Set Association Analysis

The joint analysis of gene expression and SNP genotype data using a pathway-based strategy for more robust and comprehensive inference of associations.

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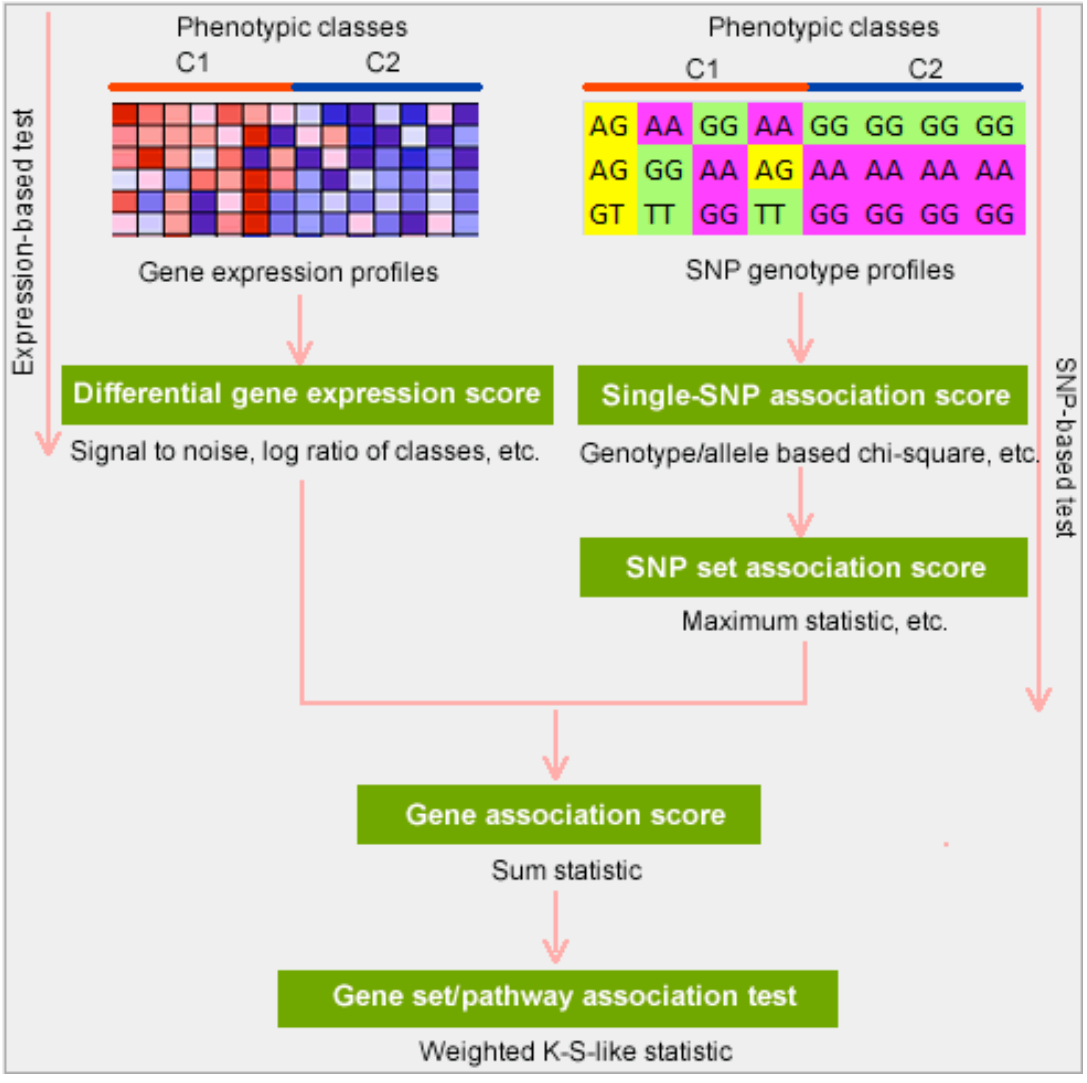

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Gene expression data $X_1, ..., X_n$ and corresponding (Categorical) phenotypic labels: $Y_1, ..., Y_n$, with $X_i \in \mathbb{R}^N$.

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Collections of a priori defined gene sets.

Evidence of differential expression

For each gene, $1, \ldots, N$, compute a differential expression score

$$r_i = \frac{\hat{\mu}_0 - \hat{\mu}_1}{\sqrt{\hat{\sigma}_1^2/n_1 + \hat{\sigma}_2^2/n_2}}.$$

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P-value calibration

$$B(p_j) = \begin{cases} = \frac{1}{-ep_j \log(p_j)} & \text{if } p_j \in (0, \frac{1}{e}] \\ 1 & \text{otherwise.} \end{cases}$$

Single SNP association score

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Simulations suggest genotype-based chi-square test (more power).

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Summary statistic: Default is maximum. Weighted average. Bayes factors.
Result: $\{s_1, ..., s_N\}$.

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$$\tilde{r}_i = \frac{r_i}{\sum_{j=1}^{N} |r_j| \times \mathbf{I}[\text{sign}(r_i) = \text{sign}(r_j)]}$$

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(3) combine evidence $c_i = \tilde{s}_i + \tilde{r}_i$.

eQTL setting (in progresss)

The previous gene association score can be thought of as

$$c_j = e(Y \mid S_j) + e(Y \mid X_j).$$

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This idea can be used for other genomic features.

Gene set association score

For a gene set S with h genes a the rank ordered association scores $\{c_{(1)}, ..., c_{(N)}\}$ compute running association score

$$\mathrm{RAS}_S(i) = \frac{1}{N_s} \sum_{j=1}^{i} |c_j| \times \mathbf{I}(j \in S) - \frac{1}{N-h} \mathbf{I}(j \notin S),$$

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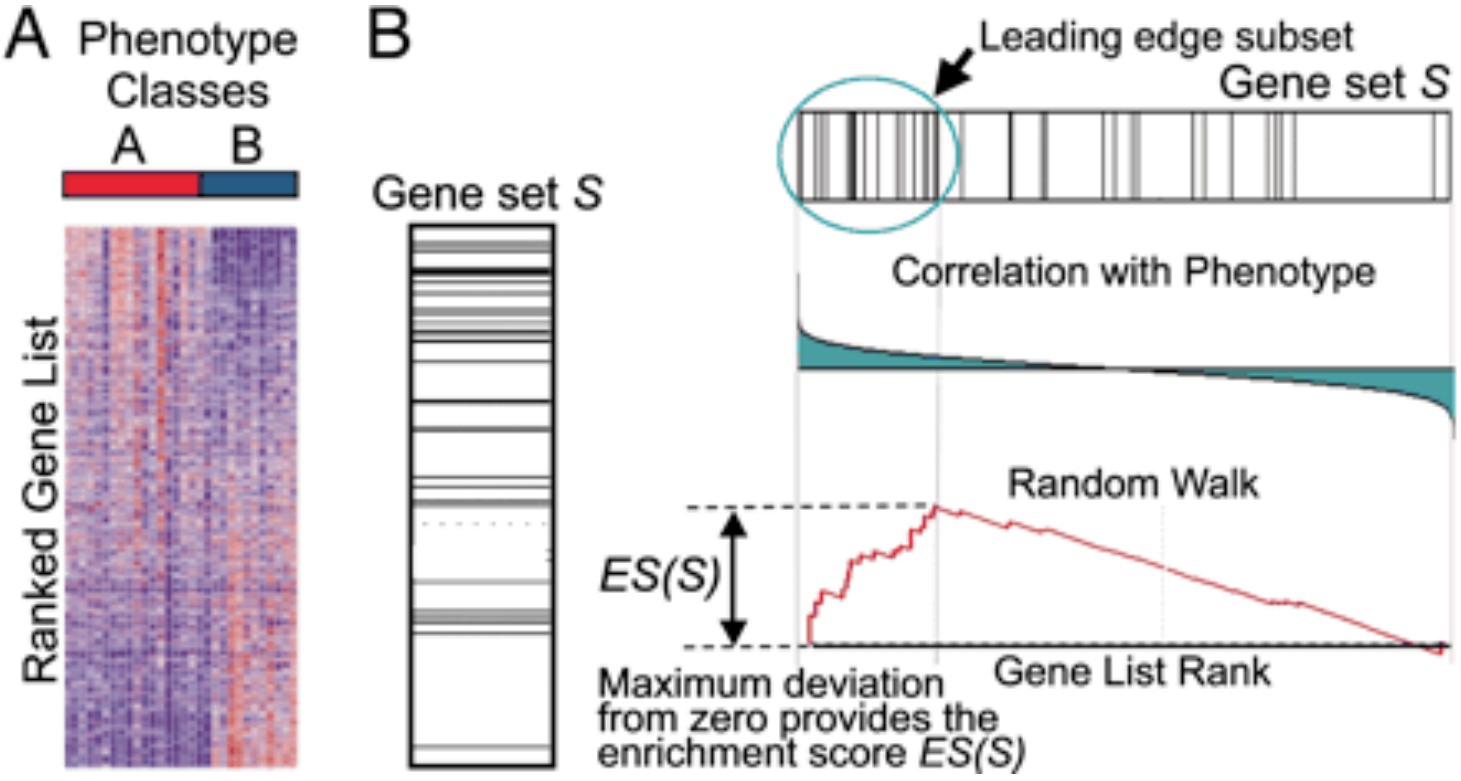
The association score $\mathrm{AS}(S)$ is the maximum deviation of $\{\mathrm{RAS}_S(i)\}$ from zero.

Gene set association score



Statistical significance and adjustment for multiple hypothesis testing

Use permutation procedure and FDR corrections. This automatically corrects for linkage structure and population.

Simulation studies

We compared four methods

(1) GSAA – SNP and expression data

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(4) two step regression model – two step regression model, filter genes with un/weakly associated SNPs, regression on remaining SNPs.

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We generated simulated expression data and SNP data

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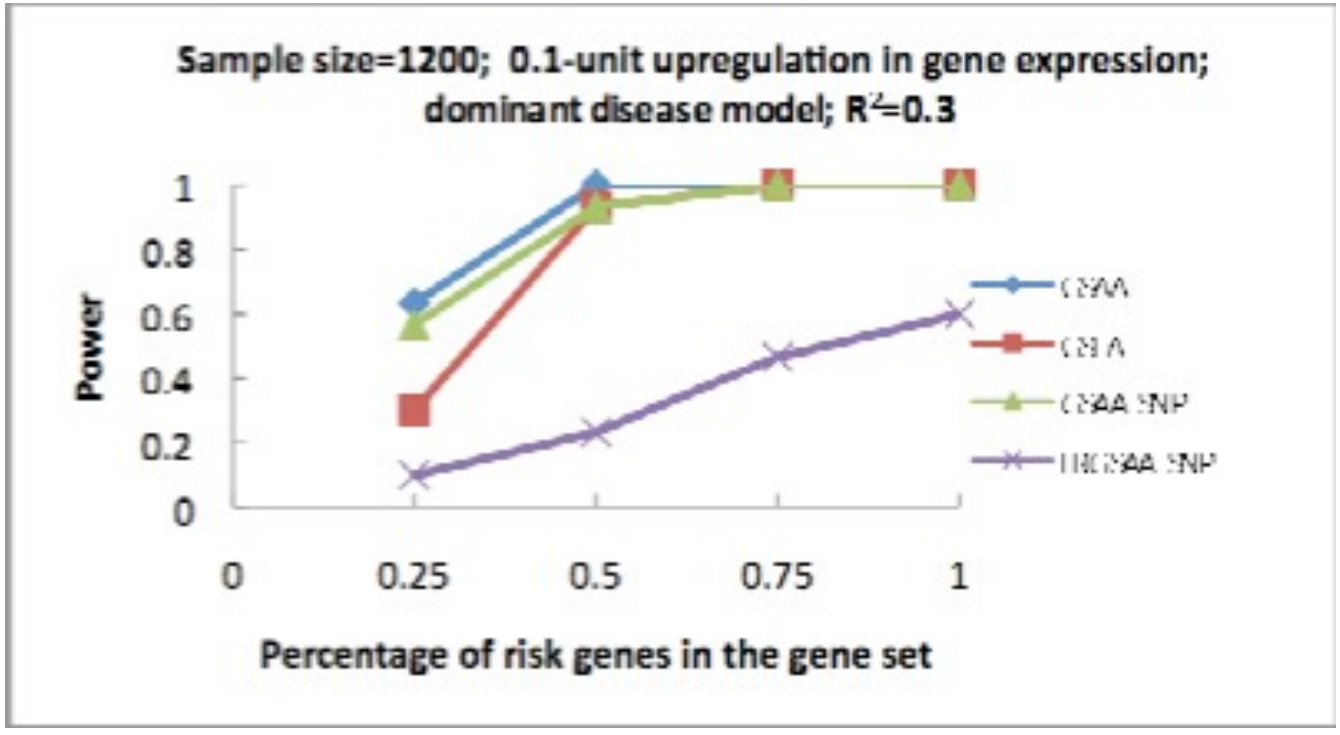
(2) Expression data was simulated using normals.

Simulated data

(1) 1000 genes, first 20 causal, 3 SNPs in each causal gene with the second marker is in LD with the disease variant with varying R^2.

(2) 100 gene sets, the first set is causal including 5-20% of causal genes.

Power calculations



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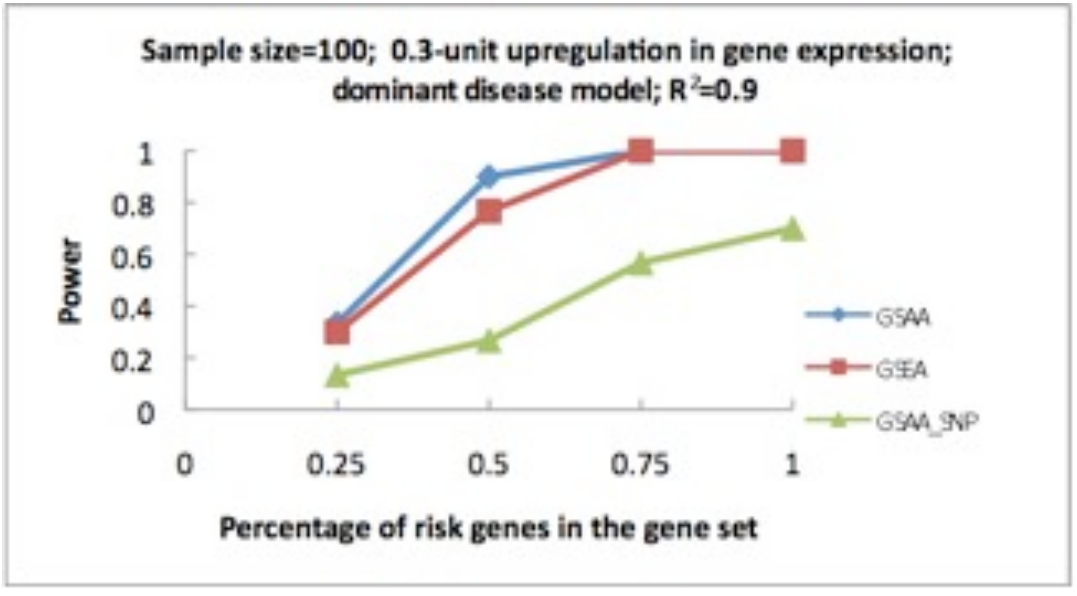

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Collection of clinical, expression, SNP, copy number, and high-throughput sequencing data.

Glioblastoma data

Expression data: 258 tumor samples and 11 normal samples
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357 "canonical pathways" from MSigDB and 658 GO gene sets.

Pathways associated

Table 2. Most significant canonical pathways associated with tumor samples with FDR≤0.25

Gene Set Name	P-value	FDR
[a,b] P53PATHWAY	0.011	0.0955
[a,b] RELAPATHWAY	0.0146	0.1482
[a] ATRBRCAPATHWAY	0.0846	0.1574
[a] HSA03030_DNA_POLYMERASE	0.1319	0.1764
[a] G1PATHWAY	0.045	0.1853
[b] CASPASEPATHWAY	0.0184	0.1949
[a,b] HSA04115_P53_SIGNALING_PATHWAY	0.0034	0.1961
[a] CELL_CYCLE_KEGG	0.0669	0.2034
[a] DNA_REPLICATION_REACTOME	0.2094	0.2035
[a] G2PATHWAY	0.0432	0.2151
INTRINSICPATHWAY	0.0476	0.2311
[a,b] ATMPATHWAY	0.1239	0.2371
STATIN_PATHWAY_PHARMGKB	0.0256	0.2403

[a] Canonical pathways related to the cell cycle, proliferation, cell cycle transitions, or checkpoints.
[b] Canonical pathways related to or contain genes involved in the induction of apoptosis.
For full results, see Table S15.

Expression and association signatures

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Top ranked genes with respect to combined expression and SNP association:

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(2) CDKN2A – locus associated in recent GWA study.

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SNP data: 1748 cases samples and 2938 controls

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Table 3. Most significant canonical pathways and GO gene sets associated with case samples with FDR≤0.25

Gene Set Name	P-value	FDR
Canonical Pathways		
PROTEASOME	0.0121	0.0539
[b] SA_MMP_CYTOKINE_CONNECTION	0.0059	0.0652
CHOLESTEROL_BIOSYNTHESIS	0.1532	0.0823
AMINOACYL_TRNA_BIOSYNTHESIS	0.0794	0.0882
PROTEASOMEPATHWAY	0.0133	0.0925
[b] LAIRPATHWAY	0.017	0.1035
[b] STEMPATHWAY	0.0113	0.111
HSA00970_AMINOACYL_TRNA_BIOSYNTHESIS	0.1063	0.1176
[b] HYPERTROPHY_MODEL	0.0171	0.1193
[b] IL6PATHWAY	0.0122	0.1196
[a] ATMPATHWAY	0.0006	0.126
[a,b] TNFR2PATHWAY	0.0075	0.139
[b] ERYTHPATHWAY	0.0551	0.1395
HSA03050_PROTEASOME	0.0102	0.1464
[a,b] NTHIPATHWAY	0.0147	0.2121
[b] NO2IL12PATHWAY	0.0541	0.216
[a,b] TIDPATHWAY	0.0177	0.2235
HSA00530_AMINOSUGARS_METABOLISM	0.0181	0.2269
[a,b] RELAPATHWAY	0.0251	0.2429
GO Gene Sets		
[b] CHEMOKINE_RECEPTOR_BINDING	0.0022	0.0138
[b] G_PROTEIN_COUPLED_RECEPTOR_BINDING	0.003	0.0261
[b] CHEMOKINE_ACTIVITY	0.0022	0.0276
PROTEIN_DOMAIN_SPECIFIC_BINDING	$<10^{-15}$	0.0543
INDUCTION_OF_APOPTOSIS_BY_INTRACELLULAR_SIGNALS	$<10^{-15}$	0.1114
[b] VIRAL_GENOME_REPLICATION	0.002	0.1253

[a] Canonical pathways including NF-κB signaling.
[b] Canonical pathways and GO gene sets related to cytokines, chemokines, and immune response.
For full results, see Table S28, S30.

Extensions

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(2) Other genomic sources: methylation, protemics ?,...

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(5) A proper model.

Software

GSAA software.

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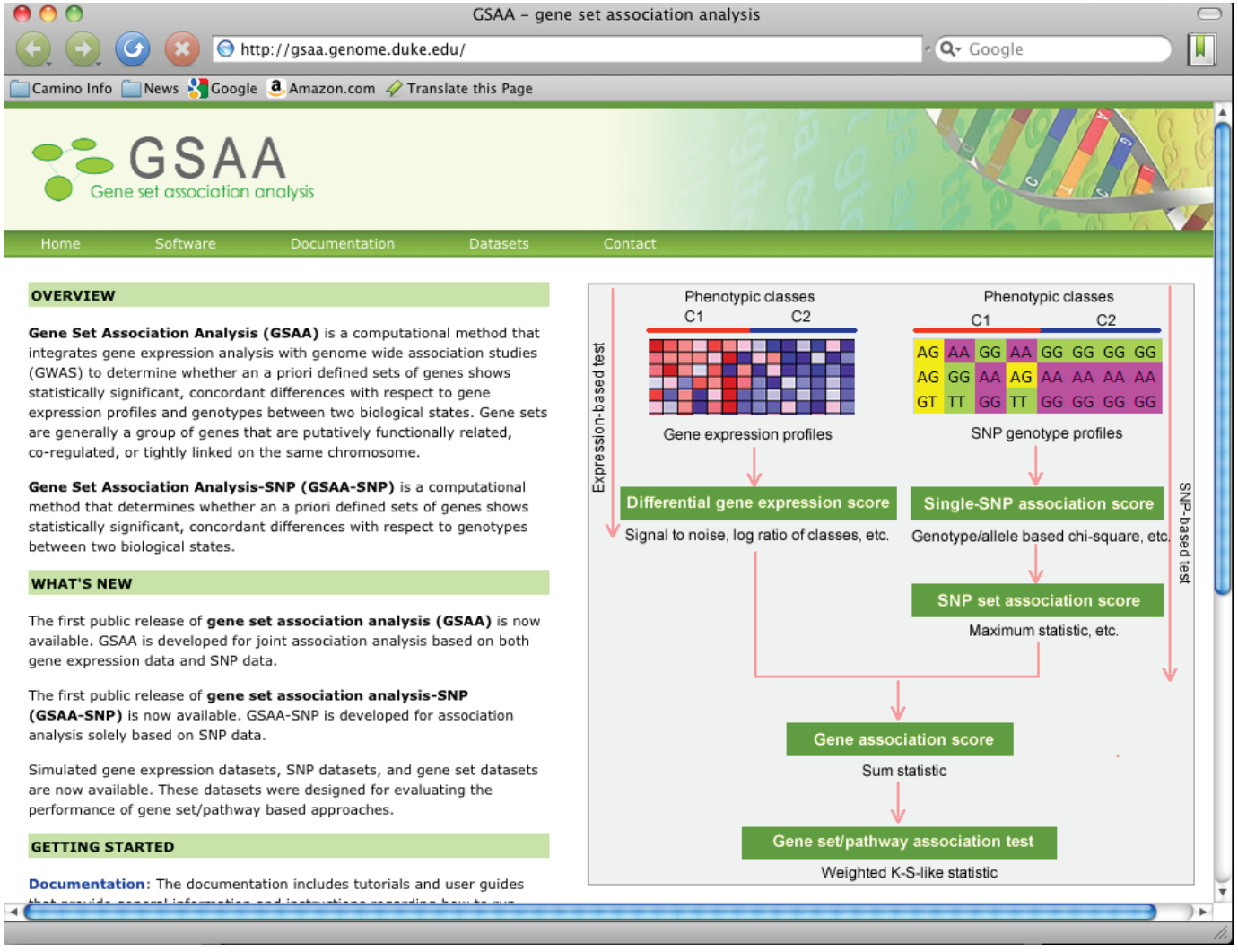

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