

metaGEM: an R package for meta-analysis of gene expression microarrays

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1 The metaGEM package

The package “metaGEM” is a collection of R functions to meta-analyze multiple studies based on gene expression microarray (GEMs) technology. It currently has implementation to perform vote counting, Fisher’s method of linear combination of p -values and random effects inverse-variance model.

This package was used in the following publication.

```
> citation("metaGEM")
```

To cite the metaGEM package in publications use:

Ramasamy A, Mondry A, Holmes CC, Altman DG. Key Issues in Conducting a Meta-Analysis of Gene Expression Microarray Datasets. PLoS Med. 2008 Sep 2;5(9):e184. PubMed ID: 18767902

A BibTeX entry for LaTeX users is

```
@article{,
  title = {Key Issues in Conducting a Meta-Analysis of Gene Expression Microarray Datasets},
  author = {Adaikalavan Ramasamy and Adrian Mondry and Chris C. Holmes and Douglas G. Altman},
  journal = {PLoS Medicine},
  number = {9},
  volume = {5},
  pages = {e184},
  year = {2008},
}
```

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2 Installation

The package `metaGEM` depends on the following R packages (`survival`, `splines`, `tools`) which are part of the default R installation. It additionally depends on the `rmeta` package for the core meta-analysis functions which can be installed via:

```
install.packages("rmeta")
```

It also requires the `multtest` package which is one of the default BioConductor packages. If not, you will need to install it using:

```
source("http://bioconductor.org/biocLite.R")
biocLite("multtest")
```

Next install the `metaGEM` package itself. In windows, one should modify the following command to reflect the path the package is in and excute it.

```
install.packages("metaGEM_0.3.9.zip", repos=NULL)
```

Similarly, in a Linux or Unix environment, one should install the package using

```
R CMD INSTALL metaGEM_0.3.9.tar.gz
```

3 Getting Started

We shall start by loading the package and the data to be used for illustrative purpose. The objective of the case study is to identify genes involved in the angiogenesis pathway that are consistently up- or down-regulated in cancers compared to normal tissue across different types of cancer.

```
> library(metaGEM)
> data(angular.GEMs)
> names(angular.GEMs)
```

[1] "Bcells.Basso"	"Bcells.Haslinger"	"Bcells.Klein"
[4] "Bladder.Dyrskjot"	"Bladder.Ramaswamy"	"BoneMarrow.Pellagati"
[7] "Brain.Ramaswamy"	"Colon.Ramaswamy"	"Gastric.Chen"
[10] "Gastric.Hippo"	"HN.Ginos"	"HN.Winter"
[13] "HN.Kuriakose"	"Kidney.Lenburg"	"Kidney.Ramaswamy"
[16] "Liver.Chen"	"Lung.Beer"	"Lung.Bhatt"
[19] "Lung.Borczuk"	"Lung.Hu"	"Lung.Jones"
[22] "Pancreas.Coulevard"	"Pancreas.Gruetzmann"	"Pancreas.Ishikawa"
[25] "Pancreas.Ramaswamy"	"Prostate.Lapointe"	"Prostate.Singh"
[28] "Prostate.Welsh"	"Thyroid.Aldred"	"Thyroid.Huang"

`angular.GEMs` is a list containing the expression data for 30 gene expression cancer studies. Here, we combined the different studies in a list for programming

convenience but this needs not to be case. We identified probes involved in the angiogenesis pathway using gene ontology ID "GO:0001525".

Each study in turn is a list of three objects:

- *expr* : the gene expression with rows representing probes and columns representing individual samples
- *class* : a vector used for two-class comparison and corresponds to the samples (same order as the columns in the *expr* matrix) with coding 0 (e.g. normal state) or 1 (e.g. diseased state).
- *keys* : a character vector that contains the mapping between gene-level ID to probe-level ID (same order as the rows in the *expr* matrix). If there are multiple gene-level IDs for the probe, separate by commas.

```
> study1 <- angio.GEMs[[1]]
> names(study1)

[1] "expr" "class" "keys"

> dim(study1$expr)

[1] 81 76

> head(study1$expr[, 1:17])
```

	GC_GSM44143	GC_GSM44144	GC_GSM44145	GC_GSM44146	GC_GSM44147	GC_GSM44148
1103_at	7.957125	7.989142	8.385903	7.866101	7.963237	7.875089
110_at	10.539679	10.736554	10.886344	10.642075	10.702474	10.687875
1114_at	8.923941	9.110819	9.334577	8.802456	8.853061	8.819157
1165_at	5.994917	5.820310	6.035808	6.080121	6.044072	6.067573
1262_s_at	6.100475	6.273634	6.209829	6.083056	6.193215	6.057253
1369_s_at	9.004378	9.348463	9.160286	8.959191	9.109953	9.981794
	GC_GSM44149	GC_GSM44150	GC_GSM44151	GC_GSM44152	BLL_GSM44194	BLL_GSM44195
1103_at	8.052673	7.794092	8.060426	7.897723	8.172545	8.015044
110_at	10.614298	10.467232	10.820543	10.635772	10.938416	10.565582
1114_at	8.997854	8.700318	9.104952	8.863807	8.924742	8.605557
1165_at	6.210225	6.071133	5.898282	6.018906	6.352702	7.379131
1262_s_at	6.304842	6.112066	6.256042	6.068427	6.552304	6.207223
1369_s_at	11.422599	12.586527	11.546107	9.901550	10.030576	8.869429
	BLL_GSM44196	BLL_GSM44197	BLL_GSM44198	BLL_GSM44199	DLBCL_GSM44204	
1103_at	8.025910	8.135513	8.243668	8.022870	8.047781	
110_at	10.425961	10.476280	10.438566	10.354283	10.545482	
1114_at	8.501332	9.027549	8.415759	8.606003	8.848800	
1165_at	6.431008	6.993048	7.797360	8.051576	6.837626	
1262_s_at	6.543764	6.178579	6.235826	6.226617	6.477566	
1369_s_at	8.833822	8.881111	9.230184	8.966454	9.065959	

```
> study1$class
```


HN.Ginos	140	140	114	178	79
HN.Winter	241	241	197	299	85
HN.Kuriakose	84	84	76	95	61
Kidney.Lenburg	190	190	152	240	83
Kidney.Ramaswamy	86	86	77	98	66
Liver.Chen	126	126	110	142	84
Lung.Beer	51	51	44	60	51
Lung.Bhatt	84	84	76	95	61
Lung.Borczuk	84	84	76	95	61
Lung.Hu	62	62	62	62	36
Lung.Jones	101	101	101	101	63
Pancreas.Coulevard	62	62	62	62	36
Pancreas.Gruetzmann	190	190	152	240	83
Pancreas.Ishikawa	190	190	152	240	83
Pancreas.Ramaswamy	86	86	77	98	66
Prostate.Lapointe	133	133	117	149	85
Prostate.Singh	84	84	76	95	61
Prostate.Welsh	81	81	73	92	61
Thyroid.Aldred	84	84	76	95	61
Thyroid.Huang	81	81	73	92	61

The object `angio.annDB` is also loaded and contains some annotation information obtained from SOURCE. In a real application, this information would normally be obtained only after the meta-analysis.

4 Combining effect sizes

In general, meta-analysis is a two stage process. The first step is to summarize each study. In the case of meta-analysis, we need to summarize each probe within a study. These study-specific estimates are then combined in the second step.

4.1 Calculating study-specific effect size

We can use `effect.sizes` to calculate the effect size for each probe within a study. We choose the Hedges' adjusted g formulation for effect size since it corrects for the bias in effect size when sample sizes are small.

```
> all.ES <- lapply(angio.GEMs, effect.sizes)
> length(all.ES)

[1] 30

> head(all.ES[[1]])
```

	n1	m1	sd1	n2	m2	sd2	diff	pooled.sd
1103_at	66	8.255745	0.3034292	10	7.984151	0.16415891	0.27159371	0.2900847
110_at	66	10.646276	0.2004456	10	10.673285	0.12396966	-0.02700839	0.1927719
1114_at	66	8.778331	0.2558879	10	8.951094	0.18820387	-0.17276316	0.2486421
1165_at	66	7.590826	0.9194767	10	6.024135	0.10575551	1.56669181	0.8625393
1262_s_at	66	6.362857	0.3340360	10	6.165884	0.09253233	0.19697316	0.3147234
1369_s_at	66	9.217344	0.3759845	10	10.102085	1.29127487	-0.88474089	0.5718061
	g	se.g						
1103_at	0.9267354	0.3480096	Hs.283749,Hs.708689					
110_at	-0.1386806	0.3395364	Hs.513044					
1114_at	-0.6877605	0.3441418	Hs.68879					
1165_at	1.7978999	0.3709183	Hs.83077					
1262_s_at	0.6194964	0.3432410	Hs.133379					
1369_s_at	-1.5315394	0.3625285	Hs.624,Hs.551925,Hs.561078					

all.ES is a list containing the following summary statistics for each probe within each study:

- *n1, m1, sd1* : The size, mean and standard deviation of group 1
- *n2, m2, sd2* : The size, mean and standard deviation of group 2
- *diff, pooled.sd* : The difference in group means ($\text{diff} = m2 - m1$) and the associated standard error.
- *g, se.g*: The effect size as formulated by the Hedges' adjusted *g* and the associated standard error.

4.2 Combining effect sizes across studies

Next, we calculate the random effects inverse-variance meta-analysis.

```
> output.REM <- combine.effect.sizes(all.ES)
> names(output.REM)

[1] "g"          "se.g"       "pooled.estimates"

> n.geneIDs <- apply(output.REM$g, 2, function(x) length(x[!is.na(x)]))
> names(n.geneIDs) <- NULL
> n.geneIDs

[1] 61 61 61 79 66 85 66 66 80 51 79 85 61 83 66 70 51 61 61 36 63 36 83 83 66 85 61 61
[29] 61 61

> head(output.REM$pooled.estimates)

      n.studies  summary se.summary      tau2    p.value
Hs.103527      20  0.24010957 0.15389738 0.34416960 0.118714346
Hs.111         27 -0.32605021 0.13525978 0.37135087 0.015928571
Hs.112408      29  0.13803905 0.10325414 0.17724565 0.181259752
```

```
Hs.11392      20 -0.71045064 0.22818338 0.89663822 0.001848779
Hs.115263     20  0.02441159 0.09926810 0.09180615 0.805747487
Hs.1239       27 -0.05388559 0.09800044 0.15097859 0.582421955
```

```
> table(output.REM$pooled.estimates$n.studies)
```

```
 1  2  3  4  5  6  7 10 11 12 13 14 15 16 17 20 23 24 27 28 29 30
 7  7  9  1  2  1  3  2  3  3  2  2  2  4  1  4  3  7  7 11  8 20
```

output.REM contains three components. The matrix `g` and `se.g` are the the study-specific effect size and the associated standard error for each gene-level ID. These are then used as inputs for random effects inverse-variance meta-analysis which is stored as the matrix component `pooled.estimates`.

4.3 Visualizing the results

We developed a simple plot `plot.sumsum`, motivated by the volcano plot concept, to summarize all the results simultaneously. But first, we merge it with the annotation information to obtain the gene symbol (if available).

```
> pooled.ES <- output.REM$pooled.estimates
> angio.annDB <- angio.annDB[, "Symbol", drop = FALSE]
> pooled.ES <- merge(pooled.ES, angio.annDB, by = 0, all.x = T)
> rownames(pooled.ES) <- pooled.ES$Row.names
> pooled.ES$Row.names <- NULL
> mylabels <- paste("\n", rownames(pooled.ES), "(", pooled.ES$Symbol,
+      ")", sep = "")
> mylabels <- gsub("\\(NA\\)", "", mylabels)
```

Figure suggests that there appears to be five statistically significant up-regulated and three statistically significant down-regulated angiogenesis genes (at 1% false discovery rate) across all 30 studies.

However, if we inspect the results closer, we can see that the top few hits were identified only in few studies.

```
> pooled.ES$p.fdr <- p.adjust(pooled.ES$p.value, method = "fdr")
> pooled.ES <- pooled.ES[order(pooled.ES$p.fdr), ]
> pooled.ES[which(pooled.ES$p.fdr < 0.01), ]
```

	n.studies	summary	se.summary	tau2	p.value	Symbol	p.fdr
Hs.696701	1	1.1933454	0.23036503	NA	2.216043e-07	<NA>	2.415487e-05
Hs.696107	1	1.0204950	0.22669491	NA	6.743649e-06	<NA>	3.675289e-04
Hs.567968	3	0.7512201	0.19139650	0.07164694	8.675081e-05	<NA>	3.151946e-03
Hs.592212	27	0.3126040	0.08401733	0.12793127	1.986642e-04	TYMP	5.413601e-03
Hs.690402	1	0.8059848	0.22287456	NA	2.988257e-04	<NA>	6.514400e-03
Hs.152913	11	-0.5997692	0.16842910	0.24867897	3.695022e-04	EMCN	6.712623e-03
Hs.154210	28	-0.6547309	0.18731413	0.83611273	4.734174e-04	S1PR1	7.371785e-03
Hs.283749	30	-0.4416104	0.12857625	0.43231827	5.933880e-04	ANG	8.084912e-03

```
> plot.sumsum(pooled.ES, cex = 0.5, labels = mylabels, lwd = 2, signif.thr = 0.05)
```

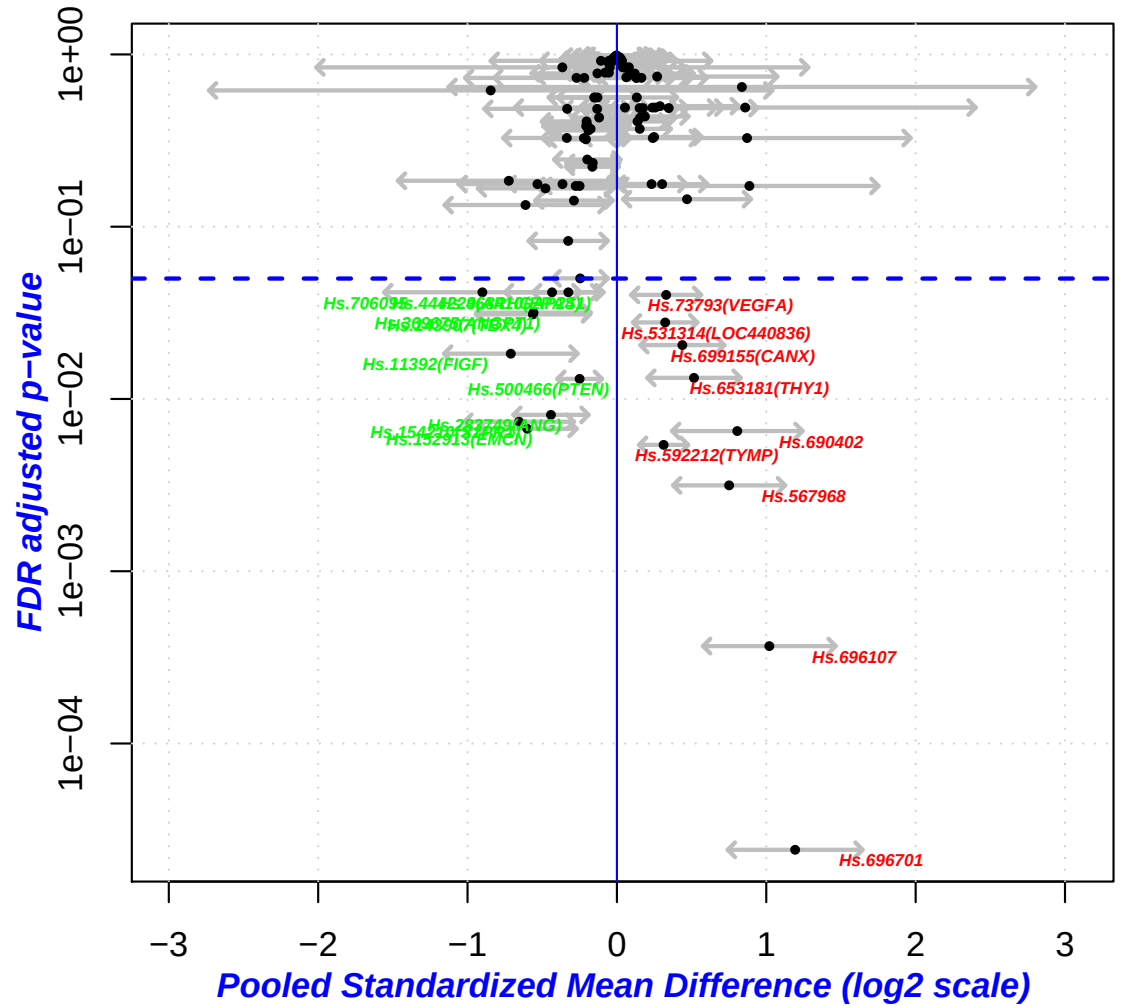


Figure 1: A Summary plot of the pooled effect size (black dots) with the 95% confidence interval (gray bars) sorted by the FDR. The Gene Symbol is given in parantheses when available.

Therefore we choose to impose an additional criteria that the probe must appear in at least five studies and relax the p-value threshold.

```
> length(w <- which(pooled.ES$p.fdr < 0.05 & pooled.ES$n.studies >= 5))
```

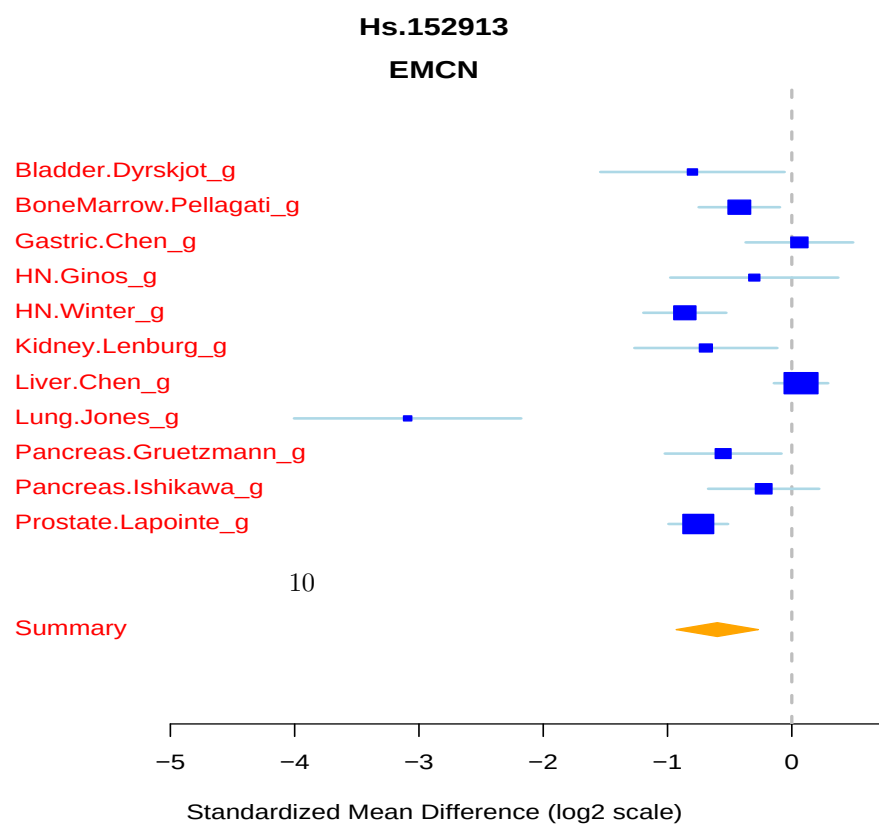
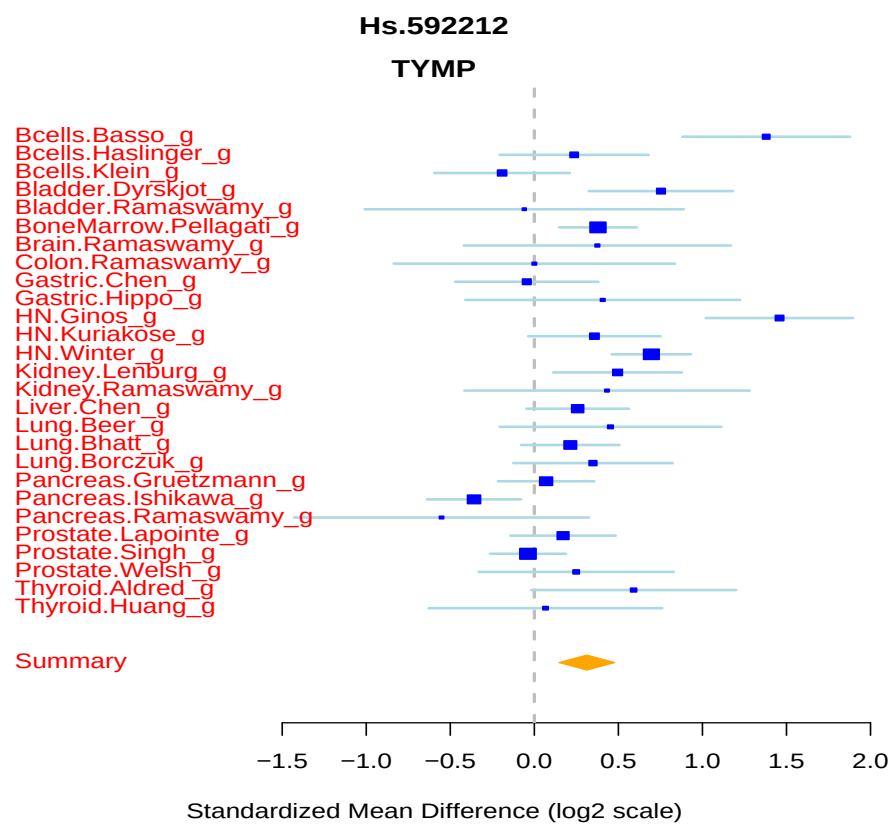
```
[1] 14
```

```
> pooled.ES[w, ]
```

	n.studies	summary	se.summary	tau2	p.value	Symbol	p.fdr
Hs.592212	27	0.3126040	0.08401733	0.1279313	0.0001986642	TYMP	0.005413601
Hs.152913	11	-0.5997692	0.16842910	0.2486790	0.0003695022	EMCN	0.006712623
Hs.154210	28	-0.6547309	0.18731413	0.8361127	0.0004734174	S1PR1	0.007371785
Hs.283749	30	-0.4416104	0.12857625	0.4323183	0.0005933880	ANG	0.008084912
Hs.500466	30	-0.2495422	0.07634018	0.1240698	0.0010799759	PTEN	0.013079708
Hs.653181	30	0.5162059	0.15957086	0.6474617	0.0012165844	THY1	0.013260770
Hs.11392	20	-0.7104506	0.22818338	0.8966382	0.0018487793	FIGF	0.018319722
Hs.699155	30	0.4381103	0.14348038	0.5058356	0.0022622580	CANX	0.020548843
Hs.531314	24	0.3231964	0.11005045	0.1858239	0.0033161615	LOC440836	0.027804739
Hs.143907	10	-0.5622203	0.19522056	0.2585621	0.0039777178	TBX4	0.030969375
Hs.369675	29	-0.5583001	0.19584308	1.0148108	0.0043615979	ANGPT1	0.031694278
Hs.73793	30	0.3296404	0.11972405	0.3630968	0.0058991564	VEGFA	0.040188003
Hs.444229	13	-0.4342368	0.16080713	0.2764305	0.0069264923	ARHGAP24	0.041612265
Hs.468410	30	-0.3252423	0.12058280	0.3680008	0.0069914199	EPAS1	0.041612265

Next, we can use forest plots to identify the contribution of each study, identify possible outliers and subgroup effect. Plotting the top three hits, for example, shows that the summary effect size for Hs.154210 (S1PR1) appears to be largely due to the lung cancer studies. Proving that such a subgrouping effect exists would require further work.

```
> tophits <- rownames(pooled.ES[w, ])
> tophits.symbol <- as.character(pooled.ES[w, "Symbol"])
> for (i in 1:3) {
+   forest.plot(output.REM, key = tophits[i])
+   title(main = tophits.symbol[i], line = 0)
+ }
```



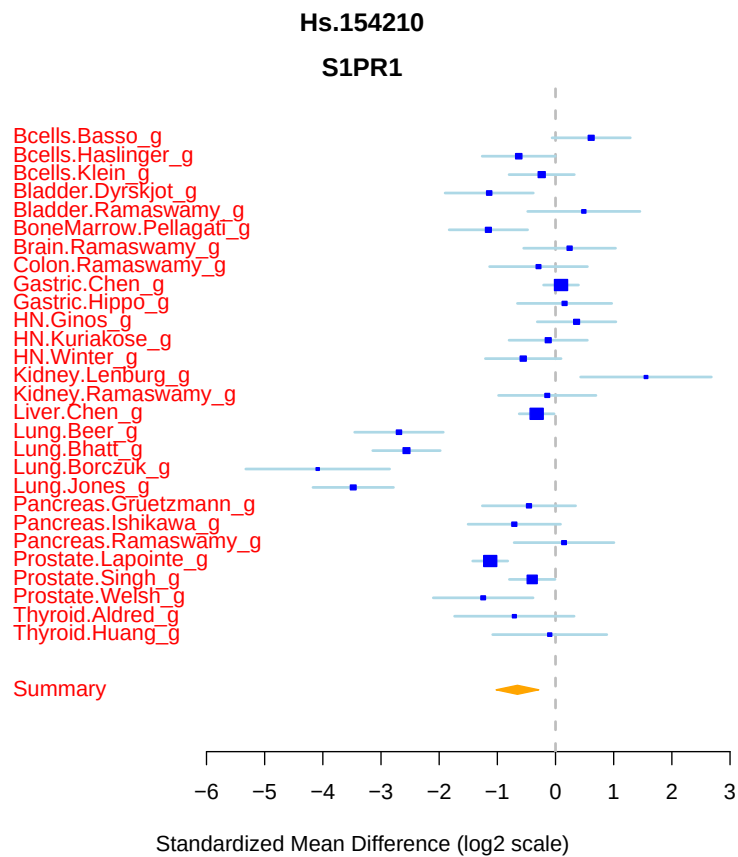


Figure 2: Forest plots for the three most statistically significant genes identified from the meta-analysis that was present in at least 5/30 studies.

4.4 ASIDE: How the function `summarize.effect.sizes` works

This section may be safely skipped and details what happens to each study in the first few steps of the `combine.effect.sizes` function. First, we create an artificial example:

```
> expr1 <- matrix(round(rnorm(35), digits = 2), nr = 5, nc = 7)
> rownames(expr1) <- paste("probe", 1:5, sep = "")
> colnames(expr1) <- c(paste("normal", 1:3, sep = ""), paste("tumor",
+       1:4, sep = ""))
> study1 <- list(expr = expr1, class = rep(0:1, c(3, 4)), keys = c("A",
+       "B", "A,C", NA, "E"))
> study1
```

```
$expr
      normal1 normal2 normal3 tumor1 tumor2 tumor3 tumor4
probe1    0.88   -0.98    0.44   0.34   1.54   1.09  -0.19
probe2   -0.94   -0.53   -0.56  -1.53    0.03   0.76   0.47
probe3   -0.72    0.18    0.10  -0.74  -0.14  -0.05   0.09
probe4   -0.70    1.81    1.08   0.33   1.95  -0.25   0.73
probe5   -0.27   -0.02    0.99   0.36   0.83   0.22   0.44
```

```
$class
[1] 0 0 0 1 1 1 1
```

```
$keys
[1] "A"   "B"   "A,C" NA    "E"
```

```
> es1 <- effect.sizes(study1)
> es1[, c("g", "se.g", "keys")]
```

```
      g      se.g keys
probe1 0.57179696 0.7979704 A
probe2 0.63858524 0.8062046 B
probe3 -0.12587114 0.7654555 A,C
probe4 -0.03091414 0.7638648 <NA>
probe5 0.41238925 0.7817427 E
```

First, discard any probes that could not be mapped to Gene IDs.

```
> bad <- which(is.na(es1$g) | is.na(es1$keys) | es1$keys == "NA")
> es1.clean <- es1[setdiff(1:nrow(es1), bad), ]
> es1.clean[, c("g", "se.g", "keys")]
```

```
      g      se.g keys
probe1 0.5717970 0.7979704 A
probe2 0.6385852 0.8062046 B
probe3 -0.1258711 0.7654555 A,C
probe5 0.4123893 0.7817427 E
```

Next, we need to replace probes that map to multiple Gene IDs with a new record for each Gene ID. This happens for probe 3 in this example.

```
> es1.expanded <- expand.df(es1.clean)
> es1.expanded[, c("g", "se.g", "keys")]
```

	g	se.g	keys
probe1	0.5717970	0.7979704	A
probe2	0.6385852	0.8062046	B
probe3	-0.1258711	0.7654555	A
probe3.1	-0.1258711	0.7654555	C
probe5	0.4123893	0.7817427	E

Finally, if a Gene ID is identified multiple times within a study, we need to summarize it to get a single estimate per Gene ID. Here, we use the fixed effect inverse variance (the default) to summarize.

```
> es1.summarized <- summ.eff.within(es1.expanded)
> es1.summarized
```

	g	se.g
A	0.2084596	0.5523960
B	0.6385852	0.8062046
C	-0.1258711	0.7654555
E	0.4123893	0.7817427

Here Gene A is identified twice and the output for Gene A should be identical to:

```
> w <- which(es1.expanded$keys == "A")
> meta.summaries(es1.expanded$g[w], es1.expanded$se.g[w])
```

Fixed-effects meta-analysis

Call: meta.summaries(d = es1.expanded\$g[w], se = es1.expanded\$se.g[w])

Summary effect=0.208 95% CI (-0.874, 1.29)

Estimated heterogeneity variance: 0 p= 0.528

We can repeat the steps above for the other studies and combine the effect sizes using the `multimerge` function which is equivalent to an iterative `merge` on the rownames (i.e. using `by=0` argument).

5 Vote counting and combining p -values

Vote counting involves counting the number of studies in which a gene was declared as significant. We can also combine the p -values using Fisher's sum of logs methods for a given gene which accounts for the strength of significance. For both methods, we need to first calculate a measure of significance for each gene in each study.

5.1 Calculating study-specific significance

We can use `ttest.Pvalues` or `ttest.Qvalues` to calculate the nominal p -values or the Q -values (the FDR-adjusted p -values) from a t -test statistics with equal variance assumption. These functions return the one-sided significance testing for up- and down-regulation of the gene. Here we take the Q -values as the significance measure.

```
> all.Qvals <- lapply(angio.GEMs, ttest.Qvalues)
> length(all.Qvals)

[1] 30

> head(all.Qvals[[1]])
```

	Q.up	Q.down	keys
1103_at	1.696097e-02	0.9999995329	Hs.283749,Hs.708689
110_at	1.000000e+00	0.7256864230	Hs.513044
1114_at	1.000000e+00	0.0941102585	Hs.68879
1165_at	3.002087e-05	0.9999995329	Hs.83077
1262_s_at	9.999838e-02	0.9999995329	Hs.133379
1369_s_at	1.000000e+00	0.0001609331	Hs.624,Hs.551925,Hs.561078

5.2 Fisher's sum of logs method

This method sums the logarithm of the one-sided hypothesis testing significance (p -values or Q -values) across k studies for a given gene. One advantage is that we can account for the strength in evidence rather than choosing an arbitrary threshold for declaring significance as we do in vote counting.

The p -value for the test statistic can be calculated using permutation method or by using the asymptotic distribution of chi-square with $2k$ degrees of freedom. Our function `sum.of.logs` calculates the Fisher statistics (minus twice the sum of log of significances) and calculates the asymptotical p -values for both alternative hypotheses.

```
> output.Fisher <- sum.of.logs(all.Qvals)
> head(output.Fisher)
```

	F.stat.up	F.pval.up	F.stat.down	F.pval.down
Hs.103527	73.852309	0.000891894	25.03625	9.690188e-01
Hs.111	25.743055	0.999610064	150.65753	4.570851e-11
Hs.112408	47.127637	0.845547939	32.28585	9.975224e-01
Hs.11392	7.627488	0.999999995	243.75721	4.888361e-31
Hs.115263	23.673572	0.981231036	20.80779	9.947378e-01
Hs.1239	43.803800	0.837798858	56.96026	3.655263e-01

5.3 Vote counting

In this method, we count the number of studies in which a gene reaches a certain statistical significance. However, to do this, we need to choose a value for the statistical significance. We choose a threshold of 0.10 for this exercise. The function `count.votes` implements vote counting procedure and returns two ordered list - one each for testing the alternative hypothesis that genes are up-regulated and down-regulation.

```
> output.VC <- count.votes(all.Qvals, vote.threshold = 0.1)
> head(output.VC)
```

	yes.down	valid.down	yes.up	valid.up
Hs.103527	1	20	7	20
Hs.111	9	27	2	27
Hs.112408	2	29	5	29
Hs.11392	7	20	0	20
Hs.115263	1	20	2	20
Hs.1239	4	27	3	27

The output above shows that, for example, the gene Hs.103527 was significantly up-regulated in 1/20 studies and was significantly down-regulated in 7/20 studies.

6 Combining ranks

Not yet implemented in this package. However, users may be interested in the RankProd package.

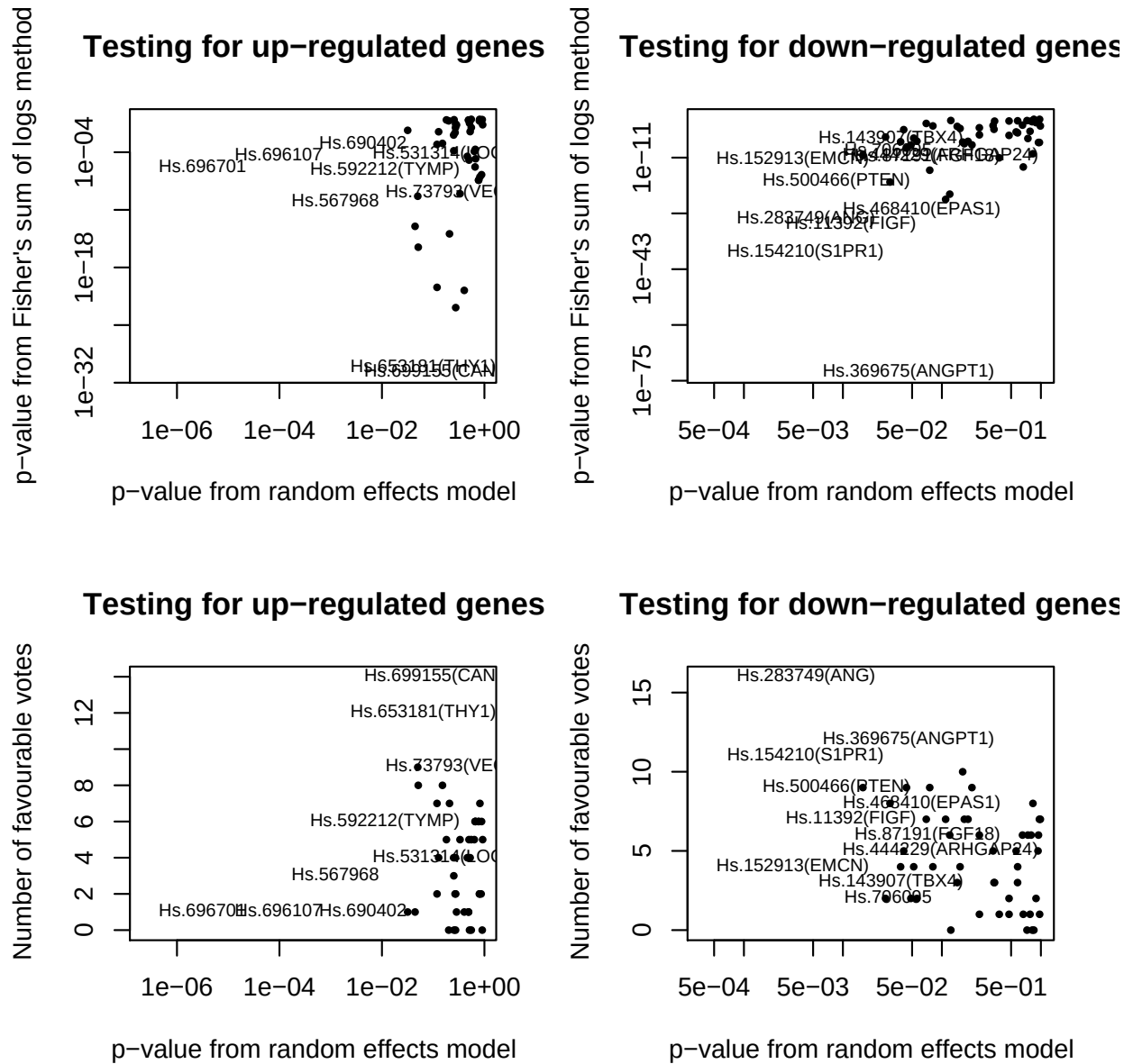
7 Comparison of results

To conclude, we can do a quick and dirty comparison of the outputs. For each alternative hypothesis, we plot the p -value from the random effects model against the Fisher's sum of logs method and also vote counting as follows. We

label the points which are significant at the nominal p-value of 0.01 using the random effects model.

```
> out <- cbind(pooled.ES, output.VC[rownames(pooled.ES), ], output.Fisher[rownames(pooled.ES),
+ ])
> rownames(out) <- paste(rownames(out), "(", out$Symbol, ")", sep = "")
> rownames(out) <- gsub("\\(NA\\)", "", rownames(out))
> out.pos <- out[which(out$summary > 0), ]
> out.neg <- out[which(out$summary < 0), ]
> myplot <- function(x, y, x.thres = 0.01, labels, cex = 0.75, ...) {
+   plot(x, y, type = "n", ...)
+   w <- which(x < x.thres)
+   points(x[-w], y[-w], cex = cex, pch = 20)
+   text(x[w], y[w], labels = labels[w], cex = cex, pos = 4)
+ }
> lab1 <- "p-value from random effects model"
> lab2 <- "p-value from Fisher's sum of logs method"
> lab3 <- "Number of favourable votes"
> main1 <- "Testing for up-regulated genes"
> main2 <- "Testing for down-regulated genes"

> par(mfrow = c(2, 2))
> myplot(out.pos$p.value, out.pos$F.pval.up, labels = rownames(out.pos),
+   log = "xy", xlab = lab1, ylab = lab2, main = main1)
> myplot(out.neg$p.value, out.neg$F.pval.down, labels = rownames(out.neg),
+   log = "xy", xlab = lab1, ylab = lab2, main = main2)
> myplot(out.pos$p.value, out.pos$yes.up, labels = rownames(out.pos),
+   log = "x", xlab = lab1, ylab = lab3, main = main1)
> myplot(out.neg$p.value, out.neg$yes.down, labels = rownames(out.neg),
+   log = "x", xlab = lab1, ylab = lab3, main = main2)
```



As you can see for the alternative hypothesis for up-regulated genes (left column of figures), the genes CANX and THY1 have a significant p -values for random effects model and Fisher's method as well as having a high number of favourable votes; making them worthy of further investigation. Similarly, we can prioritize ANG, ANGPT1 and S1PR1 for investigation of the down-regulated alternative hypothesis.

```
> w <- which(out$Symbol %in% c("CANX", "THY1", "ANG", "ANGPT1", "S1PR1"))
> out[w, ]
```

	n.studies	summary	se.summary	tau2	p.value	Symbol
Hs.154210(S1PR1)	28	-0.6547309	0.1873141	0.8361127	0.0004734174	S1PR1
Hs.283749(ANG)	30	-0.4416104	0.1285763	0.4323183	0.0005933880	ANG
Hs.653181(THY1)	30	0.5162059	0.1595709	0.6474617	0.0012165844	THY1
Hs.699155(CANX)	30	0.4381103	0.1434804	0.5058356	0.0022622580	CANX
Hs.369675(ANGPT1)	29	-0.5583001	0.1958431	1.0148108	0.0043615979	ANGPT1

	p.fdr	yes.down	valid.down	yes.up	valid.up	F.stat.up
Hs.154210(S1PR1)	0.007371785	11	28	1	28	19.81023
Hs.283749(ANG)	0.008084912	16	30	2	30	39.66767
Hs.653181(THY1)	0.013260770	3	30	12	30	284.55167
Hs.699155(CANX)	0.020548843	4	30	14	30	287.93036
Hs.369675(ANGPT1)	0.031694278	12	29	4	29	87.86476

	F.pval.up	F.stat.down	F.pval.down
Hs.154210(S1PR1)	9.999981e-01	320.97880	7.758202e-39
Hs.283749(ANG)	9.801849e-01	275.44456	2.367018e-29
Hs.653181(THY1)	6.348750e-31	44.96753	9.257646e-01
Hs.699155(CANX)	1.645901e-31	54.10322	6.899125e-01
Hs.369675(ANGPT1)	6.896912e-03	509.49172	2.006653e-73