Genomics of Prostate Cancer Health Disparity- Beyond the Gene List

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Yes, I brought the band: FAMU Marching 100

Prostate Cancer

- Most commonly diagnosed male cancer
- African American Males have highest morbidity and mortality rates than any other racial and ethnic groups
- Annual CaP incidence for AA was 59% higher than that of White Males (2001-2005).
- Overall CaP incidence down, but CaP decline in AA lags behind that of White males
- Hence, CaP is a health disparity for AA men

- ***Cause of this disparity still unclear…***

Factors thought to Contribute to CaP Disparity

CAG repeats
Inadequate knowledge
Lower socioeconomics
Limited access to health care
Less likely to ID early symptoms
Poor CaP survival
Present at late stage
High Grade CaP
More aggressive CaP tumors
Different Disease Biology
Higher testosterone
Age , Race (non-modifiable factors)

Odedina, Infectious Agents & Cancer 2009, 4(Suppl):S2

Prostate Cancer

We favor the Hypothesis:

- Differences in Gene expression may explain CaP health disparity

Identified 97 Candidate Genes

- **Microarray comparison of prostate tumor gene expression in African-American and Caucasian American males: a pilot project study.**
- Reams RR, Agrawal D, Davis MB, Yoder S, Odedina FT, Kumar N, Higginbotham JM, Akinremi T, Suther S, Soliman KF.
- Infect Agent Cancer. 2009 Feb 10;4 Suppl 1:S3.PMID: 19208208

Identification of differentially expresed genes

- Filtering criteria of at least +/- 2.0 fold change AND $p< 0.00001$

Description of PCa Tumor Tissue

- This pilot study made use of (three) 3+3 CaP tumor & non-tumor (normal) matched control samples from African American Males and (three) 3+3 PCa tumor & matched control samples from White American males.

- Samples were obtained from Dr. Deepak Agrawal, a collaborator from H. Lee Moffitt Center via IRB approval The limitation of this study is the small number of PCa samples available

Microarray Platform

- Gene Expression data were measured using the Affymetrix GeneChip HG-U133 plus 2 arrays containing 54,000 probe sets.

- Total of 12 samples were used 3 tumor & 3 normal from AAM; 3 tumor and 3 normal from WAM.
- Data from cel files was uploade to R-bioconductor (LIMMA package or SAM used)

Experimental Design II

Case matched ratios

Caucasian

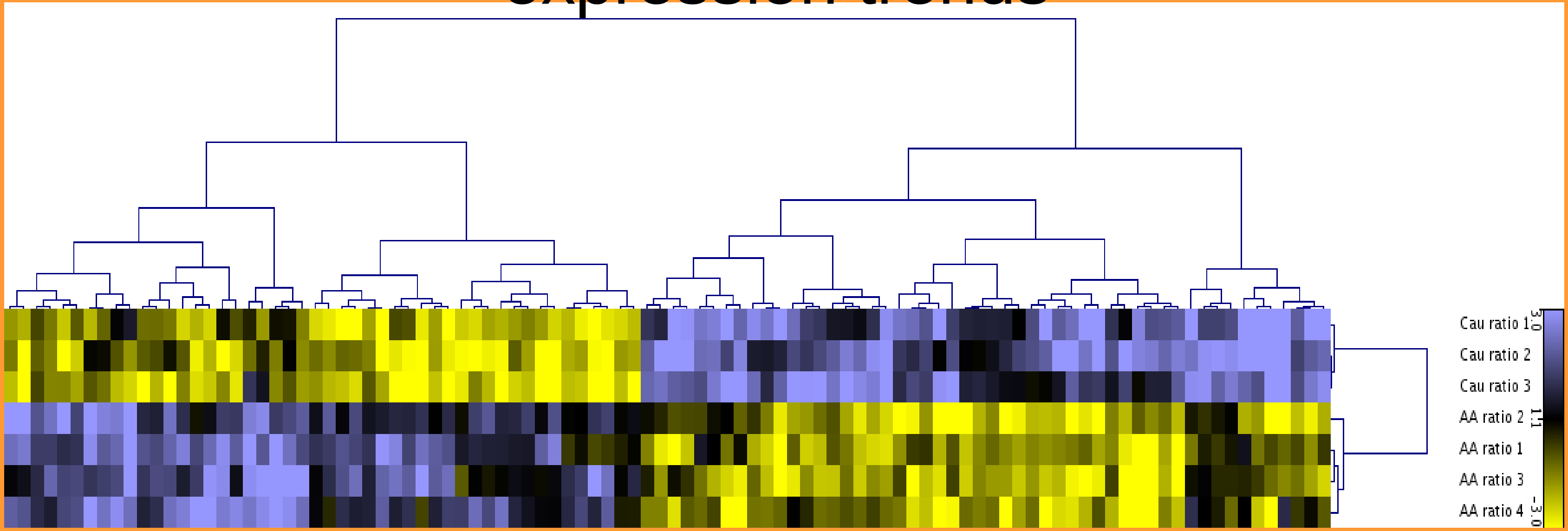
Tumor / Normal

Comparison of ratios

African-American

Tumor / Normal

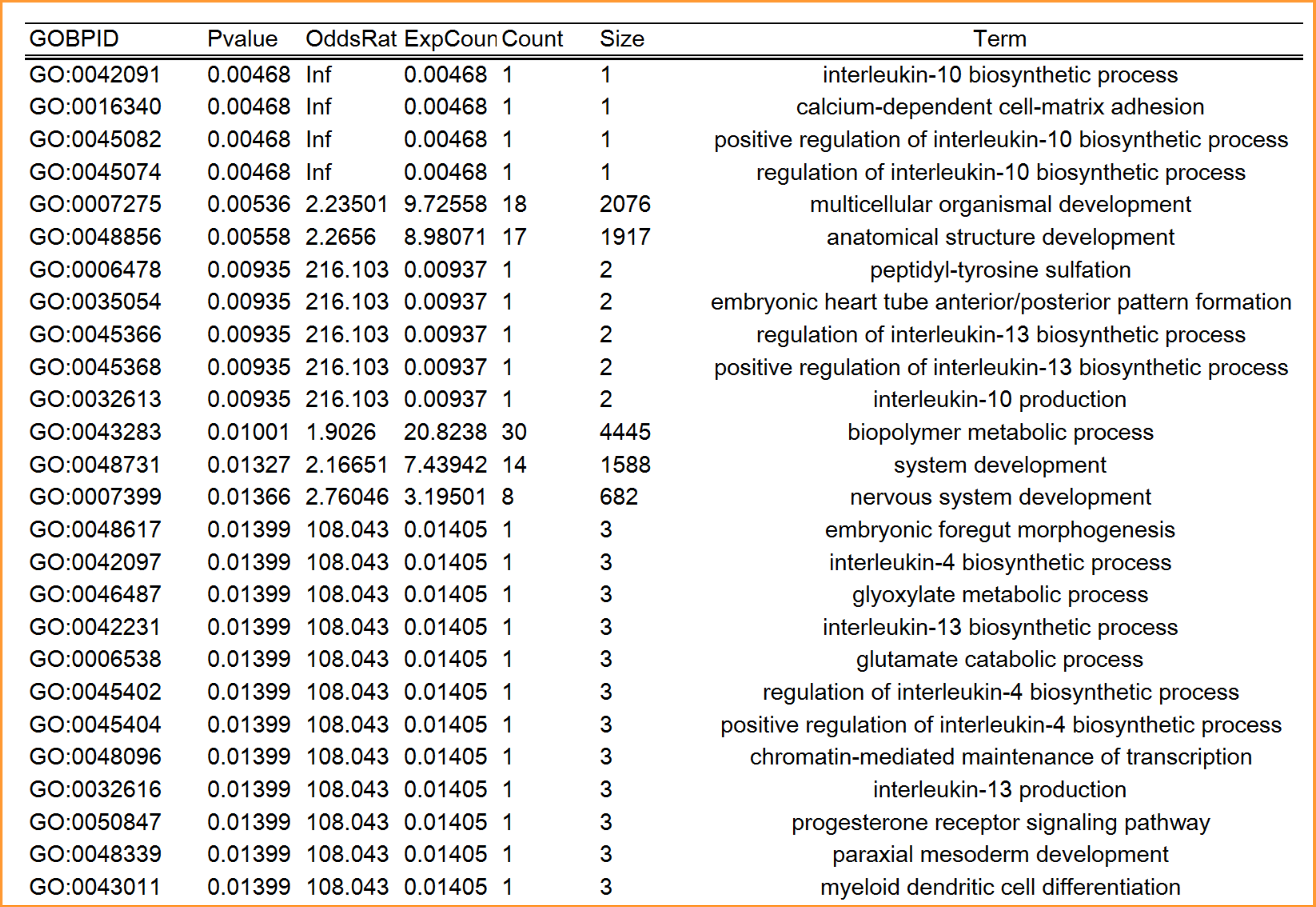
Case-matched ratios ~ test for race specific expression trends



chromosome 9 open reading frame 93	-5.288819	***cholinergic receptor, nicotinic, alpha 10***	-2.851774
helicase (DNA) B	4.651818	***paired-like homeodomain transcription factor 1***	3.531298
cholinergic receptor, muscarinic 3	-5.047906	***chromosome 9 open reading frame 62***	-3.983288
zyg-11 homolog A (C. elegans)	-5.491622	***chromosome 21 open reading frame 88***	2.890642
ring finger protein 187	-5.379997	***tubulin tyrosine ligase-like family, member 5***	4.073312
→ ***histone deacetylase 11***	-4.487602	***transcription factor 12 (HTF4, helix-loop-helix transcri***	-2.795597
hypothetical protein LOC440295	4.920586	→ ***progesterone receptor***	-2.783023
interferon regulatory factor 4	-4.214245	→ ***myeloid/lymphoid or mixed-lineage leukemia 4***	-3.80314
paired box gene 5 (B-cell lineage specific activator)	3.166453	***NSFL1 (p97) cofactor (p47)***	-4.411559
homeobox D4	-3.859365	***ring finger protein 168***	4.556035
alanine-glyoxylate aminotransferase	-4.773391	***hypothetical protein FLJ32658***	-3.835421
contactin 4	4.053437	***glypican 5***	-3.178348
transcription factor 3 (E2A immunoglobulin enhancer	-3.119406	***Friend leukemia virus integration 1***	2.434884
potassium voltage-gated channel, KQT-like subfamily,	-5.316092	***heterogeneous nuclear ribonucleoprotein D (AU-rich***	3.095944
zinc finger protein 650	-4.522508	***ADAM metallopeptidase with thrombospondin type 1***	3.204275
WD repeat domain 32	-4.752269	***myelin-associated oligodendrocyte basic protein***	2.572629
inscuteable homolog (Drosophila)	-3.820665	***Ran GTPase activating protein 1***	-3.898153
chromosome 15 open reading frame 26	-4.805246	***delta-like 3 (Drosophila)***	2.556212
WD repeat domain 60	-4.202883	***chemokine (C-X-C motif) ligand 2***	-4.644899
transient receptor potential cation channel, subfamily	4.572568	***G protein-coupled receptor 98***	-3.110733
DEAD (Asp-Glu-Ala-As) box polypeptide 19B	4.220601	***BUB3 budding uninhibited by benzimidazoles 3 homo***	-3.065752

Beyond the Gene List

Gene Ontology Enrichment



GOBPID	Pvalue	OddsRat	ExpCoun	Count	Size	Term
GO:0042091	0.00468	Inf	0.00468	1	1	interleukin-10 biosynthetic process
GO:0016340	0.00468	Inf	0.00468	1	1	calcium-dependent cell-matrix adhesion
GO:0045082	0.00468	Inf	0.00468	1	1	positive regulation of interleukin-10 biosynthetic process
GO:0045074	0.00468	Inf	0.00468	1	1	regulation of interleukin-10 biosynthetic process
GO:0007275	0.00536	2.23501	9.72558	18	2076	multicellular organismal development
GO:0048856	0.00558	2.2656	8.98071	17	1917	anatomical structure development
GO:0006478	0.00935	216.103	0.00937	1	2	peptidyl-tyrosine sulfation
GO:0035054	0.00935	216.103	0.00937	1	2	embryonic heart tube anterior/posterior pattern formation
GO:0045366	0.00935	216.103	0.00937	1	2	regulation of interleukin-13 biosynthetic process
GO:0045368	0.00935	216.103	0.00937	1	2	positive regulation of interleukin-13 biosynthetic process
GO:0032613	0.00935	216.103	0.00937	1	2	interleukin-10 production
GO:0043283	0.01001	1.9026	20.8238	30	4445	biopolymer metabolic process
GO:0048731	0.01327	2.16651	7.43942	14	1588	system development
GO:0007399	0.01366	2.76046	3.19501	8	682	nervous system development
GO:0048617	0.01399	108.043	0.01405	1	3	embryonic foregut morphogenesis
GO:0042097	0.01399	108.043	0.01405	1	3	interleukin-4 biosynthetic process
GO:0046487	0.01399	108.043	0.01405	1	3	glyoxylate metabolic process
GO:0042231	0.01399	108.043	0.01405	1	3	interleukin-13 biosynthetic process
GO:0006538	0.01399	108.043	0.01405	1	3	glutamate catabolic process
GO:0045402	0.01399	108.043	0.01405	1	3	regulation of interleukin-4 biosynthetic process
GO:0045404	0.01399	108.043	0.01405	1	3	positive regulation of interleukin-4 biosynthetic process
GO:0048096	0.01399	108.043	0.01405	1	3	chromatin-mediated maintenance of transcription
GO:0032616	0.01399	108.043	0.01405	1	3	interleukin-13 production
GO:0050847	0.01399	108.043	0.01405	1	3	progesterone receptor signaling pathway
GO:0048339	0.01399	108.043	0.01405	1	3	paraxial mesoderm development
GO:0043011	0.01399	108.043	0.01405	1	3	myeloid dendritic cell differentiation

Beyond the Gene List

New Bioinformatics Collaborator

Krishna Rani Kalari (Mayo Rochester):
Performed genotype-phenotype, SNP and expression transcript levels, correlations using HapMap Yorubi Population on the 97 genes previously revealed to be differentially expressed in BM tumors compared to WM tumors

Beyond The Gene List

Results

26527 genotype-phenotype associations were obtained with a p-value < 10-3, of which 17542/ 26527 associations has a p-value <10-4 (table with association results is attached below).

Genome-wide associations with a p-value < 10-3 were obtained for making Manhattan plots. Association and map files were created as input files for the Haploview software to generate Manhattan plots.

Figure 1: Results of genome-wide association SNPs with candidate prostate cancer genes using HapMap lymphoblastoid cell lines in YRI population. X-axis consists of different chromosomes and Y-axis consists of negative log p-value for association.

Association results in lymphoblastoid cell lines show that RanGAP1 gene which is a key regulator of the RAN GTP/GDP cycle that is located on chromosome 22 may be involved with

Association Results in YRI lymphoblastoid cell lines

RanGAP1 gene is located on chromosome 22 and may be involved with ABCD3 gene located on Chromosome 1

****

RanGAP1 gene, key regulator of the RAN GTP/GDP cycle

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ABCD3 gene which is ATP-Binding cassette, subfamily member

Association Results in YRI lymphoblastoid cell lines

STXBP2 gene is located on chromosome 19 and may be involved with TMTC2 gene located on Chromosome 12

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STXBP2 gene, syntaxin binding protein

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TMTC2 gene a transmembrane tetraccopeptide repeat containing 2 gene

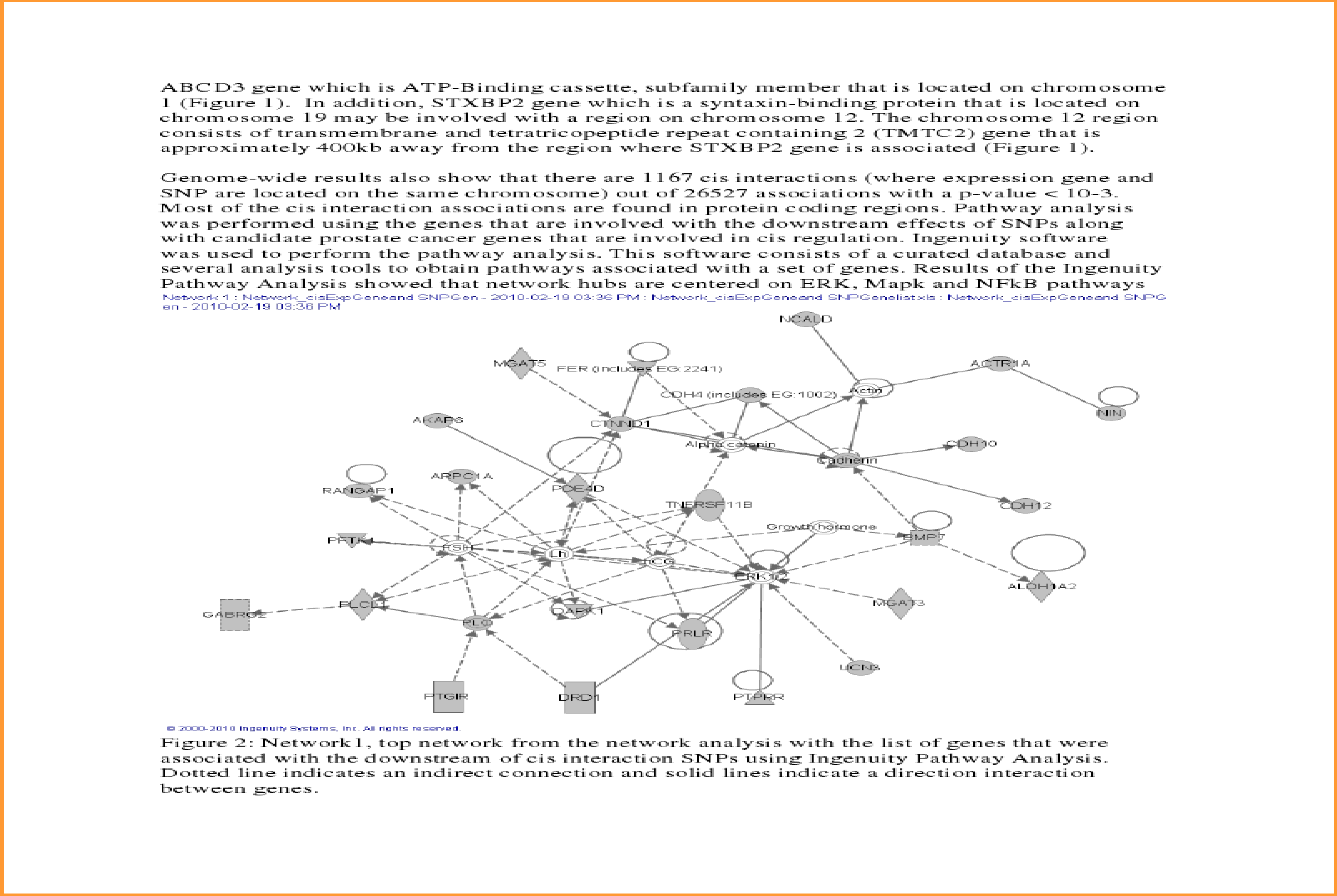
Association Results in YRI lymphoblastoid cell lines

Genome-wide results show 1167 cis interactions, where expression gene and SNP are located on the same chromosome ($p < 0.03$) . Most cis interactions in **coding region**; possible functional role in gene expressionhence

Ingenuity Pathway Analysis Revealed 3 top Networks Hubs

1. ERK
2. MAPK
3. NFkB

ERK



ABCD3 gene which is ATP-Binding cassette, subfamily member that is located on chromosome 1 (Figure 1). In addition, STXBP2 gene which is a syntaxin-binding protein that is located on chromosome 19 may be involved with a region on chromosome 12. The chromosome 12 region consists of transmembrane and tetratricopeptide repeat containing 2 (TMTC2) gene that is approximately 400kb away from the region where STXBP2 gene is associated (Figure 1).

Genome-wide results also show that there are 1167 cis interactions (where expression gene and SNP are located on the same chromosome) out of 26527 associations with a p-value < 10-3. Most of the cis interaction associations are found in protein coding regions. Pathway analysis was performed using the genes that are involved with the downstream effects of SNPs along with candidate prostate cancer genes that are involved in cis regulation. Ingenuity software was used to perform the pathway analysis. This software consists of a curated database and several analysis tools to obtain pathways associated with a set of genes. Results of the Ingenuity Pathway Analysis showed that network hubs are centered on ERK, Mapk and NFkB pathways

Network 1 : Network_cisExpGeneand SNPGen - 2010-02-19 03:36 PM : Network_cisExpGeneand SNPGenelist.xls : Network_cisExpGeneand SNPGen - 2010-02-19 03:36 PM

Figure 2: Network1, top network from the network analysis with the list of genes that were associated with the downstream of cis interaction SNPs using Ingenuity Pathway Analysis. Dotted line indicates an indirect connection and solid lines indicate a direction interaction between genes.

Attendance at This NCIBI

Much needed, to gain knowledge in current state of the art bioinformatic tools , Metabolomics, Proteomics

Collaorations to use NCIBI Tools to further probe this 97 candidte gene list ….

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Nagi, Kumar
Deepak Agrawal
Sean Yoder
Microarray Core Facility
Tissue Bank

DoD FAMU Moffitt Prostate
Cancer Training Center Grant

YOU ARE INVITED TO ATTEND!!
NCI Sponsored/Univ of Florida Hosted
The Science of Global Prostate Cancer Disparities in Black Men
Aug. 27-29, 2010
Crowne Plaza
Jacksonville, FL
Welcome

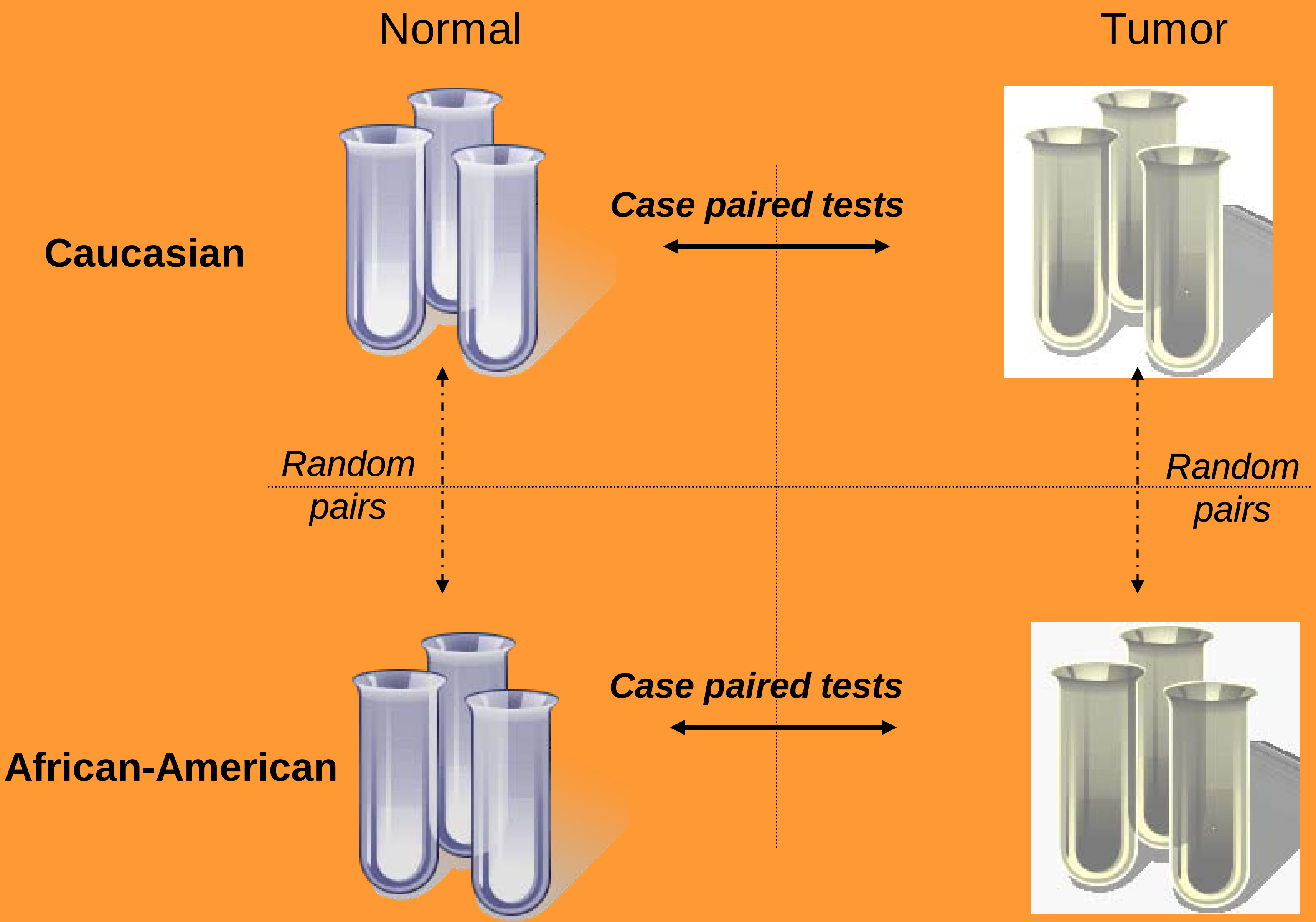
The Science of Global Prostate Cancer Disparities in Black Men "Bridging Gaps Through Research, Education, and Outreach Worldwide

http://conferences.dce.ufl.edu/docs/GPC/UF_GPCD_Agenda.pdf

1st Biennial Conference Highlights

- Global Collaborations Among Cancer Scientists from UK, Bahamas, Africa and USA, clinicians, survivors, and advocates
- To better understand the etiology of CaP among at-risk Black Men, develop interventions to address CaP disparities
- Prof Folake Odedina won an NCI grant award to host conference and bring in international scientists
- CME credits

Experimental Design I



Normal

Tumor

Caucasian

Case paired tests

Random pairs

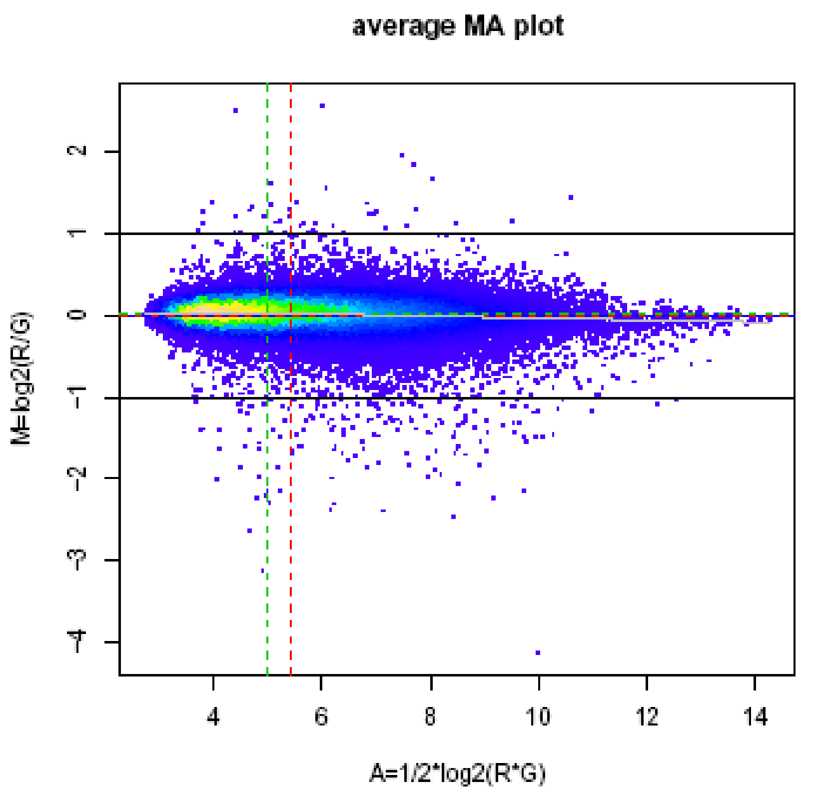
Random pairs

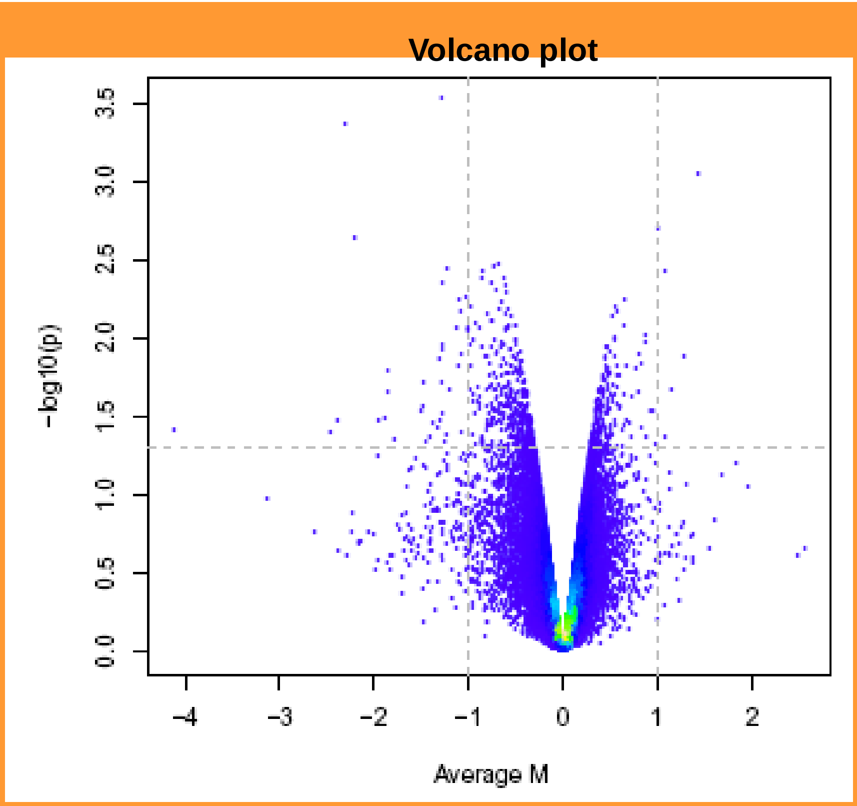
African-American

Case paired tests

CA tumor vs. normal
Case Paired t-test analysis

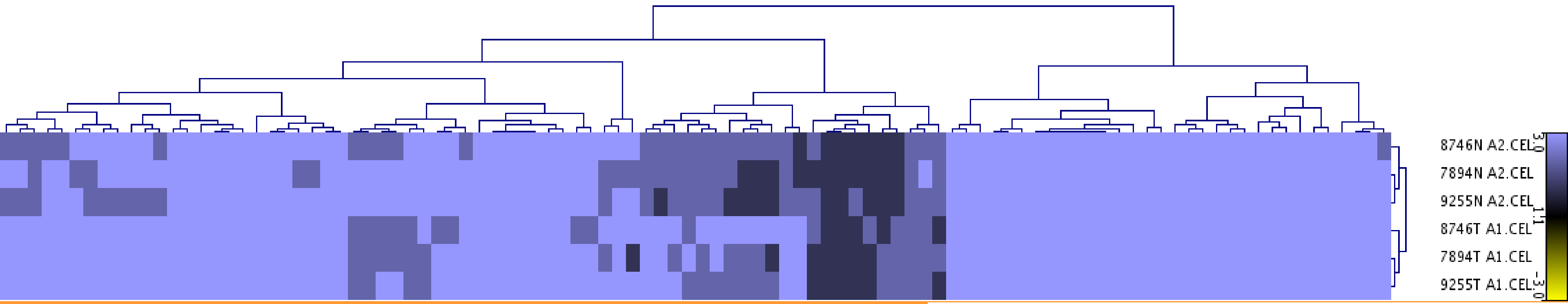
chloride channel, calcium activated, family member 4	-1.278013
TATA element modulatory factor 1	0.467335
protocadherin 8	-2.204783
hemoglobin, gamma A	-0.795291
olfactomedin 1	0.664676
spondin 2, extracellular matrix protein	1.433004
hemoglobin, gamma G	-0.72237
influenza virus NS1A binding protein	-0.451927
cystatin SN	-2.305485
hypothetical protein LOC441476	0.36533

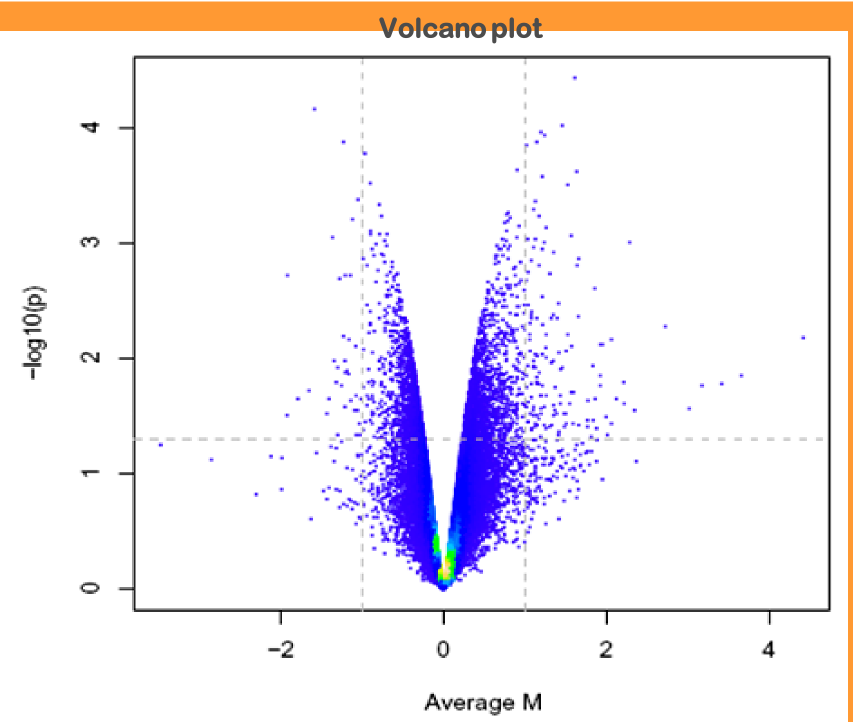


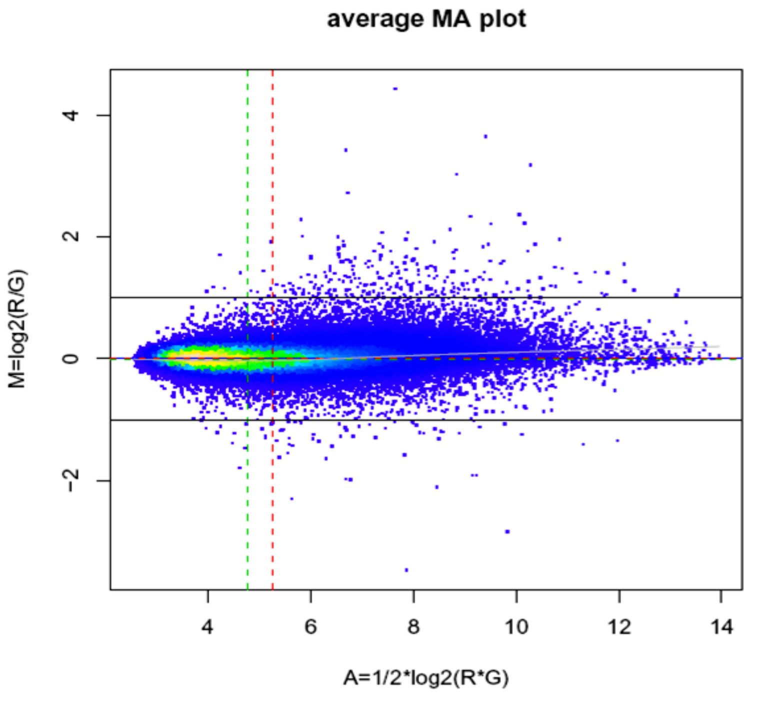


African American tumor vs. normal Case Paired t-test analysis

leukemia inhibitory factor receptor alpha	1.612491
aldehyde oxidase 1	-1.582018
acyl-CoA synthetase medium-chain family member 3	1.462871
natriuretic peptide receptor C/guanylate cyclase C (a	1.198831
histone cluster 1, H2bg	1.245253
	1.148056
RAD23 homolog B (S. cerevisiae)	-1.22417
insulin-like growth factor 1 (somatomedin C)	1.025239
paired related homeobox 1	-0.966881
prostate-specific non-coding gene	0.918045

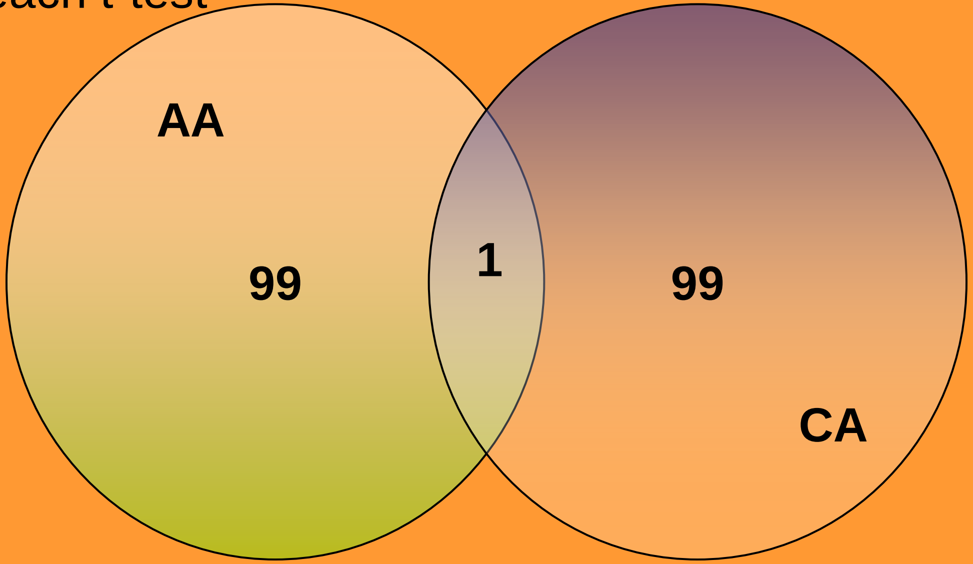


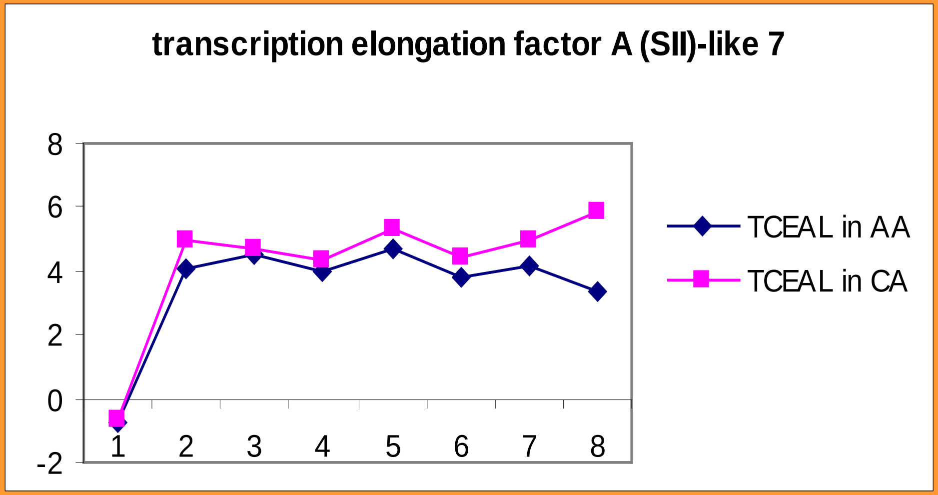


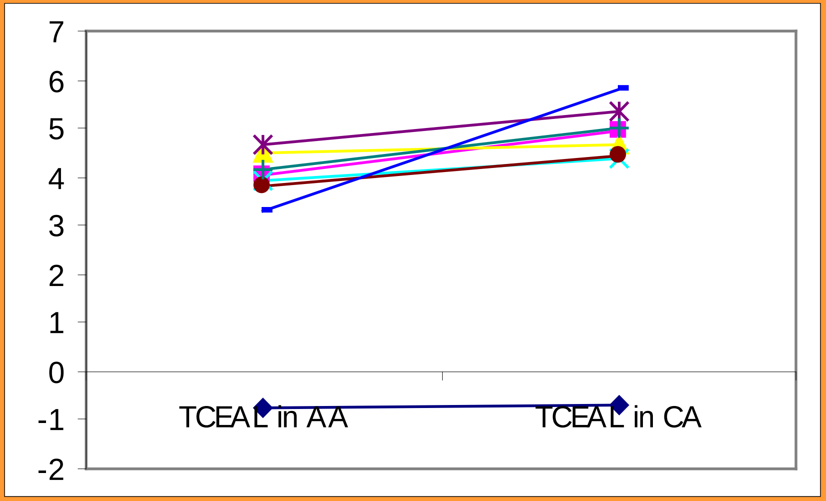


Overlaps case paired t-test Af Am. & Caucasian genelists

Top 100 genes from each t-test

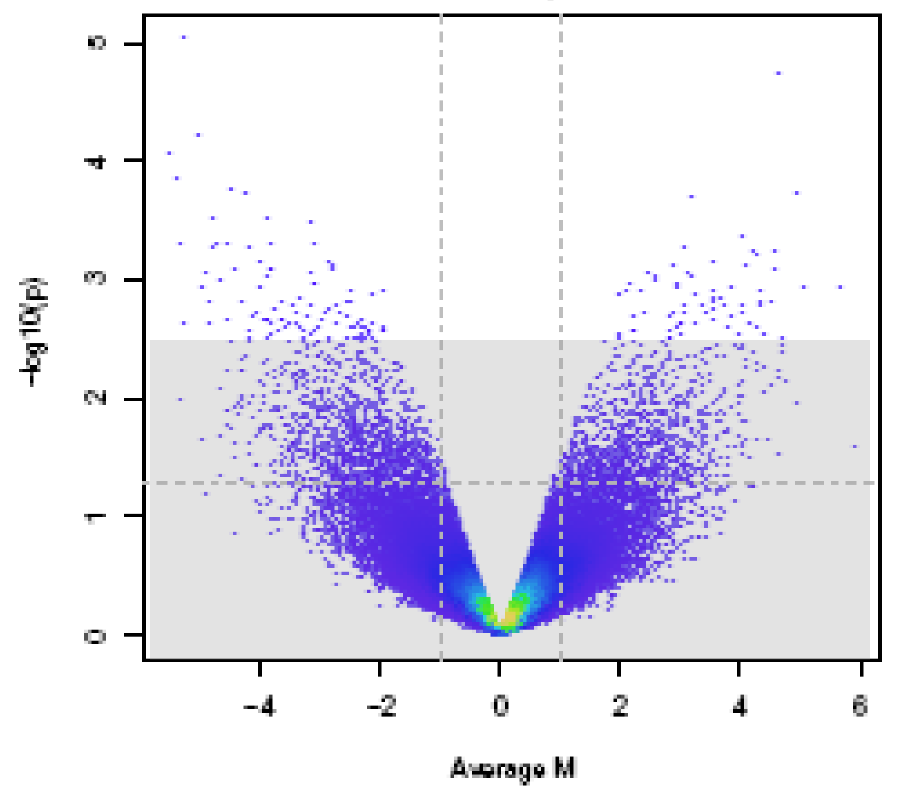






Case-matched ratios ~ Race group test

Volcano plot



Modified MAplot