Discussion/ Debate: Meet the Professor Workshop; Demystifying Molecular Techniques for Thyroid Cancer Diagnosis and Treatment

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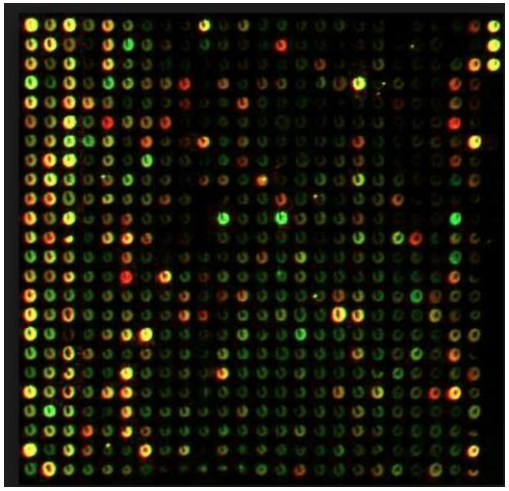
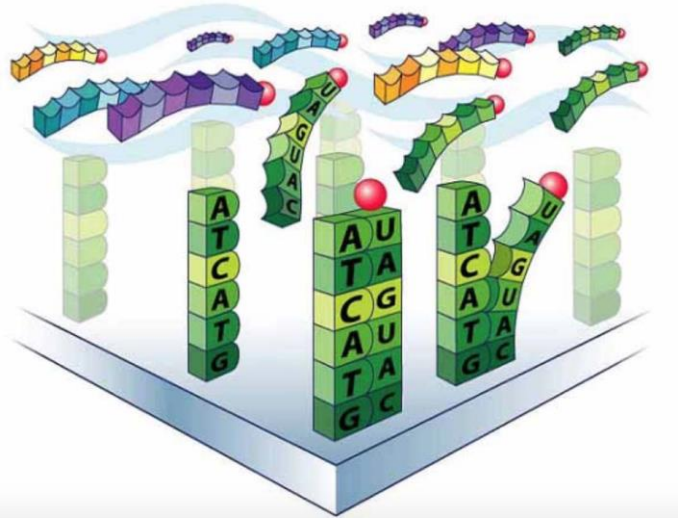
Disclosures

- Asuragen, consultant (former)
- Interpace, consultant (former)
- Rosetta Genomics, consultant (starting)

Gene Expression Profiling Early 2000's

- Field was largely initiated by the Director's Challenge program of the NCI, 1999
- Spotted DNA microarrays
  - Competitive hybridization
- Oligo DNA microarrays
- Michigan program project
  - Lung, ovary and colon cancer
- RNA sequencing

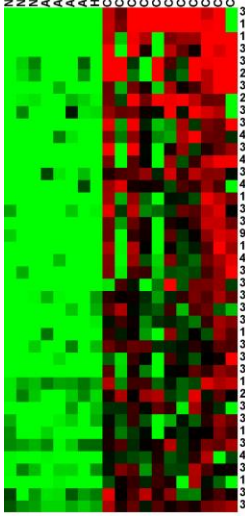




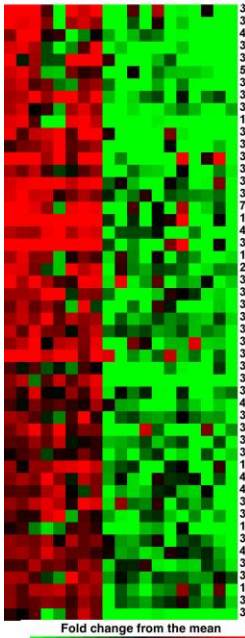
2003

Gene Expression Profiling Possesses Diagnostic Potential

Differential gene expression: adrenal adenomas and carcinomas

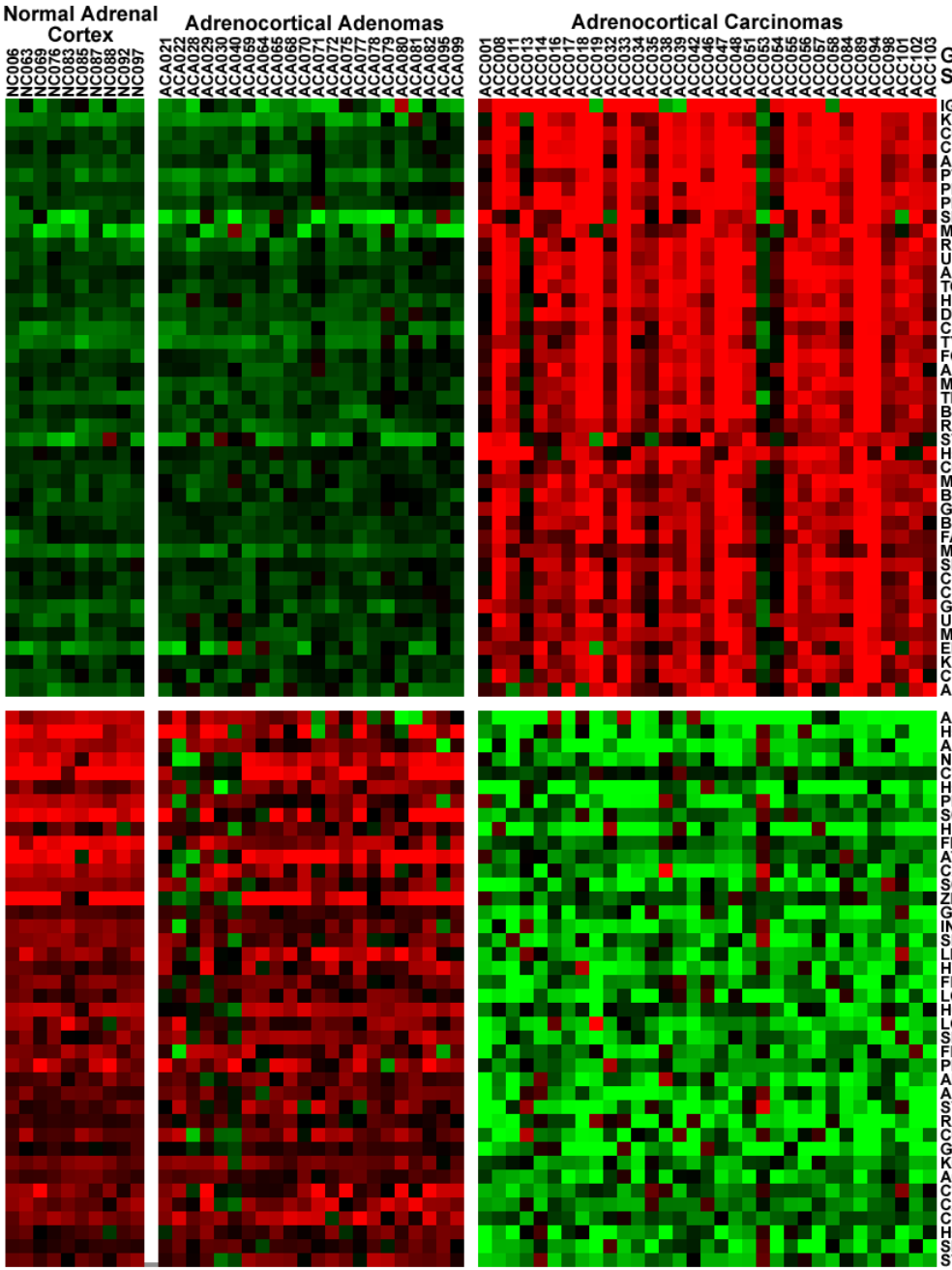


Probe-Set	Gene Symbol	Unigene Title	Fold change
36782_s_at	IGF2	insulin-like growth factor 2 (somatomedin A)	105.5
1591_s_at	IGF2	insulin-like growth factor 2 (somatomedin A)	40.9
1651_at	UBCH10	ubiquitin carrier protein E2-C	32.4
38116_at	KIAA0101	KIAA0101 gene product	28.2
34342_s_at	SPP1	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	20.9
2092_s_at	SPP1	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	16.6
39109_at	C20ORF1	chromosome 20 open reading frame 1	15.6
2079_s_at	IGF2	insulin-like growth factor 2 (somatomedin A)	14.9
39542_at	ENC1	ectodermal-neural cortex (with BTB-like domain)	11.4
39230_at	DJ742C19.2	phorbolin (similar to apolipoprotein B mRNA editing protein)	10.8
41060_at	CCNE1	cyclin E1	10.4
32263_at	CCNB2	cyclin B2	8.88
40145_at	TOP2A	topoisomerase (DNA) II alpha (170kD)	8.75
37050_r_at	TOM34	translocase of outer mitochondrial membrane 34	8.70
40328_at	TWIST	twist (Drosophila) homolog (acrocephalosyndactyly 3; Saethre-Chotzen syndrome)	8.44
1803_at	CDC2	cell division cycle 2, G1 to S and G2 to M	7.70
37263_at	GGH	gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase)	7.22
39677_at	KIAA0186	KIAA0186 gene product	7.09
904_s_at	TOP2A	topoisomerase (DNA) II alpha (170kD)	6.86
1599_at	CDKN3	cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)	6.84
40726_at	KNSL1	kinesin-like 1	6.68
34715_at	FOXM1	forkhead box M1	6.40
39945_at	FAP	fibroblast activation protein, alpha	6.17
36909_at	WEE1	wee1+ (S. pombe) homolog	5.49
37883_1_at	AF038169	hypothetical protein	5.30
34878_at	SMC4L1	SMC4 (structural maintenance of chromosomes 4, yeast)-like 1	5.27
1801_at	BARD1	BRCA1 associated RING domain 1	5.23
31845_at	ELF4	E74-like factor 4 (ets domain transcription factor)	5.20
35699_at	BUB1B	budding uninhibited by benzimidazoles 1 (yeast homolog), beta	4.89
34852_g_at	STK15	serine/threonine kinase 15	4.68
1505_at	TYMS	thymidylate synthetase	4.61
2057_g_at	FGFR1	fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)	4.44
37461_at	ANGPT2	angiopoietin 2	4.37
35314_at	KIAA0159	chromosome condensation-related SMC-associated protein 1	4.09
1721_g_at	MAD2L1	MAD2 (mitotic arrest deficient, yeast, homolog)-like 1	3.96
38065_at	HMG2	high-mobility group (nonhistone chromosomal) protein 2	3.54
41401_at	CSRP2	cysteine and glycine-rich protein 2	3.44
34390_at	P4HA2	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), alpha polypeptide II	3.44
1951_at	ANGPT2	angiopoietin 2	3.38
38656_s_at	MGC5576	hypothetical protein MGC5576	3.37
39337_at	H2AFZ	H2A histone family, member Z	3.27



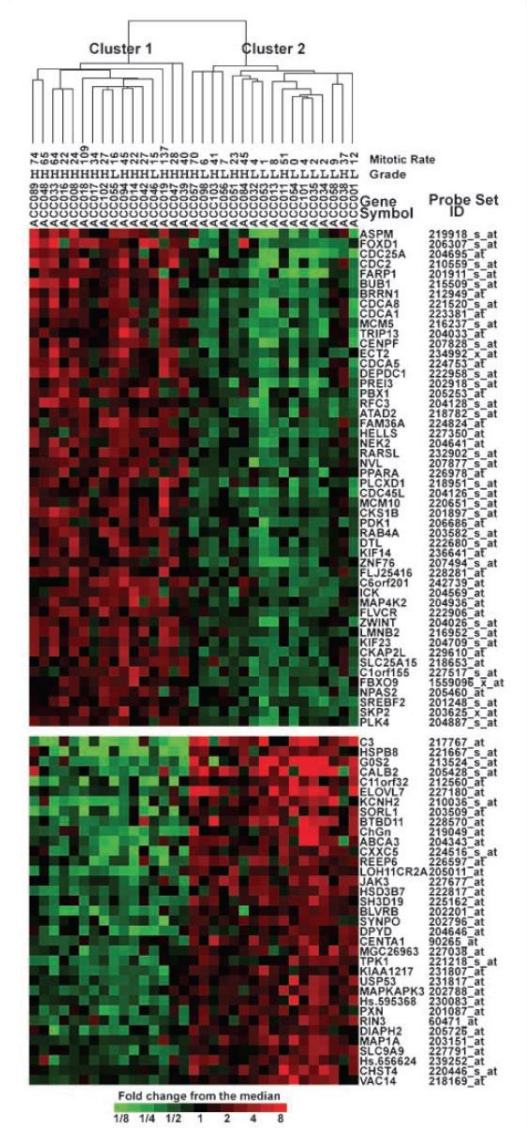
34637_f_at	ADH1	alcohol dehydrogenase 1 (class I), alpha polypeptide	0.04
35730_at	ADH2	alcohol dehydrogenase 2 (class I), beta polypeptide	0.06
40578_s_at	TMOD	tropomodulin	0.07
32666_at	SDF1	stromal cell-derived factor 1	0.07
36271_at	KIAA1024	KIAA1024 protein	0.08
533_g_at	PTHR1	parathyroid hormone receptor 1	0.10
38786_at	NULL	Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 248114	0.10
33118_at	SEMA3B	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3B	0.10
38026_at	FBLN1	fibulin 1	0.11
1736_at	IGFBP6	insulin-like growth factor binding protein 6	0.11
32056_at	PNUTL2	peanut (Drosophila)-like 2	0.13
34042_at	CHAD	chondroadherin	0.14
36512_at	AADAC	arylacetamide deacetylase (esterase)	0.15
37972_at	DNASE1L3	deoxyribonuclease I-like 3	0.15
37599_at	AOX1	aldehyde oxidase 1	0.15
37674_at	ALAS1	aminolevulinate, delta-, synthase 1	0.15
770_at	GPX3	glutathione peroxidase 3 (plasma)	0.16
1412_g_at	CYP11B1	cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1	0.16
40527_at	KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1	0.16
34548_at	CYP11B1	cytochrome P450, subfamily XIB (steroid 11-beta-hydroxylase), polypeptide 1	0.16
1731_at	PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	0.17
216_at	PTGDS	prostaglandin D2 synthase (21kD, brain)	0.18
36661_s_at	CD14	CD14 antigen	0.18
39295_s_at	ARGBP2	Arg/Abl-interacting protein ArgBP2	0.18
37765_at	LMOD1	leiomodin 1 (smooth muscle)	0.18
37191_at	KIAA0273	KIAA0273 gene product	0.19
38053_s_at	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	0.19
39545_at	CDKN1C	cyclin-dependent kinase inhibitor 1C (p57, Kip2)	0.20
34002_at	HSD3B2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	0.21
33235_at	KIAA0938	KIAA0938 protein	0.21
32077_s_at	KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1	0.23
36495_at	FBP1	fructose-1,6-bisphosphatase 1	0.23
40685_at	ALDH7	aldehyde dehydrogenase 7	0.23
37405_at	SELENBP1	selenium binding protein 1	0.23
32174_at	SLC9A3R1	solute carrier family 9 (sodium/hydrogen exchanger), isoform 3 regulatory factor 1	0.23
38042_at	G6PD	glucose-6-phosphate dehydrogenase	0.24
38844_at	PDK2	pyruvate dehydrogenase kinase, isoenzyme 2	0.25
1930_at	ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	0.26
41755_at	KIAA0977	KIAA0977 protein	0.26
41473_at	EPHX2	epoxide hydrolase 2, cytoplasmic	0.27
38487_at	KIAA0246	KIAA0246 protein	0.27
36275_at	Hs.29596	Homo sapiens mRNA from chromosome 5q21-22, clone:FBR89	0.27
1501_at	IGF1	insulin-like growth factor 1 (somatomedin C)	0.28
39438_at	CREBL2	cAMP responsive element binding protein-like 2	0.28
40409_at	ALDH10	aldehyde dehydrogenase 10 (fatty aldehyde dehydrogenase)	0.28
33197_at	MYO7A	myosin VIIA (Usher syndrome 1B (autosomal recessive, severe))	0.29
38406_f_at	PTGDS	prostaglandin D2 synthase (21kD, brain)	0.30
1327_s_at	MAP3K5	mitogen-activated protein kinase kinase kinase 5	0.32
38119_at	GYPC	glycophorin C (Gerbich blood group)	0.33
39842_at	CRLF1	cytokine receptor-like factor 1	0.33

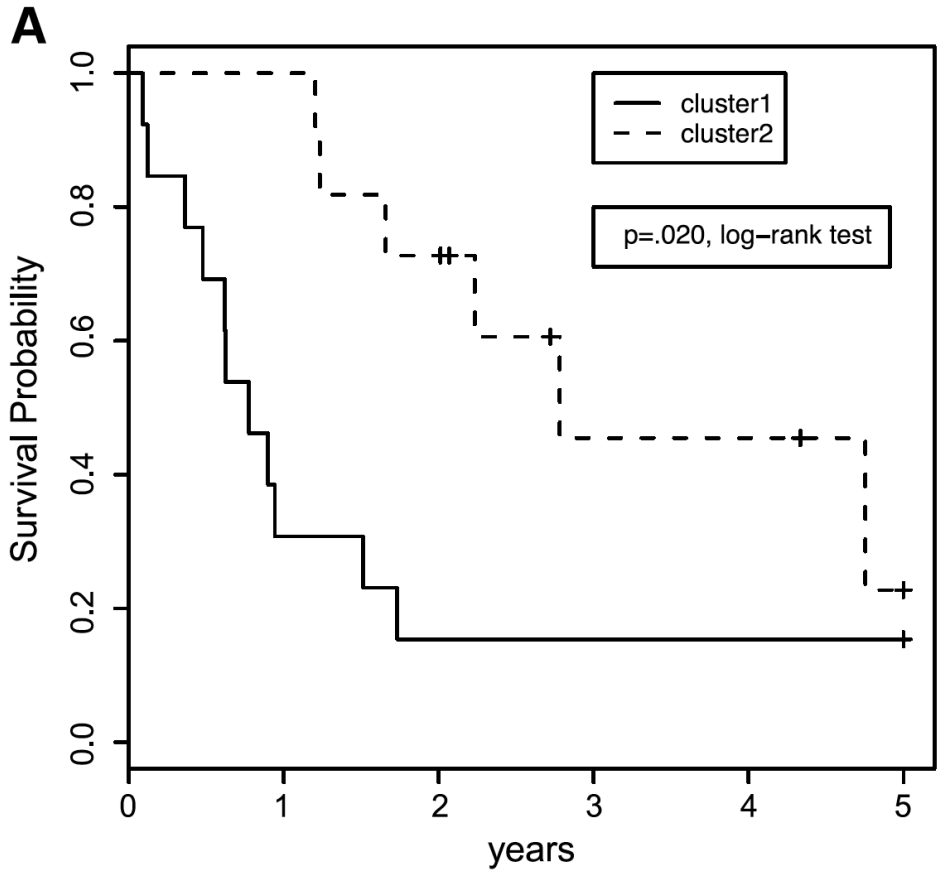
2009



Gene Symbol	Gene Title	Probe Set ID
IGF2	insulin-like growth factor 2 (somatomedin A)	210881_s_at
KIAA0101	KIAA0101	202503_s_at
CCNB2	cyclin B2	202705_at
CDC2	cell division cycle 2, G1 to S and G2 to M	203213_at
ASPM	asp (abnormal spindle)-like, microcephaly associated (Drosophila)	219918_s_at
PTTG1	pituitary tumor-transforming 1	203554_x_at
PBK	PDZ binding kinase	219148_at
PRC1	protein regulator of cytokinesis 1	218009_s_at
SPP1	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I)	209875_s_at
MGC16121	hypothetical protein MGC16121	227488_at
RRM2	ribonucleotide reductase M2 polypeptide	201890_at
UBE2C	ubiquitin-conjugating enzyme E2C	202954_at
ANLN	anillin, actin binding protein (scraps homolog, Drosophila)	222608_s_at
TOP2A	topoisomerase (DNA) II alpha 170kDa	201292_at
HMMR	hyaluronan-mediated motility receptor (RHAMM)	207165_at
DTL	denticleless homolog (Drosophila)	218585_s_at
CDKN3	cyclin-dependent kinase inhibitor 3	1555758_a_at
TYMS	thymidylate synthetase	202589_at
FOXM1	forkhead box M1	202580_x_at
APOBEC3B	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B	206632_s_at
MELK	maternal embryonic leucine zipper kinase	204825_at
TPX2	TPX2, microtubule-associated, homolog (Xenopus laevis)	210052_s_at
BIRC5	baculoviral IAP repeat-containing 5 (survivin)	202095_s_at
RACGAP1	Rac GTPase activating protein 1	222077_s_at
STC1	Stanniocalcin 1	230746_s_at
Hs.211461	CDNA FLJ39613 fis, clone SKNSH2009357	1564220_a_at
CCNB1	cyclin B1	214710_s_at
MGC24665	hypothetical protein MGC24665	226456_at
BUB1B	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	203755_at
GINS1	GINS complex subunit 1 (Psf1 homolog)	206102_at
BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	209642_at
FAM83D	family with sequence similarity 83, member D	225687_at
MYBL1	v-myb myeloblastosis viral oncogene homolog (avian)-like 1	213906_at
SHCBP1	SHC SH2-domain binding protein 1	219493_at
CDC20	CDC20 cell division cycle 20 homolog (S. cerevisiae)	202870_s_at
CEP55	centrosomal protein 55kDa	218542_at
GAS2L3	Growth arrest-specific 2 like 3	238756_at
UBE2T	ubiquitin-conjugating enzyme E2T (putative)	223229_at
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	203362_s_at
ENC1	ectodermal-neural cortex (with BTB-like domain)	201341_at
KIF20A	kinesin family member 20A	218755_at
CDCA8	cell division cycle associated 8	221520_s_at
AK3L1	adenylate kinase 3-like 1	204348_s_at
AADAC	arylacetamide deacetylase (esterase)	205969_at
Hs.69297	Transcribed locus	238835_at
ADH1B	alcohol dehydrogenase IB (class I), beta polypeptide	209613_s_at
NR4A2	nuclear receptor subfamily 4, group A, member 2	204622_x_at
CTH	cystathionase (cystathionine gamma-lyase)	217127_at
Hs.444595	MRNA; cDNA DKFZp779F2345	238780_s_at
PDGFRA	platelet-derived growth factor receptor, alpha polypeptide	203131_at
SCNN1A	sodium channel, nonvoltage-gated 1 alpha	203453_at
H19	H19, imprinted maternally expressed untranslated mRNA	224646_x_at
FMO2	flavin containing monooxygenase 2	228268_at
ATP1B3	ATPase, Na+/K+ transporting, beta 3 polypeptide	242836_at
CXCL12	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	209687_at
SORBS2	Sorbin and SH3 domain containing 2	227826_s_at
ZNF331	zinc finger protein 331	227613_at
GPR98	G protein-coupled receptor 98	223582_at
INMT	indolethylamine N-methyltransferase	224061_at
SLC16A9	solute carrier family 16 (monocarboxylic acid transporters), member 9	227506_at
LMOD1	leiomodin 1 (smooth muscle)	203766_s_at
HTR2B	5-hydroxytryptamine (serotonin) receptor 2B	206638_at
FNDC4	fibronectin type III domain containing 4	218843_at
LOC440606	similar to novel 3-beta hydroxysteroid dehydrogenase family member	215665_at
HOXA5	homeobox A5	213844_at
LOC389895	similar to CG4768-PA	240312_at
SEMA6A	sema domain, transmembrane domain, and cytoplasmic domain, 6A	215028_at
FBLN1	fibulin 1	202995_s_at
PHYHIP	phytanoyl-CoA 2-hydroxylase interacting protein	205325_at
AMDHD1	amidohydrolase domain containing 1	229596_at
ABLIM1	actin binding LIM protein 1	200965_s_at
SYTL5	Synaptotagmin-like 5	242093_at
RAB34	RAB34, member RAS oncogene family	1555630_a_at
COL4A3	collagen, type IV, alpha 3 (Goodpasture antigen)	222073_at
GIPC2	GIPC PDZ domain containing family, member 2	219970_at
KCNQ1	potassium voltage-gated channel, KQT-like subfamily, member 1	211217_s_at
AOX1	aldehyde oxidase 1	205082_s_at
C7orf10	chromosome 7 open reading frame 10	219655_at
CAB39L	calcium binding protein 39-like	225915_at
CHAD	chondroadherin	206869_at
Hs.595279	Transcribed locus	238521_at
SEPT4	septin 4	210657_s_at
ST6GALNAC5	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 5	220979_s_at

Gene Expression Profiling Possesses Prognostic Power

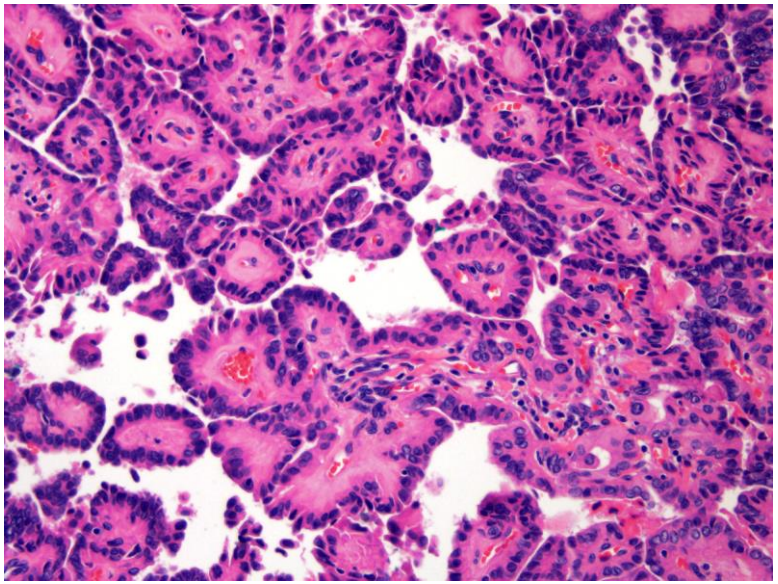




Adrenocortical tumors are easy compared to thyroid tumors

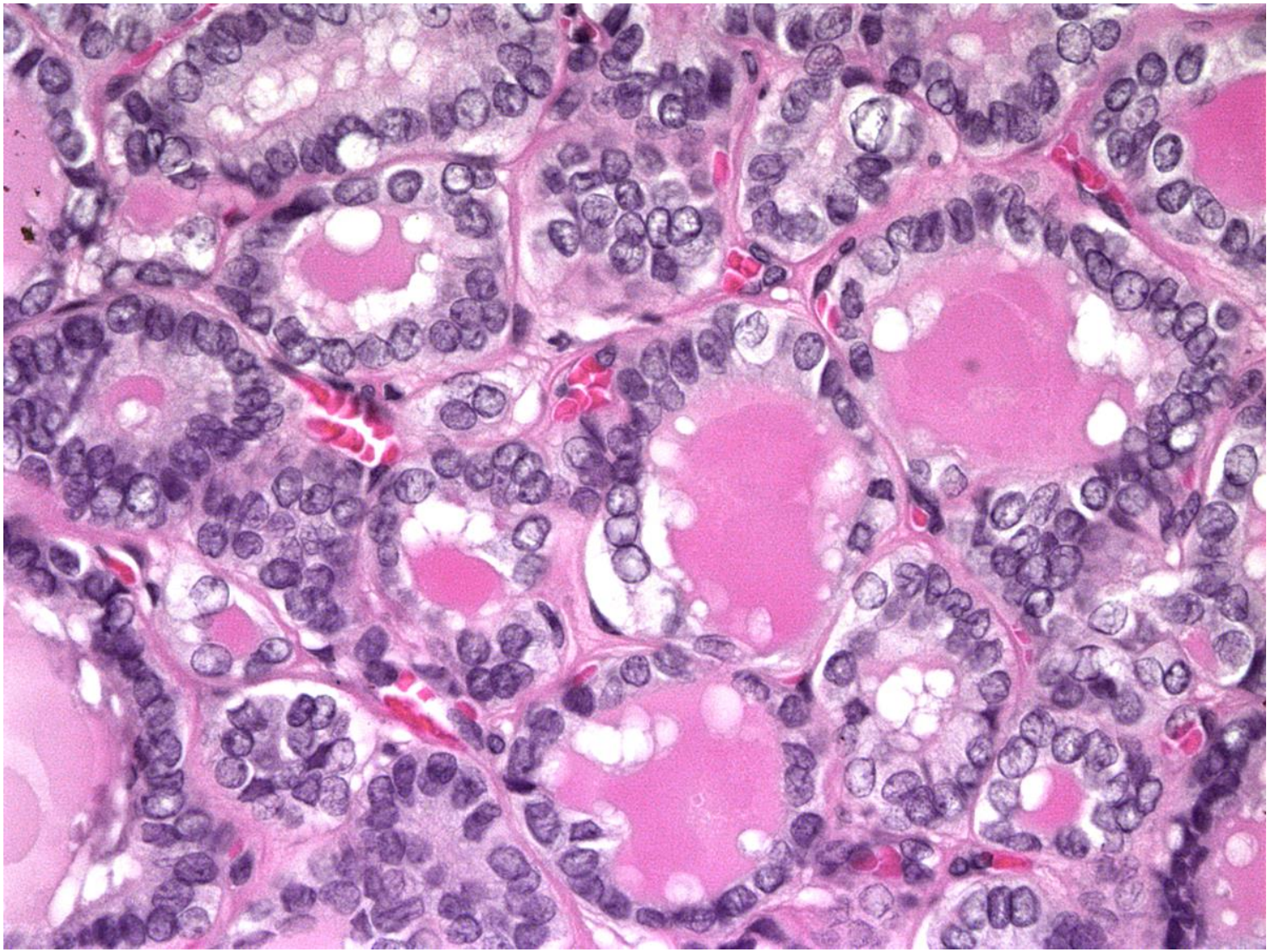
- Tumor classification schemes differ
  - Thyroid has more diagnostic entities
- Pathologists do not agree on thyroid diagnoses even after resection
  - Especially true for follicular patterned lesions

"True" papillary carcinoma



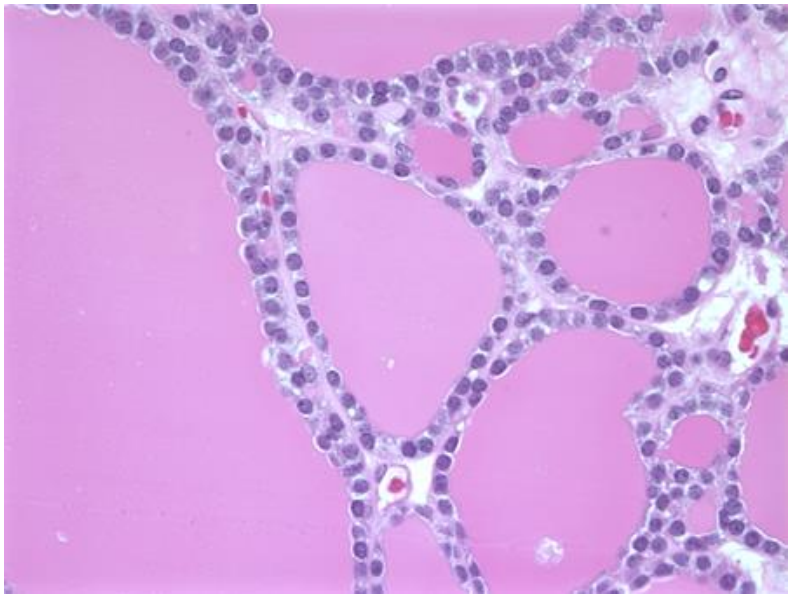
High agreement amongst pathologists

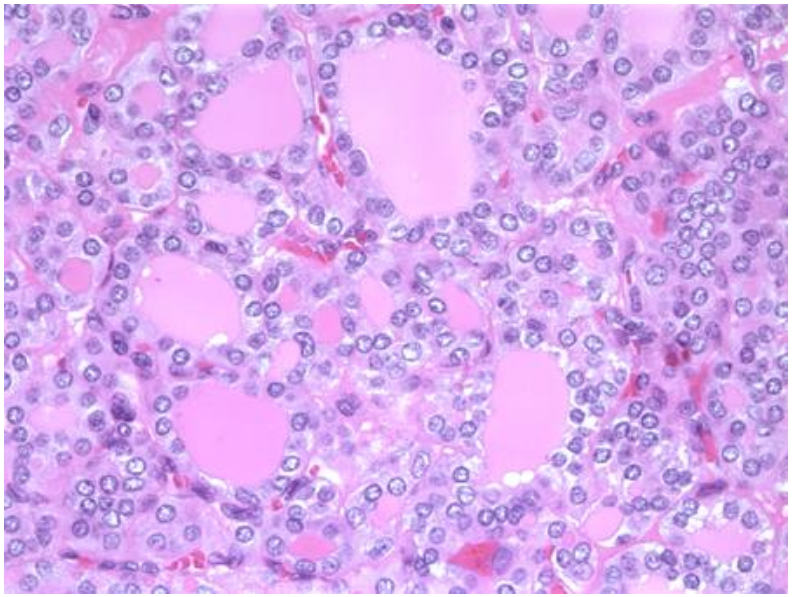
Follicular Variant of Papillary Carcinoma (or NIFTP)



High agreement amongst pathologists

Follicular lesions - ? Carcinoma (or NIFTP)





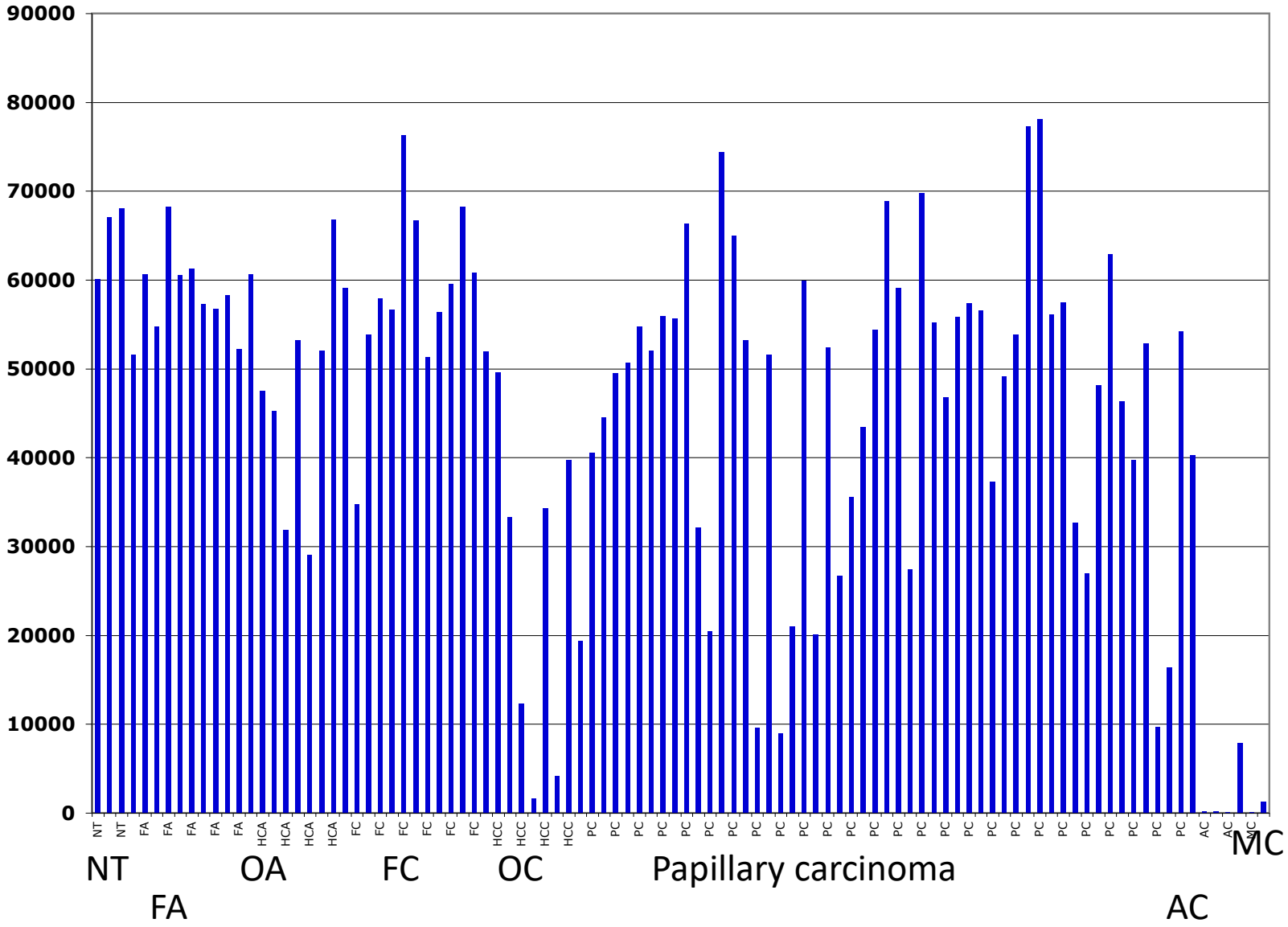
Source of anguish and disagreement amongst pathologists

Biggest source of consultation cases

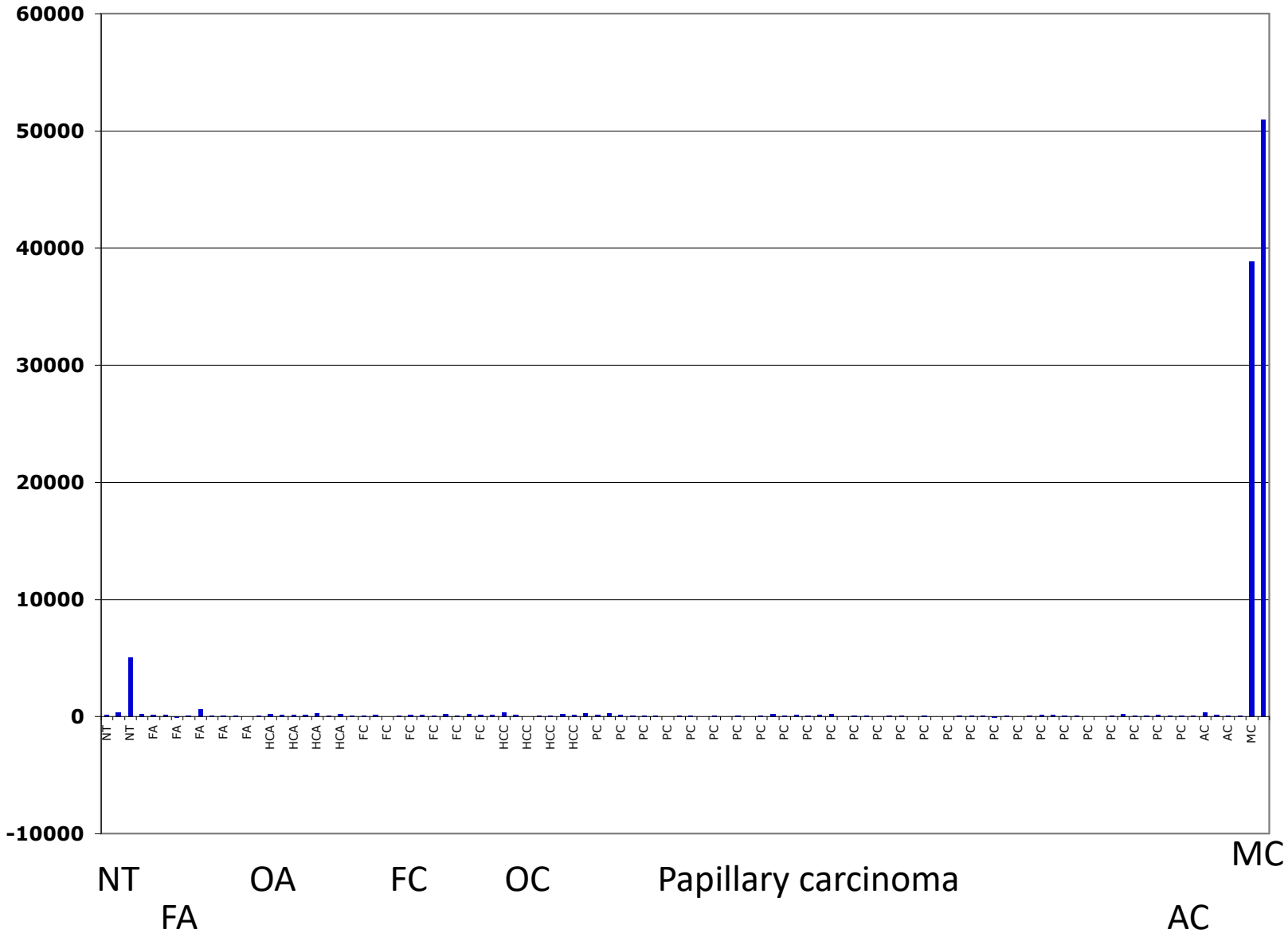
2004
Gene Expression Study of 95 Thyroid Tumors and 4 Normals

- To create a genome-wide gene expression dataset that spans the entire spectrum of thyroid neoplasia
- Affymetrix arrays
- To classify the tumors using statistical techniques
- Apply knowledge of pathology and genotype

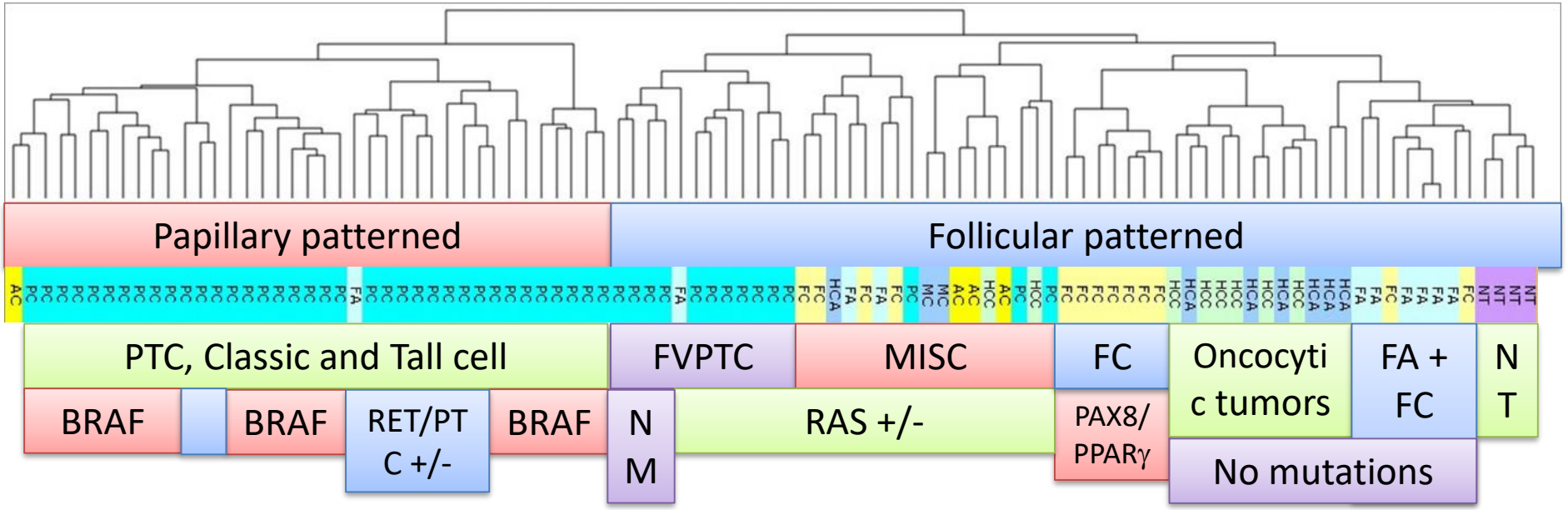
Thyroglobulin Expression in Thyroid Tumors



Calcitonin Expression in Thyroid Tumors



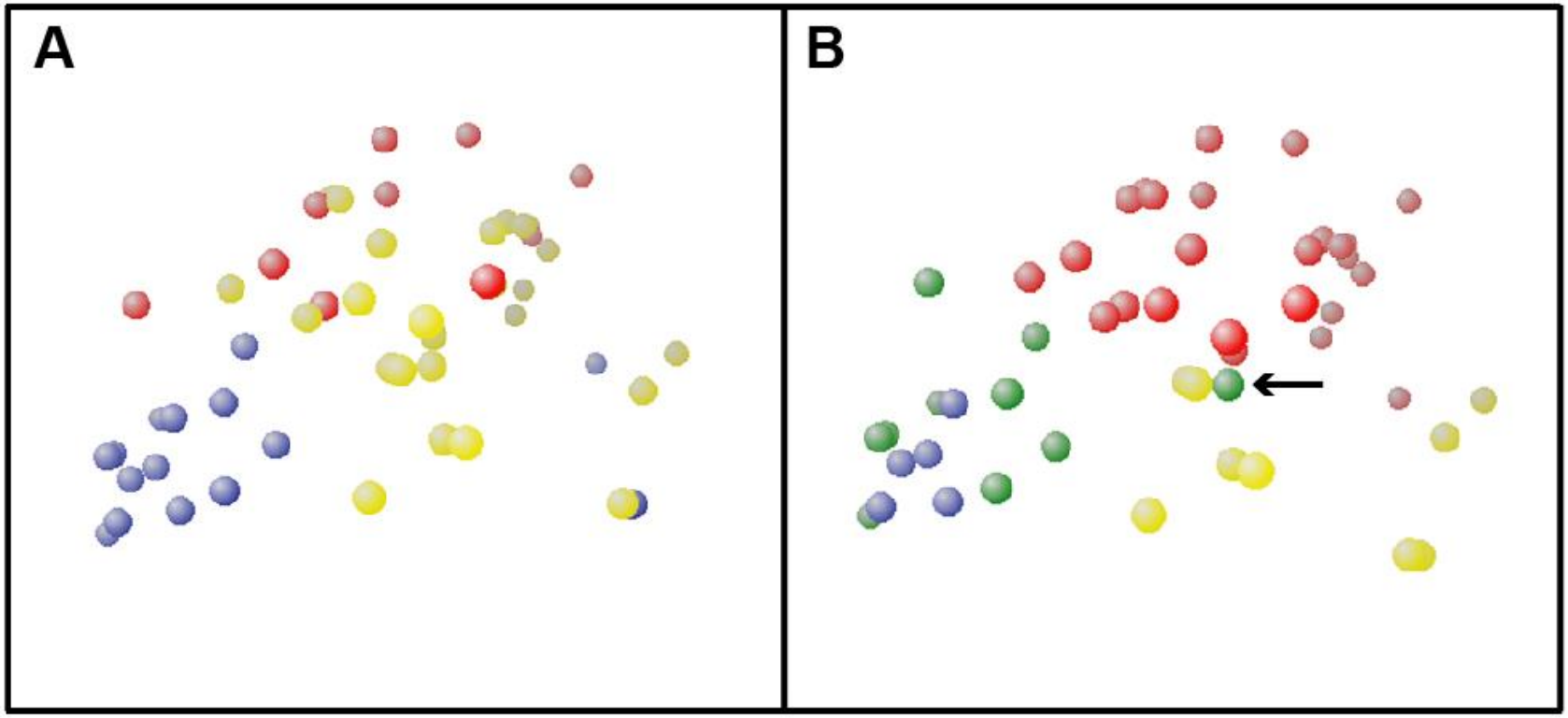
Strong correlation between gene expression and genotype



Confirmed by TCGA study of PTC (Cell, 2014)

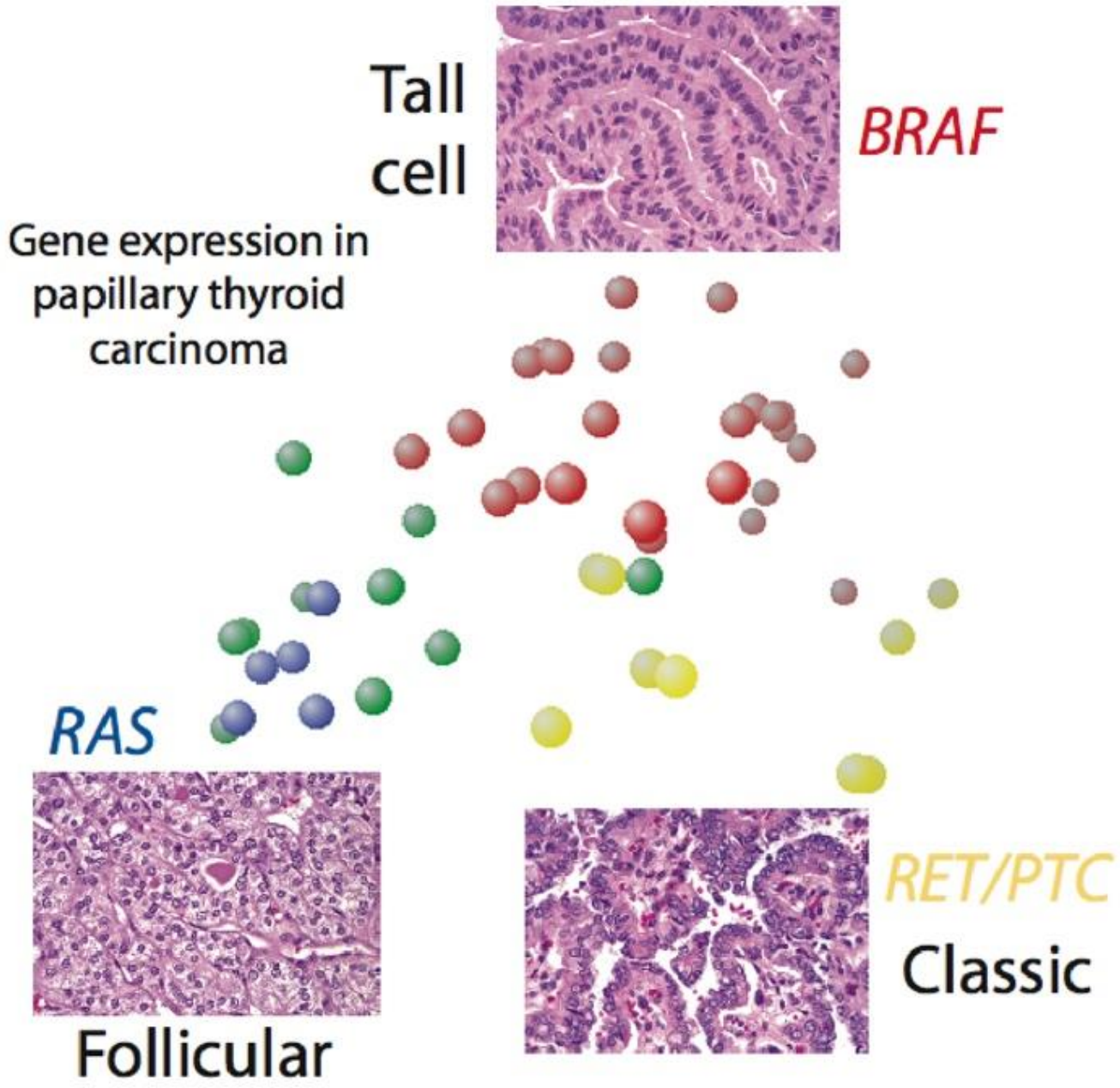
Molecular classification of papillary thyroid carcinoma: distinct *BRAF*, *RAS*, and *RET/PTC* mutation-specific gene expression profiles discovered by DNA microarray analysis

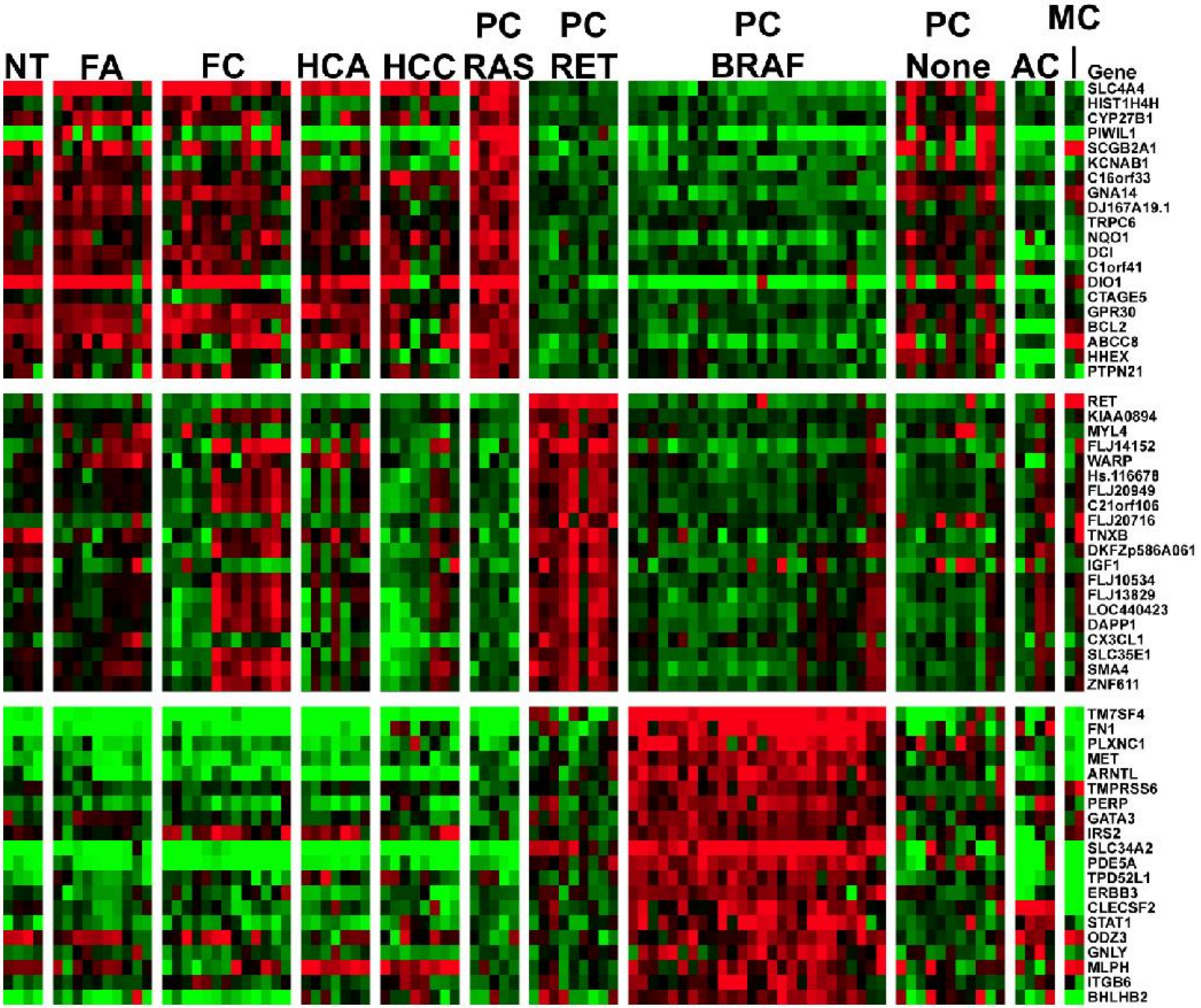
Thomas J Giordano*[,1], Rork Kuick[2], Dafydd G Thomas[1,3], David E Misek[2], Michelle Vinco[1], Donita Sanders[1], Zhaowen Zhu[4], Raffaele Ciampi[4], Michael Roh[1], Kerby Shedden[5], Paul Gauger[6], Gerard Doherty[6], Norman W Thompson[6], Samir Hanash[2], Ronald J Koenig[3] and Yuri E Nikiforov[4]



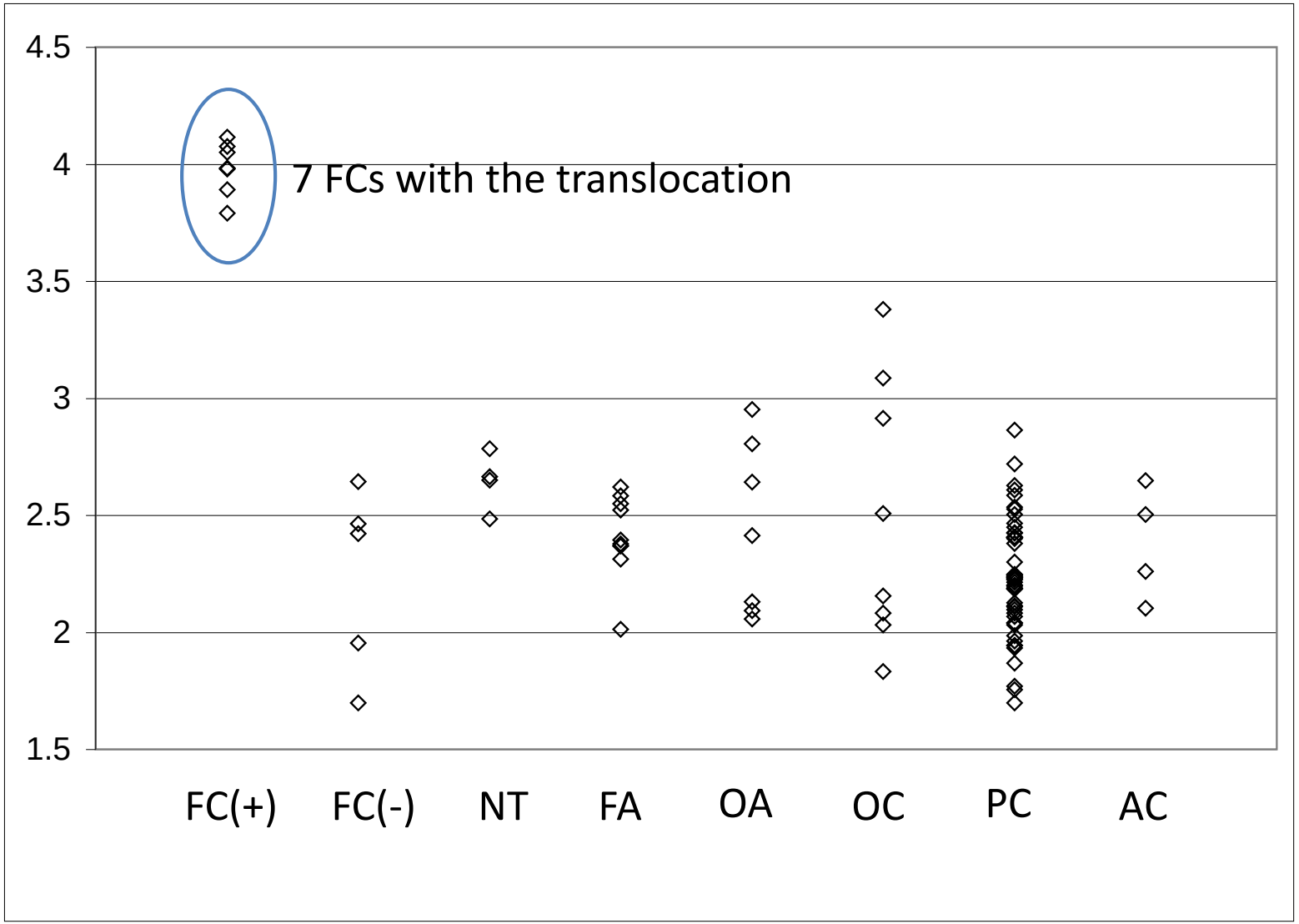
Morphology

Mutation

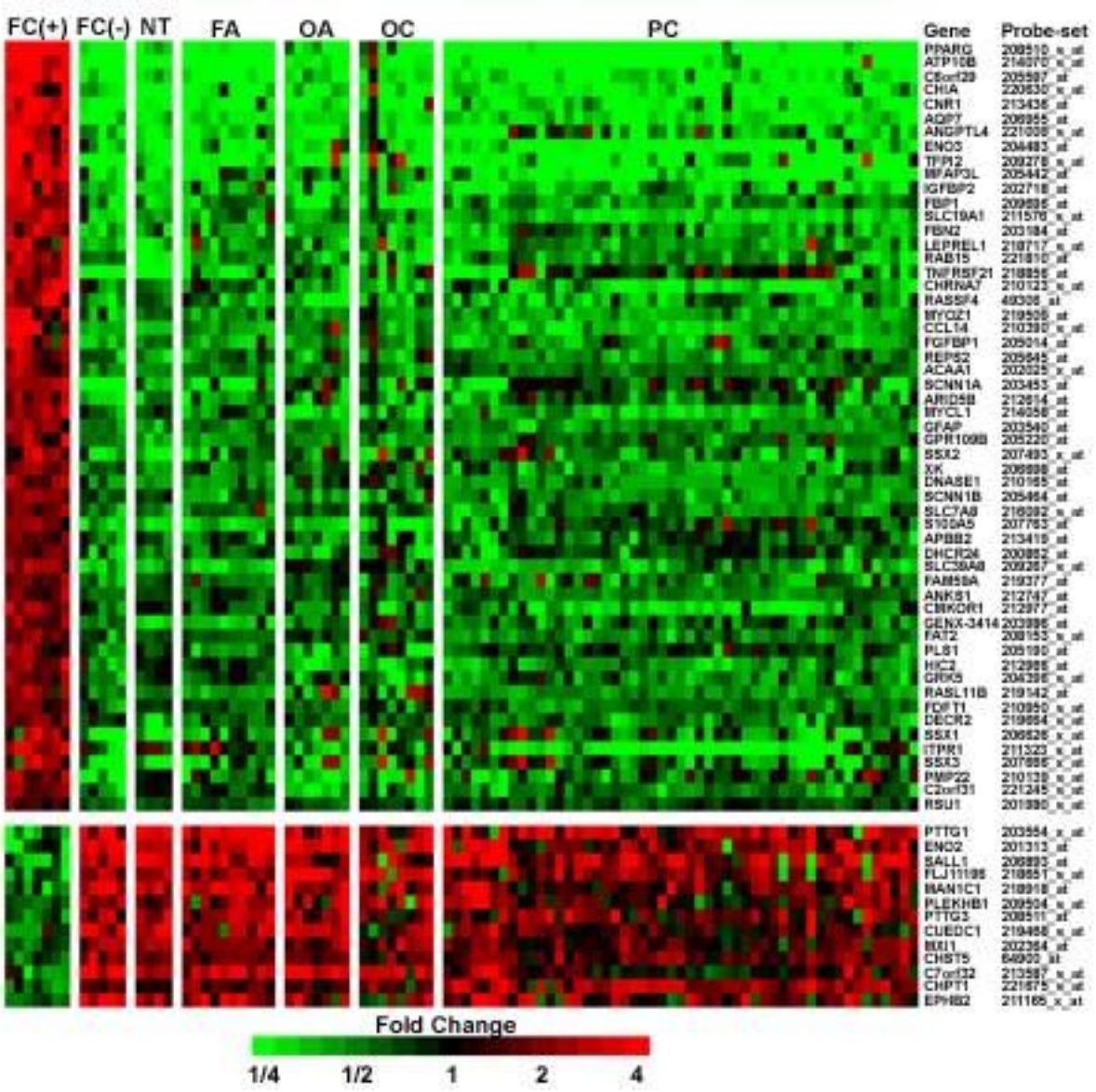

Tall cell
BRAF
Gene expression in papillary thyroid carcinoma
RAS
Follicular
RET/PTC
Classic


NT
FA
FC
HCA
HCC
PC RAS
PC RET
PC BRAF
PC None
AC
MC
Gene
SLC4A4
HIST1H4H
CYP27B1
PIWIL1
SCGB2A1
KCNAB1
C16orf33
GNA14
DJ167A19.1
TRPC6
NQO1
DCI
C1orf41
DIO1
CTAGE5
GPR30
BCL2
ABCC8
HHEX
PTPN21
RET
KIAA0894
MYL4
FLJ14152
WARP
Hs.116678
FLJ20949
C21orf106
FLJ20716
TNXB
DKFZp586A061
IGF1
FLJ10534
FLJ13829
LOC440423
DAPP1
CX3CL1
SLC35E1
SMA4
ZNF611
TM7SF4
FN1
PLXNC1
MET
ARNTL
TMPRSS6
PERP
GATA3
IRS2
SLC34A2
PDE5A
TPD52L1
ERBB3
CLECSF2
STAT1
ODZ3
GNLY
MLPH
ITGB6
BHLHB2

PPARγ gene expression is a marker of the *PAX8-PPARγ* translocation



Distinct Gene Expression Profile Driven by PAX8/PPARγ Fusion



"Afirma" Gene Expression Classifier

- Based on the abundant literature, it is not surprising that a gene expression based classifier could be successfully developed
- Not surprising that you can identify a BRAF-V600E signature
- Designed to identify benign nodules so that patients can avoid surgery
- 142 gene panel performed on thyroid FNA samples using Affymetrix arrays

"Afirma" Gene Expression Classifier Series of Studies

- Analytical validation
- Clinical validation
- Clinical utility
- Cost effective

- Variation in performance across institutions
  - Differences in patient populations with distinct prevalence of malignancy
  - Differences in cytology practice
  - Differences in surgical pathology practice

Nice model for test assessment

Institutional prevalence of malignancy of indeterminate thyroid cytology is necessary but insufficient to accurately interpret molecular marker tests

Pablo Valderrabano[1], Marino E Leon[2], Barbara A Centeno[2], Kristen J Otto[1], Laila Khazai[2], Judith C McCaffrey[1], Jeffery S Russell[1] and **Bryan McIver[1]**

[1]Department of Head and Neck, and Endocrine Oncology and [2]Department of Anatomic Pathology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida, USA

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- Calculated the institutional PoM for each category of the Bethesda system (Bethesda) on all thyroid nodules with cytological evaluation
- Assessing the institutional performance of each test is necessary along with PoM individualization

Field has Matured and Will Continue to Evolve

Molecular Testing of Thyroid Nodules

A Review of Current Available Tests for Fine-Needle Aspiration Specimens

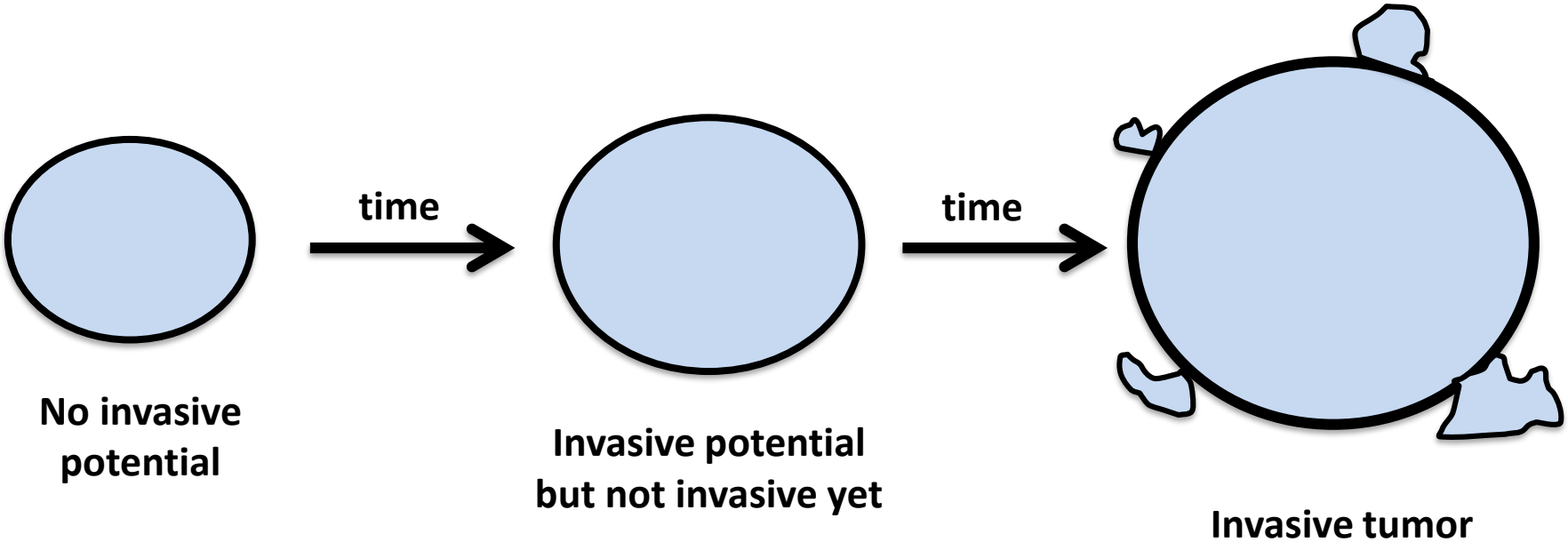
Ming Zhang, MD, PhD; Oscar Lin, MD, PhD

Table 2. Comparison of Currently Available Molecular Tests for Indeterminate Thyroid Cytopathology Fine-Needle Aspiration Specimens

	Afirma[a]	ThyGenX[b]	ThyroMIR[b]	ThyroSeq[c]
Methodology	mRNA gene expression	Multiplex PCR by sequence-specific probes	microRNA expression	Next-generation sequencing
Test report	Benign/suspicious	Specific gene mutation/ translocation	Negative/positive	Specific gene mutation/ translocation
Specimen collection	2 dedicated FNA passes	1 dedicated FNA pass with at least 50 ng of cellular material	same as ThyraMIR	1–2 drops from first pass if adequate cellularity on smear slide
Strength	High NPV	High PPV	Good NPV and PPV when combined with ThyGenX	High NPV and PPV
Limitation	Low PPV	Low NPV	Limited validation data	Limited validation data
Cost[d]	$4875 for Afirma GEC and MTC $975 for Afirma MTC alone $475 for Afirma BRAF alone	$1675 for ThyGenX alone	$3300 for ThyraMIR (reflex test)	$3200

In DTC, strong correlation between gene expression and genotype

Source of discrepancy: Evolution of RAS mutant tumor

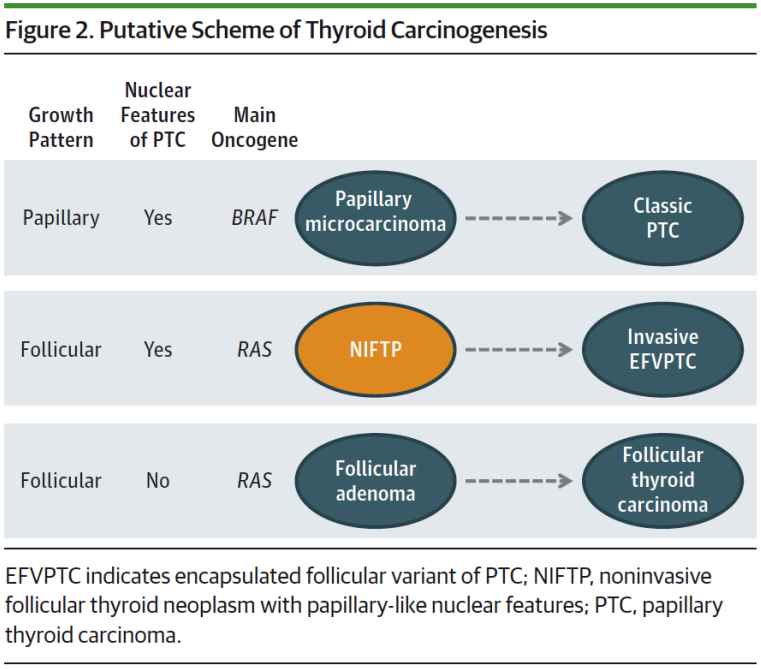


Gene expression diagnosis - malignant

Surgical pathology diagnosis - adenoma

Source of discrepancy: NIFTP

- We purposely did not considered NIFPT to be a cancer
- But not absolutely benign
- RAS mutation enriched
- Biology may be that of an *in situ* cancer
  - Gene expression classifiers may be positive
- Complicates and affects the performance characteristics of these assays



Summary

- **Gene expression classifiers and genotyping are complementary approaches that reflect the same underlying biology**
- Each approach has benefits and disadvantages
  - Gene expression can perform well in cases of unusual mutations, e.g. rare *BRAF* fusions
  - Genotyping has to expand as new mutations are found (e.g. TCGA)
- Both approaches have common challenges
  - Problem of RAS mutant follicular neoplasms
    - (FVPTV and NIFTP)
  - Inter-observer variability amongst pathologists
- Combination of genotyping and gene expression will be advantageous