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Altered patterns of gene expression distinguishing ascending aortic aneurysms from abdominal aortic aneurysms: Complementary DNA expression profiling in the molecular characterization of aortic disease

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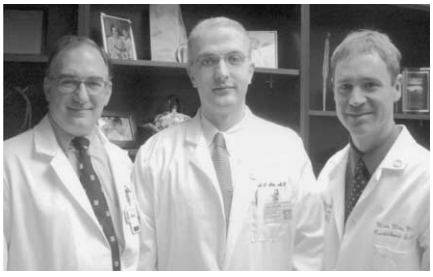

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Objectives: The purpose of this study was to profile altered patterns of gene expression that characterize degenerative ascending thoracic aortic aneurysms and to compare these patterns with those observed for infrarenal abdominal aortic aneurysms.

Methods: Full-thickness aortic wall tissues were obtained during surgical repair of degenerative thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms (n = 4 each), with normal thoracic and abdominal aortas from organ transplant donors used as control preparations. Radiolabeled complementary DNA was prepared for each specimen and hybridized to complementary DNA microarrays, and differential levels of gene expression between aneurysmal and normal aortic tissues at each site were assessed by parametric statistics.

Results: Of 1185 genes examined, 112 (9.5%) were differentially expressed ($P < .05$) between thoracic aortic aneurysms and normal thoracic aorta, with 105 increased and 7 decreased. There were 104 genes (8.8%) differentially expressed between infrarenal abdominal aortic aneurysms and normal abdominal aorta (65 increased and 39 decreased). Quantitative increases in expression for 97 genes were unique to thoracic aortic aneurysms, whereas increases for 61 genes were unique to infrarenal abdominal aortic aneurysms. Although 8 gene products were significantly altered in both thoracic and infrarenal abdominal aortic aneurysms, these changes were directionally concordant for only 4 (matrix metalloproteinase 9/gelatinase B, v-*yes*-1 oncogene, mitogen-activated protein kinase 9, and intercellular adhesion molecule 1/CD54). Results for 9 genes were independently confirmed by quantitative reverse transcriptase–polymerase chain reaction.

Conclusions: Thoracic aortic aneurysms and infrarenal abdominal aortic aneurysms exhibit distinct patterns of gene expression relative to normal aorta from the same sites, with most alterations being unique to each disease. Degenerative aneurysms arising in different locations are thus characterized by a high degree of molecular heterogeneity, reflecting different pathophysiologic mechanisms.

Degenerative aortic aneurysms represent a relatively common condition responsible for considerable cardiovascular morbidity and mortality. Although aneurysms may arise at any site along the course of the aorta, they most frequently occur in the infrarenal abdominal aorta or the descending portion of the thoracic aorta. The ascending thoracic aorta is another common location for aortic aneurysms, which may develop in association with hypertension and spontaneous (type A) aortic dissection, congenital valvular abnormalities (eg, bicuspid

aortic valves[1-3]), and inherited connective tissue disorders (eg, Ehlers-Danlos syndrome type IV,[4] Marfan syndrome,[5] and other fibrillinopathies[6,7]). Genetic linkage studies have also revealed several new chromosomal loci associated with familial forms of thoracic aortic aneurysm and dissection, including a locus at 11q23.2-q24 and another at 5q13-14.[8,9] Although candidate genes at each of these loci have been identified and examined, the genes responsible for aortic disease in these families have not yet been identified. Thus, despite significant progress during the past decade toward understanding the pathogenesis of aneurysmal disease, the specific factors causing aneurysmal degeneration in various locations remain unresolved.

Although it might be assumed that the pathophysiology of aortic aneurysms is homogeneous regardless of location, the histopathologic features of thoracic aortic aneurysms (TAAs) are quite different from those that characterize abdominal aortic aneurysms (AAAs). The microscopic findings in TAAs are most frequently described by the term "cystic medial necrosis," reflecting a noninflammatory loss of medial smooth muscle cells (SMCs), fragmentation of elastic fibers, and mucoid degeneration.[10,11] In contrast, the histopathologic features of AAAs are dominated by severe intimal atherosclerosis, chronic transmural inflammation, and destructive remodeling of the elastic media.[12,13] Although it is clear that each of these pathologic patterns can be associated with aneurysm formation, it is uncertain how they are related to the underlying etiology and pathophysiology of aortic disease arising in these two different locations.[14]

Further evidence suggests that the cellular and molecular mechanisms underlying various forms of aneurysmal disease may also be different. Studies on human and experimental AAAs have focused on increased expression and tissue localization of elastin- and collagen-degrading enzymes, particularly matrix metalloproteinases (MMPs), cysteine proteases, and their respective inhibitors.[15-18] Genes encoding a number of proinflammatory cytokines, chemotactic factors, and cell adhesion molecules have also been implicated in AAAs,[19] and depletion of vascular SMCs may have an important influence the process of vascular remodeling that occurs during aneurysmal degeneration.[20,21] Studies focusing on TAAs have indicated that cystic medial necrosis is also associated with elastin degradation and fragmentation,[22,23] SMC depletion and apoptosis,[24] and increased expression of at least some MMPs.[25,26]. However, the absence of a significant inflammatory response implies alternative mechanisms of aneurysm formation in TAAs versus AAAs, perhaps related to the different embryologic origin of cells populating the ascending and infrarenal aorta, differing structural properties and propensities toward atherosclerotic degeneration, or the distinct hemodynamic conditions that distinguish these two areas. Despite ongoing investigations regarding the fundamental mechanisms responsible for aneurysmal degeneration, the spectrum of molecular alterations that may occur in aneurysms at different sites remains unknown.

The development of microchip- and membrane-based complementary DNA (cDNA) arrays has recently made it possible to examine simultaneous expression of thousands of gene products in the same experiment.[27-31] Microarray techniques have been applied to diverse disease processes, including human atherosclerosis and AAAs, yielding valuable insights into the pathologic profiles of altered gene expression in these conditions.[32-34] Given the potential discoveries that might arise from more comprehensive understanding of altered gene expression in different forms of aneurysmal disease, the purpose of this study was to establish a transcriptional profile of aortic wall gene expression that occurs in TAAs relative to normal ascending aorta (NTA). A second goal of this study was to compare altered patterns of gene expression between TAAs and AAAs to determine the similarities and differences that may exist between these two conditions.

Methods

Aortic Tissues

Full-thickness aortic wall specimens were obtained from 4 patients undergoing elective surgical repair of asymptomatic TAAs (2 men and 2 women, ages 49, 78, 82, and 52 years, mean age 65.3 ± 4.3 years). The mean aneurysm size was 6.0 ± 0.4 cm by computed tomography (5.0, 5.0, 8.0, and 6.0 cm). None of these lesions were associated with aortic dissection, congenital aortic valve abnormalities, inherited connective tissue disorders, or familial forms of aneurysmal disease, and no patients had AAAs. For comparison, 4 full-thickness specimens of NTA were obtained from cadaveric organ transplant donors without visible evidence of atherosclerosis (2 men and 2 women, ages 25, 40, 59, and 38 years, mean age 40.5 ± 3.5 years).

A second series of full-thickness aortic wall specimens was obtained from 4 patients undergoing elective repair of asymptomatic AAAs, including 2 men and 2 women (ages 84, 80, 76, and 57 years, mean age 74.3 ± 6.0 years). The mean aneurysm size was 5.7 ± 0.3 cm by computed tomography (5.5, 5.1, 6.3, and 6.0 cm). None of the patients had "inflammatory" AAAs or aneurysms elsewhere. For comparison, specimens of normal infrarenal abdominal aorta (NAA) without visible evidence of atherosclerosis were obtained from 4 cadaveric organ transplant donors (3 men and 1 woman, ages 43, 18, 14, and 36 years, mean age 27.8 ± 7.0 years).

All tissue specimens were snap-frozen in liquid nitrogen immediately on procurement and stored at −70°C before nucleic acid extraction. All tissues were obtained with approval by the Washington University School of Medicine human research subjects committee.

Light Microscopy

Representative aortic tissue samples corresponding to each of the four groups were fixed in 10% neutral buffered formalin and

processed for routine embedding in paraffin. Sections (5 μm) were stained with Verhoeff–van Geisen stain for elastin and viewed by light microscopy.

RNA Samples

Each aortic tissue sample (n = 16) was pulverized under liquid nitrogen, and total RNA was isolated with Trizol reagent (Gibco BRL, Grand Island, NY).[35,36] RNA samples were further extracted with phenol and chloroform to eliminate excess protein, and residual DNA contamination was eliminated with RQ1 ribonuclease-free deoxyribonuclease (Promega Corporation, Madison, Wis). In some cases the samples were enriched for messenger RNA by incubation with streptavidin-coated magnetic beads and biotinylated oligodeoxythymidine (Atlas Pure Total RNA Labeling System; Clontech, Palo Alto, Calif).

Preparation of Radiolabeled cDNA and Membrane Hybridization

Radiolabeled cDNA was prepared with reagents and protocols provided with the AtlasArray Human 1.2 I cDNA Expression Array kit from CLONTECH (CLONTECH Laboratories, Inc, Palo Alto, Calif), as previously described elsewhere.[33] For each of the 16 samples, 5 μg total RNA was incubated with a mixture of array-specific oligonucleotide primers and 400 units of Superscript II reverse transcriptase (Gibco) in the presence of deoxyadenosine triphosphate labeled with phosphorus 32 (Amersham Pharmacia Biotech, Piscataway, NJ). Labeled cDNA samples were purified by column chromatography to remove unincorporated isotope before use. Each cDNA sample was mixed into hybridization buffer (ExpressHyb; CLONTECH) and then incubated overnight at 68°C with a nylon membrane containing bound cDNA clones corresponding to 1185 known genes (AtlasArray Human 1.2 I; CLONTECH). Membranes were washed according to the manufacturer's protocol, exposed to a phosphor screen (Eastman Kodak Company, Rochester, NY), and subsequently scanned (Molecular Dynamics, Inc, Sunnyvale, Calif). Densitometry readings for each gene were obtained with AtlasImage 1.01a (CLONTECH) and adjusted for the background density adjacent to each immobilized target cDNA on the same membrane.[33]

Data Analysis

The relative signal intensity for each gene on a given membrane was calculated as a fraction of the total signal intensity for all genes on the same membrane. The entire data set of adjusted intensity levels was entered into a commercially available data analysis and display platform specifically designed for high-density genomic expression studies (GeneSpring 4.0.4; Silicon Genetics, Redwood, Calif). Data were thereafter accessible for both mathematic computations and a number of different color graphic analytic display options, including the construction of experimental gene trees (dendrograms), self-organizing maps, statistical comparisons, and hierarchic cluster analysis (for reviews of these techniques, see Eisen and colleagues[29] and Lockhart and coworkers[31] and references contained therein).

For each gene represented on the microarray, the mean ratio of expression (or fold change) in aneurysmal tissue was calculated by comparison with the mean values obtained for normal aorta from the same location (TAAs vs NTA and AAAs vs NAA). The data set was then examined with a parametric comparisons test to identify genes exhibiting different levels of expression between aneurysms and normal aorta.[29,31] The two lists of differentially expressed genes were then compared to identify similarities and differences in the patterns of altered gene expression between TAAs and AAAs. To examine the consistency of changes in gene expression within the specimens from each group, the upper limit of normal expression was determined as the mean plus 2 SD of values obtained for the 4 normal aorta specimens. For genes reported to be expressed at increased levels, the number of individual aneurysm specimens exhibiting expression above the upper limit of normal was determined and recorded as a percentage of the total (n = 4) for each group. A similar analysis was performed for genes reported to be expressed at decreased levels, by determining the percentage of aneurysm specimens in which expression was below the lower limit of normal (mean minus 2 SD).

Reverse Transcription–Quantitative Polymerase Chain Reaction

To independently confirm results obtained by cDNA microarray analysis, the relative expression patterns of 9 selected genes were also measured by reverse transcription–quantitative polymerase chain reaction (RT-qPCR) assays. Reverse transcription was performed with 1 μg total RNA isolated from each aortic tissue sample (n = 16) along with a GeneAmp 2400 thermal cycler and reagents from PE Biosystems (5.5-mmol/L magnesium chloride, 0.5-mol/L deoxyribonucleoside triphosphates, 1.25-U/μL MultiScribe Reverse Transcriptase, and 2.5-μmol/L random hexamers; PE Biosystems, Foster City, Calif). The cDNA samples were used for real-time detection of PCR amplification with SYBR Green, a fluorescent DNA binding dye (GeneAmp 5700 Sequence Detection System; PE Biosystems), as previously described elsewhere.[36] Primer pairs were selected with PrimerExpress version 1.6 software (PE Biosystems) to amplify the following gene products: MMP-9/gelatinase-B (GenBank J05070), interleukin (IL) 6 signal transducer *gp130* (GenBank M57230), v-*yes*-1 Yamaguchi sarcoma virus–related oncogene (GenBank M16038), apolipoprotein E (GenBank M12529), IL-8 (GenBank Y00787), IL-1β (GenBank K02770), tumor necrosis factor (TNF) α (GenBank X01394), vascular cell adhesion molecule 1 (GenBank M30257), cathepsin D (GenBank M11233), and β-actin (GenBank X00351).

All real-time polymerase chain reactions (50 μL) were performed in duplicate with 10 ng cDNA and kit reagents, with initial incubations at 50°C (2 minutes) and 95°C (10 minutes) for AmpErase and AmpliTaq Gold activation, respectively, followed by 40 cycles of polymerase chain reaction (95°C for 15 seconds alternating with 60°C for 1 minute). Direct detection of reaction products was monitored by measuring the increase in fluorescence caused by the binding of SYBR Green I to double-stranded DNA products, along with an internal reference standard (ROX). Fluorescence signals were analyzed with the GeneAmp 5700 Sequence Detection System software (SDS version 1.3; PE Biosystems) according to the manufacturer's recommendations. All quantitative results were normalized to the mean concentration of β-actin messenger RNA to account for variability in the quality of total RNA and the efficiency of reverse transcription between samples. For each gene, statistical differences in expression between TAAs

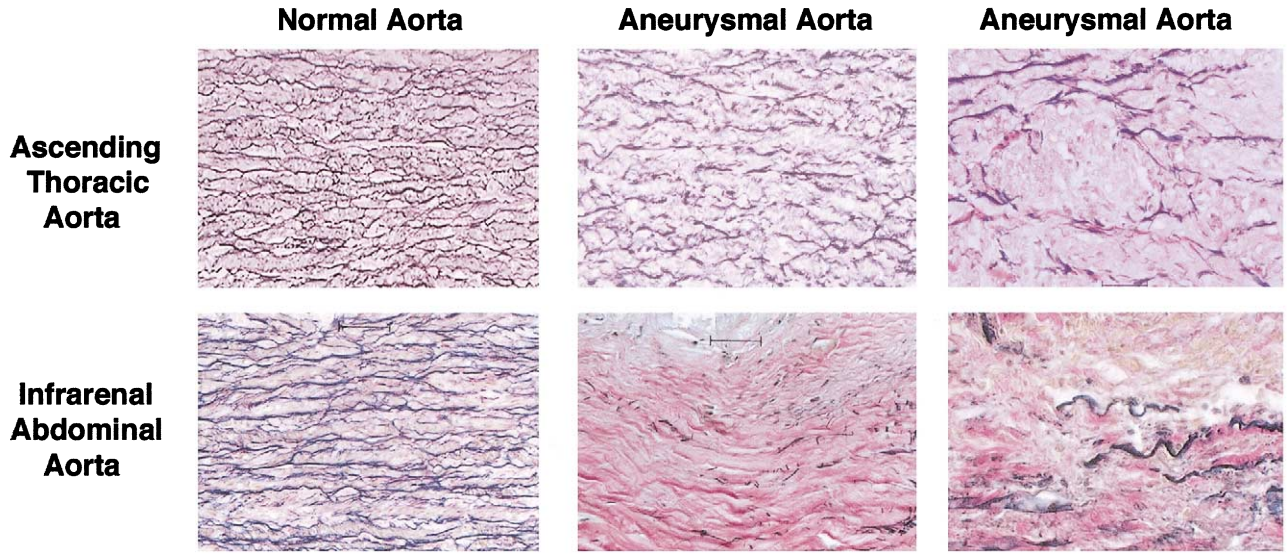

Figure 1. Histopathology of aortic aneurysms. Representative sections of aortic wall tissue stained with Verhoeff–van Geisen for elastin *(dark purple fibers).* Compared with NTA, TAAs exhibited disruption, fragmentation and disorganization of medial elastic fibers in absence of significant inflammatory changes, along with other characteristic features of cystic medial necrosis *(upper panels).* AAAs exhibited more extreme destruction of medial elastin and replacement by fibrocollagenous extracellular matrix *(pink),* along with depletion of medial SMCs and mononuclear inflammatory cell infiltration in direct association with areas of elastic fiber degeneration *(lower panels).*

and NTA and between AAAs and NAA were analyzed by the paired Student *t* test.

Results

Morphologic Appearance of Aneurysmal Tissues

Light microscopy of TAA tissues revealed disruption and disorganization of medial elastic fibers in the absence of significant inflammatory changes, along with areas of ground substance accumulation considered characteristic of cystic medial necrosis (Figure 1). In contrast, AAAs were characterized by more extreme destruction of medial elastic fibers with replacement by a fibrocollagenous extracellular matrix and depletion of medial SMCs. AAAs also exhibited infiltration of the media and adventitia by mononuclear inflammatory cells, often in direct association with degenerating elastic fibers (Figure 1).

Microarray Data

Representative hybridization blots for microarrays prepared from specimens of aneurysmal and normal thoracic aorta are shown in Figure 2. After phosphoimaging to quantify the relative expression level of each gene, data were corrected for the adjacent background intensity and normalized as a fraction of the total intensity observed on the entire blot. The complete data sets (adjusted intensities for each gene on each of 16 different arrays) were then entered into the GeneSpring program for further analysis. Figure 2, *B,* illustrates a color display map depicting the relative levels of expression for all 1185 genes on the array in each sample of TAAs and NTA.

Gene Expression Profiles of Aneurysmal Tissues

One hundred twelve of 1185 genes represented on the microarray (9.5%) were differentially expressed between TAAs and NTA ($P < .05$), with 105 exhibiting a significant increase in expression and 7 exhibiting a significant decrease (Table 1). The magnitude of increased expression was at least 10-fold greater in TAAs than NTA for 17 of these transcripts, with the greatest increases observed for protein kinase C (PKC) ζ (>100-fold), uracil-DNA glycosidase (44-fold), lymphotoxin β (41-fold), TNF-α (27-fold), and the TNF receptor superfamily member CD27 (18-fold). With regard to the consistency of these results, the alterations described were observed in at least 3 of the 4 individual TAA specimens for 95 of the 112 genes (85%), including 92 of the 105 genes (88%) exhibiting increased expression and 3 of the 7 genes (43%) exhibiting decreased expression (Table 1).

One hundred four of 1185 genes (8.8%) were differentially expressed between AAAs and NAA ($P < .05$), with 65 exhibiting a significant increase in expression and 39 exhibiting a significant decrease (Table 2). Only 6 of these transcripts were increased greater than 10-fold between

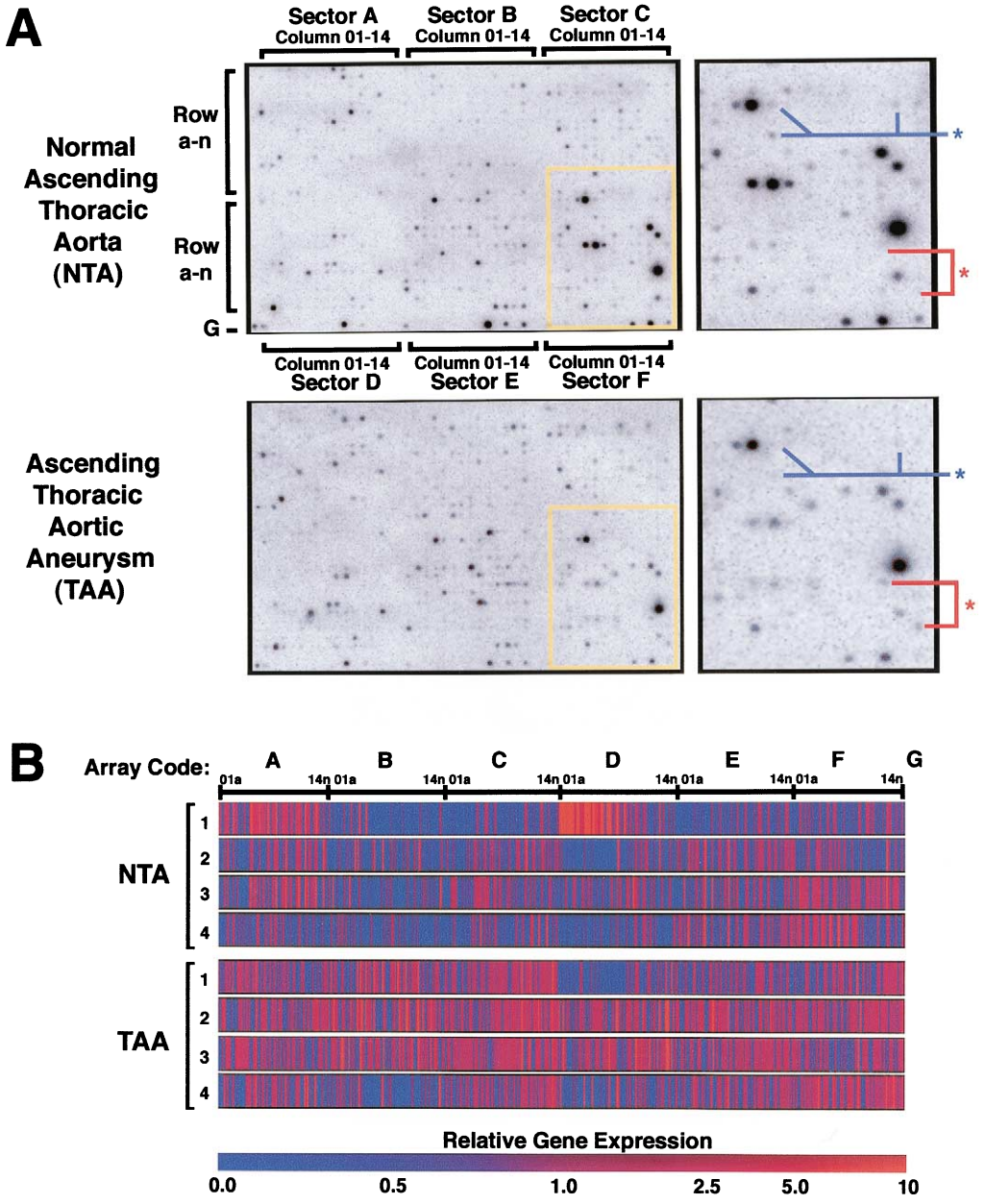

Figure 2. Microarray analysis of gene expression during aneurysm development. A, Representative autoradiographs for AtlasArray Human 1.2 I membranes (CLONTECH) blotted with phosphorus 32–labeled cDNA derived from NTA and TAA. Each blot contains bound cDNA clones from 1185 distinct gene products that can be identified by superimposing orientation grid provided by manufacturer, with each gene located by sectors *A* through *F,* columns *01* through *14,* and rows *a* through *n* (*sector G* denotes panel of 9 housekeeping genes; see manufacturer website for further details at http://atlasinfo.clontech.com). Differences in gene expression between blots can be visualized in higher resolution panels from the areas highlighted in *yellow*, as shown on *far right* (*blue asterisks* denote 3 genes exhibiting decreased expression in TAAs vs NTA and *red asterisks* denote 2 genes exhibiting increased expression). B, Color display illustrating relative levels of expression for all 1185 genes represented on AtlasArray Human 1.2 I microarray as generated by GeneSpring version 4.0.4 software (Silicon Genetics). Data shown reflect levels of relative gene expression (normalized relative intensities) for each individual gene in 4 specimens of NTA and 4 TAAs, where each gene is represented by single vertical band oriented according to array code shown *above.* Color bar *(below)* indicates spectrum of normalized relative intensities shown on color display, ranging from *blue* (low-level expression) to *purple* (mean expression) to *red* (high-level expression).

TABLE 1. One hundred twelve genes exhibiting altered expression in TAAs

Array code	GenBank reference	Gene or protein name	NTA	TAA	A/N ratio	*P* value	Conservation (%)
B09i	Z15108	PKC-ζ	0 ± 0	163 ± 66	>100	.048	100
C14n	X15653	Uracil-DNA glycosylase	3 ± 3	131 ± 41	43.59	.020	100
C10f	L11015	Lymphotoxin β	10 ± 10	396 ± 129	41.05	.024	100
C08f	X01394	TNF-α	23 ± 9	619 ± 240	26.63	.047	100
C03g	M63928	CD27 (TNF receptor superfamily)	106 ± 38	1927 ± 638	18.13	.029	100
B10i	L07032	PKC-θ	6 ± 6	81 ± 3	14.05	<.001	100
D13b	U31215	Glutamate receptor metabotropic 1	5 ± 5	63 ± 22	13.72	.039	75
B13c	M12154	Antigen p97 (melanotransferrin)	9 ± 9	112 ± 34	12.43	.026	75
B08j	M61906	PI-3-kinase, regulatory subunit (p85 α)	9 ± 9	101 ± 31	11.64	.029	75
B13k	M31158	Cyclic adenosine monophospate–dependent protein kinase, type IIβ	11 ± 8	130 ± 45	11.54	.041	75
B11c	M12530	Transferrin	10 ± 6	117 ± 39	11.26	.035	100
A14e	M21574	Platelet-derived growth factor receptor α	113 ± 77	1246 ± 419	11.04	.038	75
C14c	M97934	*STAT2*	30 ± 9	333 ± 47	10.95	.001	100
B03n	M28213	*RAB2* (*ras* oncogene family)	261 ± 98	2813 ± 822	10.80	.022	100
B14l	X14034	Phospholipase Cγ2	24 ± 16	258 ± 75	10.60	.023	100
D09i	Y00264	Amyloid β (A4) precursor protein	976 ± 110	10,047 ± 3463	10.30	.040	100
E10a	M62829	Early growth response 1	3330 ± 1222	33,837 ± 8606	10.16	.013	100
A03h	X59798	Cyclin D1	62 ± 26	611 ± 191	9.89	.029	75
D06j	X79067	EGF-response factor 1	1422 ± 255	13,772 ± 4690	9.68	.039	100
D13a	M55983	Deoxyribonuclease I	6 ± 6	52 ± 18	9.05	.048	50
A09g	M35410	Insulinlike growth factor binding protein 2	627 ± 156	5626 ± 1679	8.97	.025	75
F07m	J05070	MMP-9/gelatinase B	151 ± 26	1306 ± 367	8.64	.020	75
E07a	M36542	POU domain class 2 transcription factor 2	12 ± 7	98 ± 18	8.33	.004	100
B09g	U12535	EGF receptor pathway substrate 8	44 ± 27	361 ± 115	8.10	.037	75
C09f	D12614	Lymphotoxin α	15 ± 9	120 ± 23	8.03	.006	100
C01f	L34075	FK506 binding protein 12 (FRAP)	32 ± 8	252 ± 60	7.99	.011	100
E03h	M30257	Vascular cell adhesion molecule 1	70 ± 13	545 ± 81	7.81	.001	100
B03m	X80907	PI-3-kinase, regulatory subunit (p85 β)	32 ± 16	240 ± 44	7.45	.004	100
C02e	J03075	PKC substrate 80K-H	588 ± 175	4118 ± 789	7.00	.005	100
E09l	D11086	IL-2 receptor γ	262 ± 75	1787 ± 535	6.81	.030	75
B02n	X08004	*RAP1B* (*ras* oncogene family)	274 ± 140	1850 ± 470	6.75	.018	75
C13a	L11329	Dual-specificity phosphatase 2 (PAC-1)	21 ± 9	140 ± 32	6.74	.011	100
A04d	X56681	*jun* D proto-oncogene	228 ± 43	1502 ± 503	6.59	.045	75
B14m	X16316	*vav*-1 oncogene	54 ± 34	352 ± 114	6.53	.046	75
C12b	U14188	Ephrin A4	51 ± 5	316 ± 53	6.20	.003	100
C02f	U61166	SH3 p17 protein	63 ± 9	387 ± 77	6.19	.006	100
C03a	M31724	Protein tyrosine phosphatase type 1	52 ± 26	324 ± 92	6.18	.030	75
F13a	X14672	N-acetyltransferase 2	51 ± 19	314 ± 49	6.17	.003	100
C09e	AF076974	Transform/transcript domain-associated protein	175 ± 37	1079 ± 114	6.15	<.001	100
A02f	D17517	TYR03 protein tyrosine kinase	75 ± 29	459 ± 152	6.15	.047	75
B12d	M57230	IL-6 signal transducer gp130	43 ± 9	261 ± 86	6.14	.045	75
C13c	M97935	STAT-1	60 ± 31	358 ± 21	5.94	<.001	100
C08h	U39613	ICE-LAP3	22 ± 13	131 ± 22	5.81	.006	75
C10d	M23379	RAS p21 protein activator	92 ± 30	521 ± 112	5.67	.010	100
B10g	M15800	*mal,* T-cell differentiation protein	55 ± 14	302 ± 64	5.49	.009	100
C10c	X14454	Interferon regulatory factor 1	119 ± 24	651 ± 78	5.49	.001	100
F10i	K02770	IL-1β	642 ± 119	3472 ± 1080	5.41	.040	100
C14e	M23410	Junction plakoglobin	104 ± 22	563 ± 40	5.39	<.001	100
E11c	M97190	Sp2 transcription factor	146 ± 7	771 ± 243	5.28	.042	100
A04h	D13639	Cyclin D2	67 ± 22	336 ± 16	5.00	<.001	100
F13m	M11233	Cathepsin D	3975 ± 2371	19,697 ± 1763	4.96	.002	100
C13d	S76965	Cyclic adenosine monophosphate–dependent protein kinase inhibitor α	58 ± 21	284 ± 64	4.88	.015	100
C09d	X69550	Rho GDP dissociation inhibitor α	2949 ± 938	14,297 ± 1549	4.85	.001	100
G31	M11886	MHC class I C	3264 ± 1153	15,513 ± 3565	4.75	.017	75
B02g	M16038	v-*yes*-1 oncogene	127 ± 69	602 ± 162	4.75	.035	75
E03e	M64673	Heat shock transcription factor 1	746 ± 107	3507 ± 733	4.70	.010	100
E01e	L12579	Cut-like 1 (*Drosophila*)	293 ± 55	1352 ± 306	4.61	.014	100

TABLE 1. Continued

Array code	GenBank reference	Gene or protein name	NTA	TAA	A/N ratio	*P* value	Conservation (%)
E09a	M62810	Mitochondrial transcription factor 1	115 ± 39	524 ± 145	4.58	.034	75
E12e	M96824	Nucleobindin 1	2430 ± 732	10,831 ± 2299	4.46	.013	100
E01f	U04847	SWI/SNF-related member 1	615 ± 114	2605 ± 347	4.23	.002	100
B05j	L31951	Mitogen-activated protein kinase 9	70 ± 23	292 ± 53	4.18	.008	75
D09e	M65212	Catechol-O-methyltransferase	1376 ± 458	5711 ± 1545	4.15	.036	75
D12d	AF016709	Purinergic receptor P2X	18 ± 11	69 ± 18	3.93	.048	75
F14m	U41766	ADAM-9	230 ± 48	895 ± 134	3.88	.003	100
E14g	J03132	Intercellular adhesion molecule 1/CD54	1281 ± 809	4960 ± 699	3.87	.014	50
A05h	M92287	Cyclin D3	153 ± 63	588 ± 39	3.84	.001	100
B04g	U07707	Epidermal growth factor receptor pathway substrate 15	162 ± 46	601 ± 142	3.71	.025	100
F14l	U78095	Serine protease inhibitor 2	454 ± 164	1681 ± 406	3.70	.031	75
C13e	X61615	Leukemia inhibitory factor receptor	65 ± 32	230 ± 41	3.56	.019	50
C11b	U56976	Phosphodiesterase IB, calmodulin dependent	61 ± 35	213 ± 18	3.51	.008	50
B14h	U25265	Mitogen-activated protein kinase 5	116 ± 26	393 ± 108	3.40	.047	100
A09h	M77234	Ribosomal protein S3A	6819 ± 3384	22,853 ± 4397	3.35	.028	75
A02h	M25753	Cyclin B1	136 ± 29	453 ± 107	3.32	.029	75
C11g	X76981	Adenosine A3 receptor	118 ± 38	386 ± 92	3.28	.035	75
C12a	M63960	Protein phosphatase-1, catalytic subunit α	665 ± 196	2125 ± 363	3.20	.012	100
C08e	X75621	Tuberous sclerosis 2	250 ± 51	793 ± 89	3.17	.002	100
C02i	X04106	Calpain 4, small subunit (30 kd)	6392 ± 1247	20,202 ± 3665	3.16	.012	100
B05e	U12140	Neurotrophic tyrosine kinase receptor type 2	61 ± 23	191 ± 28	3.14	.011	75
C12c	L19067	v-*rel* (nuclear factor κB related) p65	196 ± 34	613 ± 131	3.13	.022	75
E13e	U02326	Neuregulin 1	90 ± 15	280 ± 75	3.11	.048	100
C03h	X96586	Sphingomyelinase activation-associated factor	171 ± 50	532 ± 125	3.11	.037	75
B06h	U09578	Mitogen-activated protein kinase-activated protein kinase 3	53 ± 26	158 ± 21	2.98	.020	50
A05c	M80359	Mitogen-activated protein/microtubule affinity-regulating kinase 3	213 ± 59	632 ± 157	2.97	.047	75
C02g	Y09392	TNF receptor superfamily member 12	139 ± 34	402 ± 73	2.88	.017	75
C07h	U20537	Caspase 6	78 ± 20	219 ± 30	2.81	.008	75
A04i	M14505	Cyclin-dependent kinase 4	275 ± 78	751 ± 156	2.73	.034	75
B07n	M28212	RAB6	243 ± 49	641 ± 145	2.64	.040	75
C07e	U17032	Rho guanosine triphosphatase activating protein 5	86 ± 15	225 ± 49	2.62	.035	75
C07j	X86779	Fas-activated serine/threonine kinase	344 ± 72	884 ± 125	2.57	.010	100
D10l	U58198	IL enhancer binding factor 1	126 ± 25	320 ± 70	2.53	.041	50
E14e	U03494	Transcription factor CP2	122 ± 29	304 ± 68	2.48	.049	50
E06i	M59911	Integrin α_3	163 ± 32	397 ± 84	2.44	.040	75
E05j	M23197	CD33 antigen (gp67)	109 ± 10	264 ± 46	2.42	.016	100
C04h	U13021	Caspase 2	88 ± 21	209 ± 21	2.38	.006	75
A04f	X16416	v-*abl*	116 ± 27	274 ± 25	2.36	.006	100
C10b	U40370	Phosphodiesterase 1A, calmodulin dependent	185 ± 50	436 ± 89	2.36	.049	75
F07k	X87212	Cathepsin C	916 ± 281	2138 ± 355	2.33	.036	50
E06k	U11814	Keratinocyte growth factor receptor	66 ± 12	151 ± 14	2.28	.003	100
D10k	AF069733	Transcriptional adaptor 3	185 ± 20	400 ± 85	2.16	.048	50
F13c	M22489	Bone morphogenetic protein 2	198 ± 67	405 ± 47	2.05	.044	50
C02l	X98093	Polymerase (DNA directed) γ	183 ± 28	366 ± 53	2.00	.022	75
F12j	M24398	Parathymosin	6374 ± 1193	12,240 ± 1778	1.92	.034	75
E04j	M21097	CD19 antigen	91 ± 17	172 ± 10	1.89	.007	75
E01j	X87838	Catenin β1 (88 kd)	353 ± 59	567 ± 54	1.61	.036	50
F06b	M11717	Heat shock 70 kd protein 1A	5558 ± 990	8556 ± 678	1.54	.047	50
E11m	U77604	Microsomal glutathione S-transferase 2	1448 ± 44	1040 ± 141	0.72	.032	0
C06n	U07418	*mut*L homolog 1 (*Escherichia coli*)	36,628 ± 4866	21,048 ± 2038	0.57	.026	25
E12l	U00672	IL-10 receptor α	180 ± 15	69 ± 24	0.38	.008	100
C05k	U32944	Dynein cytoplasmic light polypeptide	13,323 ± 983	4965 ± 1028	0.37	.001	100
A08l	M63618	Bullous pemphigoid antigen 1 (230/240 kd)	731 ± 158	227 ± 18	0.31	.019	0
F01g	M96956	Teratocarcinoma-derived growth factor 3	1296 ± 439	151 ± 59	0.12	.041	25
D11h	U32376	Discs, large (*Drosophila*) homolog 2	70 ± 23	6 ± 6	0.09	.034	75

A/N, Abnormal/normal ratio.

TABLE 2. One hundred four genes exhibiting altered expression in AAAs

Array code	GenBank reference	Gene or protein name	NAA	AAA	A/N ratio	*P* value	Conservation (%)
F07m	J05070	MMP-9/gelatinase B	96 ± 81	8317 ± 2590	86.52	.019	100
B04d	L25259	CD86 antigen (B7-2 antigen)	34 ± 34	1463 ± 237	43.42	.001	100
E10f	L36720	Bystin-like	31 ± 31	1277 ± 484	40.77	.042	75
B04c	M12529	Apolipoprotein E	993 ± 570	14,459 ± 4144	14.56	.018	100
E03i	M15395	Integrin β_2	256 ± 191	3278 ± 707	12.79	.006	100
B05k	U43408	Nonreceptor tyrosine kinase 1	65 ± 65	767 ± 235	11.87	.028	100
B03j	U09607	Janus kinase 3	359 ± 157	2999 ± 968	8.35	.036	100
E09g	M73780	Integrin β_8	430 ± 329	3443 ± 749	8.00	.010	100
F14g	Y00787	IL-8	2150 ± 950	15,494 ± 5313	7.21	.048	75
B05i	D10495	PKC-δ	458 ± 282	3226 ± 994	7.04	.037	75
E08c	X55122	GATA-3	553 ± 292	3774 ± 736	6.83	.007	100
E04h	M30640	Selectin E	392 ± 164	2566 ± 615	6.54	.014	75
C04j	M63167	v-*akt* (rac protein kinase α)	260 ± 187	1698 ± 328	6.53	.009	100
B02j	M35203	Janus kinase 1	263 ± 100	1650 ± 546	6.27	.046	75
E10b	M97676	*msh* homolog 1 (*Drosophila*)	1249 ± 335	7725 ± 2032	6.19	.020	100
B02k	AJ000512	Serum glucocorticoid-regulated kinase	590 ± 194	3478 ± 907	5.89	.021	75
E02i	L12002	Integrin α_4	256 ± 256	1462 ± 349	5.72	.032	50
E01c	U08015	Nuclear factor of activated T cells	295 ± 91	1659 ± 521	5.63	.042	75
E01d	U43188	E74-like factor 2	345 ± 164	1892 ± 521	5.48	.030	100
C04k	U45880	Baculoviral IAP repeat-containing 4	278 ± 98	1512 ± 435	5.44	.032	75
A11l	M81750	Myeloid cell nuclear differentiation antigen	2331 ± 911	12,077 ± 708	5.18	<.001	100
E06e	D28468	Albumin D box-binding protein	1429 ± 661	7399 ± 722	5.18	.001	100
E04a	M16937	Homeo box B7	1121 ± 604	5650 ± 1728	5.04	.048	75
B13d	D10924	CXC chemokine receptor-4 (fusin)	2254 ± 588	11,172 ± 1767	4.96	.003	100
E05g	D83542	Cadherin 15 (M-cadherin)	571 ± 465	2704 ± 722	4.73	.048	50
E06h	M34064	Cadherin 2 (N-cadherin)	435 ± 264	2018 ± 456	4.64	.024	75
F08k	X07549	Cathepsin H	2914 ± 1216	13,310 ± 3460	4.57	.030	75
C10a	L14778	Calcineurin A α	1411 ± 717	6394 ± 1268	4.53	.014	75
F12f	M74178	Macrophage stimulating 1	2307 ± 906	10297 ± 1592	4.46	.005	100
B07g	AF055581	Lymphocyte adaptor protein	647 ± 267	2865 ± 634	4.42	.018	75
E05c	U10324	IL enhancer binding factor 3 (90 kd)	844 ± 440	3712 ± 705	4.40	.014	75
E09b	M97287	Special AT-rich sequence binding protein 1	1226 ± 262	5290 ± 1192	4.31	.016	100
B01e	U08839	Urinary-type plasminogen activator receptor	500 ± 418	2155 ± 305	4.31	.019	50
E11g	M14219	Decorin	3497 ± 1447	14,855 ± 4031	4.25	.038	75
E08d	M58603	Nuclear factor κB enhancer p105	805 ± 330	3234 ± 534	4.02	.008	75
E14i	Y00796	Integrin α_L (CD11a antigen)	3269 ± 898	12,634 ± 1824	3.86	.004	100
E06d	AF072825	*raf*-responsive zincfinger protein	491 ± 293	1893 ± 321	3.86	.018	75
E07b	M93255	Friend leukemia virus integration 1	3564 ± 958	13,673 ± 2286	3.84	.007	100
B06d	M62424	Coagulation factor II (thrombin) receptor	2657 ± 929	10,182 ± 593	3.83	>.001	100
A09c	K00650	v-*fos*	2290 ± 819	8080 ± 1584	3.53	.018	75
B05j	L31951	Mitogen-activated protein kinase 9	479 ± 195	1629 ± 427	3.40	.050	50
A12l	D38305	Transducer of ERBB2-1	935 ± 512	3170 ± 679	3.39	.039	50
B02m	U86453	PI-3-kinase δ polypeptide	626 ± 246	2103 ± 494	3.36	.037	75
B05l	U08316	Ribosomal S6 kinase 3	1095 ± 440	3671 ± 595	3.35	.013	75
E09d	D45132	Zinc-finger DNA-binding protein	519 ± 156	1735 ± 164	3.34	.002	100
B05d	D10925	Chemokine (C-C motif) receptor 1	419 ± 194	1343 ± 273	3.21	.033	75
C02k	L09210	Inducible nitric oxide synthase (nitric oxide synthase 2)	381 ± 196	1168 ± 242	3.07	.045	50
B03l	L07597	Ribosomal S6 kinase 1	963 ± 413	2914 ± 420	3.03	.016	75
E08b	M96944	Paired box gene 5	1491 ± 566	4393 ± 600	2.95	.013	50
B04k	L32976	Mitogen-activated protein kinase 11	1474 ± 878	4337 ± 198	2.94	.020	0
B04m	U78576	PI-4-phosphate 5-kinase type 1 α	684 ± 367	2010 ± 324	2.94	.035	50
E14g	J03132	Intercellular adhesion molecule 1/CD54	6563 ± 2678	18,441 ± 2215	2.81	.014	50
F14j	Z71621	Wingless-type MMTV integration site 2B	3676 ± 1447	10,078 ± 1651	2.74	.027	75
F13k	D45248	Proteasome activator subunit 2	4268 ± 1256	11,121 ± 2172	2.61	.034	75
E07e	L11672	Zinc finger protein 91 (HPF7, HTF10)	3639 ± 1152	9444 ± 683	2.60	.005	75
B07m	X15014	v-*ral*	1573 ± 296	3966 ± 708	2.52	.021	75
B05n	M28211	*RAB4* (*ras* oncogene family)	335 ± 107	823 ± 150	2.46	.038	50
F06d	M29645	Insulinlike growth factor 2 (somatomedin A)	1221 ± 433	2954 ± 358	2.42	.022	25

TABLE 2. Continued

Array code	GenBank reference	Gene or protein name	NAA	AAA	A/N ratio	*P* value	Conservation (%)
E05b	M87503	Interferon-stimulated transcription factor 3 γ	5644 ± 1379	13,606 ± 966	2.41	.003	100
E03c	U08853	Transcription factor LCR-F1	884 ± 310	2080 ± 244	2.35	.023	25
B02g	M16038	v-*yes*-1 oncogene	1707 ± 587	3953 ± 534	2.32	.030	25
E08h	M59040	CD44 antigen	3073 ± 991	7094 ± 521	2.31	.011	25
E02c	U08191	RκB	1264 ± 117	2857 ± 378	2.26	.007	100
F10e	M21121	RANTES	7363 ± 1838	15,804 ± 2850	2.15	.047	50
E01k	M59818	CSF-3 receptor	2319 ± 167	4320 ± 547	1.86	.013	100
B12d	M57230	IL-6 signal transducer gp130	3567 ± 536	1377 ± 579	0.39	.032	75
D01n	L04282	Zinc-finger protein 148	4260 ± 225	1677 ± 679	0.39	.011	50
D11n	D26120	Zinc-finger protein 162	2567 ± 525	977 ± 382	0.38	.050	25
D07l	M73077	Glucocorticoid receptor DNA binding factor 1	2510 ± 434	896 ± 394	0.36	.033	50
D09j	U65928	COP9 subunit 5	4236 ± 1085	1288 ± 484	0.30	.048	25
B08m	X06820	*ras* homolog gene family, member B	15,578 ± 3944	4710 ± 1925	0.30	.048	0
B08n	U02081	Guanine nucleotide regulatory protein	4416 ± 1051	1235 ± 614	0.28	.040	25
D03i	U18840	Myelin oligodendrocyte glycoprotein	2782 ± 645	769 ± 493	0.28	.048	50
D07j	L01042	TATA element modulatory factor 1	2915 ± 749	758 ± 439	0.26	.047	50
A09i	M84489	Mitogen-activated protein kinase 1	1686 ± 133	437 ± 336	0.26	.014	75
D12m	L14754	Immunoglobulin μ binding protein 2	1859 ± 469	428 ± 247	0.23	.036	50
B09d	L37882	Frizzled homolog 2 (*Drosophila*)	905 ± 126	198 ± 159	0.22	.013	75
B14h	U25265	Mitogen-activated protein kinase 5	1860 ± 370	392 ± 220	0.21	.014	50
F12l	Z81326	Protease inhibitor 12 (neuroserpin)	1511 ± 293	305 ± 305	0.20	.029	75
A08g	M28882	Melanoma adhesion molecule	8631 ± 2691	1687 ± 782	0.20	.048	25
D10l	U58198	IL enhancer binding factor-1	1738 ± 475	333 ± 155	0.19	.031	25
D13j	J03161	Serum response factor	2648 ± 312	496 ± 279	0.19	.002	100
D13m	M88163	SWI/SNF homolog	2547 ± 422	459 ± 302	0.18	.007	75
D05l	M21535	v-*ets* (avian erythroblastosis virus oncogene)	3703 ± 863	666 ± 431	0.18	.020	50
D07k	AF069736	PCAF-associated factor 65β	2215 ± 530	332 ± 231	0.15	.017	50
D10e	X52836	Tryptophan hydroxylase	11,706 ± 1092	1424 ± 517	0.12	<.001	100
B10e	L36645	EphA4	866 ± 291	97 ± 97	0.11	.046	75
D01b	M60974	Growth arrest and DNA damage inducible 45	9267 ± 2748	1027 ± 799	0.11	.028	25
F05j	L15344	IL-14	514 ± 145	49 ± 49	0.10	.023	75
B10k	M34181	Cyclic adenosine monophosphate–dependent protein kinase β	1806 ± 367	154 ± 104	0.09	.005	75
D04n	Z30094	General transcription factor IIH polypeptide 2	2069 ± 712	159 ± 151	0.08	.039	50
F11l	D38595	Plasma kallikrein-sensitive glycoprotein	2216 ± 596	168 ± 144	0.08	.016	50
D10m	M95809	General transcription factor IIH polypeptide 1	2523 ± 350	180 ± 97	0.07	.001	100
D14k	L19871	Activating transcription factor 3	3866 ± 1008	252 ± 252	0.07	.013	75
B12g	U48959	Myosin light chain kinase	16,148 ± 4217	770 ± 287	0.05	.011	25
B11g	D84476	Mitogen-activated protein kinase 5	3229 ± 1203	137 ± 81	0.04	.043	50
B11l	L36151	PI-4-kinase α	360 ± 57	14 ± 14	0.04	.001	100
F13a	X14672	N-acetyltransferase-2	827 ± 320	25 ± 25	0.03	.047	75
B13g	X69490	Titin	2412 ± 686	70 ± 57	0.03	.014	50
D11j	U35143	Retinoblastoma-binding protein 7	2351 ± 516	60 ± 60	0.03	.005	100
B12e	L11695	Transforming growth factor β receptor I	1715 ± 599	35 ± 35	0.02	.031	75
D13k	U00115	B-cell CLL/lymphoma-6	2004 ± 517	35 ± 34	0.02	.009	50
D05g	U26662	Neuronal pentraxin II	2413 ± 880	27 ± 23	0.01	.035	50
F01m	D45027	R3H domain	498 ± 201	0 ± 0	0	.048	100

A/N, Abnormal/normal ratio.

AAAs and NAA, including MMP-9/gelatinase B (86-fold), CD86/B7-2 antigen (43-fold), bystin-like (41-fold), apolipoprotein E (15-fold), integrin β_2 (13-fold), and nonreceptor tyrosine kinase 1 (12-fold). Sixty-six of the 104 genes (63%) exhibited the alterations described in at least 3 of the 4 individual AAA specimens, including 50 of the 66 genes (76%) exhibiting increased expression and 17 of the 39 genes (44%) exhibiting decreased expression (Table 2).

Parallel analyses between aneurysmal and normal aortic tissues from each location are summarized by the Venn diagram shown in Figure 3, illustrating that the patterns of altered gene expression were quite dissimilar between TAAs and AAAs. It is notable that quantitative increases in expression for 97 genes were found to be unique to TAAs, whereas increases in expression for 61 genes were found to be unique to AAAs; similarly, decreases in expression for 7

genes were unique to TAAs and decreases in 35 gene products were unique to AAAs. Although 8 additional transcripts were significantly altered in *both* TAAs and AAAs, these alterations were *directionally concordant* for only 4 genes: MMP-9/gelatinase B, v-*yes*-1, mitogen-activated protein kinase-9, and intracellular adhesion molecule 1/CD54 (all increased in both TAAs and AAAs). In contrast, the remaining genes were altered in a *directionally discordant* manner, with all 4 being significantly increased in TAAs and significantly decreased in AAAs (N-acetyltransferase-2, IL-6 signal transducer gp130, mitogen-activated protein kinase kinase-5, and IL enhancer binding factor-1).

RT-qPCR

To validate the results of cDNA microarray analysis, the patterns of messenger RNA expression for a series of selected genes were independently examined with real-time RT-qPCR. As shown in Table 3, this revealed significant increases in expression of MMP-9/gelatinase B and v-*yes*-1 oncogene in both TAAs and AAAs; increased expression of TNF-α, IL-1β, vascular cell adhesion molecule 1, and cathepsin D in TAAs but not in AAAs; increased expression of apolipoprotein E and IL-8 in AAAs but not in TAAs; and increased expression of IL-6 signal transducer gp130 in TAAs with decreased expression in AAAs. In each case, the significance and magnitude of the alterations in expression as detected by RT-qPCR corresponded with the results of the microarray analysis. Thus there was a high degree of consistency between the quantitative results obtained with the two independent techniques.

Discussion

To better understand cellular and molecular changes that accompany aneurysm development, cDNA microarrays have been used to profile differences in gene expression between normal human aorta and AAAs.[33,34] To the best of our knowledge, this investigation represents the first application of high-throughput transcriptional profiling to degenerative aneurysms of the ascending thoracic aorta, thereby providing some initial insights into molecular similarities and differences that may exist between TAAs and AAAs.

The most important observations arising from this study are as follows: (1) TAAs are characterized by significant alterations in expression for at least 112 genes relative to NTA; (2) the altered pattern of gene expression observed in TAAs is distinguishable from that observed in AAAs with respect to at least 208 genes, of which 101 are increased and 7 decreased in TAAs but not in AAAs and 61 are increased and 39 decreased in AAAs but not TAAs; (3) at least 8 genes exhibit altered expression in *both* TAAs and AAAs, including 4 with directionally concordant and 4 with directionally discordant changes; and (4) the greatest increases in

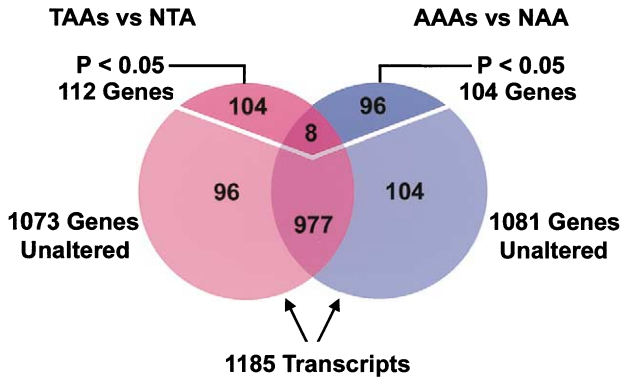

Figure 3. Number and distribution of genes exhibiting altered levels of expression in aortic aneurysms. Venn diagram showing number and distribution of 1185 genes for which significantly altered ($P < .05$) or unaltered ($P > .05$) levels of expression were detected in TAAs versus NTA *(red)* and in AAAs versus NAA *(blue)* by parametric comparisons test. Numbers of genes exhibiting altered or unchanged levels of expression in both conditions are illustrated by areas of overlap *(purple).*

gene expression in TAAs were observed for the ζ and θ isoforms of PKC, uracil-DNA glycosylase (a DNA repair enzyme), lymphotoxin-β, TNF-α, and CD27 (TNF receptor superfamily member-7), whereas the greatest changes in AAAs were for MMP-9/gelatinase B, CD86/B7-2 antigen, bystin-like, apolipoprotein E, integrins β_2 and β_8, nonreceptor tyrosine kinase 1, Janus kinase 3, IL-8, and PKC-δ. These findings support the hypothesis that TAAs and AAAs are fundamentally different pathophysiologic entities at a molecular level, probably reflecting distinct mechanisms of disease.

In a study of this nature, it is tempting to speculate that individual genes exhibiting altered levels of expression in aneurysmal tissues might be related to the pathophysiologic events underlying aneurysm formation. However, it is important to emphasize first that in this study we examined only established "end-stage" aneurysmal tissues from lesions advanced enough to require elective repair, and second that these aneurysmal tissues were evaluated against normal aortic tissues from comparatively young, healthy, cadaveric organ transplant donors as control preparations. The relative levels of gene expression found in these tissues thus may reflect alterations related to differences in age, microscopic atherosclerotic vascular disease, hemodynamic stresses, and other systemic factors, as well as the presence of aneurysmal degeneration. Furthermore, the alterations described in these established aneurysms do not necessarily provide insight into transcriptional alterations that might occur at earlier stages of disease, only those present in lesions that have enlarged enough to require repair. Although altered gene expression in these tissues may actually be more relevant to the potential clinical complications of

TABLE 3. Changes in aortic wall gene expression measured by RT-qPCR versus cDNA microarrays (n = 9 genes)

Gene or protein name	Thoracic	*P* value	Abdominal	*P* value
MMP-9/gelatinase B				
Aneurysmal expression (mean ± SD, arbitrary units)	$2.71 \pm 0.34 \times 10^{-2}$		$1.16 \pm 0.20 \times 10^{-1}$	
Normal expression (mean ± SD, arbitrary units)	$0.86 \pm 0.34 \times 10^{-2}$		$0.10 \pm 0.07 \times 10^{-1}$	
RT-qPCR aneurysmal/normal ratio	3.13	<.001	11.8	<.001
Microarray aneurysmal/normal ratio	8.64	.020	86.5	.019
IL-6 signal transducer gp130				
Aneurysmal expression (mean ± SD, arbitrary units)	$5.74 \pm 0.85 \times 10^{1}$		$6.02 \pm 0.93 \times 10^{1}$	
Normal expression (mean ± SD, arbitrary units)	$3.54 \pm 0.56 \times 10^{1}$		$1.13 \pm 0.21 \times 10^{2}$	
RT-qPCR aneurysmal/normal ratio	1.62	.041	0.53	.038
Microarray aneurysmal/normal ratio	6.14	.045	0.39	.032
v-*yes*-1 oncogene				
Aneurysmal expression (mean ± SD, arbitrary units)	$7.11 \pm 0.93 \times 10^{-1}$		$8.68 \pm 1.12 \times 10^{-2}$	
Normal expression (mean ± SD, arbitrary units)	$4.21 \pm 0.69 \times 10^{-1}$		$2.73 \pm 0.74 \times 10^{-2}$	
RT-qPCR aneurysmal/normal ratio	1.69	.019	3.17	<.001
Microarray aneurysmal/normal ratio	4.75	.035	2.32	.030
Apopliporotein E				
Aneurysmal expression (mean ± SD, arbitrary units)	$5.71 \pm 1.29 \times 10^{0}$		$8.77 \pm 1.98 \times 10^{-1}$	
Normal expression (mean ± SD, arbitrary units)	$2.33 \pm 1.39 \times 10^{0}$		$7.05 \pm 1.51 \times 10^{-2}$	
RT-qPCR aneurysmal/normal ratio	2.45	>.05	12.44	<.001
Microarray aneurysmal/normal ratio	2.69	>.05	14.56	.018
IL-8				
Aneurysmal expression (mean ± SD, arbitrary units)	$3.33 \pm 0.30 \times 10^{0}$		$1.16 \pm 0.23 \times 10^{-1}$	
Normal expression (mean ± SD, arbitrary units)	$5.80 \pm 0.15 \times 10^{0}$		$5.17 \pm 1.55 \times 10^{-2}$	
RT-qPCR aneurysmal/normal ratio	0.57	>.05	2.24	<.001
Microarray aneurysmal/normal ratio	1.01	>.05	7.21	.048
IL-1β				
Aneurysmal expression (mean ± SD, arbitrary units)	$2.12 \pm 0.36 \times 10^{-2}$		$5.92 \pm 0.90 \times 10^{-3}$	
Normal expression (mean ± SD, arbitrary units)	$5.86 \pm 0.73 \times 10^{-3}$		$1.54 \pm 0.50 \times 10^{-2}$	
RT-qPCR aneurysmal/normal ratio	3.61	<.001	0.38	>.05
Microarray aneurysmal/normal ratio	5.41	.040	1.01	>.05
TNF-α				
Aneurysmal expression (mean ± SD, arbitrary units)	$3.51 \pm 1.36 \times 10^{-3}$		$1.47 \pm 0.31 \times 10^{-4}$	
Normal expression (mean ± SD, arbitrary units)	$2.31 \pm 1.50 \times 10^{-4}$		$5.86 \pm 2.30 \times 10^{-5}$	
RT-qPCR aneurysmal/normal ratio	15.24	.024	2.50	>.05
Microarray aneurysmal/normal ratio	26.63	.047	3.16	>.05
Vascular cellular adhesion molecule 1				
Aneurysmal expression (mean ± SD, arbitrary units)	$1.29 \pm 0.10 \times 10^{0}$		$2.65 \pm 0.88 \times 10^{-1}$	
Normal expression (mean ± SD, arbitrary units)	$5.57 \pm 0.76 \times 10^{-1}$		$6.27 \pm 1.32 \times 10^{-1}$	
RT-qPCR aneurysmal/normal ratio	2.31	<.001	0.42	>.05
Microarray aneurysmal/normal ratio	7.81	.001	1.94	>.05
Cathepsin D				
Aneurysmal expression (mean ± SD, arbitrary units)	$4.51 \pm 1.28 \times 10^{-1}$		$1.52 \pm 0.94 \times 10^{-1}$	
Normal expression (mean ± SD, arbitrary units)	$1.60 \pm 0.56 \times 10^{-1}$		$3.53 \pm 2.94 \times 10^{-2}$	
RT-qPCR aneurysmal/normal ratio	2.82	.045	4.29	>.05
Microarray aneurysmal/normal ratio	4.96	.002	1.34	>.05

aneurysmal disease (expansion and rupture) than those that might exist during early stages of aneurysm formation, it is also clear that the transcriptional alterations identified in this study will need to be confirmed at the protein level, proceeding thereafter to functional assessment of their relevance in in vivo model systems. Nonetheless, with these limitations in mind, the expression profiles derived from this study provide a valuable start toward evaluating gene expression patterns that characterize established aneurysmal disease in the thoracic and abdominal aorta and, perhaps of equal importance, determining how such patterns differ between TAAs and AAAs.

Not surprisingly, many of the genes altered in TAAs and AAAs are involved in intracellular signal transduction and transcriptional activation pathways. The differences between TAAs and AAAs may therefore reflect either the presence or absence of different cell types or the dominance of different cellular activation and signaling pathways in each disease. For example, two particular isoforms of PKC were expressed at relatively high levels in TAAs versus

NTA, PKC-ζ and PKC-θ (>100-fold and 14-fold, respectively), whereas AAAs were associated with a 7-fold increase in expression of PKC-δ but no significant alterations in other PKC isoforms. Although regulation of PKC activity occurs largely through phosphorylation events by upstream mediators of cell activation, the dominant presence of certain isoforms may influence downstream events by determining how cells respond to PKC activation.[37,38]

With respect to other functionally-related groups of genes, Table 1 reveals that TAAs were associated with pronounced increases in the expression of genes involved in regulating cell survival, proliferation, and programmed cell death, including the DNA repair enzyme uracil-DNA glycosylase (44-fold),[39] lymphotoxins α (8-fold) and β (41-fold),[40] TNF-α (27-fold), CD27 (a TNF receptor superfamily member, 18-fold),[41] platelet-derived growth factor receptor α (11-fold), early growth response 1 (10-fold),[42], cyclins D2 (5-fold) and D3 (4-fold), jun D (7-fold), IL-1β (5-fold), and the IL-1β converting enzyme homolog ICE-LAP3 (6-fold).[43] If verified at the protein level, the increased local production of soluble cytotoxic mediators, such as TNF-α and IL-1β, may be particularly relevant to the medial SMC dysfunction and depletion observed in TAAs. Indeed, several reports have suggested that programmed cell death may play a role in the medial degeneration observed in TAAs,[24] and it is recognized that both TNF-α and IL-1β are potent inducers of apoptosis and nitric oxide production in cultured vascular SMCs, particularly when acting in combination.[44,45]

Table 2 demonstrates that AAAs were associated with increased expression of a number of genes related to atherosclerosis and chronic inflammation that were not increased in TAAs, such as those encoding the T-cell costimulatory molecule CD86/B7-2 antigen (43-fold),[46] apoplipoprotein E (15-fold), IL-8 (7-fold), GATA-3 (7-fold),[47] nuclear factor of activated T cells (6-fold),[48,49] myeloid cell nuclear differentiating antigen (5-fold), macrophage stimulating 1 (4-fold), nuclear factor κB p105 (4-fold), inducible nitric oxide synthase (nitric oxide synthase II, 3-fold), urinary-type plasminogen activator receptor (4-fold), and thrombin receptor (4-fold). Genes encoding a number of cell adhesion molecules were also selectively increased in AAAs, such as integrins β_2 (13-fold), β_8 (8-fold), α_4 (6-fold), and α_L (4-fold), as well as selectin E (6-fold). Furthermore, it is notable that AAAs were associated with significantly decreased expression of growth arrest and DNA damage inducible 45 (9-fold decrease),[50] smooth muscle and nonmuscle myosin light chain kinase (20-fold decrease), and transforming growth factor β receptor type I (50-fold decrease), which may all reflect the pronounced SMC depletion in AAAs.

Perhaps the most important alteration common to both TAAs and AAAs was the marked increase in expression of MMP-9/gelatinase B (9-fold in TAAs vs NTA and 86-fold in AAAs vs NAA), an observation consistent with previous studies demonstrating that production of MMP-9 is increased in TAAs and dissections and in AAAs.[51-53] MMP-9 is thought to contribute to elastin degradation in aneurysmal disease, because it exhibits enzymatic activity against elastic fibers and other extracellular matrix proteins and because it is produced by medial SMC and aneurysm-infiltrating macrophages.[52,53] It has also been shown that MMP-9 is overexpressed in various experimental animal models of AAAs, that MMP inhibitors suppress development of experimental AAAs in rats and mice, and that mice lacking MMP-9 are resistant to aneurysm development.[35,54-57] In the context of these observations, it appears likely that increased expression of MMP-9 plays a central role in the destructive remodeling of the elastic media associated with aortic aneurysms, regardless of their location or initiating etiology.

Although the clinical significance of this work is yet unknown, the altered patterns of gene expression identified here will provide a valuable foundation for further investigations into the pathobiology of aortic aneurysmal disease. This study also demonstrates that significant heterogeneity exists between TAAs and AAAs at the molecular level and illustrates the use of high-throughput cDNA microarrays to generate novel information. Further applications of gene expression profiling can be expected to substantially enhance our understanding of the diverse processes involved in aneurysmal degeneration.

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Discussion

Dr Frank W. Sellke *(Boston, Mass).* Can you comment on how you performed the biopsies to make sure that there was consistency between the TAA and AAA specimens? Because there is so much, how do I put it, grungy material in aneurysms of both types, especially AAAs, how did you take this into consideration? Also, can you comment on the statistical analysis specific for cDNA array technology?

Dr Absi. Thank you, Dr Sellke. With respect to the biopsies, I obtained TAA specimens from the attending surgeons who were performing the procedure, basically as a ring of tissue removed when they had completed aneurysm repair. This tissue was snap-frozen immediately in liquid nitrogen, and at a later time I extracted the messenger RNA. AAA repair was basically performed by endoaneurysmorrhaphy, so in these cases I obtained a slice of tissue along the whole length of aneurysm itself.

With respect to the statistical analysis, it is true that we did not have a high number of blots. In going over the statistical analysis, especially that provided with the software, our approach was in agreement with literature reports indicating that with 5 blots one can achieve reproducible results. Just because you are dealing with 1185 genes, the parametric analysis must be done with some adjustments in a special kind of analysis of variance, such as the Kruskal-Wallis test, which can be of help when you have a relatively small number of samples.

Dr Michael J. Reardon *(Houston, Tex).* Congratulations on a nice study and a nice presentation. I did have one question about your normal samples. Were they from donors, or were they from the recipients in transplants? Because if they came from donors, they clearly came from much younger people, and there may be altered gene expression with age. We have started taking some plugs from the ascending aorta from coronaries to use those as normal specimens. Would you comment on your normal specimens?

Dr Absi. Thank you, Dr Reardon. I am glad that you brought that up. That is an important issue, and we did not mean to minimize it during the presentation. The pathophysiology of aneurysmal disease is thought to include numerous factors, age and atherosclerosis among them. We chose to use donors for our normal controls, who were young, because we did not want to bias against any of these factors, namely age, in that situation. Age may make a difference and at least some of the results we obtained may be due to age. For this reason we are also planning in the future to compare results in aneurysms with age-matched samples obtained from patients undergoing coronary artery bypass grafting. However, if we had selected or stratified for age in this initial study we would have tended to bias against it, and we did not want to do that here.

Dr Anthony L. Estrera *(Houston, Tex).* This was a nice study. My question is in relation to gender predominance. In our experience in Houston, our patients with thoracoabdominal and ascending arch repairs are primarily male, with a ratio of about 3:2; whereas in our infrarenal replacements there is a greater male predominance, about a 10:1 ratio. Do you have a comment? I appreciate the small numbers in your study, but that would be something to look at in the future.

Dr Absi. We simply chose our patients randomly over a certain period of time during the conduct of the study, without selection for gender, and agree that this will be an important issue to examine in the future.

Dr Larry R. Kaiser *(Philadelphia, Pa).* You mentioned that apolipoprotein E was 14-fold upregulated in, I think, the AAAs. Are you concerned that the high concentration of apolipoprotein E was due to contamination, perhaps by infiltrating foam cells in the arterial wall, and did you use laser capture microdissection to remove some of these foam cells and infiltrating leukocytes that might have interfered?

Dr Absi. No, we did not use that technique, as this study was not designed to identify the cell types or locations where the changes in gene expression occurred, but only to provide a broad survey of changes in gene expression within the tissue samples as a whole. There are clearly a variety of different cell types present within aneurysm tissues, including macrophages and vascular smooth muscle cells, but the cells in which the changes in gene expression were occurring were not examined in this initial investigation. This is another promising approach for the future.