

Supplementary material on-line

Novel pathways in pathobiology of human abdominal aortic aneurysms

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This file contains 3 tables (Table S1, S2 and S3) and Fig. S1

Table S1. Human infrarenal abdominal aortic tissue samples used in the study

Case ID	Classification	Sex	Age (y)	Cause of Death	Micro-array	PCR Array	Western Blot	IHC
A1-F	AAA	F	82		√*			
A2-F	AAA	F	68		√*			
A3-F	AAA	F	64		√*			
A2-M	AAA	M	63		√*			
A3-M	AAA	M	67		√*			
A4-M	AAA	M	63		√*			
GHS020200	AAA	M	82		√			
GHS020400	AAA	M	76		√			
GHS200306	AAA	M	70		√			
GHS200308	AAA	M	74		√			
WSU052	AAA	M	70					√
WSU075	AAA	M	67					√
WSU068	AAA	M	72					√
GHS01	AAA	M	67			√		√
GHS02	AAA	F	63			√		
GHS05	AAA	M	64			√		√
GHS07	AAA	F	65			√	√	
GHS11	AAA	F	61			√		
GHS12	AAA	M	70			√		
GHS13	AAA	M	78			√		
GHS14	AAA	M	75			√		
GHS15	AAA	M	66			√		
GHS19	AAA	M	66			√		
GHS32	AAA	C	64				√	
GHS48	AAA	C	65				√	
GHS68	AAA	C	61				√	
GHS200210	AAA	M	60			√		
GHS200212	AAA	M	73			√		
GHS200214	AAA	M	60			√		
GHS200219	AAA	M	67			√		
GHS200305	AAA	M	64			√		
C1-F	Control	F	74	Cancer	√*			
C2-F	Control	F	52	Cancer	√*			
C3-F	Control	F	84	Aortic arch dissection	√*			
C1-M	Control	M	65	Peritonitis	√*			
C2-M	Control	M	59	Cancer	√*			
C3-M	Control	M	52	Liver cirrhosis	√*			
C4-M	Control	M	73	Cancer	√*			
GHS200105	Control	M	21	Trauma	√			
GHS200401	Control	F	53	Cancer	√			
GHS024200	Control	M	53	NA	√			
NDRI56735	Control	F	54	Cancer	√		√	
NDRI56724	Control	F	77	Natural		√		
NDRI57017	Control	F	78	Cardiovascular		√	√	

NDRI57054	Control	M	69	Cardiovascular	√	√
NDRI57179	Control	F	57	Respiratory failure	√	
NDRI57110	Control	M	69	Cardiovascular	√	
NDRI66274	Control	M	61	Cardiovascular	√	
NDRI66279	Control	M	64	Cancer	√	
NDRI66324	Control	M	67	Cancer	√	
NDRI66747	Control	M	57	Cardiovascular	√	
NDRI66799	Control	M	67	Respiratory	√	
ME-01-05	Control	M	53	NA		√
ME-02-05	Control	M	78	Cardiovascular	√	√
ME-05-01	Control	F	69	Trauma	√	√
ME-05-03	Control	M	54	Cardiovascular	√	
ME-05-05	Control	F	59	Cardiovascular		√
ME-10-01	Control	F	88	Trauma	√	
ME-10-03	Control	M	44	Overdose	√	

NA, Not available.

All donors were Caucasian.

Samples were stored in RNAlater (Ambion, Austin, TX), in phosphate-buffered formalin (and embedded in paraffin), or snap-frozen in liquid nitrogen.

*These samples were used in the previous microarray study [1].

For the microarray experiment [1], the summary statistics are:

- AAA group ($n = 6$: mean age = 67.8 +/- 7.3, Median = 65.5, Male:Female = 3:3)
- Control group ($n = 7$): mean age = 65.6 +/- 12.1, Median = 65, Male:Female = 4:3)
- Comparison of ages in the study groups: $P = 0.69$ (two-tailed t-test, unequal variance)

For the second microarray experiment reported in this study, the summary statistics are:

- AAA group ($N = 4$): mean age = 75 +/- 5.0, Median = 74, Male:Female = 4:0)
- Control group ($N = 4$): mean age = 45.3 +/- 16.2, Median = 53, Male:Female = 2:2)
- Comparison of ages in the study groups: $p = 0.03$ (two-tailed t-test, unequal variance)

For the PCRArrays, the summary statistics are:

- AAA group ($n = 15$): mean age = 66.7 +/- 5.4; median = 66; Male:Female = 12:3)
- Control group ($n = 15$): mean age = 66.6 +/- 11.1; median = 67; Male:Female = 10:5)
- Comparison of ages in the study groups: $P = 0.98$ (two-tailed t-test, unequal variance)

Table S2. Design of custom PCRArray, the “AAA-chip”, with 43 genes of interest and 5 controls

Gene Symbol	Gene ID	RefSeq Number*	RT ² Catalog Number	Size of PCR Band (bp)	Reference Position [†]	Splice Variant Amplicons [§]
ADCY7	113	NM_001114	PPH02794	173	5285	NA
ARL4C	10123	NM_005737	PPH11184	166	610	NA
BLNK	29760	NM_013314	PPH06241	85	1322	NM001114094
CCL4	6351	NM_002984	PPH00563	93	512	NA
CCL8	6355	NM_005623	PPH01167	154	1035	NA
CD4	920	NM_000616	PPH01629	183	2548	NA
CD48	962	NM_001778	PPH07024	162	561	NA
CD83	9308	NM_004233	PPH05590	169	2379	NM001040280
CSRP2	1466	NM_001321	PPH07753	114	398	NA
CXCR4	7852	NM_003467	PPH00621	150	192	NM001008540
CYTIP	9595	NM_004288	PPH05576	141	905	NA
DUSP6	1848	NM_001946	PPH02573	188	1362	NM0022652
FCGR2B	2213	NM_004001	PPH02368	79	1607	NM001002273, NM001002274, NM001002275
FOSB	2354	NM_006732	PPH00157	117	820	NM001114171

GATM	2628	NM_001482	PPH09960	84	1682	NA
GPR65	8477	NM_003608	PPH12137	105	578	NA
HSPB2	3316	NM_001541	PPH01204	109	320	NA
IL10RA	3587	NM_001558	PPH00591	101	3400	NR026691
ITGA10	8515	NM_003637	PPH00635	92	3433	NA
ITGA5	3678	NM_002205	PPH00176	87	2690	NA
LARP6	55323	NM_197958	PPH19780	117	153	NM018357
LCP2	3937	NM_005565	PPH01634	154	1664	NA
LDOC1	23641	NM_012317	PPH09280	83	170	NA
LMOD1	25802	NM_012134	PPH10165	122	3126	NA
LYZ	4069	NM_000239	PPH14748	105	295	NA
MARCKS	4082	NM_002356	PPH05579	158	1457	NA
MFGE8	4240	NM_005928	PPH07218	88	1217	NM001114614
MX2	4600	NM_002463	PPH01326	164	2818	NA
NCF4	4689	NM_000631	PPH14176	174	1312	NM013416
NPTX2	4885	NM_002523	PPH07197	149	1278	NA
NTRK3	4916	NM_002530	PPH01551	173	1584	NM001007156, NM001012338
PDE8B	8622	NM_003719	PPH10098	92	2432	NM001029851, NM00102985, NM001029853, NM001029854
PLEK	5341	NM_002664	PPH10056	177	795	NA
PRUNE2	158471	NM_138818	PPH17589	87	698	NM015225, XM001715010, XM001719626, XM001719633, XM001722398, XM001722406
PTPRC	5788	NM_002838	PPH01510	64	193	NM080923, NM080922, NM080921
RAMP1	10267	NM_005855	PPH02548	99	291	NA
RASL12	51285	NM_016563	PPH08720	191	875	NA
SAMSN1	64092	NM_022136	PPH05800	117	1186	NA
SMARCD3	6604	NM_003078	PPH21185	85	1610	NM001003802, NM001003801
SMTN	6525	NM_006932	PPH12727	92	2867	NM134270, NM134269
TMOD1	7111	NM_003275	PPH06917	67	1079	NA
TPM2	7169	NM_003289	PPH16441	90	656	NM213674, NM001145822
ZBTB16	7704	NM_006006	PPH06320	157	2202	NM001018011
GAPDH		NM_002046	PPH00150			
RPL13A		NM_012423	PPH01020			
HGDC		SA_00105	PPH65835			Human Genomic DNA Contamination
RTC		SA_00104	PPX63340			Reverse Transcription Control
PPC		SA_00103	PPX63339			Positive PCR Control

*The RefSeq Accession number refers to the representative sequence used to design the primers.

[†]The Reference Position is a position within the sequence of the amplicon relative to the start of the relevant RefSeq sequence.

[§]The primers used for the assay can also generate amplicons from the listed splice variants.

Information was generated from: <http://sabiosciences.com/RT2PCR.php>. Assays can be purchased from SABioscience-Qiagen.

A custom PCRArray was designed with 43 genes of interest selected from our two microarray studies [1], and run on RNA samples isolated from a set of 15 human AAA samples and 15 control abdominal aorta samples (Supplementary material online, Table S1). cDNA synthesis was performed using the RT² first strand kit (SABiosciences-Qiagen, Frederick, MD). Expression of the 43 genes was analyzed in a 96-well plate using a custom RT² Profiler PCR array (SABiosciences-Qiagen, Frederick, MD). The assays were run according to manufacturer's instructions (SABiosciences-Qiagen, Frederick, MD) in a 7500 Fast Real Time PCR system (Applied Biosystems, Carlsbad, CA). Two housekeeping genes (GAPDH and RPL13A) were measured to standardize the results. After PCR, amplification plots were inspected and baselines and threshold values were set according to the manufacturer's recommendations (SABiosciences-Qiagen, Frederick, MD). The threshold cycle numbers (C_T) were computed for each well. For calculating the ΔC_T values for each well and processing the results further, the online analysis tool provided by SABiosciences-Qiagen was used. Briefly, the amplification C_T values from the housekeeping genes were subtracted from the gene-of-interest C_T values for each sample to calculate the ΔC_T .

Table S3. Primary antibodies used for Western blots and immunohistochemical staining

Gene Symbol	Protein name	Antibody catalog number	Vendor	Species	WB Dilution	IHC Dilution
<i>ACTB</i>	ACTB	A1978	Sigma	Mouse monoclonal	1:1000	
<i>PTPRC</i>	PTPRC	HPA000440	Sigma-Aldrich	Rabbit	1:100	1:50
<i>PTPRC</i>	CD45	MS-355-R7	Thermo	polyclonal Mouse monoclonal	1:200	1:200
<i>CD4</i>	CD4	M7310	Dako	Mouse	1:200	
<i>CD4</i>	CD4	790-4423	Ventana	monoclonal Rabbit monoclonal		as provided by company
<i>GATM</i>	GATM	HPA026077	Sigma-Aldrich	Rabbit polyclonal	1:200	1:115
<i>ITGA5</i>	ITGA5	AB1928	Chemicon	Rabbit polyclonal		1:188
<i>ITGA10*</i>	ITGA10	sc-161740	Santa Cruz	Goat polyclonal	1:200	
<i>LYZ†</i>	LYZ	A0099	DAKO	Rabbit polyclonal		1:401
<i>FOSB</i>	FOSB	sc-48	Santa Cruz	Rabbit polyclonal		1:240
<i>MFGE8*</i>	MFGE8	sc-8029	Santa Cruz	Mouse monoclonal		1:100
<i>BLNK</i>	BLNK	sc-15345	Santa Cruz	Rabbit polyclonal		1:51
<i>DUSP6</i>	MKP-3	sc-28902	Santa Cruz	Rabbit polyclonal		1:80
<i>CXCR4</i>	Fusin	sc-9046	Santa Cruz	Rabbit polyclonal		1:120
<i>PLEK</i>	PLEK	sc-100813	Santa Cruz	Mouse monoclonal		1:50

WB, western blot; IHC, immunohistochemical staining

* Antibody did not perform well and no results are reported here.

† For staining with LYZ antibody tissues were pre-treated with proteinase K according to DAKO protocol.

Reference:

- 1 Lenk GM, Tromp G, Weinsheimer S, Gatalica Z, Berguer R, Kuivaniemi H: Whole genome expression profiling reveals a significant role for immune function in human abdominal aortic aneurysms. *BMC Genomics* 2007;8:237.

Fig. 1S (on next page). A network of the 38 differentially expressed genes in AAA. The analysis, which was carried out with Ingenuity Pathways Analysis software version 9.0 (IPA, Ingenuity® Systems, Mountain View, CA, www.ingenuity.com), yielded four networks, which were then merged. The differentially expressed genes are highlighted in grey. Molecules are represented as nodes, and the biological relationship between two nodes is represented as a line. All lines are supported by at least one literature citation or from canonical information stored in the Ingenuity Pathways Knowledge Base. Nodes are displayed using various shapes that represent the functional class of the gene product. Lines are displayed with various labels that describe the nature of the relationship between the nodes (e.g., P for phosphorylation, T for transcription).

