Identification of key genes associated with the human abdominal aortic aneurysm based on the gene expression profile

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Abstract. The present study was aimed at screening the key genes associated with abdominal aortic aneurysm (AAA) in the neck, and to investigate the molecular mechanism underlying the development of AAA. The gene expression profile, GSE47472, including 14 AAA neck samples and eight donor controls, was downloaded from the Gene Expression Omnibus database. The total AAA samples were grouped into two types to avoid bias. Differentially expressed genes (DEGs) were screened in patients with AAA and subsequently compared with donor controls using linear models for microarray data, or the Limma package in R, followed by gene ontology enrichment analysis. Furthermore, a protein-protein interaction (PPI) network based on the DEGs was constructed to detect highly connected regions using a Cytoscape plugin. In total, 388 DEGs in the AAA samples were identified. These DEGs were predominantly associated with limb development, including embryonic limb development and appendage development. Nuclear receptor co-repressor 1 (NCOR1), histone 4 (H4), E2F transcription factor 4 (E2F4) and hepatocyte nuclear factor 4α (HNF4A) were the four transcription factors associated with AAA. Furthermore, HNF4A indirectly interacted with the other three transcription factors. Additionally, six clusters were selected from the PPI network. The DEG screening process and the construction of an interaction network enabled an understanding of the mechanism of AAA to be gleaned. HNF4A may exert an important role in AAA development through its interactions with the three other transcription factors (E2F4, NCOR1 and H4), and the mechanism of this coordinated regulation of the transcription factors in AAA may provide a suitable target for the development of therapeutic intervention strategies.

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Abbreviations: AAA, abdominal aortic aneurysm; MMP, matrix metalloproteinase; DEG, differentially expressed gene; GEO, gene expression omnibus; MDS, multi-dimensional scaling; FDR, false discovery rate; FC, fold change

Key words: abdominal aortic aneurysm, differentially expressed genes, protein-protein interaction, function analysis, transcription factors

Introduction

Abdominal aortic aneurysm (AAA) is characterized by permanent, localized dilations of the abdominal aorta, which are defined as having diameters 1.5 times greater than normal (or which measure >3 cm) (1). Aortic rupture is the most serious clinical condition resulting from the progression of AAA (2). Almost 80% of patients who experience aortic rupture succumb to mortality (3). Since the majority of aneurysms are usually asymptomatic until rupture occurs, diagnosis is therefore problematic, and no preventative therapies are currently available for patients to effectively limit the progression of AAA (2,4). Therefore, it is important to investigate the mechanisms of AAA initiation and progression in order to assist diagnostic applications and to develop therapeutic options.

AAA is considered to be a particular, localized form of atherothrombosis (5). It shares the usual risk factors with occlusive atherothrombosis, including an increasing age, male gender, smoking, possible genetic susceptibility and low high density lipoprotein-to-cholesterol levels (6). The pathogenesis of AAA is complicated and multifactorial. Unique hemodynamic forces, which particularly impact on the infrarenal area, and variations in the content of elastin and collagen in different parts of the aorta rendered the infrarenal part of the abdominal aorta highly susceptible to AAA development (2). Numerous animal models and clinical studies reported that the initiation of AAA involves an inflammatory response, which is often enforced by the upregulation of adhesion molecules (7-9). The degradation of the extracellular matrix, which is caused by an increased activity of matrix metalloproteinases (MMPs) and serine proteases, and smooth muscle cell apoptosis are the predominant features associated with the progression of

AAA (10,11). Previous genome-wide studies using microarrays have investigated the pathogenesis of AAA (12-14). The involvement of the immune system in AAA formation and progression was also reported in previous studies (15,16), however, the molecular mechanisms leading to the development and progression of AAA remain to be fully elucidated.

Using the identical gene expression profile, Biros *et al* (17) demonstrated that immune pathways are upregulated within the undilated aorta proximal to an AAA. In the present study, the differentially expressed genes (DEGs) featured in the gene expression profile in AAA necks were analyzed. Furthermore, a function and pathway-enrichment analysis was performed on the DEGs, and a protein-protein interaction network (PPI) was constructed to identify those DEGs which have a central role in AAA. The present study also aimed to gain further insights into the molecular mechanisms underlying the development of AAA. Understanding these molecular mechanisms may assist in developing the understanding of the pathogenesis of AAA, and to translate these pathogenic activities into therapeutic applications.

Materials and methods

Microarray data and data pre-processing. The gene expression profile of GSE47472 was downloaded from the Gene Expression Omnibus (GEO) (17) in the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/geo/) based on the platform of GPL10558 (or the Illumina HumanHT-12 V4.0 expression beadchip). A total of 22 data biopsies were obtained from the AAA neck samples, comprising 14 AAA samples from patients undergoing open AAA repair and eight normal samples from beating heart organ donors following brain mortality. The original data were pre-processed using the beadarray package in R language (version 2.18.0; http://bioconductor.org/packages/release/bioc/html/beadarray.html) (18), and normalized using the quantile method (19). Boxplots of the raw and normalized data were produced.

Screening of DEGs. Multi-dimensional scaling (MDS), which was constructed with the poltMDS (20) function in the linear models for microarray data (Limma) (21) package (version 3.24.15; http://www.bioconductor.org/packages/release/bioc/html/limma.html) was used to investigate the association of the samples as a measure of quality control. From the results of the MDS procedure, the AAA samples were separated into types A and B. The DEGs in the AAA sample types A and B were identified using the Limma package and were compared with the controls. The common DEGs, which featured consistent changes in their expression levels, were selected as the targets for further analysis. The false discovery rate (FDR) was calculated for multiple testing correction using the Benjamini and Hochberg method (22). The threshold for the DEGs was set as the log fold change (FC)>1 and FDR≤0.01. Pearson's correlation coefficient was used to examine the associations between these DEGs (23).

Enrichment analysis of the DEGs. The probe sets, which featured differential expression between the controls and the AAA samples, were annotated to Ensembl gene identifiers (IDs) for ID mapping using the database for annotation, visualization and integrated discovery (DAVID) tool (version 6.7; http://david.abcc.Ncifcrf.gov/ (24,25). Gene ontology (GO; http: //www. geneontology. org/) (26) and Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/pathway.html) analyses were performed on the selected lists of genes. The threshold was set as $P \leq 0.05$.

Constructing an interaction network and functional analysis. Following ID mapping, all selected genes were exported into Cytoscape plugin (27) using the BisoGenet module (28) to create network visualizations. The source of the interaction network database was the Biomolecular Interaction Network Database (BIND) (29). Subsequently, a cluster analysis on the resulting network was performed with the Plugin, ClusterONE (http://apps.cytoscape.org/apps/clusterone) (30) program, using a $P<0.05$ as a cut-off. The significant GO categories of the DEGs in the subnetworks were analyzed using the DAVID tool.

Results

Data pre-processing. The raw data downloaded from the GEO databases were normalized (Fig. 1A). The median values of each sample were almost at the identical level, suggesting that the data were eligible for further analysis. An MDS plot was constructed as a means of visualizing the data. This was performed for all the probes available. The AAA samples were observed to cluster into two groups, types A and B (Fig. 1B).

Screening of the DEGs. Comparing the AAA type A samples with the donor controls yielded the identification of 1,584 DEGs (Fig. 1C). Comparing the AAA type B samples with the donor controls yielded 984 DEGs in total (Fig. 1D). A total of 340 genes were identified as being DEGs common to each group, as revealed by the Venn diagram (Fig. 2A), and therefore, 338 DEGs exhibited a consistent change. An assessment of the correlation of the 338 DEGs revealed a positive pattern of correlation, with a Pearson's correlation coefficient (r) of 0.974 ($P<2.2^{-16}$). The top three upregulated and downregulated DEGs, with the highest logFC, are listed in Table I. Subsequently, the expression pattern of the DEGs was determined. From the heat map shown in Fig. 2, it was observed that the expression pattern of the DEGs enabled the AAA samples to be distinguished from the donor control samples. Furthermore, the AAA sample types A and B were consistent.

GO and KEGG enrichment analyses. The results of the GO and KEGG enrichment analyses of the common DEGs using the DAVID tool are presented in Fig. 3. A total of 18 significantly enriched categories were identified, which comprised 15 biological processes, two molecular functions and one KEGG pathway, grouped and annotated manually. The DEGs were predominantly associated with limb development, including embryonic limb morphogenesis and appendage development. The tyrosine kinase signaling pathway was also significantly enriched.

Table I. Differentially expressed genes in Type A and Type B.

Probe ID	Expression change	Gene symbol	Type A		Type B	
			logFC	adj.P.Val	logFC	adj.P.Val
ILMN_1899549	Upregulated	NA	3.369751	0.008994	3.112059	0.001856
ILMN_1717168	Upregulated	PCDHGA4	2.753187	$1.33e^{-10}$	2.225494	$5.06e^{-11}$
ILMN_2177965	Upregulated	RPS19BP1	2.678406	$4.24e^{-8}$	2.365836	$6.39e^{-9}$
ILMN_2231051	Downregulated	TCP11L2	-3.164650	$6.34e^{-8}$	-1.003280	0.001986
ILMN_1787591	Downregulated	xpa	-3.391970	$8.9e^{-8}$	-1.162970	0.001270
ILMN_1717733	Downregulated	NA	-4.015280	$3.99e^{-11}$	-2.217670	$5.8e^{-9}$

Positive values for logFC represent upregulated and negative values represent downregulated genes, ID, identifier; FC, fold change, adj.P.Val, adjusted P-value.

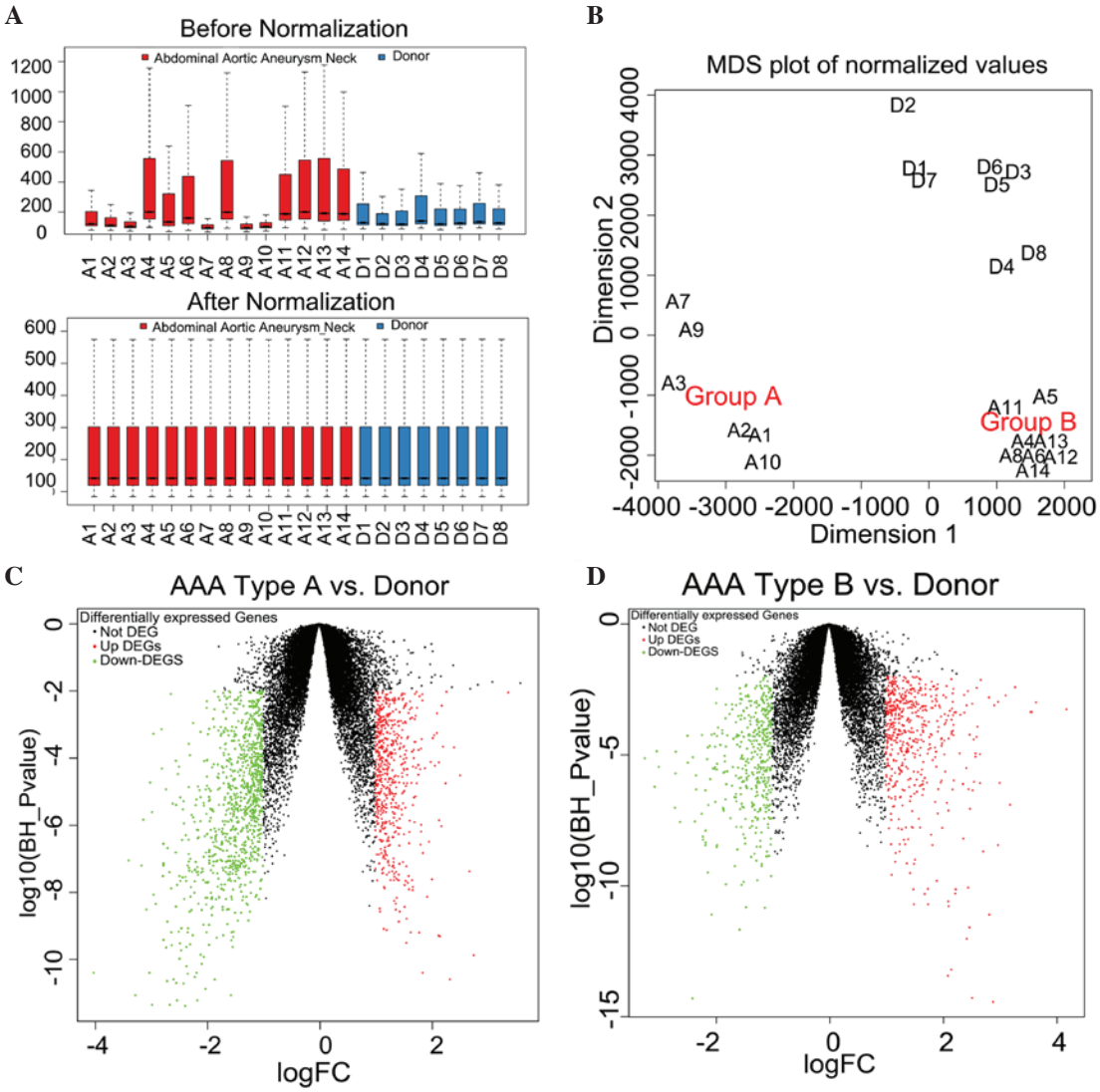

Figure 1. Data preprocessing and screening of the DEGs (A) The normalized expressed value data are shown. The black line featured in each of the colored boxes represents the median of each set of data, which determines the degree of standardization of the data through its distribution. Following normalization, the black lines in the boxes are almost in a straight line, indicating a good degree of standardization. (B) An MDS plot of the summarized microarray data following normalization. The array weights, calculated with the design matrix, reflect the association between the samples. Volcano plots are shown of the $\log_{10}$false discovery rate against the logFC for each gene of (C) AAA type A, vs. control and (D) AAA type B, vs. control. The FC and the statistical significance were plotted on the x- and y-axes, respectively. The genes, which are statistically significantly upregulated, are shown in red and those, which are statistically significantly downregulated, are shown in green. MDS, multidimensional scaling; FC, fold change; DEG, differentially expressed gene; AAA, abdominal aortic aneurysm.

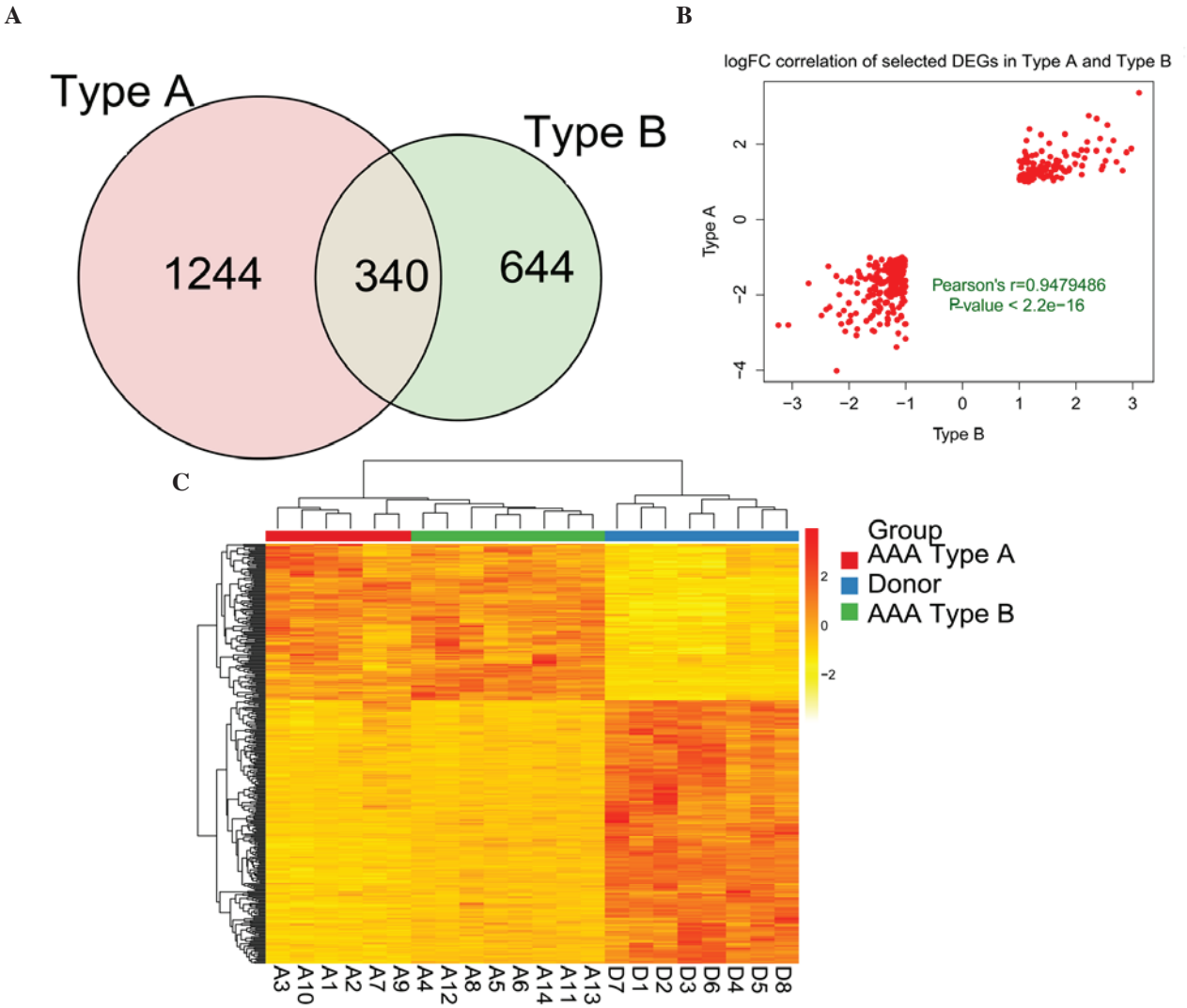

Figure 2. Identification of the DEGs as the analysis target. (A) A Venn diagram demonstrating a comparison of the AAA type A and type B samples, vs. the control. Overlapping probes are indicated as DEGs in types A and B. (B) The correlation of the selected probe sets with a correlation coefficient of r=0.974 and $P<2.2^{-16}$. (C) Heat maps of the genes, which are significantly differentially expressed in types A and B. DEG, differentially expressed gene; AAA, abdominal aortic aneurysm.

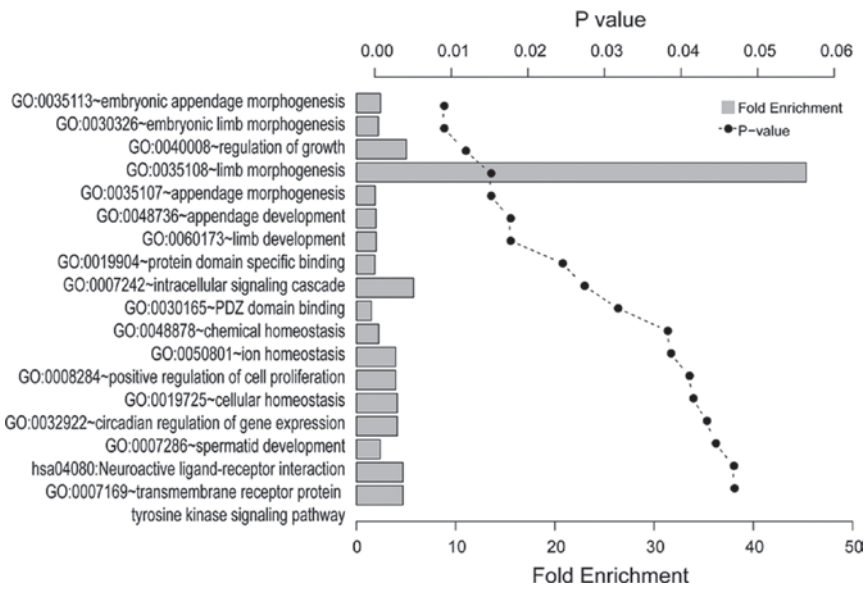

Figure 3. Enriched GO terms of the differentially expressed genes. A total of 15 biological processes, two molecular functions and a Kyoto Encyclopedia of Genes and Genomes pathway were enriched. GO, gene ontology.

Table II. Noteworthy clusters in the network.

Cluster	GO ID	Nodes	Density	Quality	P-value	Description	Genes in cluster
1	0043565	6	0.733	0.917	0.003	Sequence-specific DNA binding	BACH2, JUN, MAKF, MAFG, ATF7, MAFB
2	0045665	4	0.667	1	0.011	Negative regulation of neuron differentiation	PBX1, PAX6, IPO13, HXB
3	0031143	4	0.667	1	0.011	Pseudopodium	ACTN2, MYOZ2, MYOZ1, LDB3
4	0007242	3	0.667	1	0.026	Intracellular signaling cascade	NDKB, ITGB1BP1, KRIT1
5	0030117	3	0.667	1	0.026	Membrane coat	AP3D1, AP3S2, AP3S1
6	0001759	3	0.667	1	0.026	Induction of an organ	FGR1, FGF1, FGF2

GO, gene ontology; ID, identifier.

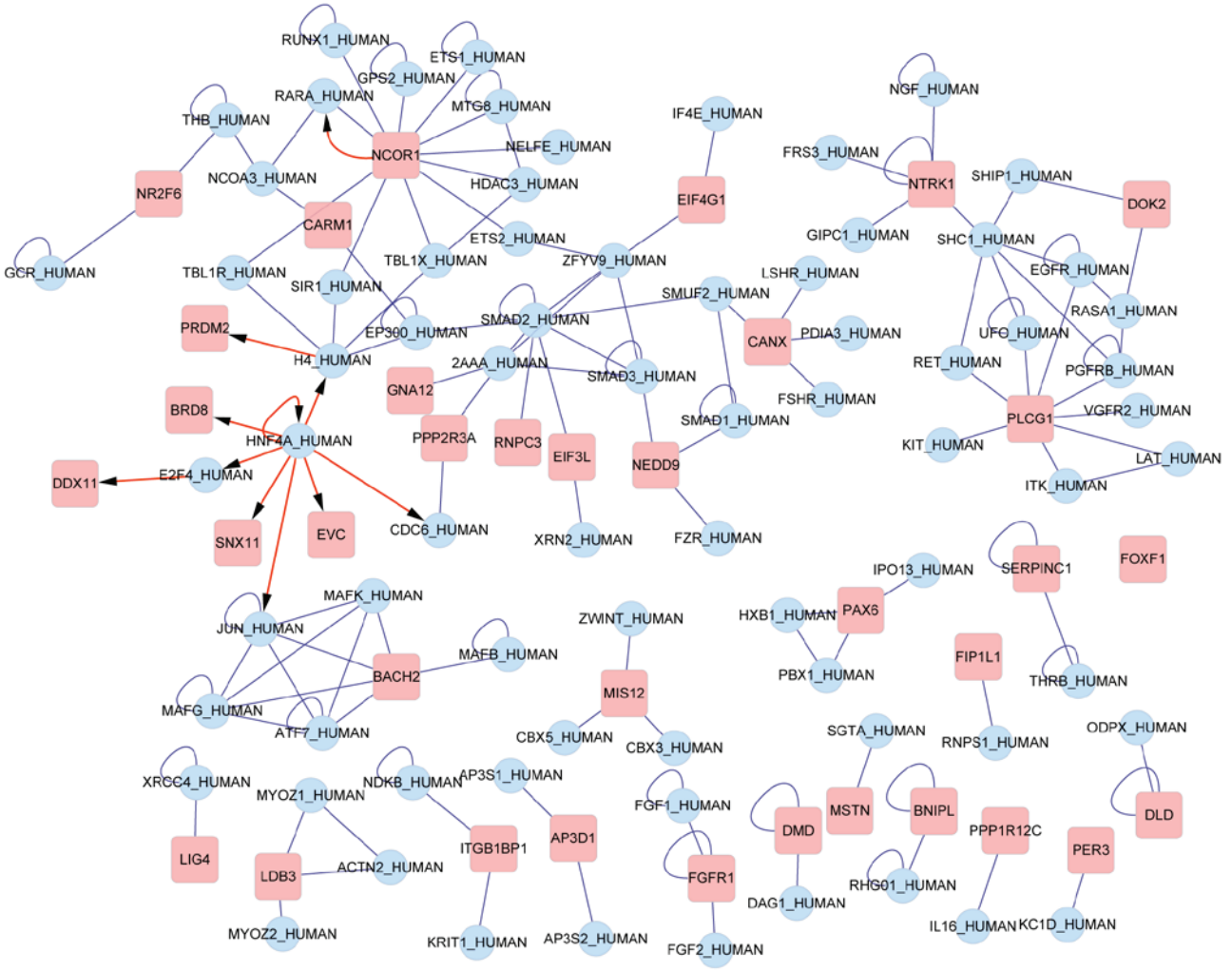

Figure 4. Interaction network of the DEGs. The nodes shown in pink represent the DEGs and those in blue represent the proteins interacting with the DEGs. The red arrows indicate interactions between DNA and protein, and the blue lines denote interactions between proteins. DEG, differentially expressed gene.

Interaction network and functional analysis. The 338 probe sets, which were retained, were mapped, in total, to 297 gene symbols. Interactions between the genes were searched for in BIND and the results are presented in Fig. 4. The transcriptional factors nuclear receptor corepressor 1 (NCOR1), histone 4 (H4), E2F transcription factor 4 (E2F4) and hepatocyte nuclear factor 4α (HNF4A) were identified in the network to regulate other DEGs. Six noteworthy clusters emerged from the clustering analysis (Table II) and the subnetwork diagram is presented in Fig. 5.

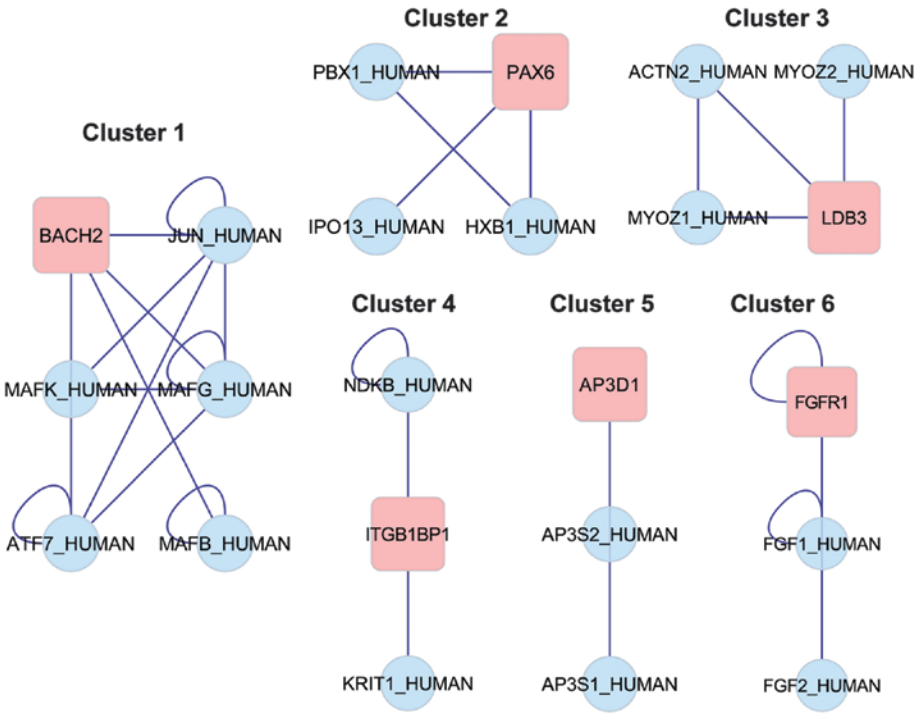

Figure 5. Schematic of six highly connected clusters extracted from the protein-protein interaction networks.

Discussion

AAA is a complex disease, of which the pathobiology remains to be fully elucidated (2). Previous studies have revealed that the disease has a marked genetic component (12,14,31). Assessing gene expression profiling in cases of disease may reveal the underlying changes in gene activity, which contribute to the disease and enable targets for therapeutic intervention to be identified. In the present study, the gene expression profile downloaded from the GEO database was used to examine the mechanism of AAA development. A total of 340 genes were identified to be commonly DEGs in AAA samples of type A compared with donor controls and AAA samples of type B, among which 388 genes exhibited consistent changes. The analysis of the DEGs indicated that they were predominantly involved in limb development. The PPI network analysis revealed that four transcription factors regulated these DEGs in the network, and that NCOR1, H4 and E2F4 were clustered across the network in association with HNF4A. Furthermore, c-jun proto-oncogene (JUN) and its downstream components, which are regulated upstream in the pathway by HNF4A, were enriched in cluster 1.

NCOR1, which mediates transcriptional repression by certain nuclear receptors, is a part of a complex, which promotes histone deacetylation and the formation of repressive chromatin structures, and which may impede the access of basal transcription factors (32). MMPs exert a significant role in the degradation of the extracellular matrix of the vessel wall, which gradually leads to the formation of AAA (33). Mannello *et al* (34) demonstrated that MMP-3 may degrade NCOR1 to prevent transcriptional repression of the connective tissue growth factor promoter. In line with previous studies, NCOR1 may exert a critical role in the development of AAA, based on the PPI network analysis.

H4 is a core histone of the nucleosome, which functions in nucleosomal wrapping and occupies a central role in transcriptional regulation, DNA repair, DNA replication and chromosomal stability (35). Santos-Rosa *et al* (36) reported that the histone code set up by post-translational histone modifications also respond to DNA damage, which is frequently observed in cancer cells. HNF4A is a transcriptionally controlled transcription factor, and it may be essential for the development of the kidney, liver and intestine (37,38). A previous study revealed that phospholipase A_2 g10-deficent mice were protected from angiotensin-II-induced aortic aneurysms (39). Guan *et al* (40) demonstrated that the transcription levels of *Pla2g12b* were regulated by the transcription factor HNF4A and its co-activator. Furthermore, Soutoglou *et al* (41) reported that HNF4A may interact with histone acetyltransferases, a process which is dependent on the acetylation status of the histones. In this context, and based on the results of the present study, it was postulated that there may be an interaction between the expression of H4 and HNF4A in the development of AAA.

E2F4 is a transcriptional activator, which binds DNA co-operatively with differentially regulated transcription factor proteins, whose products are involved in DNA replication and in cell cycle regulation (42). The transcription factor DRTF1/E2F complex functions in the control of cell-cycle progression from G1 to S phase (43). Tung *et al* (44) identified genes, which are involved in cell cycle regulation in AAA, using a membrane-based complementary DNA expression array. Talianidis *et al* (45) demonstrated that complex interactions between HNF4 bound to the proximal promoter and SP1 bound to multiple distal regulatory sites may lead to transcriptional activation of liver-specific human apolipoprotein CIII gene. SP1 is a cellular transcription factor involved in a wide variety of processes, and it has been determined

to bind to different promoters to regulate transcription (46). In addition, Price *et al* (47) determined that differences in allelic expression of the MMP-2 gene, which exerts a major role in AAA formation, were attributed to the elimination of SP1 binding. Furthermore, the expression levels of HNF4A as a hepatocyte marker for hepatic differentiation were increased in the early G1 phase in human embryonic stem cells during their cell cycle (48). Therefore, an important interaction may occur between HNF4A and E2F4 in the pathogenesis of AAA.

A previous study revealed that HNF4A may be a key upstream mediator of JUN, whereas JUN was intimately associated with BTB and CNC homology 1, basic leucine zipper transcription factor 2 (BACH2), which is involved in the lymphocyte signaling pathway in cluster 1 (49). Henderson *et al* (50) demonstrated the death of smooth muscle cells and the expression of mediators of apoptosis by T lymphocytes in human AAA. A previous study revealed that the inhibition of c-Jun N-terminal kinase caused regression of AAA. In this context, it is surmised that JUN may interact with BACH2 in the progression of AAA.

In conclusion, genes which are critical in the development of AAA have been assessed based on the microarray data. HNF4A exerts an important role in the development of AAA through the interactions made with three other transcription factors (E2F4, NCOR1 and H4), and the coordinated regulation of these transcription factors may represent potential novel targets for the mechanism underlying the development of AAA. The present study offers novel insights into the pathobiology of AAA. Further studies are required to confirm these intriguing results in terms of the possible associations with the transcriptional factors.

References

1. Golledge J, Muller J, Daugherty A and Norman P: Abdominal aortic aneurysm: Pathogenesis and implications for management. Arterioscler Thromb Vasc Biol 26: 2605-2613, 2006.
2. Maegdefessel L, Dalman RL and Tsao PS: Pathogenesis of abdominal aortic aneurysms: MicroRNAs, proteases, genetic associations. Annu Rev Med 65: 49-62, 2014.
3. Verhoeven EL, Kapma MR, Groen H, Tielliu IF, Zeebregts CJ, Bekkema F and van den Dungen JJ: Mortality of ruptured abdominal aortic aneurysm treated with open or endovascular repair. J Vasc Surg 48: 1396-1400, 2008.
4. Golledge J and Norman PE: Current status of medical management for abdominal aortic aneurysm. Atherosclerosis 217: 57-63, 2011.
5. Sakalihasan N, Limet R and Defawe O: Abdominal aortic aneurysm. The Lancet 365: 1577-1589, 2005.
6. Michel JB, Martin-Ventura JL, Egido J, Sakalihasan N, Treska V, Lindholt J, Allaire E, Thorsteinsdottir U, Cockerill G and Swedenborg J; FAD EU Consortium: Novel aspects of the pathogenesis of aneurysms of the abdominal aorta in humans. Cardiovasc Res 90: 18-27, 2011.
7. Thomas M, Gavrila D, McCormick ML, Miller FJ Jr, Daugherty A, Cassis LA, Dellsperger KC and Weintraub NL: Deletion of p47phox attenuates angiotensin II-induced abdominal aortic aneurysm formation in apolipoprotein E-deficient mice. Circulation 114: 404-413, 2006.
8. Sharma AK, Lu G, Jester A, Johnston WF, Zhao Y, Hajzus VA, Saadatzadeh MR, Su G, Bhamidipati CM, Mehta GS, *et al*: Experimental abdominal aortic aneurysm formation is mediated by IL-17 and attenuated by mesenchymal stem cell treatment. Circulation 126 (Suppl 1): S38-S45, 2012.
9. Harrison SC, Smith AJ, Jones GT, Swerdlow DI, Rampuri R, Bown MJ, Aneurysm Consortium, Folkersen L, Baas AF, de Borst GJ, *et al*: Interleukin-6 receptor pathways in abdominal aortic aneurysm. Eur Heart J 34: 3707-3716, 2013.
10. Kunieda T, Minamino T, Nishi J, Tateno K, Oyama T, Katsuno T, Miyauchi H, Orimo M, Okada S, Takamura M, *et al*: Angiotensin II induces premature senescence of vascular smooth muscle cells and accelerates the development of atherosclerosis via a p21-dependent pathway. Circulation 114: 953-960, 2006.
11. Manning MW, Cassis LA and Daugherty A: Differential effects of doxycycline, a broad-spectrum matrix metalloproteinase inhibitor, on angiotensin II-induced atherosclerosis and abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol 23: 483-488, 2003.
12. Hinterseher I, Tromp G and Kuivaniemi H: Genes and abdominal aortic aneurysm. Ann Vasc Surg 25: 388-412, 2011.
13. Hinterseher I, Erdman R, Elmore JR, Stahl E, Pahl MC, Derr K, Golden A, Lillvis JH, Cindric MC, Jackson K, *et al*: Novel pathways in the pathobiology of human abdominal aortic aneurysms. Pathobiology 80: 1-10, 2013.
14. Golledge J and Kuivaniemi H: Genetics of abdominal aortic aneurysm. Curr Opin Cardiol 28: 290-296, 2013.
15. Duftner C, Seiler R, Dejaco C, Fraedrich G and Schirmer M: Increasing evidence for immune-mediated processes and new therapeutic approaches in abdominal aortic aneurysms-a review. Ann N Y Acad Sci 1085: 331-338, 2006.
16. Kuivaniemi H, Platsoucas CD and Tilson MD III: Aortic aneurysms: An immune disease with a strong genetic component. Circulation 117: 242-252, 2008.
17. Biros E, Moran CS, Rush CM, Gäbel G, Schreurs C, Lindeman JH, Walker PJ, Nataatmadja M, West M, Holdt LM, *et al*: Differential gene expression in the proximal neck of human abdominal aortic aneurysm. Atherosclerosis 233: 211-218, 2014.
18. Dunning MJ, Smith ML, Ritchie ME and Tavaré S: Beadarray: R classes and methods for Illumina bead-based data. Bioinformatics 23: 2183-2184, 2007.
19. Bolstad BM: Probe level quantile normalization of high density oligonucleotide array data. Unpublished manuscript, 2001.
20. Choi J: Guide: A desktop application for analysing gene expression data. BMC Genomics 14: 688, 2013.
21. Smyth GK: Limma: Linear models for microarray data. In: Bioinformatics and computational biology solutions using R and Bioconductor. Springer, New York, 397-420, 2005.
22. Benjamini Y and Hochberg Y: Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B Stat Methodol 57: 289-300, 1995.
23. Makova KD and Li WH: Divergence in the spatial pattern of gene expression between human duplicate genes. Genome Res 7: 1638-1645, 2003.
24. Huang da W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4: 44-57, 2009.
25. Huang da W, Sherman BT and Lempicki RA: Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37: 1-13, 2009.
26. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, *et al*: Gene ontology: Tool for the unification of biology. The gene ontology consortium. Nat Genet 25: 25-29, 2000.
27. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res 13: 2498-2504, 2003.
28. Martin A, Ochagavia ME, Rabasa LC, Miranda J, Fernandez-de-Cossio J and Bringas R: BisoGenet: A new tool for gene network building, visualization and analysis. BMC Bioinformatics 11: 91, 2010.
29. Bader GD, Betel D and Hogue CW: BIND: The biomolecular interaction network database. Nucleic Acids Res 31: 248-250, 2003.
30. Nepusz T, Yu H and Paccanaro A: Detecting overlapping protein complexes in protein-protein interaction networks. Nat Methods 9: 471-472, 2012.
31. Estrelinha M, Hinterseher I and Kuivaniemi H: Gene expression studies in human abdominal aortic aneurysm. Rev Vasc Med 2: 77-82, 2014.
32. Yoon HG, Chan DW, Reynolds AB, Qin J and Wong J: N-CoR mediates DNA methylation-dependent repression through a methyl CpG binding protein Kaiso. Mol Cell 12: 723-734, 2003.
33. Saratzis A, Abbas AA, Kiskinis D, Melas N, Saratzis N and Kitas GD: Abdominal aortic aneurysm: A review of the genetic basis. Angiology 62: 18-32, 2011.
34. Mannello F and Medda V: Nuclear localization of matrix metalloproteinases. Prog Histochem Cytochem 47: 27-58, 2012.

35. Turner BM, Birley AJ and Lavender J: Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. Cell 69: 375-384, 1992.
36. Santos-Rosa H and Caldas C: Chromatin modifier enzymes, the histone code and cancer. Eur J Cancer 41: 2381-2402, 2005.
37. Kanazawa T, Konno A, Hashimoto Y and Kon Y: Hepatocyte nuclear factor 4 alpha is associated with survival of the condensed mesenchyme in the developing mouse kidney. Dev Dyn 239: 1145-1154, 2010.
38. Sandovici I, Smith NH, Nitert MD, Ackers-Johnson M, Uribe-Lewis S, Ito Y, Jones RH, Marquez VE, Cairns W, Tadayyon M, *et al*: Maternal diet and aging alter the epigenetic control of a promoter-enhancer interaction at the Hnf4a gene in rat pancreatic islets. Proc Natl Acad Sci USA 108: 5449-5454, 2011.
39. Zack M, Boyanovsky BB, Shridas P, Bailey W, Forrest K, Howatt DA, Gelb MH, de Beer FC, Daugherty A and Webb NR: Group X secretory phospholipase A(2) augments angiotensin II-induced inflammatory responses and abdominal aortic aneurysm formation in apoE-deficient mice. Atherosclerosis 214: 58-64, 2011.
40. Guan M, Qu L, Tan W, Chen L and Wong CW: Hepatocyte nuclear factor-4 alpha regulates liver triglyceride metabolism in part through secreted phospholipase A2 GXIIB. Hepatology 53: 458-466, 2011.
41. Soutoglou E, Katrakili N and Talianidis I: Acetylation regulates transcription factor activity at multiple levels. Mol Cell 5: 745-751, 2000.
42. Beijersbergen RL, Kerkhoven RM, Zhu L, Carlée L, Voorhoeve PM and Bernards R: E2F-4, a new member of the E2F gene family, has oncogenic activity and associates with p107 *in vivo*. Genes Dev 8: 2680-2690, 1994.
43. Zheng N, Fraenkel E, Pabo CO and Pavletich NP: Structural basis of DNA recognition by the heterodimeric cell cycle transcription factor E2F-DP. Genes Dev 13: 666-674, 1999.
44. Tung WS, Lee JK and Thompson RW: Simultaneous analysis of 1176 gene products in normal human aorta and abdominal aortic aneurysms using a membrane-based complementary DNA expression array. J Vasc Surg 34: 143-150, 2001.
45. Talianidis I, Tambakaki A, Toursounova J and Zannis VI: Complex interactions between SP1 bound to multiple distal regulatory sites and HNF-4 bound to the proximal promoter lead to transcriptional activation of liver-specific human APOCIII gene. Biochemistry 34: 10298-10309, 1995.
46. Cook T, Gebelein B and Urrutia R: Sp1 and its likes: Biochemical and functional predictions for a growing family of zinc finger transcription factors. Ann N Y Acad Sci 880: 94-102, 1999.
47. Price SJ, Greaves DR and Watkins H: Identification of novel, functional genetic variants in the human matrix metalloproteinase-2 gene: Role of Sp1 in allele-specific transcriptional regulation. J Biol Chem 276: 7549-7558, 2001.
48. Pauklin S and Vallier L: The cell-cycle state of stem cells determines cell fate propensity. Cell 155: 135-147, 2013.
49. Cooper MD and Alder MN: The evolution of adaptive immune systems. Cell 124: 815-822, 2006.
50. Henderson EL, Geng YJ, Sukhova GK, Whittemore AD, Knox J and Libby P: Death of smooth muscle cells and expression of mediators of apoptosis by T lymphocytes in human abdominal aortic aneurysms. Circulation 99: 96-104, 1999.