Abstract. Abdominal aortic aneurysm (AAA) is one of the most significant causes of morbidity and mortality in populations aged >65 years worldwide. However, the underlying mechanisms of AAA based on the competitive endogenous RNA (ceRNA) hypothesis have remained elusive. In the present study, differently expressed long non-coding RNA (lncRNA)-microRNA (miRNA)-mRNA networks in AAA were constructed by analyzing public datasets, including GSE7084, GSE24194 from rats and that of a previous study. A total of 1,219 mRNAs, 2,093 lncRNAs and 57 miRNAs were identified to differently express in AAA. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses were performed to explore the potential roles of differently expressed lncRNAs based on their regulating mRNAs. Based on the ceRNA hypothesis, lncRNA-miRNA-mRNA networks in AAA were, for the first time, constructed at a system-wide level. The present study identified 5 upregulated lncRNAs [nuclear paraspeckle assembly transcript 1, cyclin-dependent kinase inhibitor 2B antisense RNA 1, small Cajal body-specific RNA 10, AC005224.4 and SUMO1/sentrin/SMT3-specific peptidase 3-eukaryotic translation initiation factor 4A1] and the downregulated zinc ribbon domain containing 1 antisense RNA 1 as key lncRNAs in ceRNA networks. To the best of our knowledge, the present study was the first to screen ceRNA networks in AAA. In addition, key lncRNA-mRNA-biological processes analysis indicated that these key lncRNAs were involved in regulating signal transduction, protein amino acid phosphorylation, immune response, transcription, development and cell differentiation. The present study provides novel clues to explore the molecular mechanisms of AAA progression in terms of lncRNA implication.

Introduction

Abdominal aortic aneurysm (AAA) is defined as a maximum infrarenal abdominal aortic aneurysm with a diameter of ≥3.0 cm and is characterized by permanent, localized dilations of the abdominal aorta (1,2). AAA is one of the most significant cause of morbidity and mortality in populations aged >65 years worldwide (3,4). Acute rupture is the most dangerous clinical consequence of AAA progression and causes ~80% of associated deaths in the US (5). Pathologic features of AAA include vascular smooth muscle cell apoptosis, infiltration of inflammatory cells, loss of integrity of the arterial wall, increase of oxidative stress and significant matrix degradation (6). However, the precise molecular mechanisms underlying the progression of AAA progression still remain elusive. Therefore, it is important to elucidate the etiological mechanisms of AAA progression to develop novel targets for the diagnosis, treatment, and prognostication of AAA patients.

Long non-coding (lnc)RNAs are a class of non-coding RNAs of >200 nucleotides in length, which have little or no protein-coding function (7). It has been discovered that lncRNAs have important roles in the progression of numerous human diseases, including AAA (8). For instance, Yang et al (9) identified 3,688 differentially expressed lncRNAs between AAA and normal tissues. lncRNAs regulate the protein expression at epigenetic, transcriptional and post-translational levels (10-13). One of the most well-known mechanisms of lncRNAs is their action as competing endogenous RNA (ceRNA) (14). The ceRNA hypothesis, which was proposed by Tay et al (15), holds that pseudogenes, lncRNAs, circular RNAs and mRNAs may impair micro (mi)RNA activity through sequestration, thereby upregulating miRNA target gene expression. Franco-Zorrilla et al (16) reported for the first time that non-coding RNA interferon-β promoter stimulator 1 promoted phosphate metabolism (PHO2) protein in plants by sequestering miR-399 and preventing it from inhibiting the stability and translation of PHO2 mRNA. Poliseno et al (17) also reported that certain protein-coding genes and their pseudogenes contain the same evolutionarily conserved miRNA binding sites in their 3’-untranslated

Correspondence to: Professor Hongkun Zhang, Department of Vascular Surgery, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou, Zhejiang 310003, P.R. China
E-mail: 1198050@zju.edu.cn

Key words: long non-coding RNA, abdominal aortic aneurysm, competitive endogenous RNA network, endogenous RNA, biomarkers
regions, and that they regulate their respective expression levels by competing for miRNA binding. Emerging studies have indicated that ceRNAs act as important regulators in different types of disease, including cancer, cardiac fibrosis, rheumatoid arthritis and type 2 diabetes mellitus (18-20). Thus, constructing ceRNA networks provides a novel perspective to explore the function of yet uncharacterized lncRNAs involved in AAA progression.

In the present study, differentially expressed RNAs, miRNA and mRNAs in AAA were identified from data provided by the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO), and lncRNA-miRNA-mRNA networks were constructed. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were also performed to explore the potential roles of differentially expressed lncRNAs in AAA. The present study aimed to provide useful information to identify novel lncRNAs as biomarkers for AAA.

Materials and methods

Microarray data. In the present study, two public datasets, GSE7084 (21) and that provided by Yang et al (9) were analyzed to identify differentially expressed mRNAs in AAA. The GSE7084 dataset was downloaded from the NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/). The dataset from Yang et al (9) was downloaded from the supplementary information of their publication. To further identify differentially expressed lncRNAs in AAA, the dataset by Yang et al (9) was analyzed, providing 896 upregulated and 1,197 downregulated lncRNAs. Differently expressed miRNAs were determined from the GSE24194 dataset from rats (22), which was downloaded from the NCBI GEO website. A t-test (23) in the Limma package (24) in R (25) was used to identify differentially expressed genes between normal and AAA samples. The threshold for the differentially expressed genes (DEGs) was set as a corrected P-value of <0.05 and |log2 fold-change (FC)|≥1.

GO and KEGG pathway analysis. To identify functions of DEGs in AAA, GO term enrichment analysis in the categories biological process, cellular component and molecular function was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID; https://david.ncifcrf.gov/). KEGG pathway enrichment analysis was also performed to identify pathways enriched by DEGs in AAA using DAVID. The P-value was calculated by hypergeometric distribution and a pathway with P<0.05 was considered as significant.

Construction of lncRNA-miRNA-mRNA network. The StarBase dataset was used to identify potentially dysregulated lncRNA-miRNA pairs. Next, miRcode (26), StarBase (27) and the TargetsScan database (http://www.targetscan.org) were used to identify miRNA-mRNA pairs. Finally, lncRNA-miRNA-mRNA networks were constructed. In the present study, only downregulated miRNAs and upregulated mRNAs were integrated into the ceRNA network for upregulated lncRNAs, while only upregulated miRNAs and downregulated mRNAs were integrated into the ceRNA network for downregulated lncRNAs.

Results

Identification of differentially expressed lncRNAs, mRNAs and miRNAs in AAA. In the present study, two public datasets, GSE7084 (21) and that of Yang et al (9), were analyzed to identify differentially expressed miRNAs (Fig. 1A and B). miRNAs with a FC of ≥2 or ≤0.5 and P<0.05 were selected as differentially expressed miRNAs. A total of 2,292 upregulated and 2,501 downregulated miRNAs were identified from the GSE7084 dataset. In the dataset from Yang et al (9), 1,501 upregulated and 969 downregulated miRNAs were identified. In total, the two datasets had 722 upregulated and 497 downregulated mRNAs in common (Fig. 1C and D). To identify differentially expressed lncRNAs, the dataset from Yang et al (9) was re-analyzed, and 896 upregulated and 1,197 downregulated lncRNAs were obtained (Fig. 1E). Furthermore, miRNAs with a FC of ≥2 or ≤0.5 and P<0.05 were selected as differentially expressed miRNAs. A total of 21 upregulated miRNAs and 36 downregulated miRNAs were selected for further ceRNA network construction by analyzing the GSE24194 dataset obtained from rats (Fig. 1F). Hierarchical clustering provided systematic variations in the expression of mRNAs, miRNAs and lncRNAs in AAA.

Functional prediction of differentially expressed mRNAs in AAA. GO and KEGG pathway analyses were performed to explore the function of the 1,219 differentially expressed mRNAs using DAVID. The results indicated that the upregulated mRNAs were enriched in GO terms including immune response, signal transduction, inflammatory response, chemotaxis, cell adhesion, cell-cell signaling, protein amino acid phosphorylation, proteolyis, cellular defense response, regulation of transcription and apoptosis (Fig. 2A), while the downregulated genes were enriched in GO terms including regulation of transcription, oxidation/reduction, cell adhesion, signal transduction, ion transport, protein amino acid phosphorylation, development, nervous system development, muscle development and cell-matrix adhesion (Fig. 2B).

KEGG pathway analysis revealed that the upregulated mRNAs were mainly involved in cytokine-cytokine receptor interaction, hematopoietic cell lineages, the B-cell receptor signaling pathway, cell adhesion molecules, leukocyte transendothelial migration, the T-cell receptor signaling pathway, graft-versus-host disease, Toll-like receptor signaling pathway and the mitogen-activated protein kinase signaling pathway (Fig. 2C), while the downregulated genes were involved in regulation of the actin cytoskeleton, calcium signaling pathway, focal adhesion, neuroactive ligand-receptor interaction, tight junctions, tryptophan metabolism, and valine, leucine and isoleucine degradation (Fig. 2D).

Construction of lncRNA-miRNA-mRNA networks in AAA. To explore the functions of lncRNAs acting as miRNA sponges in AAA, two lncRNA-mediated ceRNA networks were first constructed by using bioinformatics analysis. First, the StarBase dataset was used to identify potentially dysregulated lncRNA-miRNA pairs. Next, miRcode (26), StarBase (27) and the TargetsScan database were used to identify miRNA-mRNA pairs. Finally, lncRNA-miRNA-mRNA networks were constructed. In the present study, only downregulated miRNAs
and upregulated mRNAs were integrated into the ceRNA network mediated by upregulated lncRNAs, while, only upregulated miRNAs and downregulated mRNAs were integrated into the ceRNA network mediated by downregulated lncRNAs.

As presented in Fig. 3, the upregulated lncRNA-miRNA-mRNA network contained 38 lncRNA nodes, 374 mRNA nodes, 27 miRNA nodes and 2,021 edges. In addition, the downregulated lncRNA-miRNA-mRNA network
Identification of key lncRNA-mRNA-biological processes sub-network. According to the ceRNA network analysis, all node degrees were calculated to identify hub lncRNAs, which have critical roles in biological networks. Five upregulated lncRNAs [nuclear paraspeckle assembly transcript 1 (NEAT1), cyclin-dependent kinase inhibitor 2B (CDKN2B)-antisense RNA 1 (AS1), small Cajal body-specific RNA 10 (SCARNA10), AC005224.4 and SUMO1/sentrin/SMT3-specific peptidase 3 (SENP3)-eukaryotic translation initiation factor 4A1 (EIF4A1)] and the downregulated zinc ribbon domain containing 1 (ZNRD1-AS1) were identified as key lncRNAs in the ceRNA networks and interacted with >5 different miRNAs (Fig. 4).

Furthermore, key lncRNA-mRNA-biological processes analysis indicated that NEAT1, CDKN2B-AS1, SCARNA10, AC005224.4 and SENP3-EIF4A1 were involved in regulating signal transduction, protein amino acid phosphorylation and immune response (Fig. 5A). ZNRD1-AS1 was associated with the regulation of transcription, development, and cell differentiation (Fig. 5B).

Discussion
AAA is one of the most significant causes of morbidity and mortality in populations aged >65 years worldwide (3,4). Previous studies reported that various proteins, including mitochondrial uncoupling protein-2, c-Jun N-terminal kinase and matrix metalloproteinase-9 and miRNAs, including miRNA-103a and miRNA-516a-5p, were associated with AAA progression (28). Of note, the underlying mechanisms of AAA have remained to be fully elucidated. lncRNAs are a class of non-coding RNAs with no protein-coding function (7). Several studies have focused on exploring the roles of lncRNAs in aortic aneurysm disease. Hypoxia-inducible factor 1α-AS1 was the first lncRNA reported to be involved in the pathogenesis of thoracic aortic aneurysm (8). Yang et al (9) identified 3,688 differentially expressed lncRNAs between
AAA and normal tissues. However, the molecular functions and detailed mechanisms of lncRNAs in AAA remain largely elusive. In the present study, DEGs in AAA were explored by analyzing a series of public datasets, including GSE7084, the dataset from Yang et al. (9) and GSE24194 obtained from rats. A total of 1,219 mRNAs, 2,093 lncRNAs and 57 miRNAs were identified to differently express in AAA.

The ceRNA hypothesis was first proposed by Salmena et al. (18) in 2011. This hypothesis holds that lncRNAs act as miRNA ‘sponges’ to promote the expression of target genes of miRNAs, which provided a novel basis for exploring the molecular functions of lncRNAs. Emerging studies have reported that ceRNAs act as important regulators in different types of disease, including cancer, cardiac fibrosis and rheumatoid arthritis (20). For instance, Karreth (29) reported that the B-Raf pseudogene functions as a competitive endogenous RNA and induces lymphoma in vivo. In the present study, deregulated lncRNA-miRNA-mRNA networks in AAA were constructed. The results indicated that several key lncRNAs served important roles in regulating AAA progression by regulating a series of miRNAs and mRNAs. To the best of our knowledge, the present study was the first to screen ceRNA networks in AAA.

To date, only few studies have focused on exploring the roles of lncRNAs in AAA. In the present study, hub lncRNAs with critical roles in biological networks were identified. The upregulated lncRNAs NEAT1, CDKN2B-AS1, SCARNA10, AC005224.4 and SENP3-EIF4A1, and the downregulated ZNRD1-AS1 were identified as key lncRNAs in the ceRNA networks and interacted with >5 different miRNAs. Furthermore, key lncRNA-mRNA-biological processes analysis revealed that NEAT1, CDKN2B-AS1, SCARNA10,
AC005224.4 and SENP3-EIF4A1 were involved in regulating signal transduction, protein amino acid phosphorylation and immune response, while ZNRD1-AS1 was associated with regulation of transcription, development and cell differentiation. Of these key lncRNAs, NEAT1 was reported to be overexpressed in different types of solid tumor, including lung cancer, oesophageal cancer, colorectal cancer and hepatocellular carcinoma (30-33). CDKN2B-AS1 was reported to interact with protein regulator of cytokinesis 1 and -2, leading to epigenetic silencing of CDKN2B (34). CDKN2B-AS1 has key roles in the progression of several cancer types (35-37), intracranial aneurysm (38), type-2 diabetes (39,40), periodontitis (41,42), Alzheimer’s disease (43,44), endometriosis (45), frailty in the elderly (46) and glaucoma (36,47).

Several limitations of the present study should be noted. First, further validation of key lncRNA expression levels in AAA samples is required. Although the datasets used in the present study have been reported and validated, further study to confirm the differences in lncRNA expression between AAA and normal samples is required. Another limitation is that the public datasets for analyzing AAA-associated lncRNAs were limited. An urgent requirement remains to identify differently expressed lncRNAs in AAA by using high-throughput methods, including lncRNA expression array and RNA sequencing. Finally, additional functional investigations of these lncRNAs on AAA progression are still required.

Based on the ceRNA hypothesis, the present study was the first to construct lncRNA-miRNA-mRNA networks in AAA, to the best of our knowledge. In the present study, a total of 1,219 mRNAs, 2,093 lncRNAs and 57 miRNAs were identified to be differently expressed in AAA based on analyzing public datasets including GSE7084, the dataset by Yang et al (9) and GSE24194. The present study further identified 5 upregulated lncRNAs (NEAT1, CDKN2B-AS1, SCARNA10, AC005224.4 and SENP3-EIF4A1) and the downregulated ZNRD1-AS1 as key lncRNAs in the ceRNA networks. Key lncRNA-mRNA-biological processes analysis indicated that these key lncRNAs are involved in regulating signal transduction, protein amino acid phosphorylation, immune response, transcription, development and cell differentiation. The present study provides novel clues for exploring the mechanistic involvement of lncRNA in AAA progression.
Figure 5. Identification of key lncRNA-mRNA-biological processes subnetwork. (A) Key upregulated lncRNA-mRNA-biological processes analysis indicated that NEAT1, CDKN2B-AS1, SCARNA10, AC005224.4 and SENP3-EIF4A1 are involved in regulating signal transduction, protein amino acid phosphorylation and immune response. (B) The downregulated gene ZNRD1-AS1 was associated with regulation of transcription, development, and cell differentiation. lncRNA, long non-coding RNA; NEAT1, nuclear paraspeckle assembly transcript 1; CDKN2B, cyclin-dependent kinase inhibitor 2B; AS1, antisense RNA 1; SCARNA10, small Cajal body-specific RNA 10; SENP3, SUMO1/sentrin/SMT3-specific peptidase 3; EIF4A1, eukaryotic translation initiation factor 4A1; ZNRD1-AS1, zinc ribbon domain containing 1.
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Availability of data and materials

High-resolution versions of Figs. 3 and 4 are available on request. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

HZ, LT and DL contributed to the conception and design of the study. LT and XH developed the methodology. YH collected the samples. ZW analyzed and interpreted the data. LT, DL and HZ wrote, reviewed and revised the manuscript. The final version of the manuscript has been read and approved by all authors.

Ethical approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Alcorn HG, Wolfson SK, Jr, Sutton-Tyrrell K, Kuller LH and O’Leary D: Risk factors for abdominal aortic aneurysms in older adults enrolled in the cardiovascular health study. Arterioscler Thromb Vasc Biol 16: 963-970, 1996.
2. Humphrey JD and Taylor CA: Intracranial and abdominal aortic aneurysms: Similarities, differences, and need for a new class of computational models. Annu Rev Biomed Eng 10: 221-246, 2008.
3. Hallett JW, Jr, Marshall DM, Pettersson TM, Gray DT, Bower TC, Cherry KJ, Jr, Ghvxiczi P and Paierlere PC: Graft-related complications after abdominal aortic aneurysm repair: Reassessment from a 36-year population-based experience. J Vasc Surg 25: 277-284; discussion 285-276, 1997.
4. Johansson G and Swedenborg J: Ruptured abdominal aortic aneurysms: A study of incidence and mortality. Br J Surg 73: 101-103, 1986.
5. Maegleflessel L, Azuma J, Toh R, Deng A, Merk DR, Raesidana A, Leeper NJ, Raaz U, Scholemierich AM, McConnell MV, et al: MicroRNA-21 blocks abdominal aortic aneurysm development and nicotine-augmented expansion. Sci Transl Med 4: 122ra122, 2012.
6. Miyake T and Morishita R: Pharmacological treatment of abdominal aortic aneurysm. Cardiovasc Res 83: 436-443, 2009.
7. Huarte M: The emerging role of IncRNAs in cancer. Nat Med 21: 1253-1261, 2015.
8. Duggirala A, Delougou F, Angelini TG, Smith T, Caputo M, Rajakaruna C and Emanuelli C: Non coding RNAs in aortic aneurysmal disease. Front Genet 6: 125, 2015.
9. Yang YG, Li MX, Kou L, Zhou Y, Qin YW, Liu XJ and Chen Z: Long noncoding RNA expression signatures of abdominal aortic aneurysm revealed by microarray. Biomed Environ Sci 29: 713-723, 2016.
10. Geisler S and Coller J: RNA in unexpected places: Long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol 14: 699-712, 2013.
11. Gibney ER and Nolan CM: Epigenetics and gene expression. Clin Exp Dermatol 105: 4-13, 2010.
12. Wilusz JE, Sunwoo H and Spector DL: Long noncoding RNAs: Functional surprises from the RNA world. Genes Dev 23: 1494-1504, 2009.
13. Wan X, Huang W, Yang S, Zhang Y, Pu H, Fu F, Huang Y, Wu H, Li T and Li Y: Identification of androgen-responsive IncRNAs as diagnostic and prognostic markers for prostate cancer. Oncotarget 7: 60503-60518, 2016.
14. Guo LL, Song CH, Wang P, Dai LP, Zhang JY and Wang KJ: Competing endogenous RNA networks and gastric cancer. World J Gastroenterol 21: 11680-11687, 2015.
15. Tay Y, Rinn J and Pandolfi PP: The multilayered complexity of ceRNA crosstalk and competition. Nature 505: 344-352, 2014.
16. Franco-Zorrilla JM, Valli A, Todesco M, Mateos I, Puga MI, Rubio-Somoza I, Levy A, Weigel D, Garcia JA and Paz-Ares J: Target mimicry provides a new mechanism for regulation of microRNA activity. Nat Genet 39: 1033-1037, 2007.
17. Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ and Pandolfi PP: A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. Nature 465: 1033-1038, 2010.
18. Salmena L, Poliseno L, Tay Y, Kay L and Pandolfi PP: A ceRNA hypothesis: The rosetta stone of a hidden RNA language? Cell 146: 353-358, 2011.
19. van Rooij E and Olson EN: MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles. Nat Rev Drug Discov 11: 860-872, 2012.
20. Chen Y, Li C, Tan C and Liu X: Circular RNAs: A new frontier in the study of human diseases. J Med Genet 53: 359-365, 2016.
21. Lenk GM, Tromp G, Weinsheimer S, Gatalica Z, Berguer R and Kuvianniemi H: Whole genome expression profiling reveals a significant role for immune function in human abdominal aortic aneurysms. BMC Genomics 8: 237, 2007.
22. Liu G, Huang Y, Lu X, Lu M, Huang X, Li W and Jiang M: Identification and characteristics of microRNAs with altered expression patterns in a rat model of abdominal aortic aneurysms. Tohoku J Exp Med 222: 187-193, 2010.
23. Cui X and Churchill GA: Statistical tests for differential expression in cDNA microarray experiments. Genome Biol 4: 210, 2003.
24. Diboun I, Wernisch L, Ongeno CA and Koltzenburg M: Microarray analysis after RNA amplification can detect pronounced differences in gene expression using limma. BMC Genomics 7: 252, 2006.
25. Gondro C, Porto-Neto LR and Lee SH: R for genome-wide association studies. Methods Mol Biol 1019: 1-17, 2013.
26. Jeggari A, Marks DS and Larsson E: miRcode: A map of putative microRNA target sites in the long non-coding transcriptome. Bioinformatics 28: 2062-2063, 2012.
27. Li JH, Liu S, Zhou H, Qu LH and Yang JH: starBase v2.0: Decoding miRNA-ceRNA, miRNA-mRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 42: D92-D97, 2014.
28. Moran CS, McCann M, Karan M, Norman P, Keteesna N and Golldege J: Association of osteoprotegerin with human abdominal aortic aneurysm progression. Circulation 111: 3119-3125, 2005.
29. Karreth FA, Reschke M, Ruocco A, Ng C, Chapuy B, Leopold V, Sjoberg M, Keane TM, Verma A, Alu A, et al: The BRAF pseudogene functions as a competitive endogenous RNA and induces lymphoma in vivo. Cell 161: 319-332, 2015.
30. Guo S, Chen W, Luo Y, Ren F, Zhong T, Rong M, Fang Z and Chen G: Clinical implication of long non-coding RNA NEAT1 expression in hepatocellular carcinoma patients. Int J Mol Sci 17: 1039, 2016.
31. Monsanto I, Puga MI, Mateos I, Puga MI, Lecontu A, Spector DL and Chen G: Non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol 16: 11680-11687, 2015.
33. Chen X, Kong J, Ma Z, Gao S and Feng X: Up regulation of the long non-coding RNA NEAT1 promotes esophageal squamous cell carcinoma cell progression and correlates with poor prognosis. Am J Cancer Res 5: 2808-2815, 2015.

34. Kotake Y, Nakagawa T, Kitagawa K, Suzuki S, Liu N, Kitagawa M and Xiong Y: Long non-coding RNA ANRIL is required for the PRC2 recruitment to and silencing of p15(INK4B) tumor suppressor gene. Oncogene 30: 1956-1962, 2011.

35. Yang XR, Liang XY, Pfeiffer RM, Wheeler W, Maeder D, Burdette L, Yeager M, Chanock S, Tucker MA and Goldstein AM: Associations of 9p21 variants with cutaneous malignant melanoma, nevi, and pigmentation phenotypes in melanoma-prone families with and without CDKN2A mutations. Fam Cancer 9: 625-633, 2010.

36. Falchi M, Bataille V, Hayward NK, Duffy DL, Bishop JA, Pastinen T, Cervino A, Zhao ZZ, Deloukas P, Soranzo N, et al: Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. Nat Genet 41: 915-919, 2009.

37. Sherborne AL, Hosking FJ, Prasad RB, Kumar R, Koehler R, Vijayakrishnan J, Papenemanu E, Bartram CR, Stanulla M, Schrappe M, et al: Variation in CDKN2A at 9p21.3 influences childhood acute lymphoblastic leukemia risk. Nat Genet 42: 492-494, 2010.

38. Helgadottir A, Thorleifsson G, Magnusson KP, Gretarsdottir S, Steinthorsdottir V, Manolescu A, Jones GT, Rinkel GJ, Blankensteijn JD, Ronkainen A, et al: The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet 40: 217-224, 2008.

39. Broadbent HM, Peden JF, Lorkowski S, Goel A, Ongen H, Green F, Clarke R, Collins R, Franzoni MG, Tognoni G, et al: Susceptibility to coronary artery disease and diabetes is encoded by distinct, tightly linked SNPs in the ANRIL locus on chromosome 9p. Hum Mol Genet 17: 806-814, 2008.

40. Cugino D, Gianfagna F, Santimone I, de Guaitano G, Donati MB, Iacoviello L and Di Castelnuovo A: Type 2 diabetes and polymorphisms on chromosome 9p21: A meta-analysis. Nutr Metab Cardiovasc Dis 22: 619-625, 2012.

41. Schaefer AS, Richter GM, Groessen-Schreiber B, Noack B, Nothnagel M, El Mohktari NE, Loos BG, Jepsen S and Schreiber S: Identification of a shared genetic susceptibility locus for coronary heart disease and periodontitis. Plos Genet 5: e1000378, 2009.

42. Ernst FD, Uhr K, Teumer A, Fanghänel J, Schulz S, Noack B, Gonzales J, Reichert S, Eickholz P, Holtfreter B, et al: Replication of the association of chromosomal region 9p21.3 with generalized aggressive periodontitis (gAgP) using an independent case-control cohort. BMC Med Genet 11: 119, 2010.

43. Yu JT, Yu Y, Zhang W, Wu ZC, Li Y, Zhang N and Tan L: Single nucleotide polymorphism rs1333049 on chromosome 9p21.3 is associated with Alzheimer's disease in Han chinese. Clin Chim Acta 411: 1204-1207, 2010.

44. Emanuele E, Lista S, Ghidoni R, Binetti G, Cereda C, Benussi L, Maletta R, Brunu AC and Politi P: Chromosome 9p21.3 genotype is associated with vascular dementia and Alzheimer's disease. Neurobiol Aging 32: 1231-1235, 2011.

45. Uno S, Zembutsu H, Hirasaawa A, Takahashi A, Kubo M, Akahane T, Aoki D, Kamatani N, Hirata K and Nakamura Y: A genome-wide association study identifies genetic variants in the CDKN2BAS locus associated with endometriosis in Japanese. Nat Genet 42: 707-710, 2010.

46. Melzer D, Frayling TM, Murray A, Hurst AJ, Harries LW, Song H, Khaw K, Luben R, Surtees PG, Bandinelli SS, et al: A common variant of the p16 INK4a genetic region is associated with physical function in older people. Mech Ageing Dev 128: 370-377, 2007.

47. Ramdas WD, van Koolwijk LME, Lemij HG, Pasutto F, Cee AJ, Thorleifsson G, Janssen SF, Jacoline TB, Amin N, Rivadeneira F, et al: Common genetic variants associated with open-angle glaucoma. Hum Mol Genet 20: 2464-2471, 2011.

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