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(54) Title: BIOMARKERS FOR ATHEROSCLEROTIC CARDIOVASCULAR DISEASES

(57) Abstract: A biomarker, the use of the biomarker in predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof, and a method of predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof.



BIOMARKERS FOR ATHEROSCLEROTIC CARDIOVASCULAR DISEASES

TECHNICAL FIELD

Embodiments of the present disclosure generally relate to the field of biological detection, more particularly, to a biomarker, the use of the biomarker in predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof, a method of predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof and a kit for predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof.

BACKGROUND

Atherosclerotic cardiovascular disease (ACVD), is typically caused by the accumulation of plaque on the arterial walls (i.e., atherosclerosis), particularly in the large and medium-sized arteries serving the heart. It refers to the following conditions: Coronary heart disease (CHD), cerebrovascular disease, peripheral artery disease and aortic atherosclerotic disease. These conditions have similar causes, mechanisms, and treatments.

The “gold standard” for detecting ACVD is invasive coronary angiography. However, this is costly, and can pose risk to the patient. Prior to angiography, non-invasive diagnostic modalities such as myocardial perfusion imaging (MPI) and CT-angiography may be used, however these have complications including radiation exposure, contrast agent sensitivity, and only add moderately to obstructive ACVD identification.

Current knowledge indicates the genetic, environmental factors and their interactions collaboratively induce complex phenotype and many diseases. ACVD has been increasingly investigated by GWAS (Genome-wide association study) in recent years and revealed 10.6% of the inherent cause by 46 common variations (Ehret, G. B. et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 478, 103-109, incorporated herein by reference). However, our knowledge on the contribution of genes to disease still need further.

Our “forgotten organ”, gut microbiota, plays a crucial role on our health in many aspects, such as intaking energy from food, producing important metabolites, promoting the development and maturity of immune system, and protecting the host from pathogen infection *et, al*. Recent studies suggested the flora dysbiosis, chronic inflammatory and metabolic abnormality exist in the intestine of some metabolic diseases like diabetes, obesity and the coronary artery disease. A recent research indicates gut microbes could metabolize the red meat ingredients (L-carnitine,

phosphatidyl-choline, cholesterol) into TMA, which would be further oxidized into TMAO in the liver to arise the oxidization reaction in blood vessel to lead inflammatory and lipid deposition, ultimately resulting in atherosclerosis and coronary heart disease (Koeth, R. A. et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature medicine* 19, 576-585, incorporated herein by reference). These studies suggested the dysbiosis of gut microbial content may strongly influenced the pathogenesis of ACVD by inducing the human metabolic abnormality. However, the lack of a large cohort for metagenomics characterization of this major group of ACVD has impeded further investigations on the role played by the microbiome.

SUMMARY

Embodiments of the present disclosure seek to solve at least one of the problems existing in the related art to at least some extent, or to provide a consumer with a useful commercial choice.

Embodiments of a first broad aspect of the present disclosure provide a biomarker, wherein the biomarker comprising at least one nucleotide sequence shown as SEQ ID NO: 1~3.

ATGAATTGTGAAACTGTTGCCCTCGGGTCGTTTTGCAAGTTGAAGTCGAGGAGAG
GAAAAAACA AAAAGGAGAAATACTCATGGCAGTAATTTCAATGAAACA ACTTCTTG
AGGCTGGTGTACACTTTGGTCACCAA ACTCGTCGCTGGAACCTAAGATGGCTAAGTA
CATCTTCACTGAGCGTAACGGAATCCACGTTATCGACTTGCAACAACTGTAAAATAT
GCTGACCAAGCTTACGATTTTCATGCGTGATGCAGCAGCTAACGATGCAGTTGTATTGTT
CGTTGGTACTAAGAAACAAGCCGCAGATGCAGTTGCTGAAGAAGCTGTTTCGTGCAGG
ACAATACTTCATCAACCACCGTTGGTTGGGTGGA ACTCTTACTAACTGGGGA ACTATC
CAAAAACGTATCGCTCGTTTGAAAGAAATCAAACGTATGGAAGAAGAAGGAATCTTC
GACGTTCTTCCTAAGAAAGAAGTTGCACTTCTTAATAAACAGCGTGACGCTCTTGAAA
AATTCTTGGGTGGTATCGAAGACATGCCTCGTATCCCAGATGTAATGTACGTAGTTGAC
CCACATAAAGAGCAAATCGCTGTAAAGAAGCTAAAAAATTGGGTATCCCAGTTGTAG
CGATGGTTGACACAAACACTGATCCAGACGATATCGATGTAATCATCCCAGCTAACGAT
GACGCTATCCGCGCGGTTAAACTGATCACAGCTAAATTGGCTGACGCTATCATCGAAG
GACGTCAAGGTGAAGATGCAACAGCAGTTGAAGCAGAATTTGCAGCTTCAGAAGCTC
AAGCAGACTCAATCGAAGAAATCGTTGAAGTTGTAGAAGGCGACAACGCTTAA(SEQ
ID NO:1).

TTGGCAATTAACGCACAAGAAATCAGCGCTTTAATTAAGCAACAAATTGAAAATT
TCAAACCCAATTTTGATGTGACTGAAACAGGTGTTGTAACCTATATCGGGGACGGTAT
CGCGCGTGCTCACGGCCTTGAAAATGCCATGAGTGGAGAGTTGTTGATTTTTGAAAAC

GGCTCTTATGGTATGGCTCAAACTTGGAGTCAACAGATGTTGGTATTATCATCCTAGG
TGA CTTTACAGATATCCGTGAAGGTGATACAATTCGCCGTACAGGTAAAATCATGGAA
GTCCCAATAGGTGAAAGTCTGATTGGTCGTGTTGTGGATCCTCTTGGTCGTCCAGTTG
ACGGTCTTGGAGAAATCCACACTGATAAACTCGTCCAGTTGAAGCGCCAGCTCCTG
GTGTTATGCAACGTAAGTCTGTATCAGAACCATTGCAAACCTGGTTTGAAAGCTATCGA
CGCCCTTGTACCGATTGGTCGTGGTCAACGTGAGTTGATTATCGGTGACCGTCAGACA
GGGAAAACA ACTATTGCGATTGATACAATCTTGAACCAAAAAGGTCAAGATATGATCT
GTATTTATGTCGCTATTGGACAAAAAGAATCAACAGTTCGTACGCAAGTAGAAACACT
ACGTCAGTACGGTGCCTTGGACTACACAATCGTTGTGACTGCCTCTGCTTCACAACCA
TCTCCATTGCTTTTCCTAGCTCCTTATGCTGGGGTTGCCATGGCGGAAGAATTTATGTA
CCAAGGCAAGCATGTTTTGATCGTTTATGATGATCTTTCAAACAAGCGGTGCGTTATC
GTGAACTTTCTCTCTTGCTTCGTCGTCCACCAGGTGCGTGAAGCCTTCCCAGGGGATGT
TTTCTACCTTCACAGCCGTTTGCTTGAGCGC(SEQ ID NO:2).

AAGGCACTCGAGGGAGCGCTCCGCGACCTCACCGCGATCACCGGCCAGAAGCCC
GTGACCACCCGCGCCAAGAAGTCCATCGCGCAGTTCAAGCTGCGTGAGGGCCAGGC
GATCGGTGCGCACGTACGCTTCGCGGGCGACCGCATGTGGGAGTTCCTGGATCGCCTC
CTCTCGACGGCGCTTCCCCGTATCCGTGACTTCCGCGGACTGTCCTCCAAGCAGTTCG
ACGGTCACGGCAATTACACCTTCGGTCTCACGGAACAGTCGATGTTCCACGAGATCGA
TCAGGACTCGATCGACCGAGTGCGCGGCATGGACATCACGGTTGTACCTCGGCCAC
CACCGACGAAGAGGGTCGCGCCCTTCTCCGTACCTCGGCTTCCCGTTCAAGGAGGA
CTGA(SEQ ID NO:3).

The inventor surprisingly found that the genes in gut microbiota described above can be effectively used as a biomarker for predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof.

Embodiments of a second broad aspect of the present disclosure provide use of the biomarker described above in predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof.

Embodiments of a third broad aspect of the present disclosure provide a method of predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof. According to embodiments of the present invention, the method comprising: (1) Determining the relative abundance of the biomarker described above in the samples of the subject to be tested; (2) predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof based on the relative abundance of the biomarker. The method described above can effectively predict the risk of atherosclerotic cardiovascular disease or related disorder thereof. The credibility of the

prediction result is quite high.

Embodiments of a forth broad aspect of the present disclosure provide a kit for predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof. According to embodiments of the present invention, the kit comprising: reagent configured to detecting the biomarker described above. It is found that the kit can be used for predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof effectively and easily.

The above summary of the present disclosure is not intended to describe each disclosed embodiment or every implementation of the present disclosure. The Figures and the detailed description which follow more particularly exemplify illustrative embodiments.

Additional aspects and advantages of embodiments of present disclosure will be given in part in the following descriptions, become apparent in part from the following descriptions, or be learned from the practice of the embodiments of the present disclosure.

DETAILED DESCRIPTION

Reference will be made in detail to embodiments of the present disclosure. The embodiments described herein with reference to drawings are explanatory, illustrative, and used to generally understand the present disclosure. The embodiments shall not be construed to limit the present disclosure. The same or similar elements and the elements having same or similar functions are denoted by like reference numerals throughout the descriptions.

A biomarker

Embodiments of a first broad aspect of the present disclosure provide a biomarker, wherein the biomarker comprising at least one nucleotide sequence shown as SEQ ID NO: 1~3. The inventor surprisingly found that the genes in gut microbiota described above can be effectively used as a biomarker for predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof.

Assessment and characterization of gut microbial content has become a major research area in human disease, including ACVD. To carry out analysis on gut microbial content in the ACVD, the inventors carried out a protocol for a Metagenome-Wide Association Study (MGWAS) (Qin, J. et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 490, 55–60 (2012), incorporated herein by reference) based on deep shotgun sequencing of the gut microbial DNA from 405 Chinese individuals (n = 218 ACVD, 187 healthy controls; Table 1). The inventors identified and validated 3 ACVD-associated gene markers. To explore the diagnostic value of the fecal microbial genes in relation to ACVD, the inventors constructed a random forest classifier from the 405 ACVD and control samples, with 5 repeats of 5-fold

cross-validation and identified 3 optimized diseases associated gut microbial gene markers. The inventors' data provide insight into the characteristics of the gut metagenome related to ACVD risk, a paradigm for future studies of the pathophysiological role of the gut metagenome in other relevant disorders, and the usefulness for a gut-microbiota-based approach for assessment of individuals at risk of such disorders.

Use of the biomarker

Embodiments of a second broad aspect of the present disclosure provide use of the biomarker described above in predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof.

A method of predicting the risk of ACVD

Embodiments of a third broad aspect of the present disclosure provide a method of predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof. According to embodiments of the present invention, the method comprising: (1) Determining the relative abundance of the biomarker described above in the samples of the subject to be tested; and (2) predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof based on the relative abundance of the biomarker described above. The method described above can effectively predict the risk of atherosclerotic cardiovascular disease or related disorder thereof. The credibility of the prediction result is quite high.

According to embodiments of present disclosure, wherein the relative abundance of the biomarker is obtained by means of sequencing.

According to embodiments of present disclosure, step (2) comprising comparing the relative abundance of the biomarker with a preset cutoff value. In some embodiments of present disclosure, a sufficient difference between the relative abundance greater and the preset cutoff may indicate that the subject to be tested has or is at the risk of developing the atherosclerotic cardiovascular disease or related disorder thereof. The person skilled in the art may understand that the values of the "sufficient difference" and the "preset value" may be obtained based on some control samples with known condition of the atherosclerotic cardiovascular disease or related disorder thereof. And according to another embodiment of present disclosure, the preset cutoff value is about at least 0.5.

It should be noted that the relative abundance is either a real value of the relative abundance or a probability, wherein the probability is a probability of atherosclerotic cardiovascular disease by comparing the relative abundance information of the biomarker in the sample of the subject with a training dataset using a Multivariate statistical mode.

According to embodiments of present disclosure, wherein the samples are gut microbiota,

optionally, the gut microbiota is obtained from the fecal of the subject to be tested. Gut microbiota are obtained from fecal samples of ACVD patients which is costless, safe and side-effect free. Analysis of stool promises accuracy, safety, affordability, and patient compliance. And samples of stool are transportable. The specific biomarkers can be used as a noninvasive test for early diagnosis of patients having ACVD, thus leading to a longer survival and a better quality of life.

According to embodiments of present disclosure, the method described above can be realized as followings : (1) determining the relative abundance of the biomarker described above in the samples of the subject to be tested; (2) obtain a probability of atherosclerotic cardiovascular disease by comparing the relative abundance information of the biomarker in the sample of the subject with a training dataset using a Multivariate statistical model; wherein the probability of atherosclerotic cardiovascular disease greater than a cutoff indicates that the subject to be tested has or is at the risk of developing the atherosclerotic cardiovascular disease or related disorder thereof. The training dataset is constructed based on the relative abundance information of each biomarker of a plurality of subjects having ACVD and a plurality of normal subjects using a Multivariate statistical model, alternatively the Multivariate statistical model is a randomForest model. The training dataset is a matrix with each row representing each biomarker of the biomarker set described above, each column representing samples, each cell representing relative abundance profile of a biomarker in the sample, and sample disease status is a vector, with 1 for ACVD and 0 for control. The relative abundance information of each of SEQ ID NO 1, 2 and 3 is a relative abundance information showed in Table 4. The probability of ACVD being at least 0.5 indicates that the subject to be tested has or is at the risk of developing the ACVD or related disorder.

A kit for predicting the risk of ACVD

Embodiments of a forth broad aspect of the present disclosure provide a kit for predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof. According to embodiments of the present invention, the kit comprising: reagent configured to detecting the biomarker described above. It is found that the kit can be used for predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof effectively and easily.

According to embodiments of present disclosure, wherein the reagent comprising at least one of the probe, prime, gene chip and antibody. The probe, gene chip and antibody are specific recognition of the biomarker described above and the prime are specifically amplify the biomarker described above based on polymerase chain reaction (PCR)-based assays. Compared with invasive coronary angiography, the kit are comfortable and noninvasive, so people will

participate in a given screening program more easily.

It is believed that 3 ACVD associated gene markers of intestinal microbiota are valuable for increasing metabolic diseases' detection at earlier stages due to the following. First, the markers of the present invention are more specific and sensitive as compared with conventional markers. Second, analysis of stool promises accuracy, safety, affordability, and patient compliance. And samples of stool are transportable. As compared with invasive coronary angiography, polymerase chain reaction (PCR)-based assays are comfortable and noninvasive, so people will participate in a given screening program more easily. Third, the markers of the present invention may also serve as tools for therapy monitoring in ACVD patients to detect the response to therapy.

Example

Identifying 3 biomarkers for evaluating ACVD risk

Sample collection

Samples from 405 Chinese subjects, including 218 individuals with ACVD and 187 control subjects, were collected at the Medical Research Center of Guangdong General Hospital. Individuals with ACVD showed clinical presentations of stable angina, unstable angina or acute myocardial infarction (AMI) (Table 1). ACVD diagnosis was confirmed by coronary angiography, and individuals that had $\geq 50\%$ stenosis in single or multiple vessels were included. All patients were ethnic Han Chinese with no known consanguinity, aged 40 to 80 years old. The exclusion criteria included ongoing infectious diseases, cancer, renal or hepatic failure, peripheral neuropathy, stroke, as well as use of antibiotics within one month of sample collection. All the healthy control individuals enrolled were free of clinically evident ACVD symptoms at the time of the medical examination. Demographic data and cardiovascular risk factors were collected by a questionnaire. Individuals with peripheral artery disease, known coronary artery disease or myocardial infarction, cardiomyopathy, renal failure, peripheral neuropathy, systemic disease and stroke were excluded. Fresh feces of each subject were collected the first morning after admission to the hospital and were frozen on dry ice within 30 min, and stored in -80°C freezers before further analysis.

The study was approved by the Medical Ethical Review Committee of the Guangdong General Hospital and the Institutional Review Board at BGI-Shenzhen. Informed consent was obtained from all participants.

Table 1: Baseline characteristics of ACVD cases and controls. Both age and BMI are tested

by Wilcoxon rank-sum tests while gender is tested by Fisher test.

Parameter	Cases (n=218)	Case NA	Controls (n=187)	Control NA	P-value
Age	60.8	0	60.2	7	0.094
Gender (M:F)	161:53	4	75:111	1	1.263e-12
BMI	24.54	67	24.41	7	0.0289

NOTE: the third column and fifth column are the number of people of whom information of age, gender or BMI was unknown.

DNA extraction from fecal samples

Fecal samples were thawed on ice and DNA extraction was performed using the Qiagen QIAamp DNA Stool Mini Kit (Qiagen) according to manufacturer's instructions. Extracts were treated with DNase-free RNase to eliminate RNA contamination. DNA quantity was determined using NanoDrop spectrophotometer, Qubit Fluorometer (with the Quant-iT™ dsDNA BR Assay Kit) and gel electrophoresis.

DNA library construction and sequencing of fecal samples

DNA library construction was performed following the manufacturer's instruction (Illumina). We used the same workflow as described previously 5 to perform cluster generation, template hybridization, isothermal amplification, linearization, blocking and denaturation, and hybridization of the sequencing primers. We constructed one paired-end (PE) library with insert size of 350 bp for each sample, followed by a high-throughput sequencing to obtain around 30 million PE reads of length 2x100bp. High-quality reads were obtained by filtering low-quality reads with ambiguous 'N' bases, adapter contamination and human DNA contamination from the Illumina raw reads, and by trimming low-quality terminal bases of reads simultaneously.

Metagenomic data processing and analysis

Construction of gene profile. High-quality reads were aligned to the 9,879,896 genes (9.9M gene catalog) by SOAP2 using the criterion of identity $\geq 90\%$. Sequence-based gene abundance profile was performed as previously described (Li, J. *et al.* An integrated catalog of reference genes in the human gut microbiome. *Nat. Biotechnol.* **32**, 834–841 (2014), incorporated herein by reference).

ACVD-associated genes identified by MGWAS. After filtering the 9,879,896 genes by occurrence in more than 10 samples, the inventors identified potential biomarkers for ACVD from the remaining genes by the MGWAS approach, using the minimum redundancy maximum relevance (mRMR) feature selection method (H. Peng, F. Long, C. Ding, Feature selection based

on mutual information: criteria of max-dependency, max-relevance, and min-redundancy. IEEE transactions on pattern analysis and machine intelligence 27, 1226 (Aug, 2005, incorporated herein by reference), and they selected the first 500 most power full genes.

They ranked the 500 genes by train set cross validation and test set ROC and prediction error and finally chose the 3 optimal genes (Table 2 and Table 3).

Considering the AUC and prediction error rate of all 405 samples (see Table 5 and Table 6), the inventors chose 3 most discriminatory genes, consisting of gene 3050214, 2841974, 6560409 (see Table 3).

The inventors used the 3 genes as biomarkers to test the power in separation ACVD patients and controls, respectively: they split 405 samples into training set (70% of the 405 samples) and test set (30% of the 405 samples) randomly, then calculated the prediction error rate and AUC for each biomarker in training set by cross validation, and the prediction error and AUC for each biomarker in test set were calculated by module which was built from training set (Table 4, Table 5 and Table 6).

Furthermore, the inventors also calculated the prediction error rate and AUC by the combined 3 gene set on training set and test set (Table 7), founding that the AUC was 0.796 in training set and 0.761 in test set, the error rate was 0.278 in training set and 0.322 in test set.

Table 2 : 3 most discriminant genes associated with ACVD (Enrich: 1: ACVD, 0:control)

Gene ID	P value	Enrich	ACVD Occurrence	Control Occurrence	ACVD mean abundance	Control mean abundance	Odds ratio
3050214	4.68E-19	Acvd	0.5321	0.1658	6.31E-07	1.03E-07	8.52(3.02,24.04)
2841974	2.74E-17	Acvd	0.3119	0.0642	2.18E-07	2.55E-08	34.17(7.77,150.38)
6560409	9.36E-18	Acvd	0.4633	0.1444	2.84E-07	4.92E-08	8.05(3.66,17.71)

Table 3 : SEQ ID NO. of the 3 gene markers

Gene ID	SEQ ID NO	Gene number(length)	Gene name in 9.9M gene catalog
3050214	1	861	N056A_GL0000096
2841974	2	900	ED9A_GL0080283
6560409	3	402	NLM027_GL0004265

Table 4 3 genes relative abundance profiles in 405 samples (Sample ID, N represents control, ZSL represents ACVD)

Sample ID	Gene ID			Sample ID	Gene ID			Sample ID	Gene ID			Sample ID	Gene ID		
	3050214	2841974	6560409		3050214	2841974	6560409		3050214	2841974	6560409		3050214	2841974	6560409
N1260A	0	0	0	N167	0	7.94E-08	0	ZSL302	3.14E-07	2.62E-07	3.36E-07	N135A	7.44E-08	1.07E-07	7.97E-08
N1261A	0	3.61E-08	0	N168	7.44E-08	7.12E-08	0	ZSL306	1.92E-07	3.68E-08	0	N136A	2.45E-07	6.71E-08	0
N1262A	4.05E-08	0	0	N170	0	0	0	ZSL308	0	0	7.25E-08	N137A	0	0	0
N1263A	6.58E-08	3.15E-08	1.41E-07	N171	0	0	8.00E-08	ZSL310	6.22E-08	2.98E-08	6.66E-08	N140A	3.14E-08	0	1.34E-07
N1265A	0	0	0	N172	6.86E-08	0	7.34E-08	ZSL312	0	0	8.75E-08	N142A	0	0	0
N1266A	3.00E-07	4.10E-08	0	N173	0	7.13E-08	0	ZSL313	2.05E-07	1.96E-07	1.76E-07	N143A	4.38E-08	0	0
N1267A	4.37E-08	0	0	N174	1.80E-07	1.15E-07	8.34E-07	ZSL314	1.69E-06	4.45E-07	4.53E-07	N147A	3.63E-07	1.93E-07	0
N1269A	4.58E-07	3.65E-08	1.63E-07	N175	0	0	0	ZSL315	0	4.12E-08	0	N148A	0	0	0
N1270A	0	3.89E-08	0	N176	4.17E-08	0	0	ZSL317	7.22E-08	0	0	N149A	6.60E-08	6.32E-08	0
N1271A	0	0	0	N182	0	0	8.34E-08	ZSL318	6.51E-07	3.86E-07	1.33E-07	N150A	0	0	0
N1272A	0	0	0	N184	4.78E-08	0	0	ZSL319	0	0	0	N152A	1.04E-07	6.66E-08	0
N1273A	4.06E-08	0	0	N185	3.25E-08	0	0	ZSL322	1.29E-07	3.09E-08	6.91E-08	N153A	0	0	0
N1274A	0	0	0	N186	3.71E-08	0	2.39E-07	ZSL325	9.62E-08	0	0	N155A	9.31E-08	0	0
N1275A	0	0	8.82E-08	N187	0	0	0	ZSL330	4.03E-07	4.28E-08	4.79E-07	ZSL-004	4.07E-06	1.18E-06	4.74E-07
N1276A	2.54E-07	1.22E-07	2.72E-07	N188	0	0	0	ZSL332	1.41E-07	4.50E-08	1.01E-07	ZSL-007	4.50E-07	1.72E-07	9.63E-08

N1277A	0	3.82E-08	8.56E-08	N189	2.50E-07	2.99E-08	1.34E-07	ZSL334	3.57E-07	7.59E-08	7.64E-07	ZSL-010	1.92E-07	9.19E-08	2.06E-07
N1279A	3.32E-07	0	0	N190	0	0	0	ZSL335	0	0	0	ZSL-011	1.93E-07	0	8.27E-07
N1280A	3.70E-08	0	0	N191	0	0	0	ZSL336	0	0	0	ZSL-019	1.81E-06	8.26E-08	1.48E-06
N1285A	2.31E-07	4.41E-08	0	N193	3.70E-08	3.54E-08	0	ZSL338	4.22E-08	4.04E-08	0	ZSL-022	4.08E-07	0	2.49E-07
N1287A	3.71E-08	0	0	N194	0	0	0	ZSL339	1.71E-07	6.56E-08	2.94E-07	ZSL-023	3.82E-07	0	0
N1288A	6.49E-08	1.24E-07	0	N195	0	4.53E-08	0	ZSL340	4.42E-07	7.04E-08	0	ZSL-024	5.44E-08	5.20E-08	1.16E-07
N1291A	0	3.18E-08	0	N197	0	0	8.30E-08	ZSL341A	2.95E-08	0	0	ZSL-025	1.10E-07	5.24E-08	1.53E-06
N1292A	4.76E-08	4.55E-08	0	N198	1.91E-07	0	0	ZSL343	4.23E-08	4.05E-08	9.06E-08	ZSL-028	5.82E-08	5.57E-08	6.23E-07
N1293A	0	0	0	N199	0	0	0	ZSL349	3.33E-07	0	3.56E-07	ZSL-029	0	0	0
N1294A	0	0	0	N200	7.04E-08	0	7.54E-08	ZSL350	0	0	1.12E-07	ZSL-031	9.54E-07	5.58E-07	3.41E-07
N1295A	0	5.09E-08	1.14E-07	N201	0	0	0	ZSL353	1.91E-07	3.05E-08	6.82E-08	ZSL-033	1.20E-07	0	5.14E-07
N1296A	0	0	8.76E-08	N202	0	1.39E-07	1.04E-07	ZSL359	9.00E-08	0	1.93E-07	ZSL-035	1.36E-05	2.68E-06	1.81E-06
N1297A	6.73E-08	3.22E-08	1.44E-07	N203	3.95E-08	0	0	ZSL365	5.38E-08	5.15E-08	3.46E-07	ZSL-037	2.19E-07	0	0
N1298A	0	0	0	N204	0	0	0	ZSL368	4.43E-08	0	1.90E-07	ZSL-040	2.94E-07	4.68E-08	1.03E-07
N1299A	3.62E-08	0	0	N206	2.50E-07	5.99E-08	1.34E-07	ZSL369	3.37E-07	5.37E-08	0	ZSL-042	1.97E-06	7.69E-07	8.61E-08
N1300A	3.77E-08	0	1.61E-07	N207	0	0	0	ZSL370	0	0	0	ZSL-044	8.94E-07	2.01E-07	1.13E-07
N1301A	0	3.03E-08	0	N209	4.38E-08	0	0	ZSL374	6.27E-06	4.38E-06	3.10E-06	ZSL-045	4.93E-06	1.98E-06	2.01E-07
N1302A	3.79E-08	0	0	N210	0	0	0	ZSL375	0	0	0	ZSL-046	1.12E-07	0	3.59E-07

N1307A	1.06E-07	0	0	N213	1.32E-07	0	0	ZSL376	0	0	0	0	0	ZSL-047	4.15E-07	1.70E-07	1.01E-06
N1309A	0	0	8.83E-08	N214	0	3.94E-08	0	ZSL377	1.03E-06	3.74E-07	0	0	0	ZSL-050	0	8.40E-08	0
ZSL402A	0	0	9.03E-08	N215	0	0	0	ZSL378	8.50E-07	3.58E-07	3.64E-07	1.91E-06	8.91E-08	ZSL-052	1.91E-06	8.91E-08	2.99E-07
ZSL399A	2.66E-07	8.48E-08	0	N218	1.11E-07	0	2.37E-07	ZSL379	0	0	0	7.40E-07	2.24E-07	ZSL-053	7.40E-07	2.24E-07	5.01E-07
ZSL403A	4.15E-08	0	1.78E-07	N220	0	0	0	N001A	0	6.22E-08	0	6.72E-07	1.61E-07	ZSL-055	6.72E-07	1.61E-07	3.60E-07
ZSL404A	0	0	0	N221	0	0	0	N002A	0	0	0	0	0	ZSL-057	0	0	1.06E-07
ZSL405A	1.06E-06	1.95E-07	6.13E-07	N222	3.78E-08	7.23E-08	0	N003A	0	0	0	1.45E-06	6.93E-07	ZSL-062	1.45E-06	6.93E-07	7.75E-08
ZSL408A	3.31E-08	3.16E-08	7.09E-08	N223	3.86E-08	0	0	N005A	0	0	1.26E-07	1.37E-07	3.28E-08	ZSL-063	1.37E-07	3.28E-08	7.34E-08
ZSL409A	3.69E-07	2.12E-07	7.90E-08	N224	0	0	0	N006A	0	0	0	2.16E-07	3.44E-08	ZSL-065	2.16E-07	3.44E-08	0
ZSL401A	6.13E-07	3.52E-07	8.75E-08	N225	0	0	8.45E-08	N009A	0	0	0	4.58E-08	4.38E-08	ZSL-066	4.58E-08	4.38E-08	0
ZSL415A	8.89E-08	4.25E-08	0	N226	0	0	0	N011A	0	0	0	1.36E-06	2.68E-07	ZSL-068	1.36E-06	2.68E-07	8.58E-08
ZSL416A	2.12E-07	3.39E-08	7.58E-08	N227	3.39E-08	0	7.27E-08	N013A	6.09E-07	3.71E-07	0	7.92E-08	3.79E-08	ZSL-071	7.92E-08	3.79E-08	8.48E-08
ZSL417A	1.16E-06	8.60E-07	1.28E-06	N231	1.73E-06	5.91E-07	1.76E-07	N017A	0	0	0	1.08E-07	1.38E-07	ZSL-073	1.08E-07	1.38E-07	3.08E-07
ZSL422A	6.80E-08	0	0	N232	0	0	8.69E-07	N020A	0	0	0	1.25E-05	6.52E-06	ZSL-074	1.25E-05	6.52E-06	1.14E-06
ZSL429A	0	3.11E-08	6.95E-08	N233	7.55E-08	0	0	N021A	0	0	0	2.25E-07	8.60E-08	ZSL-076	2.25E-07	8.60E-08	1.92E-07
ZSL430A	7.44E-07	1.62E-07	5.79E-07	N234	3.16E-06	8.47E-07	1.53E-06	N022A	0	0	1.19E-07	5.63E-08	0	ZSL-077	5.63E-08	0	0
ZSL433A	0	4.09E-08	5.49E-07	N235	0	0	0	N024A	1.54E-07	0	0	4.71E-07	1.50E-07	ZSL-079	4.71E-07	1.50E-07	2.24E-07
ZSL435A	0	3.92E-08	8.77E-08	N236	0	0	0	N025A	0	0	1.15E-07	0	0	ZSL-080	0	0	1.86E-07

ZSL436A	1.09E-07	6.96E-08	2.34E-07	N237	1.54E-07	3.67E-08	0	N026A	0	0	0	ZSL-083	5.34E-07	5.11E-07	1.14E-06
ZSL437A	7.63E-07	3.65E-08	3.27E-07	N239	0	0	0	N027A	7.56E-08	0	0	ZSL-084	1.59E-07	7.59E-08	8.50E-08
ZSL439A	0	0	0	N240	3.61E-08	0	0	N028A	1.07E-07	0	0	ZSL-085	4.41E-06	2.98E-06	1.59E-06
ZSL446A	4.31E-08	0	3.69E-07	N241	0	0	8.23E-08	N029A	0	0	0	ZSL-086	1.18E-05	2.30E-06	3.52E-06
ZSL450A	4.17E-08	7.98E-08	8.93E-08	N242	0	0	0	N031A	0	0	0	ZSL-088	1.19E-06	4.47E-07	1.00E-06
ZSL452A	1.54E-06	2.46E-07	1.84E-07	N244	3.69E-08	0	0	N032A	2.35E-07	0	0	ZSL-089	1.01E-06	3.86E-07	2.16E-07
ZSL453A	3.71E-08	0	7.95E-08	N245	0	0	9.64E-08	N033A	0	0	0	ZSL-091	1.44E-07	4.59E-08	4.11E-07
ZSL454A	3.41E-07	9.33E-08	0	N247	1.12E-07	0	3.19E-07	N034A	0	0	0	ZSL-092	3.80E-06	6.86E-07	4.01E-06
ZSL466A	0	0	0	N248	3.59E-08	0	0	N035A	0	0	0	ZSL-094	0	5.41E-08	0
ZSL467A	4.43E-08	0	0	N249	0	0	0	N037A	0	0	0	ZSL-095	1.20E-06	4.69E-07	5.83E-07
ZSL468A	0	0	8.72E-08	N251	0	0	0	N038A	0	0	0	ZSL-097	1.85E-06	4.83E-07	0
ZSL475A	8.74E-08	8.36E-08	0	N267	1.25E-07	0	0	N039A	0	0	0	ZSL-098	1.63E-07	1.04E-07	0
ZSL478A	1.54E-07	3.68E-08	2.47E-07	N268	7.54E-08	0	0	N040A	0	0	0	ZSL-099	0	5.68E-08	0
ZSL479A	9.35E-07	2.24E-07	1.67E-07	N281	0	0	0	N042A	2.14E-07	0	0	ZSL-103	0	0	0
ZSL480A	8.28E-08	3.96E-08	0	N287	3.31E-07	1.59E-07	0	N043A	0	0	1.09E-07	ZSL-105	6.25E-07	5.52E-07	4.12E-07
ZSL482A	4.03E-08	0	8.62E-08	N288	0	0	0	N044A	0	0	0	ZSL-111	5.65E-06	1.35E-06	3.49E-07
ZSL487A	0	0	0	ZSL229	6.04E-08	0	0	N046A	0	6.15E-08	0	ZSL-113	3.85E-07	2.63E-07	2.36E-07
ZSL488A	4.20E-08	4.02E-08	0	ZSL234	7.16E-08	0	7.66E-08	N047A	0	0	0	ZSL-115	0	4.41E-08	0

ZSL490A	4.40E-08	0	0	ZSL235	0	0	0	0	N049A	4.26E-08	0	0	0	ZSL-116	6.90E-07	5.50E-08	4.93E-07
ZSL492A	0	0	8.32E-08	ZSL236	8.27E-08	0	0	0	N051A	0	0	0	0	ZSL-120	1.91E-07	4.58E-08	1.03E-07
ZSL503A	5.09E-08	4.87E-08	0	ZSL237	0	4.36E-08	0	0	N052A	0	0	0	0	ZSL-122	1.08E-07	1.72E-07	3.84E-07
ZSL477A	1.51E-07	1.44E-07	4.53E-07	ZSL238	4.40E-08	0	0	0	N056A	5.86E-06	3.50E-07	9.41E-07	0	ZSL-123	1.05E-07	0	1.50E-07
N1308A	0	0	0	ZSL239	5.89E-07	2.41E-07	0	0	N062A	5.45E-08	0	0	0	ZSL-127	4.03E-08	0	8.63E-08
N1268A	4.04E-08	0	8.66E-08	ZSL240	1.19E-07	0	0	0	N064A	0	0	0	0	ZSL-128	2.09E-06	3.91E-08	1.75E-07
N1282A	0	0	0	ZSL248	1.11E-07	3.54E-08	3.17E-07	0	N066A	9.75E-08	0	1.04E-07	1.89E-07	ZSL-129	1.89E-07	9.02E-08	2.02E-07
N1283A	4.63E-08	0	0	ZSL252	0	0	0	0	N074A	0	0	1.12E-07	0	ZSL-131	0	0	1.13E-07
N1284A	4.04E-08	0	0	ZSL253	4.19E-08	0	0	0	N079A	0	0	1.04E-07	0	ZSL-132	0	1.02E-07	2.28E-07
N1286A	1.01E-07	1.29E-07	0	ZSL254	4.53E-07	2.71E-07	0	0	N082A	6.76E-08	0	0	2.60E-07	ZSL-133	2.60E-07	0	3.34E-07
N1290A	3.46E-08	6.62E-08	7.41E-08	ZSL261	0	0	0	0	N083A	0	0	1.29E-07	3.70E-07	ZSL-137	3.70E-07	3.54E-07	1.93E-06
ZSL495A	2.66E-07	1.27E-07	5.69E-07	ZSL265	9.83E-08	4.70E-08	0	0	N084A	1.40E-07	0	0	2.15E-07	ZSL-138	2.15E-07	1.54E-07	2.31E-07
ZSL497A	7.98E-08	0	3.42E-07	ZSL266	7.19E-08	0	2.31E-07	0	N085A	0	0	0	6.77E-07	ZSL-146	6.77E-07	2.29E-07	4.27E-07
ZSL501A	1.77E-06	7.90E-07	9.26E-07	ZSL267	4.41E-08	0	1.89E-07	0	N086A	0	0	0	3.47E-07	ZSL-151	3.47E-07	1.42E-07	3.19E-07
ZSL505A	0	0	0	ZSL270	0	0	0	0	N087A	0	0	0	5.03E-06	ZSL-153	5.03E-06	1.39E-06	8.74E-07
ZSL510A	1.82E-07	3.48E-08	0	ZSL271	0	0	0	0	N088A	4.69E-08	0	0	2.15E-06	ZSL-155	2.15E-06	3.71E-07	6.39E-08
ZSL515A	3.05E-07	0	7.27E-08	ZSL272	0	3.89E-08	0	0	N089A	0	0	0	1.08E-07	ZSL_107A	1.08E-07	1.38E-07	3.86E-07
ZSL506A	4.30E-08	0	0	ZSL273	0	0	0	0	N103A	0	0	0	0	ZSL_110A	0	0	0

ZSL512A	1.07E-06	1.19E-06	3.68E-07	ZSL275	0	0	0	0	0	N109A	0	ZSL_112A	3.32E-08	3.18E-08	1.42E-07
ZSL516A	0	0	1.13E-06	ZSL277	8.51E-08	0	9.11E-08	1.00E-07	0	N110A	7.16E-08	ZSL_130A	6.78E-07	4.63E-08	9.33E-07
ZSL517A	3.89E-08	0	8.33E-08	ZSL280	2.39E-07	1.14E-07	0	0	0	N111A	0	ZSL_135A	9.32E-08	0	9.98E-08
ZSL520A	5.93E-07	1.03E-07	1.15E-07	ZSL281	4.54E-08	0	0	0	0	N115A	0	ZSL_139A	8.07E-07	0	0
ZSL524A	1.62E-06	4.18E-07	0	ZSL282	1.12E-06	4.37E-07	4.45E-07	3.67E-08	0	N116A	0	ZSL_145A	6.07E-07	1.29E-07	4.33E-06
ZSL525A	5.06E-08	0	2.17E-07	ZSL283	3.71E-08	7.11E-08	0	0	0	N117A	0	ZSL_15A	6.35E-07	1.74E-07	7.78E-07
ZSL527A	4.68E-08	0	0	ZSL289	6.30E-07	7.09E-08	1.59E-07	0	0	N118A	0	ZSL_30A	6.61E-07	4.97E-07	5.06E-07
ZSL529A	3.88E-08	0	8.31E-08	ZSL290	4.40E-08	0	0	3.04E-08	0	N121A	0	ZSL_36A	0	6.54E-08	1.46E-07
ZSL530A	1.18E-07	3.77E-08	8.45E-08	ZSL291	2.66E-07	1.82E-07	0	9.13E-08	0	N123A	0	ZSL_56A	1.71E-07	4.09E-08	0
ZSL532A	1.40E-06	5.34E-07	9.97E-08	ZSL292	0	0	0	0	0	N124A	0	ZSL_58A	3.65E-08	3.49E-08	7.81E-08
N1264A	0	0	2.40E-07	ZSL294	3.57E-08	0	7.64E-08	1.21E-07	0	N125A	0	ZSL_67A	1.83E-07	1.46E-07	1.96E-07
ZSL444A	0	0	2.40E-07	ZSL297	3.92E-08	0	0	0	0	N127A	0	ZSL_6A	7.60E-07	2.18E-07	4.88E-07
ZSL496A	0	4.54E-08	2.03E-07	ZSL300	5.94E-07	1.90E-07	0	3.42E-08	6.54E-08	N128A	0	ZSL_82A	3.84E-08	0	0
ZSL431A	0	0	0	ZSL301	1.23E-07	3.93E-08	0	3.93E-08	3.76E-08	N129A	0	ZSL_96A	1.19E-07	7.60E-08	8.51E-08
ZSL432A	3.67E-08	3.51E-08	2.36E-07												

Table 5 Probability of ACVD calculated by the 3 genes respectively on each of the 405 samples
(Enrich: 1:ACVD; 0:control, if the probability ≥ 0.5 , the subject is at risk of ACVD)

Samples' Name	Enrich	gene_3050214_Probability	gene_2841974_Probability	gene_6560409_Probability	3 genes_Probability
N1260A	0	0	0	0	0
N1261A	0	0	0.28	0	0.29
N1262A	0	0.05	0	0	0.14
N1263A	0	0.01	0.91	0.71	0.49
N1265A	0	0	0	0	0
N1266A	0	0.95	1	0	0.74
N1267A	0	0.14	0	0	0.32
N1269A	0	1	0.76	0.4	0.93
N1270A	0	0	0.99	0	0.55
N1271A	0	0	0	0	0
N1272A	0	0	0	0	0
N1273A	0	0.05	0	0	0.12
N1274A	0	0	0	0	0
N1275A	0	0	0	0.26	0.24
N1276A	0	0.1	0.28	0.99	0.88
N1277A	0	0	0.96	1	0.76
N1279A	0	0.78	0	0	0.46
N1280A	0	0.87	0	0	0.07
N1285A	0	0.35	0.99	0	0.76
N1287A	0	0.3	0	0	0.09
N1288A	0	0.04	0.31	0	0.32
N1291A	0	0	0.9	0	0.34
N1292A	0	0.11	0.78	0	0.79
N1293A	0	0	0	0	0
N1294A	0	0	0	0	0
N1295A	0	0	1	0.73	0.75
N1296A	0	0	0	0.98	0.31
N1297A	0	0.25	0.34	0.88	0.57
N1298A	0	0	0	0	0
N1299A	0	0.28	0	0	0.02
N1300A	0	0.01	0	0.38	0.56
N1301A	0	0	0.29	0	0.11
N1302A	0	0.09	0	0	0.12
N1307A	0	0.67	0	0	0.19
N1309A	0	0	0	0.26	0.23
ZSL402A	1	0	0	0.97	0.12
ZSL399A	1	0.96	1	0	0.65
ZSL403A	1	0.75	0	0.34	0.73
ZSL404A	1	0	0	0	0

ZSL405A	1	1	0.77	1	1
ZSL408A	1	0.63	0.7	0.36	0.61
ZSL409A	1	0.77	1	0.74	0.82
ZSL401A	1	0.35	0.32	0.73	0.86
ZSL415A	1	0.9	0.98	0	0.72
ZSL416A	1	0.38	0.99	0.39	0.88
ZSL417A	1	1	0.36	0.99	0.81
ZSL422A	1	0	0	0	0.18
ZSL429A	1	0	0.76	0.97	0.53
ZSL430A	1	1	0.77	1	1
ZSL433A	1	0	0.76	1	0.85
ZSL435A	1	0	0.89	0.31	0.33
ZSL436A	1	0.99	0.94	0.98	0.95
ZSL437A	1	1	0.13	0.76	0.84
ZSL439A	1	0	0	0	0
ZSL446A	1	0.64	0	1	0.83
ZSL450A	1	0.35	0.37	0.12	0.65
ZSL452A	1	1	1	0.99	0.89
ZSL453A	1	0.63	0	0.14	0.33
ZSL454A	1	0.95	1	0	0.75
ZSL466A	1	0	0	0	0
ZSL467A	1	0.99	0	0	0.33
ZSL468A	1	0	0	0.9	0.19
ZSL475A	1	0.97	0.99	0	0.44
ZSL478A	1	0.14	0.28	0.86	0.86
ZSL479A	1	1	1	0.13	0.9
ZSL480A	1	1	0.36	0	0.73
ZSL482A	1	0.62	0	0.91	0.62
ZSL487A	1	0	0	0	0
ZSL488A	1	0.93	1	0	0.67
ZSL490A	1	0.97	0	0	0.36
ZSL492A	1	0	0	0.85	0.11
ZSL503A	1	0.86	0.91	0	0.48
ZSL477A	1	0.26	0.92	1	0.95
N1308A	0	0	0	0	0
N1268A	0	0.14	0	1	0.78
N1282A	0	0	0	0	0
N1283A	0	0.9	0	0	0.42
N1284A	0	0.14	0	0	0.16
N1286A	0	0.09	0.87	0	0.59
N1290A	0	0.09	0.34	0.25	0.55
ZSL495A	1	0.96	0.64	1	0.73
ZSL497A	1	0.95	0	1	0.88

ZSL501A	1	0.35	0.75	0.73	0.69
ZSL505A	1	0	0	0	0
ZSL510A	1	0.73	0.96	0	0.6
ZSL515A	1	0.34	0	0.31	0.36
ZSL506A	1	0.73	0	0	0.18
ZSL512A	1	1	0.98	1	0.96
ZSL516A	1	0	0	1	0.4
ZSL517A	1	0.75	0	0.35	0.66
ZSL520A	1	0.97	0.91	0.3	0.88
ZSL524A	1	0.94	1	0	0.83
ZSL525A	1	0.65	0	1	0.87
ZSL527A	1	0.02	0	0	0.2
ZSL529A	1	0.76	0	0.74	0.66
ZSL530A	1	0.97	0.67	0.34	0.93
ZSL532A	1	1	0.99	0.97	0.97
N1264A	0	0	0	0.7	0.62
ZSL444A	1	0	0	0.06	0.29
ZSL496A	1	0	0.13	1	0.84
ZSL431A	1	0	0	0	0
ZSL432A	1	0.09	0.74	0.72	0.72
N167	0	0	1	0	0.48
N168	0	0.02	0.75	0	0.43
N170	0	0	0	0	0
N171	0	0	0	0.38	0.23
N172	0	0.63	0	0.67	0.45
N173	0	0	0.34	0	0.32
N174	0	1	0.66	0.78	0.9
N175	0	0	0	0	0
N176	0	1	0	0	0.29
N182	0	0	0	0.95	0.29
N184	0	0.11	0	0	0.33
N185	0	0.87	0	0	0.11
N186	0	0.3	0	0.14	0.61
N187	0	0	0	0	0
N188	0	0	0	0	0
N189	0	0.05	0.7	0.11	0.49
N190	0	0	0	0	0
N191	0	0	0	0	0
N193	0	0.12	0.98	0	0.36
N194	0	0	0	0	0
N195	0	0	0.77	0	0.66
N197	0	0	0	0.96	0.26
N198	0	1	0	0	0.23

N199	0	0	0	0	0
N200	0	0.97	0	0.94	0.73
N201	0	0	0	0	0
N202	0	0	0.99	0.15	0.6
N203	0	0.37	0	0	0.23
N204	0	0	0	0	0
N206	0	0.05	0.11	0.1	0.75
N207	0	0	0	0	0
N209	0	0.15	0	0	0.32
N210	0	0	0	0	0
N213	0	0.89	0	0	0.14
N214	0	0	0.99	0	0.58
N215	0	0	0	0	0
N218	0	0.91	0	0.35	0.76
N220	0	0	0	0	0
N221	0	0	0	0	0
N222	0	0.02	0.15	0	0.43
N223	0	0.98	0	0	0.29
N224	0	0	0	0	0
N225	0	0	0	0.99	0.23
N226	0	0	0	0	0
N227	0	0.11	0	0.94	0.49
N231	0	1	1	1	1
N232	0	0	0	0.75	0.74
N233	0	0.02	0	0	0.23
N234	0	1	1	1	1
N235	0	0	0	0	0
N236	0	0	0	0	0
N237	0	0.78	0.94	0	0.79
N239	0	0	0	0	0
N240	0	0.12	0	0	0.01
N241	0	0	0	0.34	0.23
N242	0	0	0	0	0
N244	0	0.05	0	0	0.01
N245	0	0	0	1	0.31
N247	0	0.92	0	1	0.91
N248	0	0.66	0	0	0.07
N249	0	0	0	0	0
N251	0	0	0	0	0
N267	0	0.78	0	0	0.18
N268	0	0.02	0	0	0.23
N281	0	0	0	0	0
N287	0	0.35	1	0	0.75

N288	0	0	0	0	0
ZSL229	1	0.96	0	0	0.23
ZSL234	1	0.88	0	0.97	0.39
ZSL235	1	0	0	0	0
ZSL236	1	1	0	0	0.18
ZSL237	1	0	0.75	0	0.57
ZSL238	1	0.97	0	0	0.35
ZSL239	1	0.96	1	0	0.81
ZSL240	1	0.96	0	0	0.1
ZSL248	1	0.3	0.33	0.81	0.78
ZSL252	1	0	0	0	0
ZSL253	1	0.78	0	0	0.16
ZSL254	1	0.76	1	0	0.78
ZSL261	1	0	0	0	0
ZSL265	1	0.11	0.98	0	0.56
ZSL266	1	0.87	0	1	0.77
ZSL267	1	0.99	0	1	0.93
ZSL270	1	0	0	0	0
ZSL271	1	0	0	0	0
ZSL272	1	0	0.38	0	0.23
ZSL273	1	0	0	0	0
ZSL275	1	0	0	0	0
ZSL277	1	1	0	0.97	0.65
ZSL280	1	0	0.01	0	0.47
ZSL281	1	0.74	0	0	0.31
ZSL282	1	1	1	1	1
ZSL283	1	0.09	0.65	0	0.11
ZSL289	1	0.98	0.66	0.14	0.76
ZSL290	1	0.97	0	0	0.34
ZSL291	1	0.96	0.98	0	0.54
ZSL292	1	0	0	0	0
ZSL294	1	0.05	0	0.92	0.36
ZSL297	1	0.13	0	0	0.1
ZSL300	1	0.97	0.36	0	0.73
ZSL301	1	0.12	0.91	0	0.64
ZSL302	1	0.62	1	1	0.94
ZSL306	1	0.93	0.69	0	0.56
ZSL308	1	0	0	0.72	0.12
ZSL310	1	0.65	0.06	1	0.45
ZSL312	1	0	0	0.74	0.12
ZSL313	1	0.78	0.77	0.35	0.85
ZSL314	1	0.36	1	1	0.84
ZSL315	1	0	0.37	0	0.44

ZSL317	1	0.88	0	0	0.18
ZSL318	1	1	0.96	0	0.89
ZSL319	1	0	0	0	0
ZSL322	1	0.1	0.85	0.97	0.8
ZSL325	1	0.3	0	0	0.12
ZSL330	1	1	0.96	1	0.95
ZSL332	1	0.35	0.29	0.96	0.95
ZSL334	1	0.35	0.88	0.91	0.9
ZSL335	1	0	0	0	0
ZSL336	1	0	0	0	0
ZSL338	1	0.97	1	0	0.7
ZSL339	1	0.92	0.63	0.91	0.86
ZSL340	1	0.9	0.9	0	0.75
ZSL341A	1	0.14	0	0	0.01
ZSL343	1	0.76	1	0.97	0.9
ZSL349	1	0.12	0	1	0.9
ZSL350	1	0	0	0.31	0.14
ZSL353	1	0.39	0.12	0.99	0.69
ZSL359	1	0.73	0	1	0.86
ZSL365	1	0.76	0.35	1	0.97
ZSL368	1	0.99	0	1	0.92
ZSL369	1	0.87	1	0	0.58
ZSL370	1	0	0	0	0
ZSL374	1	0.35	1	1	0.91
ZSL375	1	0	0	0	0
ZSL376	1	0	0	0	0
ZSL377	1	1	0.77	0	0.73
ZSL378	1	1	0.88	1	0.98
ZSL379	1	0	0	0	0
N001A	0	0	0.02	0	0.28
N002A	0	0	0	0	0
N003A	0	0	0	0	0
N005A	0	0	0	0.09	0.12
N006A	0	0	0	0	0
N009A	0	0	0	0	0
N011A	0	0	0	0	0
N013A	0	1	1	0	0.94
N017A	0	0	0	0	0
N020A	0	0	0	0	0
N021A	0	0	0	0	0
N022A	0	0	0	0.88	0.13
N024A	0	0.77	0	0	0.15
N025A	0	0	0	0.92	0.24

N026A	0	0	0	0	0
N027A	0	0.02	0	0	0.24
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N029A	0	0	0	0	0
N031A	0	0	0	0	0
N032A	0	0.68	0	0	0.32
N033A	0	0	0	0	0
N034A	0	0	0	0	0
N035A	0	0	0	0	0
N037A	0	0	0	0	0
N038A	0	0	0	0	0
N039A	0	0	0	0	0
N040A	0	0	0	0	0
N042A	0	1	0	0	0.28
N043A	0	0	0	0.76	0.32
N044A	0	0	0	0	0
N046A	0	0	0.02	0	0.3
N047A	0	0	0	0	0
N049A	0	1	0	0	0.34
N051A	0	0	0	0	0
N052A	0	0	0	0	0
N056A	0	1	0.97	0.98	0.94
N062A	0	1	0	0	0.36
N064A	0	0	0	0	0
N066A	0	0.88	0	0.71	0.76
N074A	0	0	0	0.95	0.32
N079A	0	0	0	0.15	0.28
N082A	0	0.25	0	0	0.37
N083A	0	0	0	0.26	0.13
N084A	0	0.97	0	0	0.15
N085A	0	0	0	0	0
N086A	0	0	0	0	0
N087A	0	0	0	0	0
N088A	0	0.64	0	0	0.37
N089A	0	0	0	0	0
N103A	0	0	0	0	0
N109A	0	0	0	0	0
N110A	0	0.26	0	0.97	0.51
N111A	0	0	0	0	0
N115A	0	0	0	0	0
N116A	0	0.68	0	0	0.02
N117A	0	0	0	0	0
N118A	0	0	0	0	0

N121A	0	0	0.72	0	0.16
N123A	0	0.77	0	0	0.26
N124A	0	0	0	0	0
N125A	0	0.99	0	0	0.2
N127A	0	0	0	0	0
N128A	0	0.12	0.85	0	0.31
N129A	0	0.77	0.9	0	0.45
N135A	0	0.02	0.99	0.77	0.74
N136A	0	0.09	0.13	0	0.64
N137A	0	0	0	0	0
N140A	0	0.36	0	0.11	0.31
N142A	0	0	0	0	0
N143A	0	0.15	0	0	0.31
N147A	0	1	1	0	0.9
N148A	0	0	0	0	0
N149A	0	0.01	0.05	0	0.35
N150A	0	0	0	0	0
N152A	0	0.63	0.12	0	0.59
N153A	0	0	0	0	0
N155A	0	0.76	0	0	0.25
ZSL.004	1	0.97	0.98	1	0.96
ZSL.007	1	0.77	1	0.38	0.93
ZSL.010	1	0.93	1	1	0.99
ZSL.011	1	0.98	0	0.13	0.74
ZSL.019	1	0.9	0.99	0.77	0.87
ZSL.022	1	1	0	0.87	0.71
ZSL.023	1	0.97	0	0	0.36
ZSL.024	1	0.38	0.89	0.67	0.66
ZSL.025	1	0.78	0.97	0.39	0.8
ZSL.028	1	0.92	1	1	0.97
ZSL.029	1	0	0	0	0
ZSL.031	1	1	0.92	1	0.99
ZSL.033	1	0.92	0	1	0.8
ZSL.035	1	0.97	1	0.91	0.93
ZSL.037	1	0.91	0	0	0.13
ZSL.040	1	0.37	0.98	0.1	0.68
ZSL.042	1	1	0.9	0.96	0.81
ZSL.044	1	1	0.92	0.71	0.96
ZSL.045	1	0.99	1	1	0.91
ZSL.046	1	0.3	0	1	0.73
ZSL.047	1	1	1	0.76	0.91
ZSL.050	1	0	0.99	0	0.26
ZSL.052	1	1	1	0.99	0.95

ZSL.053	1	1	1	1	1
ZSL.055	1	1	0.77	1	1
ZSL.057	1	0	0	0.65	0.1
ZSL.062	1	1	0.96	1	0.89
ZSL.063	1	0.27	0.12	0.1	0.72
ZSL.065	1	0.76	0.98	0	0.57
ZSL.066	1	0.72	0.75	0	0.59
ZSL.068	1	1	1	0.36	0.99
ZSL.071	1	0.95	0.66	0.91	0.77
ZSL.073	1	0.93	0.77	0.98	0.91
ZSL.074	1	0.97	1	1	0.88
ZSL.076	1	0.77	1	1	0.91
ZSL.077	1	0.36	0	0	0.22
ZSL.079	1	0.36	0.99	1	0.93
ZSL.080	1	0	0	1	0.39
ZSL.083	1	0.99	1	1	0.95
ZSL.084	1	0.73	0.88	0.92	0.95
ZSL.085	1	0.99	1	0.35	0.8
ZSL.086	1	0.97	1	1	0.95
ZSL.088	1	1	1	0.75	0.94
ZSL.089	1	1	0.96	1	0.99
ZSL.091	1	0.76	0.75	1	0.99
ZSL.092	1	0.91	0.91	1	0.72
ZSL.094	1	0	1	0	0.43
ZSL.095	1	1	1	1	1
ZSL.097	1	0.99	1	0	0.84
ZSL.098	1	0.97	0.91	0	0.65
ZSL.099	1	0	0.98	0	0.41
ZSL.103	1	0	0	0	0
ZSL.105	1	0.97	0.92	1	0.98
ZSL.111	1	0.36	0.98	1	0.89
ZSL.113	1	1	1	0.72	0.98
ZSL.115	1	0	0.35	0	0.53
ZSL.116	1	1	1	1	0.98
ZSL.120	1	0.4	0.73	0.11	0.91
ZSL.122	1	0.93	1	1	0.99
ZSL.123	1	0.04	0	0.73	0.31
ZSL.127	1	0.61	0	0.91	0.62
ZSL.128	1	1	0.91	0.78	0.71
ZSL.129	1	0.79	1	1	0.99
ZSL.131	1	0	0	0.7	0.11
ZSL.132	1	0	0.92	1	0.76
ZSL.133	1	0.79	0	1	0.79

ZSL.137	1	0.77	0.88	0.92	0.87
ZSL.138	1	0.78	0.37	1	0.94
ZSL.146	1	1	1	1	1
ZSL.151	1	0.98	0.92	0.37	0.94
ZSL.153	1	0.97	0.98	0.12	0.86
ZSL.155	1	1	0.35	1	0.87
ZSL_107A	1	0.97	0.78	1	0.96
ZSL_110A	1	0	0	0	0
ZSL_112A	1	0.62	0.27	0.09	0.44
ZSL_130A	1	1	0.98	0.71	0.86
ZSL_135A	1	0.14	0	0.97	0.44
ZSL_139A	1	1	0	0	0.43
ZSL_145A	1	0.38	0.22	1	0.88
ZSL_15A	1	1	1	0.91	0.96
ZSL_30A	1	1	1	1	1
ZSL_36A	1	0	0.21	0.31	0.36
ZSL_56A	1	0.93	0.76	0	0.69
ZSL_58A	1	0.65	0.91	0.99	0.58
ZSL_67A	1	0.75	0.97	1	0.93
ZSL_6A	1	1	1	1	1
ZSL_82A	1	0.33	0	0	0.06
ZSL_96A	1	0.96	0.89	0.91	0.92

Table 6 : Probability of ACVD calculated by each of the 3 genes on 405 samples (70% of the 405 samples are training set and 30% of the 405 samples are test set)

Gene ID	Training AUC	Test AUC	ALL AUC	Training error	Test error	All error
3050214	0.718 (0.659,0.777)	0.744 (0.658,0.831)	0.75 (0.704,0.795)	0.317	0.314	0.309
2841974	0.72 (0.666,0.773)	0.705 (0.621,0.789)	0.722 (0.678,0.766)	0.335	0.322	0.328
6560409	0.703 (0.648,0.758)	0.754 (0.679,0.83)	0.726 (0.682,0.77)	0.373	0.306	0.333

Table 7: Probability of ACVD calculated by the combination of 3 genes on 405 samples (70% of the 405 samples are training set and 30% of the 405 samples are test set)

	Training AUC	Test AUC	ALL AUC	Training error	Test error	All
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						error
3 genes set	0.796 (0.745,0.848)	0.761 (0.674,0.847)	0.781 (0.736,0.825)	0.278	0.322	0.277

In addition, terms such as "first" and "second" are used herein for purposes of description and are not intended to indicate or imply relative importance or significance.

Reference throughout this specification to "an embodiment," "some embodiments," "one embodiment", "another example," "an example," "a specific examples," or "some examples," means that a particular feature, structure, material, or characteristic described in connection with the embodiment or example is included in at least one embodiment or example of the present disclosure. Thus, the appearances of the phrases such as "in some embodiments," "in one embodiment", "in an embodiment", "in another example, "in an example," "in a specific examples," or "in some examples," in various places throughout this specification are not necessarily referring to the same embodiment or example of the present disclosure. Furthermore, the particular features, structures, materials, or characteristics may be combined in any suitable manner in one or more embodiments or examples.

Although explanatory embodiments have been shown and described, it would be appreciated by those skilled in the art that the above embodiments cannot be construed to limit the present disclosure, and changes, alternatives, and modifications can be made in the embodiments without departing from spirit, principles and scope of the present disclosure.

Claims

What is claimed is:

1. A biomarker for predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof, comprising at least one nucleotide sequence shown as SEQ ID NO: 1~3.
2. Use of the biomarker of claim 1 in predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof.
3. A method of predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof, comprising:
 - (1) determining the relative abundance of the biomarker of claim 1 in the samples of the subject to be tested; and
 - (2) predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof based on the relative abundance of the biomarker of claim 1.
4. The method of claim 3, wherein step (2) comprising comparing the relative abundance of the biomarker with a preset cutoff value.
5. The method of claim 3, wherein the samples are gut microbiota.
6. The method of claim 5, wherein the gut microbiota is obtained from the fecal of the subject to be tested.
7. The method of claim 4, wherein the preset cutoff value is obtained based on a plurality of control samples with known conditions of atherosclerotic cardiovascular disease.
8. The method of claim 7, wherein the preset cutoff value is about at least 0.5.
9. The method of claim 3, wherein the relative abundance of the biomarker is obtained by means of sequencing.
10. A kit for predicting the risk of atherosclerotic cardiovascular disease or related disorder thereof, comprising:
 - reagent configured to detecting the biomarker of claim 1.
11. The kit of claim 10, wherein the reagent is in form of at least one of the probe, primer, gene chip and antibody.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/095350

A. CLASSIFICATION OF SUBJECT MATTER

C12Q 1/68(2018.01)i; C12N 15/11(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DWPI, SIPOABS, CPRSABS, CPEA, TWABS, CNABS, JPABS, MOABS, HKABS, CNMED, TWMED, KRABS, AUABS, DEABS, RUABS, SGABS, ILABS, FRABS, LEXIS, CNKI, ISI Web of Science, Elsevier Science, GenBank:gut microbiota, microbiome, atherosclerotic, cardiovascular, biomarker, risk, search on SEQ ID NOs: 1-3

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Yamashita T et al. "Intestinal Immunity and Gut Microbiota as Therapeutic Targets for Preventing Atherosclerotic Cardiovascular Diseases" <i>Circulation Journal</i> , Vol. 79, No. 9, 22 July 2015 (2015-07-22), pages 1882-1890	1-11
A	Whitney MF et al. "The Role of Gut-Microbiota in Atherosclerosis and Cardiovascular Disease" <i>University of Toronto Medical Journal</i> , Vol. 92, No. 3, 31 May 2015 (2015-05-31), pages 44-47	1-11
A	Wang Y and Cai J. "Gut microbiota and cardiovascular disease" <i>Basic & Clinical Medicine</i> , Vol. 37, No. 5, 31 May 2017 (2017-05-31), pages 729-733	1-11
A	US 2017065637 A1 (INTERNATIONAL NUTRITION RESEARCH COMPANY) 09 March 2017 (2017-03-09) the whole document	1-11

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

12 April 2018

Date of mailing of the international search report

25 April 2018

Name and mailing address of the ISA/CN

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LI, Yang

Facsimile No. (86-10)62019451

Telephone No. (86-10)62411031

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/095350**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2017/095350

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
US	2017065637	A1	09 March 2017	None	