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The studies on the new blood biomarkers in subclinical coronary diseases in Chinese populations

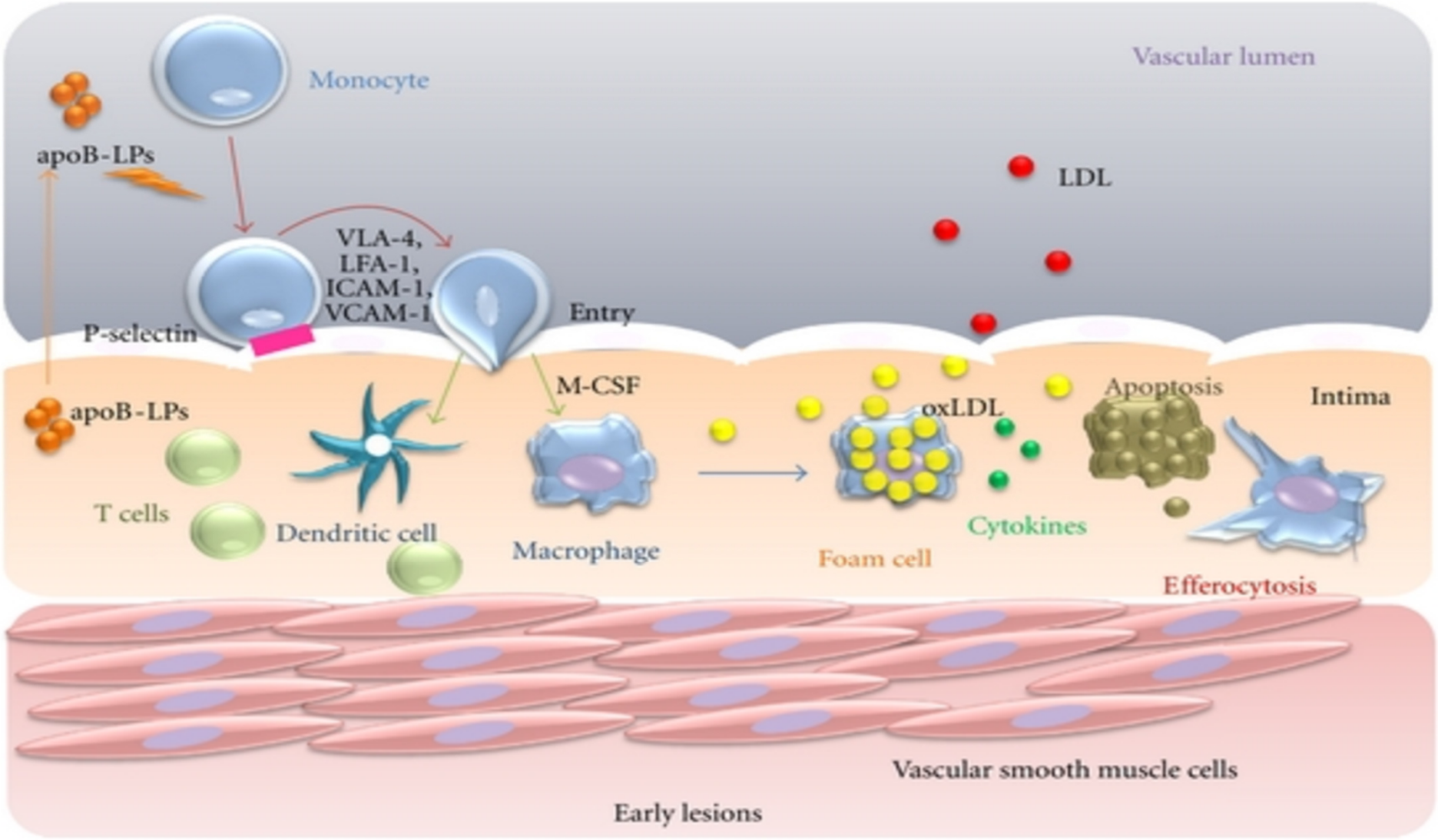
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Chinese PLA General Hospital, Beijing 100853
E-mail: tianyp61@gmail.com



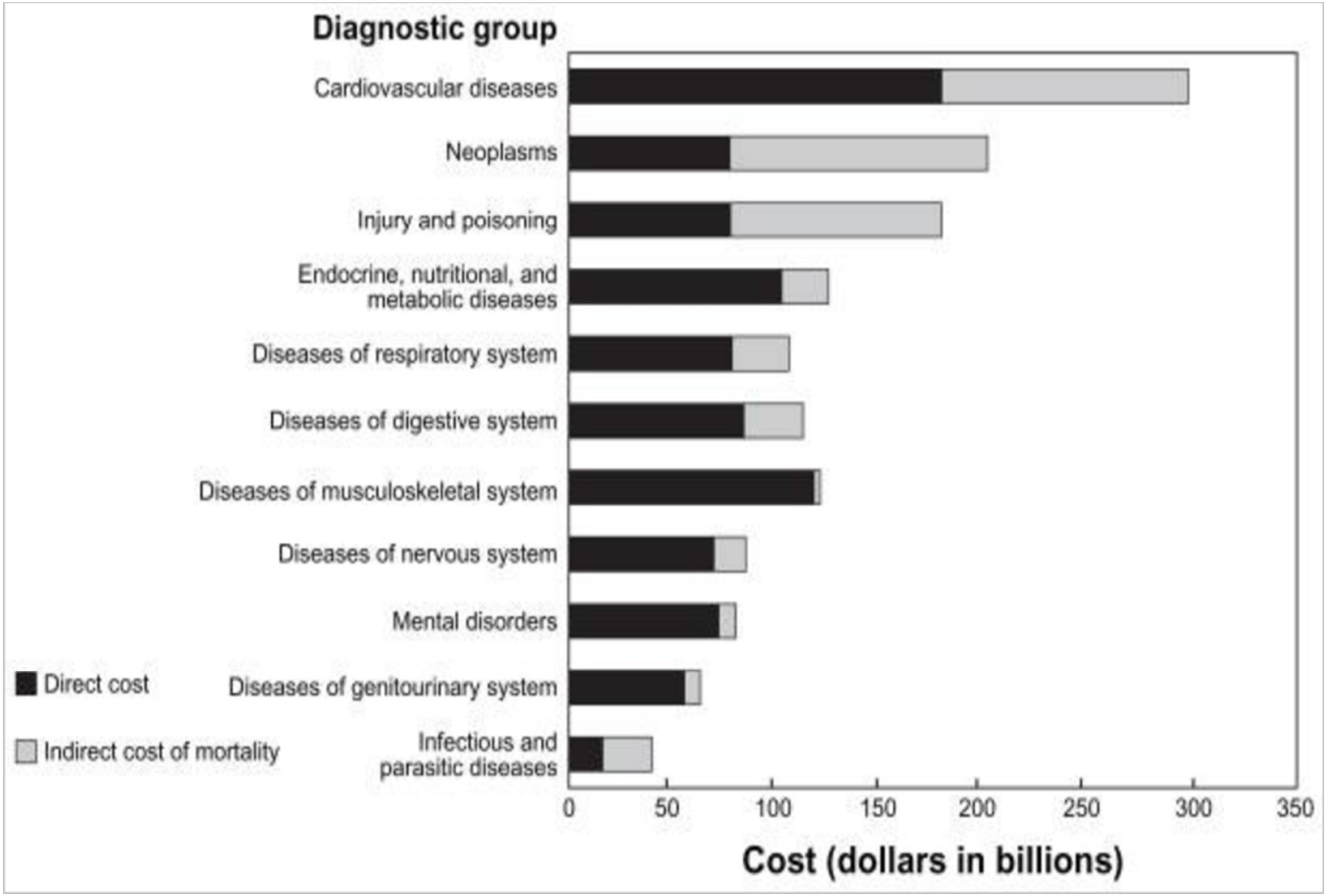
•Environment and lifestyle, their contribution to complex human diseases





The roles of M1 and M2 macrophages. Ly6C high monocytes differentiate into M1 type, classically activated macrophages that affect proteolysis and produce antibacterial products. Ly6C low monocytes differentiate into M2 type, alternatively activated macrophages that are involved in wound repair and tissue remodelling. M1 and M2 cells secrete different cytokines that function in efferocytosis and the formation of foam cells.

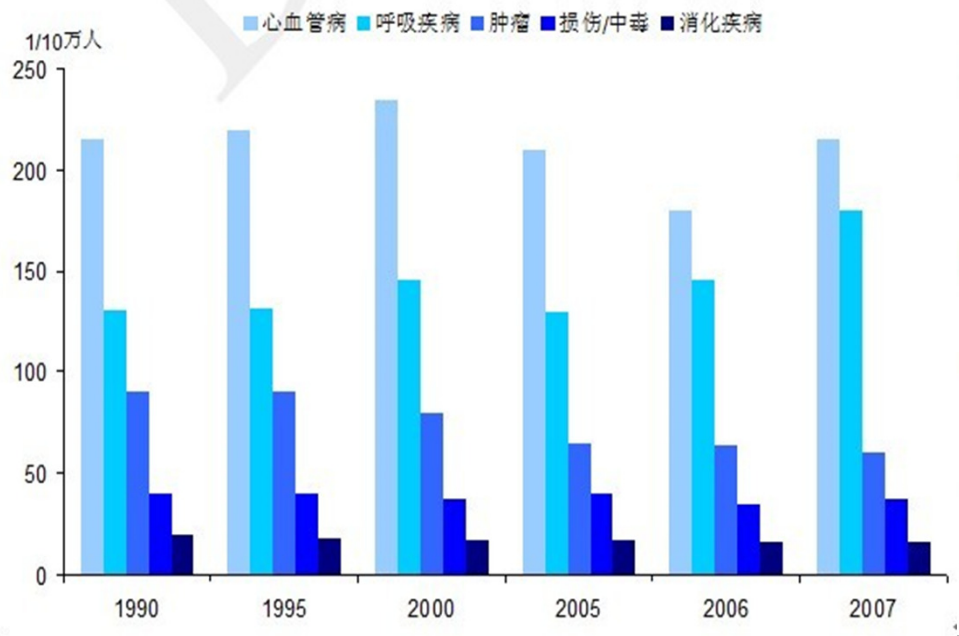
•Mediators Inflamm. 2012; 2012: 693083.



•Total economic cost of the leading diseases in the US, 2008.

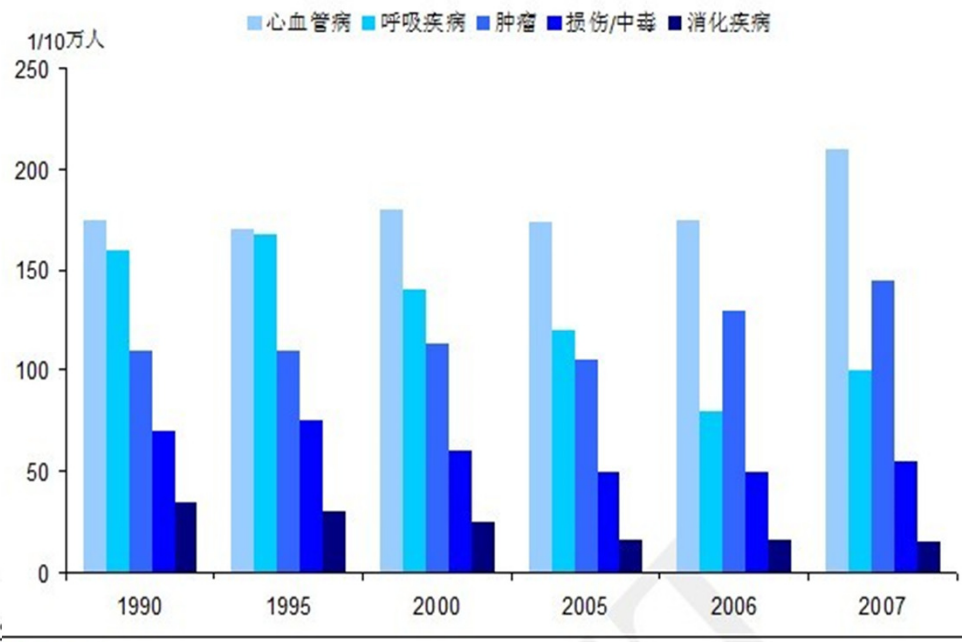


Figure 1：近 17 年中国城市居民主要疾病死亡率变化



Source：中国卫生部

Figure 2：近 17 年中国农村居民主要疾病死亡率变化



Source：中国卫生部

Biomarkers for coronary artery disease



•Cholesterol-related
•Lipoprotein(a)
•Apolipoprotein A-1
•Apolipoprotein B
•LDL particle size and number
•Triglycerides
•Cholesterol ester transfer protein
•Lipoprotein-associated phospholipase A2
•Small-dense LDL
•Paraxonase-1
•Plasma phospholipid transfer protein

•Inflammatory
•C-reactive protein/high-sensitivity C-reactive protein
•Interleukins 6, 10, 18
•Tumor necrosis factor alpha
•Intercellular adhesion molecule 1
•Myeloperoxidase
•Vascular cell adhesion molecule
•Ferritin

•Prothrombotics
•Fibrinogen
•D-dimer
•Von Willebrand factor
•Homocysteine
•Haptoglobin

•Endocrine-related
•Insulin
•Adiponectin
•Leptin
•Fasting glucose
•E-selectin
•Pro-N-terminal brain natriuretic peptide
•Chimerin
•Cystatin-C

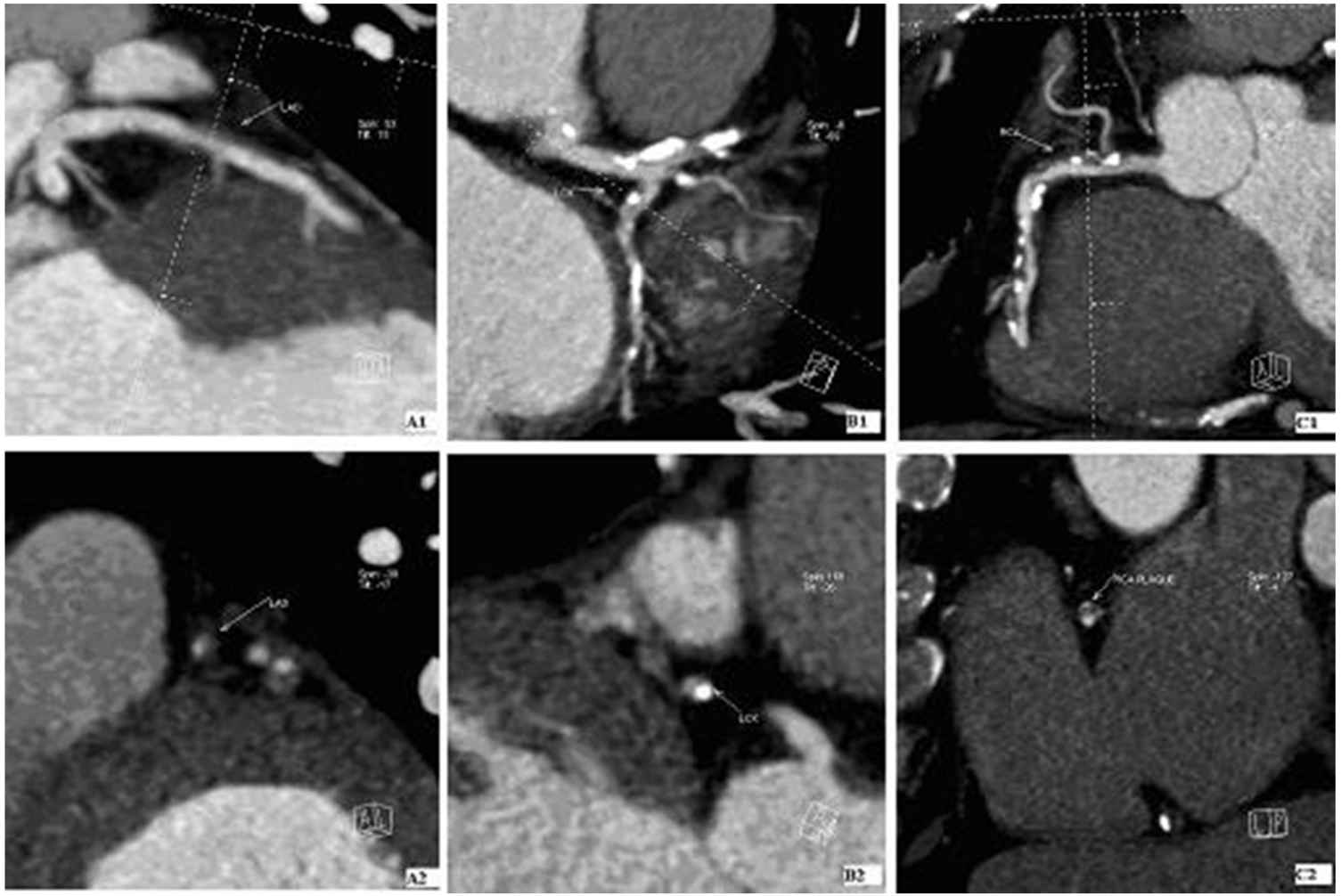
•Vascular-related
•Carotid intima-media thickness
•Coronary artery calcium score
•Ankle-brachial index

•Lifestyle
•Sedentary lifestyle
•Dietary intake

•Miscellaneous
•Sialic acid
•Des-acyl ghrelin

•Metabolic biomarkers for predicting cardiovascular disease， Vasc Health Risk Manag. 2013; 9: 37–45.

Image diagnosis



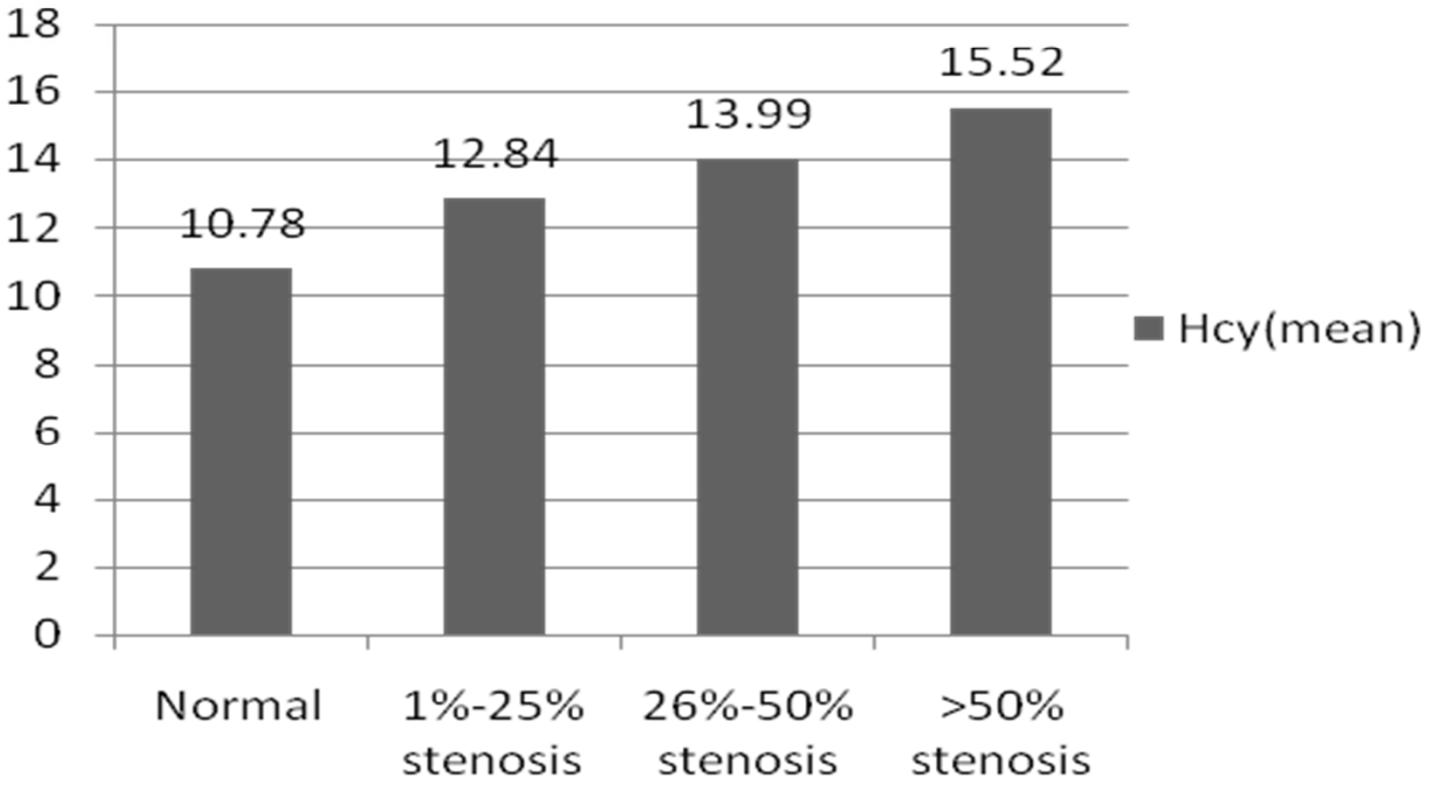
Patients



- Patients who underwent Computed tomography angiography (CTA) have been studied.
- A total of 659 were enrolled in this studies.
- All the subjects enrolled had no clinical cardiovascular disease symptoms.
- Written informed consent was obtained from participants and the ethics committee of PLA General Hospital approved present study

Demographic and clinical characteristics

	Control	1%-25% stenosis	26%-50% stenosis	>50% stenosis	P
N	235	226	127	71	
Age(years)	48.9±8.5	53.42±9.89	57.01±10.15	58.87±10.33	<0.001
Gender(male %)	75.74	79.65	73.23	78.87	0.5968
Hypertension(%)	24.68	33.19	35.43	52.11	<0.001
DM(%)	26.81	29.2	27.56	36.62	0.4431
Hyperlipidemia(%)	30.6	39.82	51.97	56.34	<0.001
Smoking(%)	20.43	26.99	29.13	36.62	0.036
Drinking (%)	21.70	23.45	23.62	28.17	0.7331
TG (mmol/L)	1.89±1.49	2.19±2.1	2.52±2.55	2.42±1.82	0.021
TC (mmol/L)	4.44±1.35	4.68±1.13	4.85±1.28	5.01±1.18	0.0013
HDL-C (mmol/L)	1.17±0.31	1.13±0.27	1.14±0.30	1.1±0.28	0.2603
LDL-C (mmol/L)	2.13±1.16	2.68±0.82	2.71±0.89	2.93±0.99	<0.001
TBIL(μmol/L)	12.16±5.84	11.8±4.78	10.45±4.89	9.98±5.12	0.0016
UA(mmol/L)	309.65±75.87	312.15±80.19	308.64±80.61	318.61±80.52	0.8290
GGT(U/L)	46.57±36.87	46.99±37.60	47.19±44.60	50.36±47.33	0.9169
Hcy(μmol/L)	10.78±4.24	12.84±6.24	13.99±5.75	15.52±9.42	<0.001



•The value of Hcy in serum and the severity of the stenosis of the trend, the serum levels of Hcy increased with the severity of stenosis

Items	1% -25% stenosis		26%-50% stenosis		>50% stenosis	
	OR (95%CI)	p value	OR (95%CI)	p value	OR (95%CI)	p value
Age	1.065(1.039-1.091)	<0.001	1.106(1.076-1.137)	<0.001	1.122(1.086-1.158)	<0.001
Hypertension	1.238(0.793-1.934)	0.347	1.338(0.789-2.268)	0.280	2.394(1.281-4.474)	0.006
Smoking	1.727(1.069-2.791)	0.026	1.775(1.006-3.132)	0.048	2.283(1.166-4.467)	0.016
TG	1.203(1.055-1.372)	0.006	1.308(1.135-1.508)	<0.001	1.283(1.087-1.515)	0.003
TC	0.928(0.769-1.121)	0.438	1.105(0.882-1.384)	0.384	1.240(0.923-1.666)	0.154
LDL-C	1.975(1.556-2.505)	<0.001	1.732(1.310-2.289)	<0.001	2.013(1.419-2.857)	<0.001
TBIL	1.008(0.971-1.046)	0.691	0.959(0.913-1.007)	0.091	0.935(0.877-0.996)	0.038
Hcy	1.082(1.033-1.132)	0.001	1.105(1.052-1.161)	<0.001	1.131(1.072-1.192)	<0.001

The relationship of risk factors and the severity of stenosis were analyzed by Logistic regression analysis

Age, smoking, serum TG, LDL-C, Hcy correlated with stenosis independently ($p<0.05$); age ($p<0.001$), blood pressure ($p=0.006$), smoking ($p=0.016$), serum TG ($p=0.003$), LDL-C ($p<0.001$), TBIL ($p=0.038$), Hcy ($p<0.001$) and severe stenosis independently



	NCP	CP	p
Items	177	247	
Age(years)	53.02±9.36	57.12±10.55	<0.001
Gender(Male%)	78.53	77.33	0.8606
Hypertension (%)	36.16	37.25	0.8988
DM (%)	32.2	28.34	0.4539
Hyperlipidemia (%)	40.68	50.2	0.0656
Smoking (%)	23.16	33.19	0.0326
Drinking (%)	22.6	25.51	0.5663
TG (mmol/L)	2.11±1.65	2.49±2.52	0.0801
TC (mmol/L)	4.64±1.18	4.89±1.19	0.0329
HDL-C (mmol/L)	1.13±0.26	1.13±0.29	0.8839
LDL-C (mmol/L)	2.57±0.82	2.91±0.94	0.0001
TBIL(μmol/L)	11.47±4.89	10.82±4.94	0.1804
UA(mmol/L)	310.44±81.44	313.43±79.53	0.7057
GGT(U/L)	46.15±39.46	48.67±42.84	0.5375
Hcy(μmol/L)	11.12±3.68	15.43±7.87	<0.001

The difference between Calcified plaque group (CP group) and non calcified plaque group (NCP group) in clinical and laboratory tests

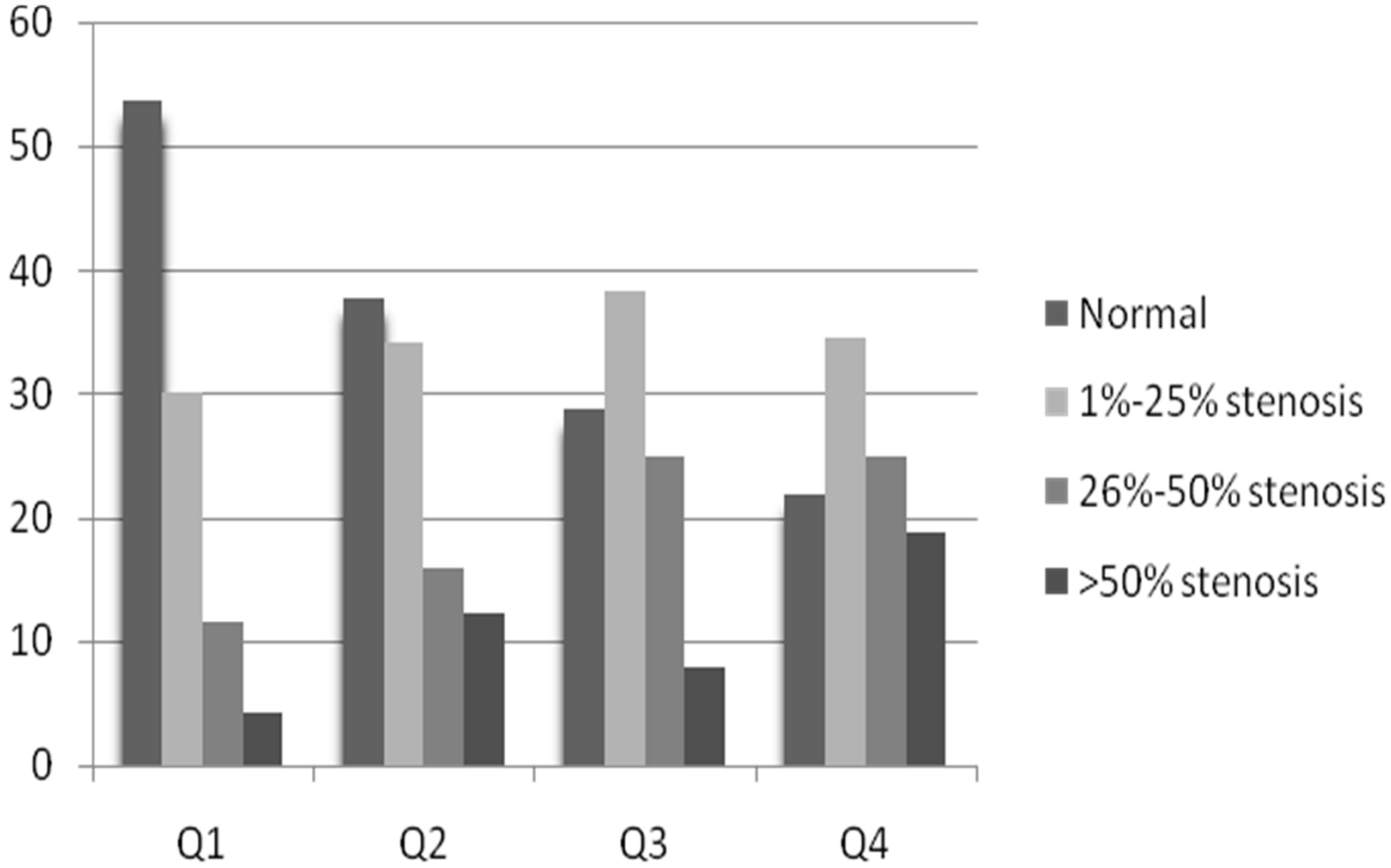
Items	calcified plaque	
	OR (95%CI)	p value
Age	1.044(1.021-1.069)	<0.001
Smoking	2.028(1.229-3.349)	0.006
TC	1.123(0.918-1.373)	0.260
LDL-C	1.534(1.165-2.02)	0.002
Hcy	1.194(1.127-1.264)	<0.001

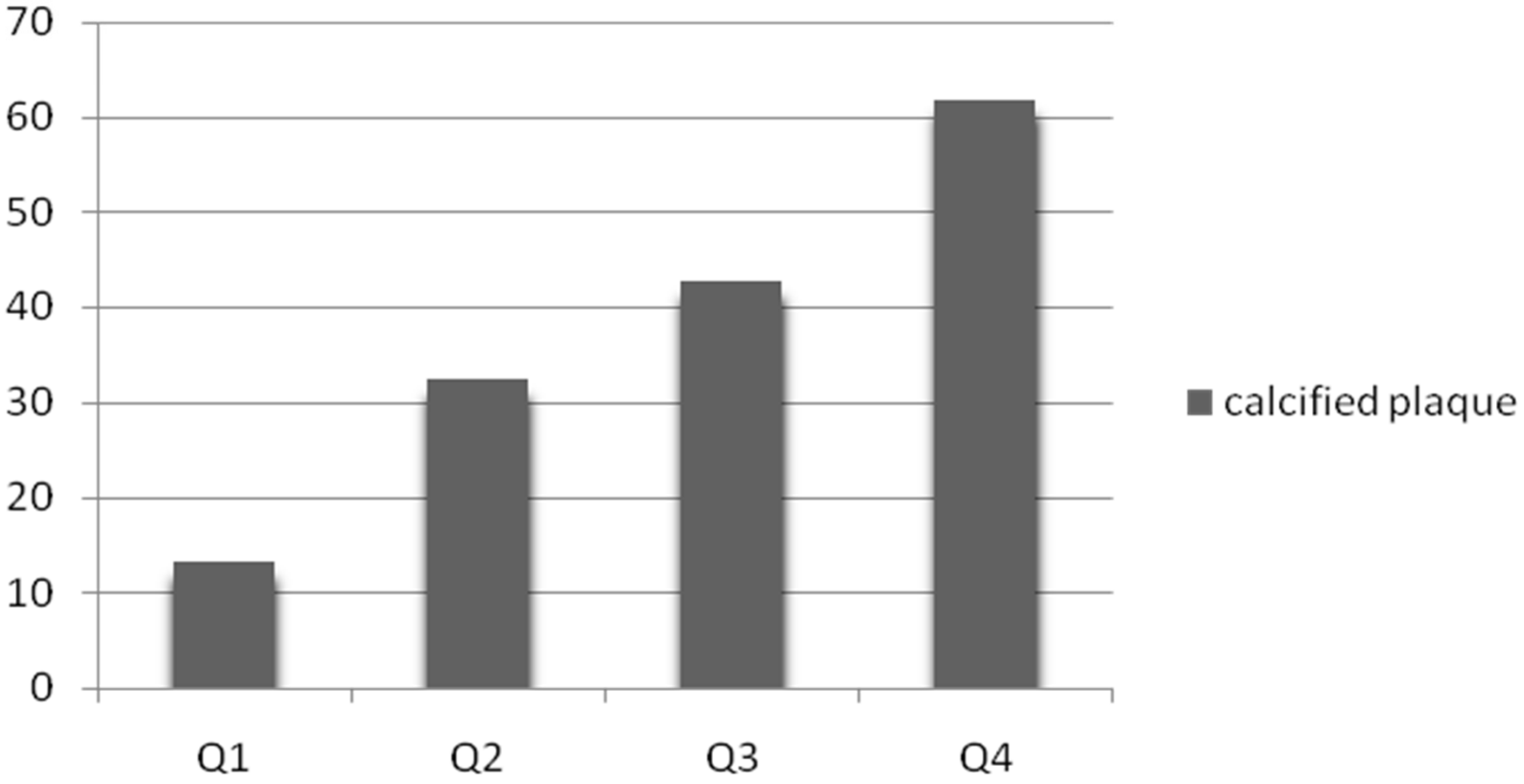
The regression analysis among cardiovascular risk factors and coronary artery calcified plaque condition showed that Hcy is the most risking factors

Quartile analysis of serum Hcy with clinical data and laboratory tests

Items	Q1(<8.79) (n=166)	Q2(8.80-11.1) (n=164)	Q3(11.17-14.6) (n=164)	Q4(>14.6) (n=165)	P
Age(years)	50.21±9.73	52.34±9.22	53.97±9.21	55.66±11.59	<0.001
Gender(male%)	77.11	77.44	79.27	73.94	0.7019
Hypertension (%)	24.7	32.32	30.49	40	0.0807
DM (%)	29.52	29.27	34.76	22.42	0.1058
Hyperlipidemia(%)	36.14	44.51	42.07	40	0.5582
Smoking (%)	23.49	22.56	24.39	26.7	0.5521
Drinking (%)	18.67	18.29	26.83	30.3	0.0148
TG (mmol/L)	2.12±2.31	2.13±2.19	2.12±1.58	2.42±2.32	0.4907
TC (mmol/L)	4.55±1.35	4.76±1.26	4.61±1.25	4.73±1.2	0.3873
HDL-C (mmol/L)	1.17±0.26	1.17±0.3	1.12±0.29	1.12±0.31	0.1820
LDL-C (mmol/L)	2.44±1.15	2.49±1.02	2.54±1.07	2.69±0.97	0.1590
TBIL(μmol/L)	11.56±5.17	11.01±5.08	12.19±5.28	10.94±5.52	0.1132
UA(mmol/L)	305.2±74.16	311.83±79.4	312.66±81.88	315.45±78.6	0.6782
GGT(U/L)	46.40±42.26	45.31±35.79	45.9±37.49	51.43±43.89	0.4582
Hcy(μmol/L)	7.41±1.06	10.04±0.69	12.69±1.08	20.32±7.45	<0.001
No plaque(%)	53.61	37.8	28.66	21.82	<0.001
Mild stenosis(%)	30.12	34.15	38.41	34.55	0.4508
Moderate stenosis(%)	11.45	15.85	25	24.85	0.0023
Severe stenosis(%)	4.22	12.20	7.93	18.79	0.0002
The calcified plaques(%)	13.25	32.32	42.69	61.82	P<0.001

Quartile analysis of serum Hcy with severity of stenosis





According to the quartile analysis of Hcy4, there were a positive correlation between serum Hcy and the number patients with calcified plaque

Logistic regression analysis showed that 15 mol/L of Hcy is the best cutoff value for differentiate severe stenosis(>50%) and calcified plaques

Item	Stenosis(>50%)	
	OR (95%CI)	p value
Hcy>15μmol/L	2.212 (1.119-4.375)	0.022

Item	Calcified Plaques	
	OR (95%CI)	p value
Hcy>15μmol/L	1.668(1.030-2.699)	0.037

Summary

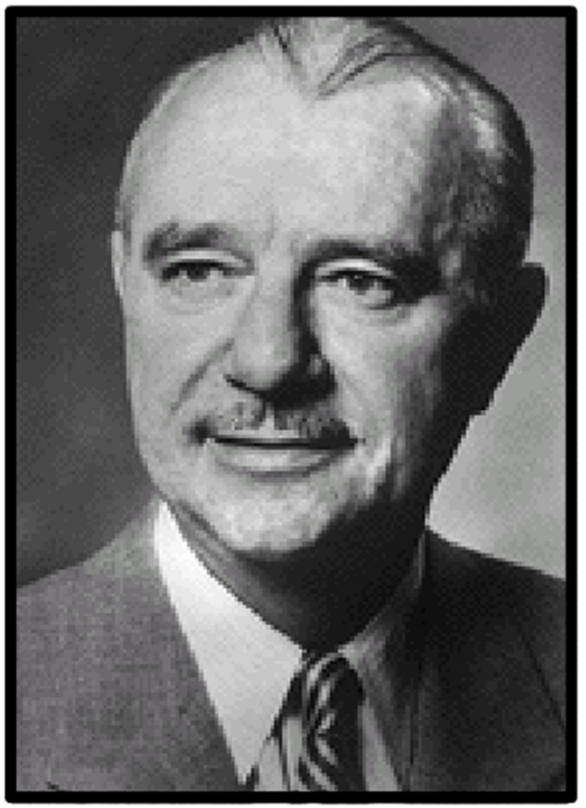


- This studies have been validated the blood traditional risk factors and coronary artery stenosis by CTA imageing examination.
- The close relationship between Hcy and coronary artery plaque and stenosis have been confirmed.
- Hcy could be used to early warning of coronary artery atherosclerotic plaque and calcification in clinical symptom free patients.

Homocysteine

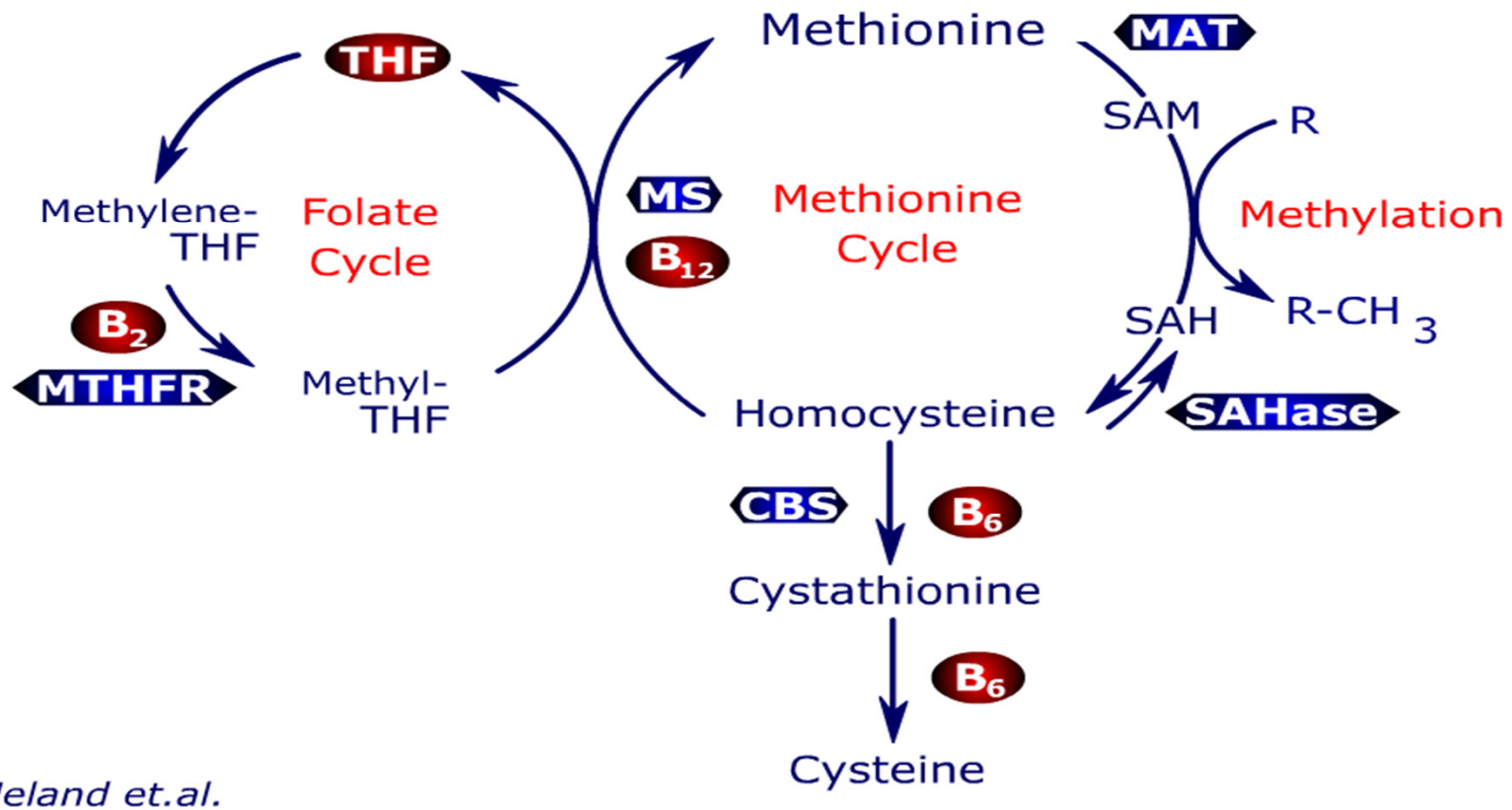
Vincent du Vigneaud

Investigated organic sulphur compounds and their metabolism, among them sulphur-containing amino acids and hormones like insulin and the pituitary hormones, oxytocin and vasopressin.
He described homocysteine for the first time in 1931. Received the Nobel Prize in Chemistry in 1955



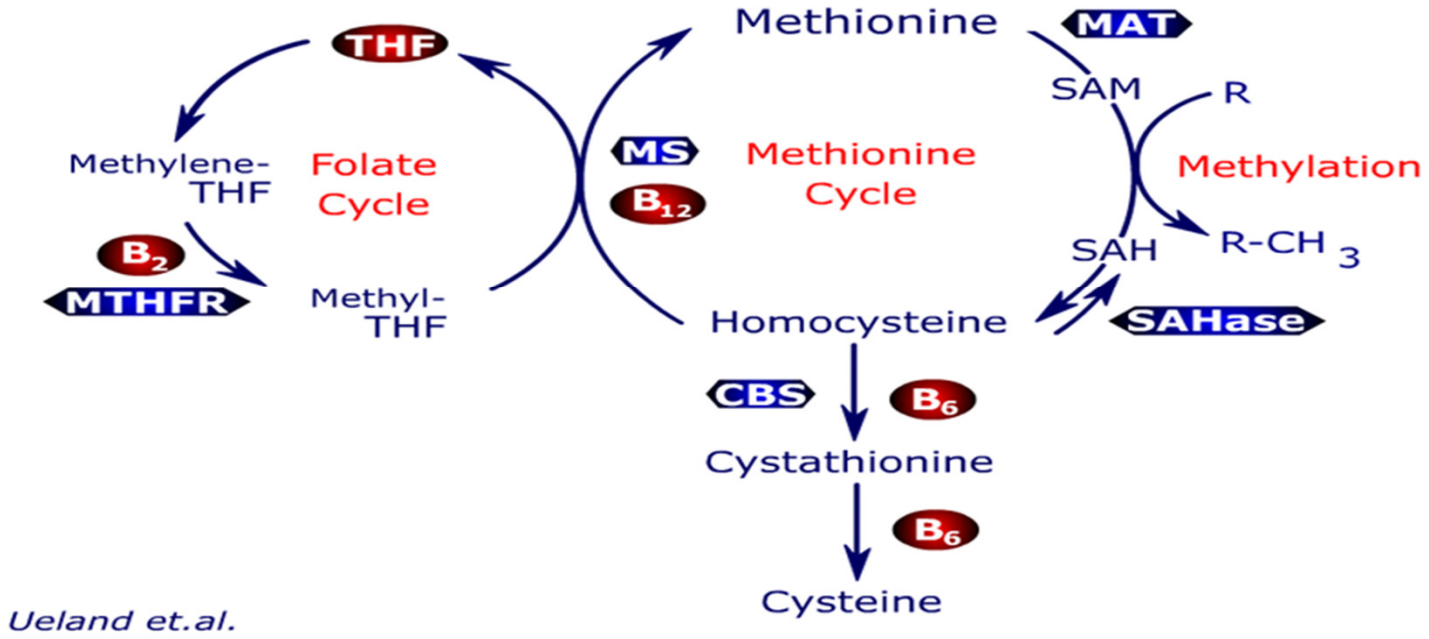
Nobel lecture Des. 1953

Homocysteine metabolism



Ueland et.al.

Homocysteine metabolism

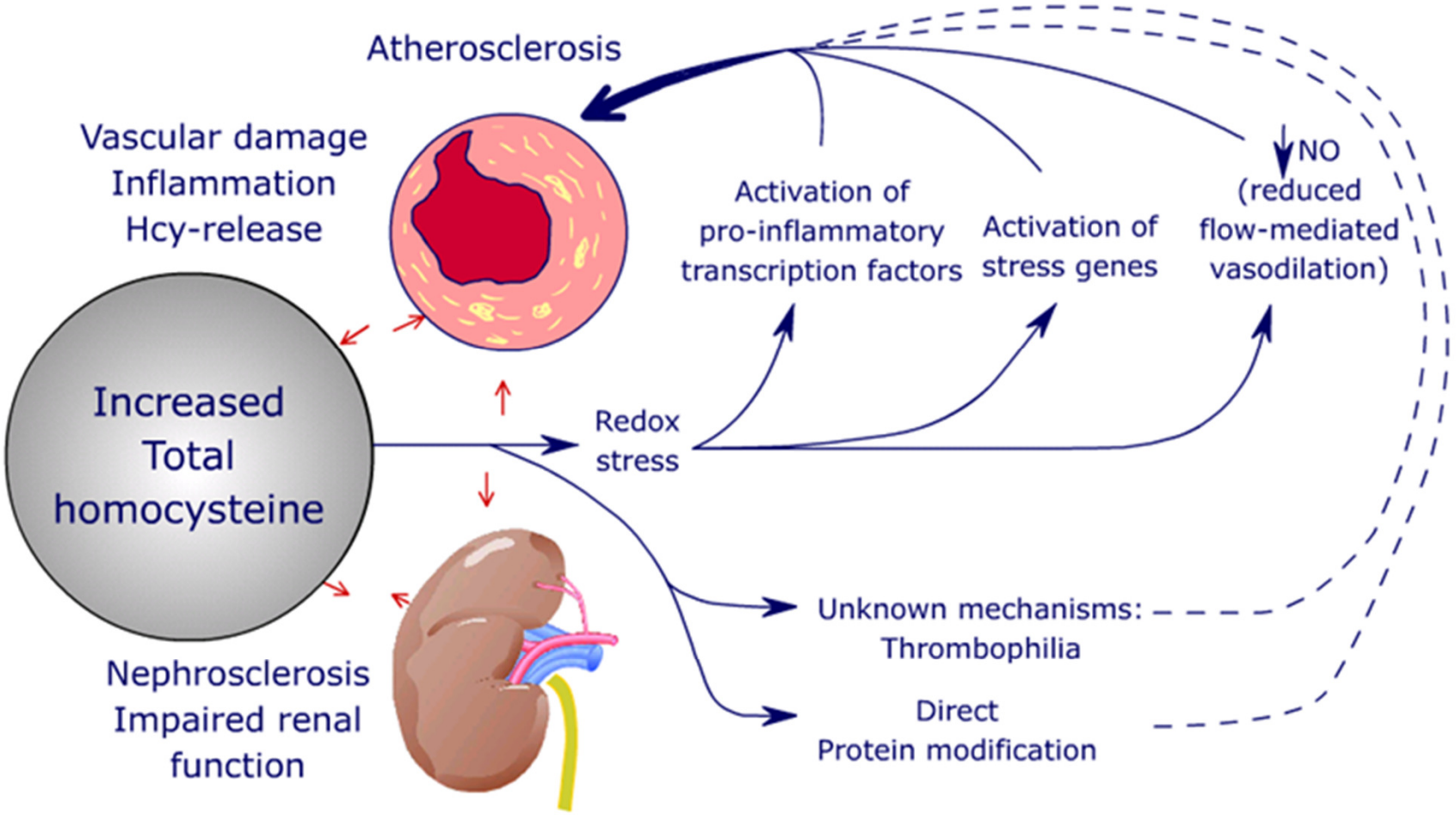


Ueland et.al.



Reduced (thiol/sulfhydryl)

Oxidised (disulfide)



Hajjar KA.
Dudman NP.
Schneede J. et al.
Brattstrøm L. and Wilcken DE.

PLAY AGAIN

RETURN



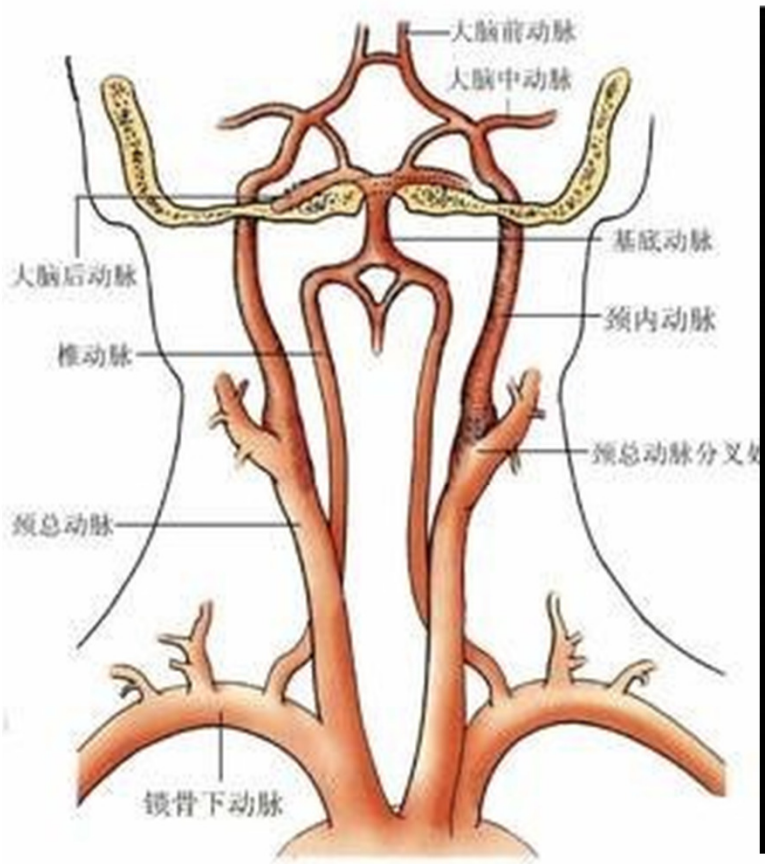
The correlation between serum lipid profile with carotid intima-media thickness and plaque

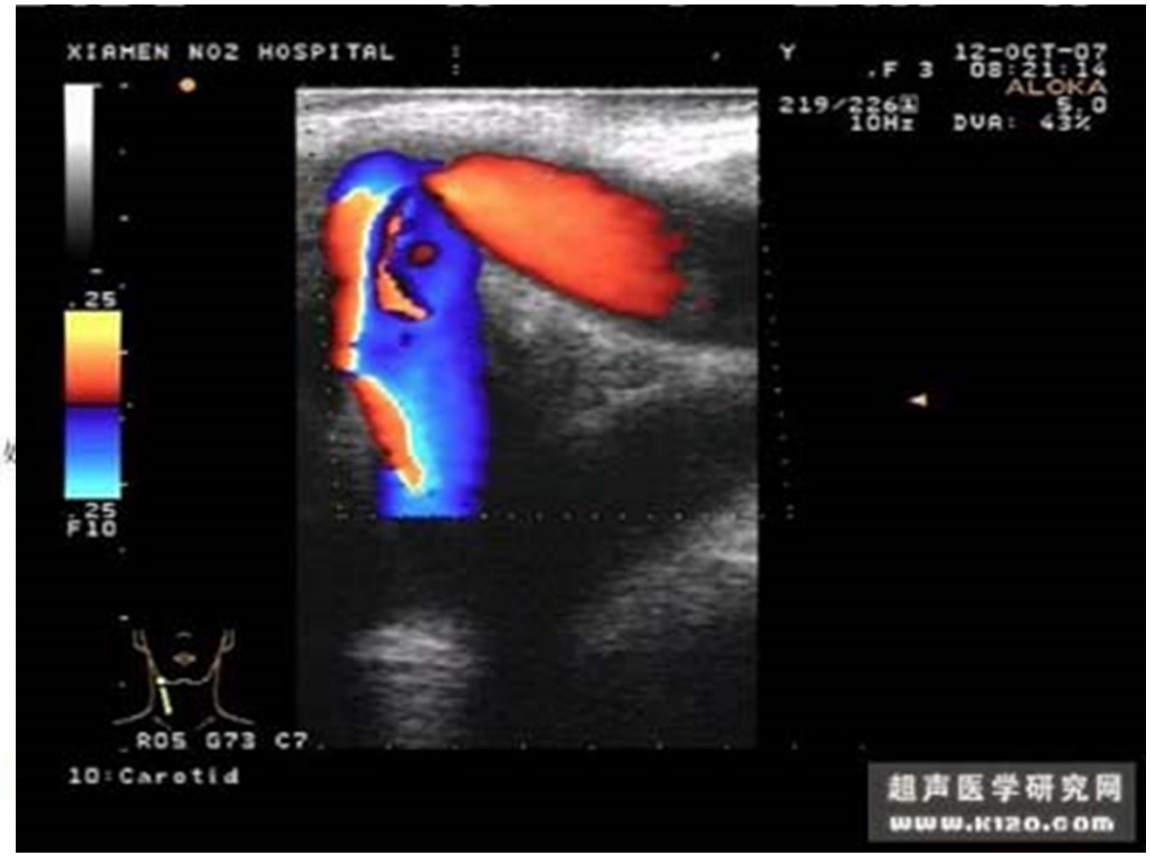
Patients



- 402 patients without apparent clinical atherosclerosis in a cross-sectional study (mean age 50.16 years; 36.07% female) have been involved.
- Demographics, anthropometrics, and laboratory data were collected.
- The presence of carotid IMT and plaque were evaluated by ultrasonography.
- Written informed consent was obtained from participants and the ethics committee of PLA General Hospital approved present study

Carotid ultrasonography





Whether there were atherosclerotic plaque have been observed, plaque was defined as >1.3mm and the ratio of thickness of peripheral vascular wall thickening of at least 50%.

Clinical characteristics



Variables	Mean ± SD or percentage
Gender, female (%)	145 (36.07)
Age (years)	50.16 ± 8.19
BMI (kg/m^2)	25.40 ± 3.41
WHR	0.89 ± 0.08
SBP (mmHg)	124.04 ± 15.42
DBP (mmHg)	82.83 ± 11.44
Fasting blood glucose (mmol/L)	5.85 ± 1.10
HbA_{1C} (%)	5.90 ± 0.67
CK (U/L)	110.62 ± 71.24
Hs-CRP (mg/dL)	0.15 ± 0.12
TC (mmol/L)	4.76 ± 0.83
TG (mmol/L)	1.86 ± 1.49
HDL-C (mmol/L)	1.29 ± 0.33
LDL-C (mmol/L)	3.10 ± 0. 80
Carotid IMT (mm)	0.84 ± 0.20
Presence of carotid plaque (%)	110 (27.40)

BMI body mass index, WHR waist-to-hip ratio, DBP diastolic blood

pressure,

SBP systolic blood pressure,

TC serum total cholesterol,

TG serum

triglycerides,

HDL-C serum high-density lipoprotein cholesterol,

LDL-C serum

low-density lipoprotein cholesterol,

CK creatine kinase,

Hs-CRP high sensitivity

C-reactive protein,

IMT intima-media thickness.

Correlation between carotid IMT and lipid parameters and other variables

Variables	Univariate		Multivariate	
	r	*p*-value	Standardized coefficient (β)	*p*-value
Gender	−0.110	0.043	NI	
Age	0.200	<0.001	0.193	<0.001
BMIWHR	0.009−0.015	0.8640.774	NINI	
SBP	0.109	0.029	0.169	<0.001
DBP	0.001	0.990	NI	
Fasting blood glucose	0.018	0.730	NI	
HbA_{1C}	0.119	0.024	NI	
CK	0.106	0.044	NI	
Hs-CRP	0.016	0.760	NI	
TC	0.126	0.017	NI	
TG	−0.018	0.736	NI	
HDL-C	−0.030	0.570	NI	
LDL-C	0.137	0.009	0.099	0.030
Non-HDL-C	0.140	0.008	NI	
TC/HDL-C ratio	0.087	0.099	NI	
LDL-C/HDL-C ratio	0.169	0.001	0.132	<0.001

Gender: Male = 1, female = 0.
Abbreviation: NI not included in the model.

Associations between lipid parameters, other variables and the presence of carotid plaque

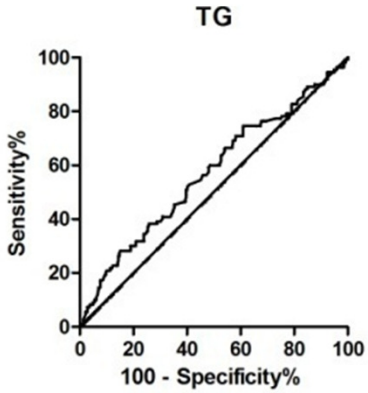
Variables	Plaque(+) (n = 110)	Plaque(−) (n = 292)	*p*-value
G/ender, female (%)	35 (31.82)	110 (37.67)	0.276
Age (years)	53.75 ± 7.67	47.46 ± 7.54	<0.001
BMI (kg/m^2)WHR	25.49 ± 3.620.90 ± 0.08	25.34 ± 3.240.89 ± 0.08	0.7430.205
SBP (mmHg)	126.46 ± 15.89	122.21 ± 14.84	0.029
DBP (mmHg)	83.66 ± 11.68	82.19 ± 11.26	0.310
Fasting blood glucose (mmol/L)	5.95 ± 1.10	5.76 ± 1.10	0.176
HbA_{1C} (%)	5.99 ± 0.67	5.84 ± 0.67	0.079
CK (U/L)	115.82 ± 88.99	106.70 ± 54.20	0.311
Hs-CRP (mg/dL)	0.16 ± 0.13	0.14 ± 0.12	0.092
TC (mmol/L)	4.87 ± 0.88	4.68 ± 0.78	0.070
TG (mmol/L)	1.96 ± 1.55	1.78 ± 1.45	0.324
HDL-C (mmol/L)	1.21 ± 0.28	1.35 ± 0.35	0.001
LDL-C (mmol/L)	3.22 ± 0. 85	3.01 ± 0. 76	0.042
Non-HDL-C (mmol/L)	3.65 ± 0.88	3.32 ± 0.76	0.002
TC/HDL-C ratio	4.19 ± 1.16	3.65 ± 1.00	<0.001
LDL-C/HDL-C ratio	2.74 ± 0.81	2.37 ± 0.80	<0.001
Mean IMT (mm)	0.84 ± 0.20	0.82 ± 0.16	0.410

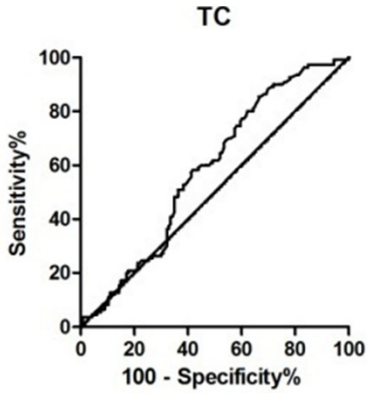
Logistic regression model for prediction of the presence of carotid plaque

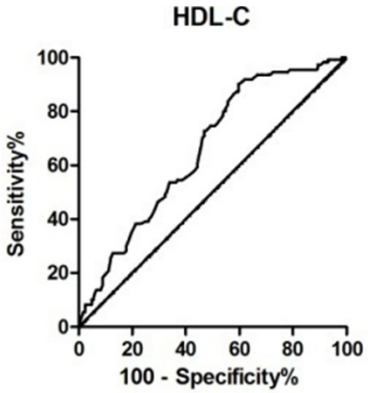
Variables	Odds ratio	95% CI	*p*-value
Age	1.121	1.082-1.161	<0.001
HDL-C	0.093	0.038-0.227	<0.001
LDL-C	1.325	1.046-1.821	0.033

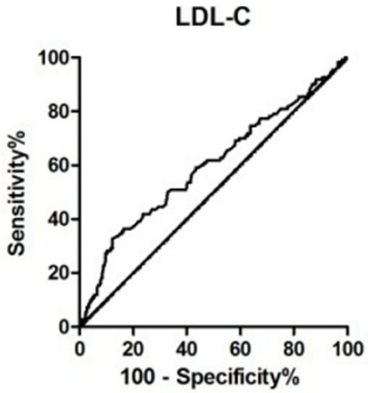
Abbreviation: CI confidence interval.

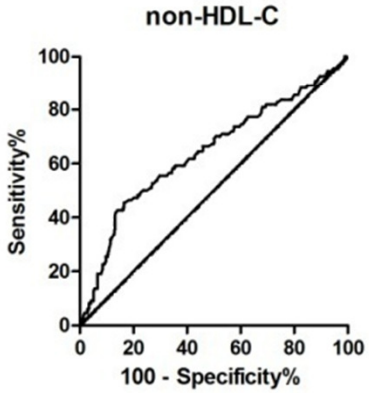
ROC curves of lipid parameters and the combination model including LDL-C and HDL-C levels for predicting the presence of carotid plaque

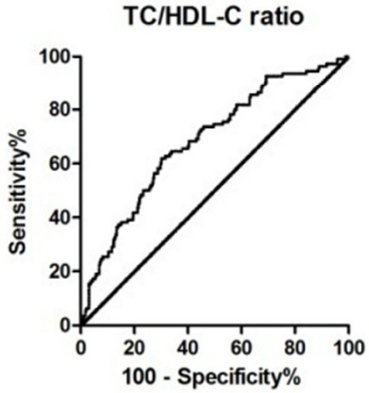


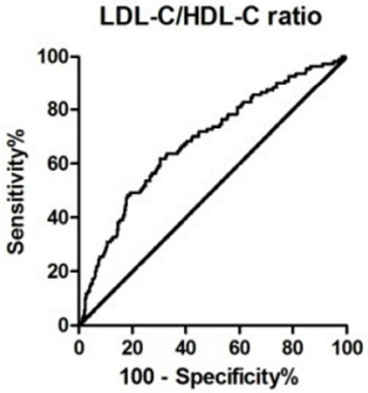


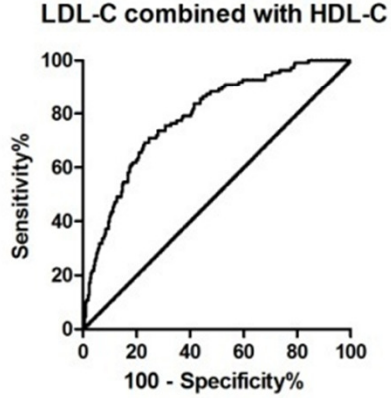












Summary



- Serum LDL-C/HDL-C ratio represents as an independent index associated with increased carotid IMT.
- LDL-C combined with HDL-C levels may be useful markers for predicting the presence of carotid plaque in the Chinese general population
- Prospective cohort studies with a larger sample size are needed to confirm this finding



Serum microRNAs might be potential biomarkers for cardiovascular diseases

Patients

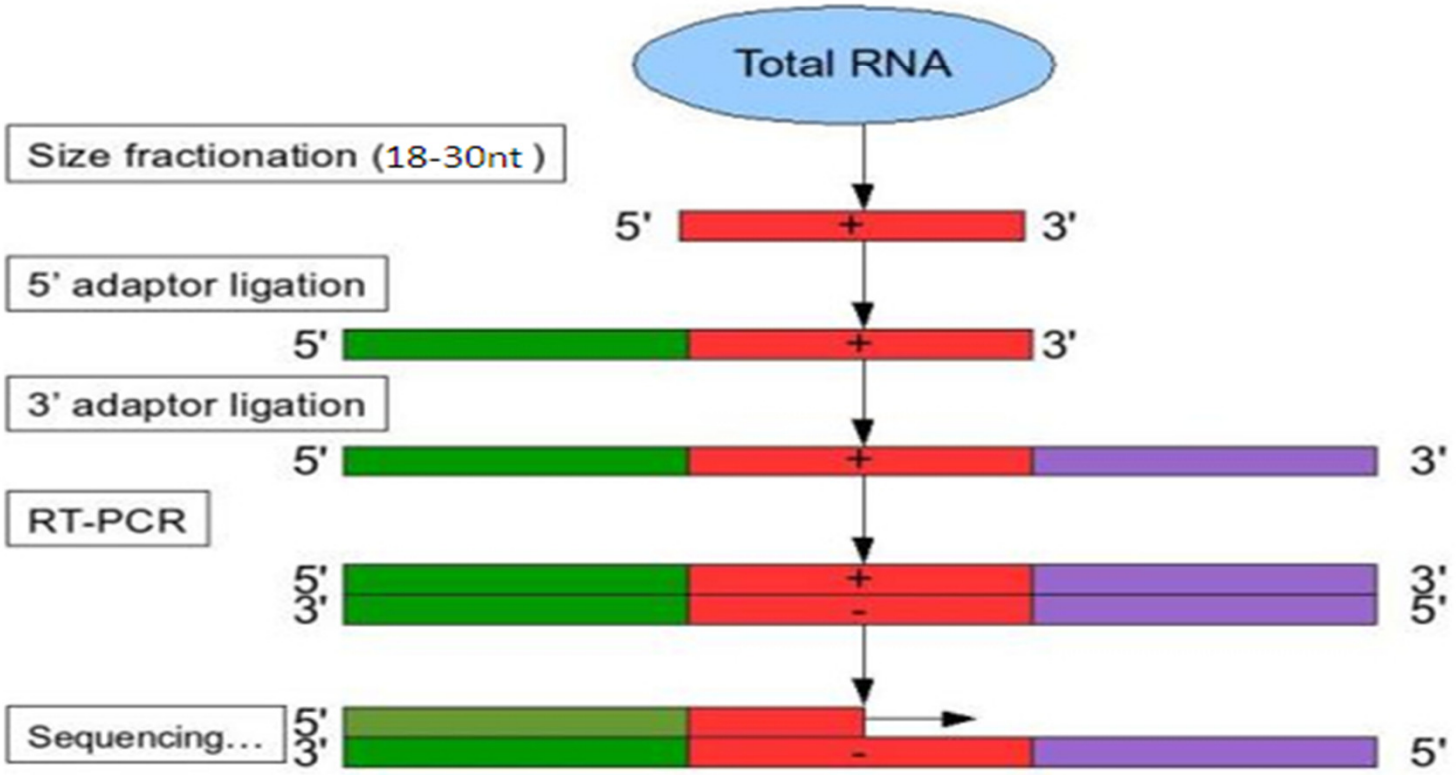


- The Study objects come from our hospital physical examination population In Dec. 2011 to Jan. 2013 year and patients.
- At the initial screening. Fifteen serum samples in normal subjects (Control) , coronary atherosclerosis (AS) , unstable angina pectoris (UAP) have been collected in each group.
- The serum pool by Solexa sequencing to determine expression miRNA table data by bioinformatics analysis.
- the purpose of this experiment is to find a new unreported miRNA which is related with cardiovascular diseases.

Diagnostic criteria



- No clinical manifestations of cardiovascular disease,
- patients with tumor, immune exclusion and other infectious diseases.
- CTA examination was not found coronary atherosclerotic plaques were classified into normal group (Control), and
- Coronary atherosclerosis group (AS) were diagnosised by CTA.
- The diagnostic criteria of UAP: with typical chest pain and ECG ST segment depression in the >Imm or ST segment elevation >3mm, ST segment change back to normal episodes were terminated or with T wave inversion.



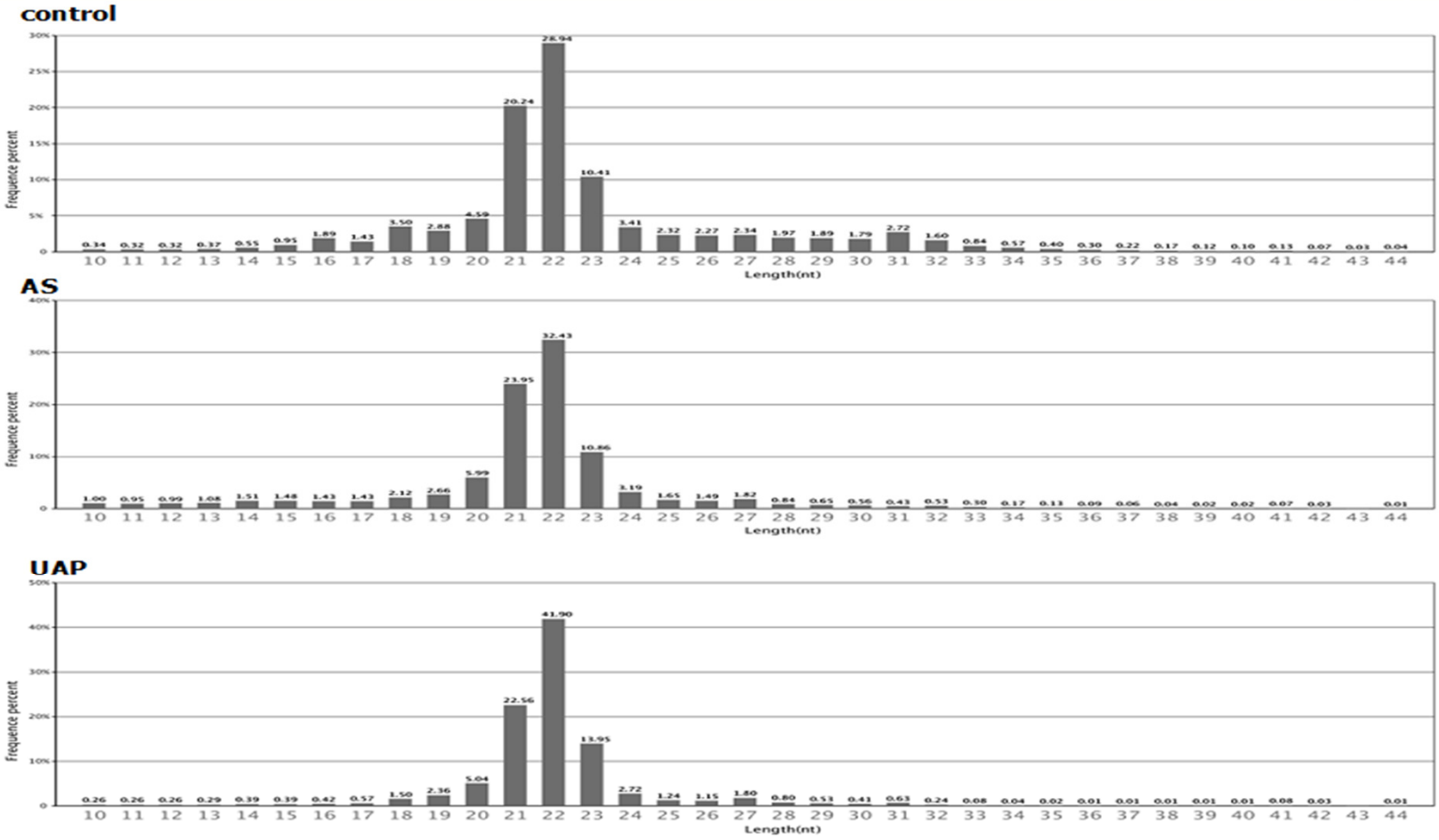
Solexa sequencing have been provided by the Shenzhen Huada gene company

Demographic and clinical characteristics

Items	Control (n=15)	AS (n=15)	UAP (n=15)	total p
Age	52.13±5.55	52.8±6.43	53±6.13	0.9185
Gender (male%)	10 (66.67%)	10 (66.67%)	10 (66.67%)	1
BMI	25.45±2.56	25.98±2.49	25.64±2.51	0.8440
Smoking(%)	6 (40%)	6 (40%)	6 (40%)	1
DM(%)	2 (13.33%)	2 (13.33%)	3 (20%)	0.8444
Hypertension (%)	4 (26.67%)	7 (46.67%)	5 (33.33%)	0.5072
Hyperlipidemia (%)	5 (33.33%)	9 (60%)	9 (60%)	0.2410
Cr (mmol/L)	71.23±10.89	73.4±12.2	75.14±14.64	0.6999

	control	AS	UAP
total_reads	9001588	9235499	8924668
high_quality	8977954(100%)	9208537(100%)	8900737(100%)
3'adapter_null	67875(0.76%)	75198(0.82%)	54070(0.61%)
insert_null	2136147(23.79%)	1152450(12.52%)	897948(10.09%)
5'adapter_contaminants	601124(6.7%)	1159414(12.59%)	52105(0.59%)
smaller_than_18nt	534627(5.95%)	929891(10.10%)	347765(3.91%)
polyA	75(0.00%)	76(0.00%)	73(0.00%)
clean_reads	5638106(62.8%)	5891508(63.98%)	7548776(84.81%)

The sequencing data quality



The length distribution of small molecules RNA in serum

Most of them are distributed in 19-24nt



	control	AS	UAP
Total	256085(100%)	195301(100%)	295319(100%)
rRNA	55298(21.59%)	35687(18.27%)	67683(22.92%)
miRNA	1246(0.49%)	1644(0.84%)	2585(0.88%)
repeat	1397(0.55%)	3454(1.77%)	3731(1.26%)
exon-sense	559(0.22%)	1076(0.55%)	3320(1.12%)
exon-antisense	56(0.02%)	627(0.32%)	143(0.05%)
intron-sense	355(0.14%)	864(0.44%)	1365(0.46%)
intron-antisense	250(0.1%)	627(0.32%)	600(0.2%)
srpRNA	14(0.01%)	14(0.01%)	29(0.01%)
snRNA	878(0.34%)	798(0.41%)	1364(0.46%)
snoRNA	159(0.06%)	143(0.07%)	378(0.13%)
scRNA	574(0.22%)	611(0.31%)	933(0.32%)
tRNA	12510(4.89%)	7961(4.08%)	16375(5.54%)
unannotated	182789(71.38%)	1423362.88(72.88%)	196813(66.64%)

•Solexa sequencing of small RNA classification

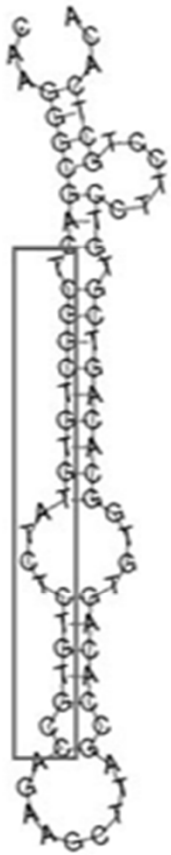
MIREAP software have been used to predict the new miRNA. A total of 263 new miRNA is predicted, but most of the content is very low (< 20 reads). In order to avoid the fracture of RNA and other pollution, we choose the next step verification in an arbitrary set of content > 30 reads miRNA. After miRbase 20.0 screening, three new miRNA have been selected for the next step of verification, it was N1(74), N2(75) and N3(344). The 3 miRNA concentration show in the following.

ID	counts (reads)		
	control	AS	UAP
N1	0	344	63
N2	136	391	259
N3	12	73	54

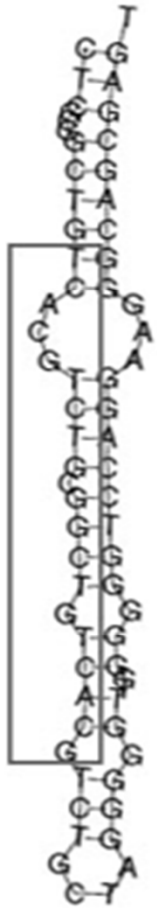
N1



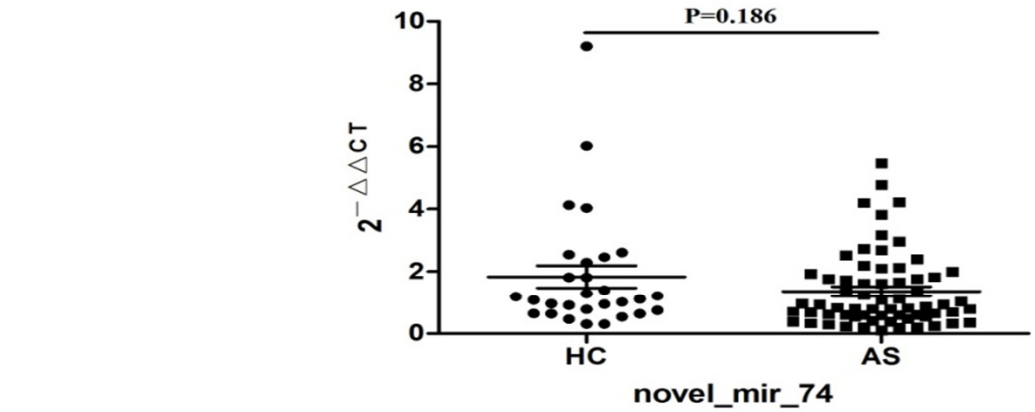
N2

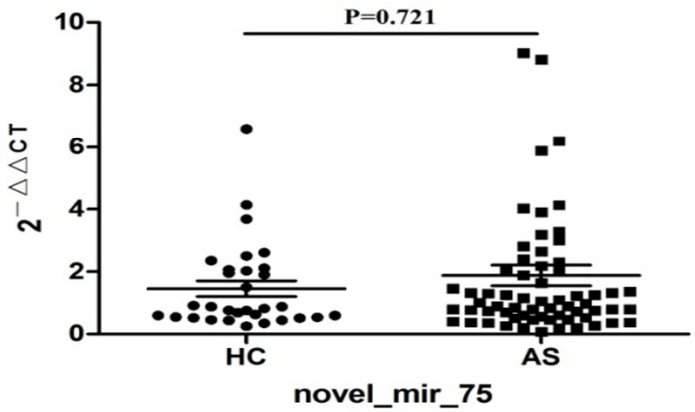


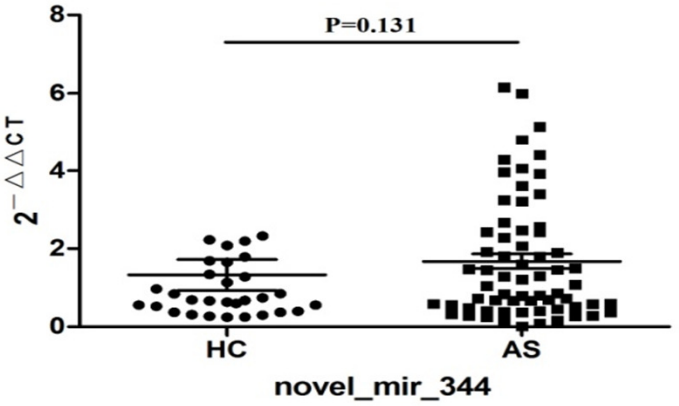
N3



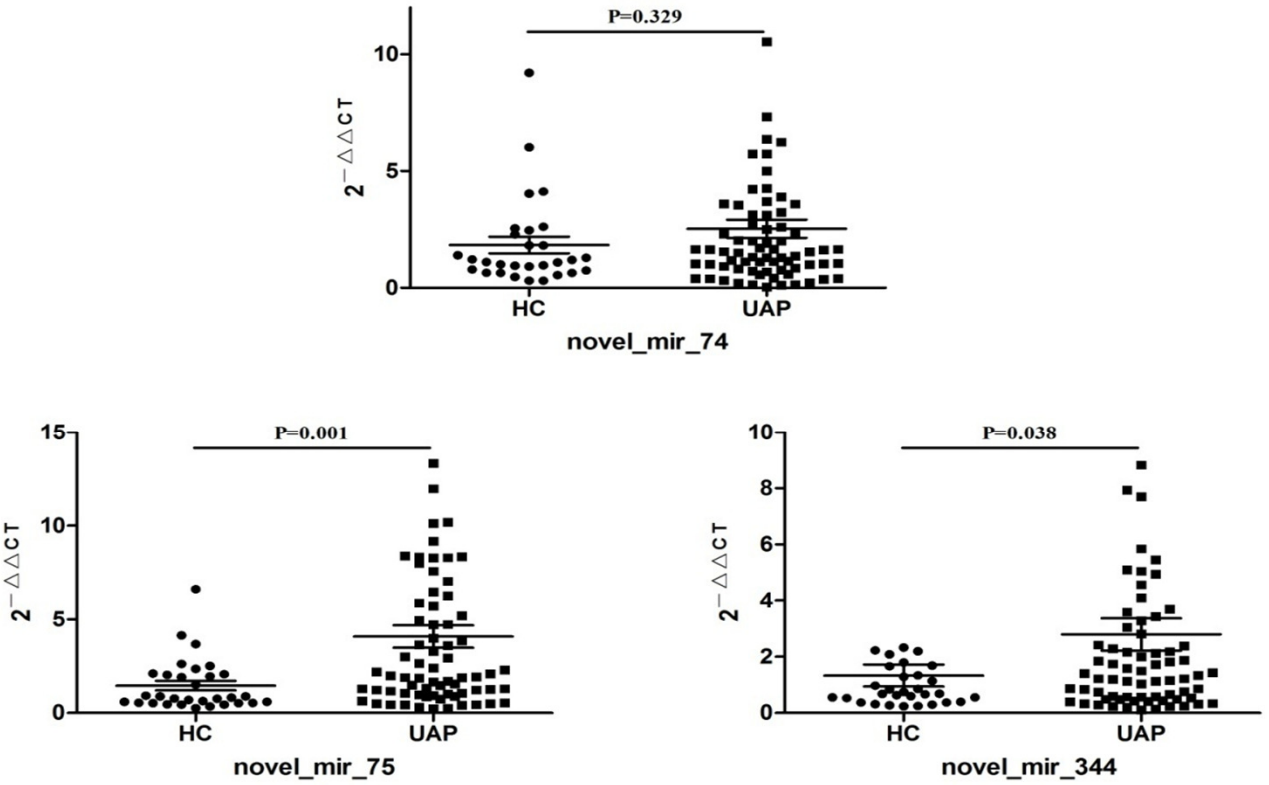
The predicted stucture



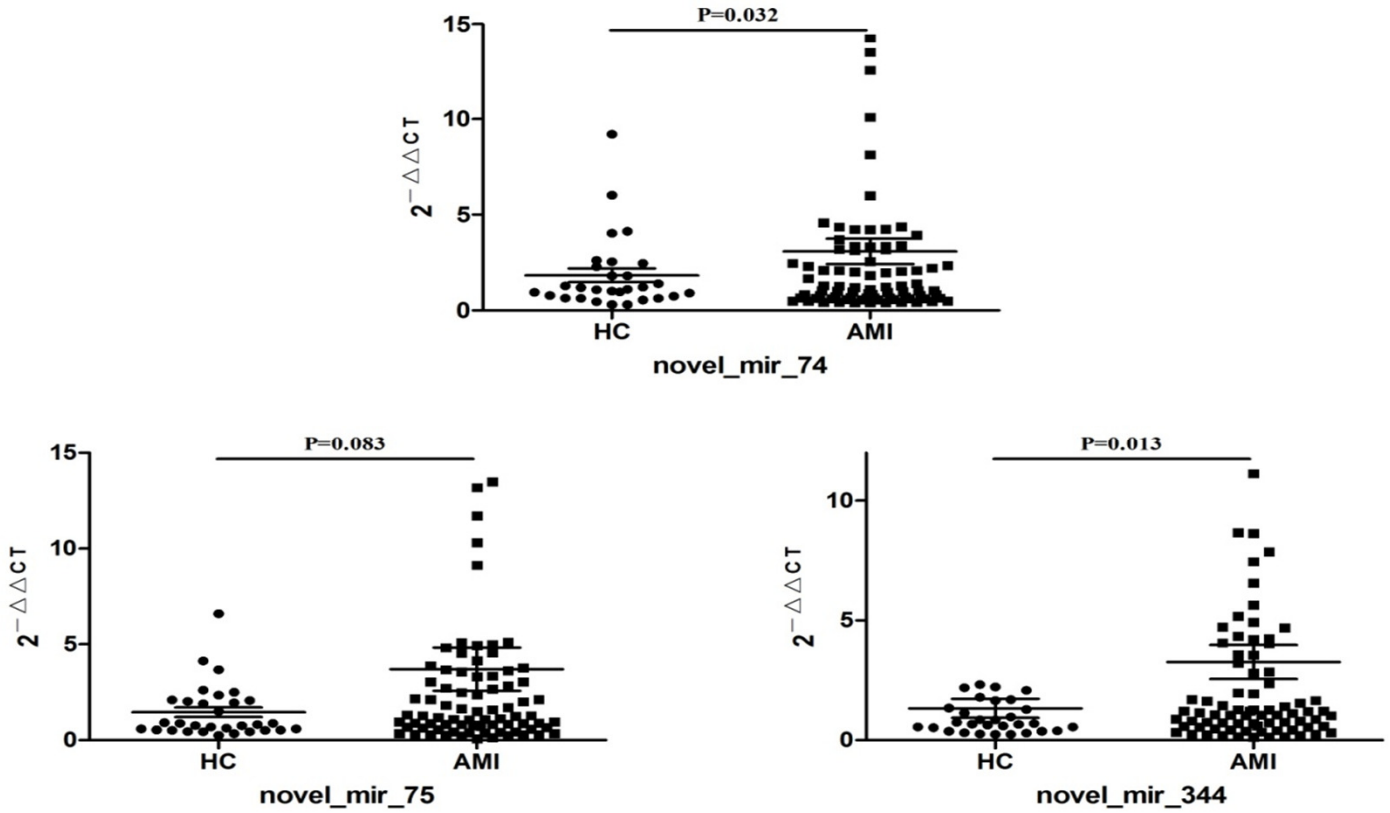




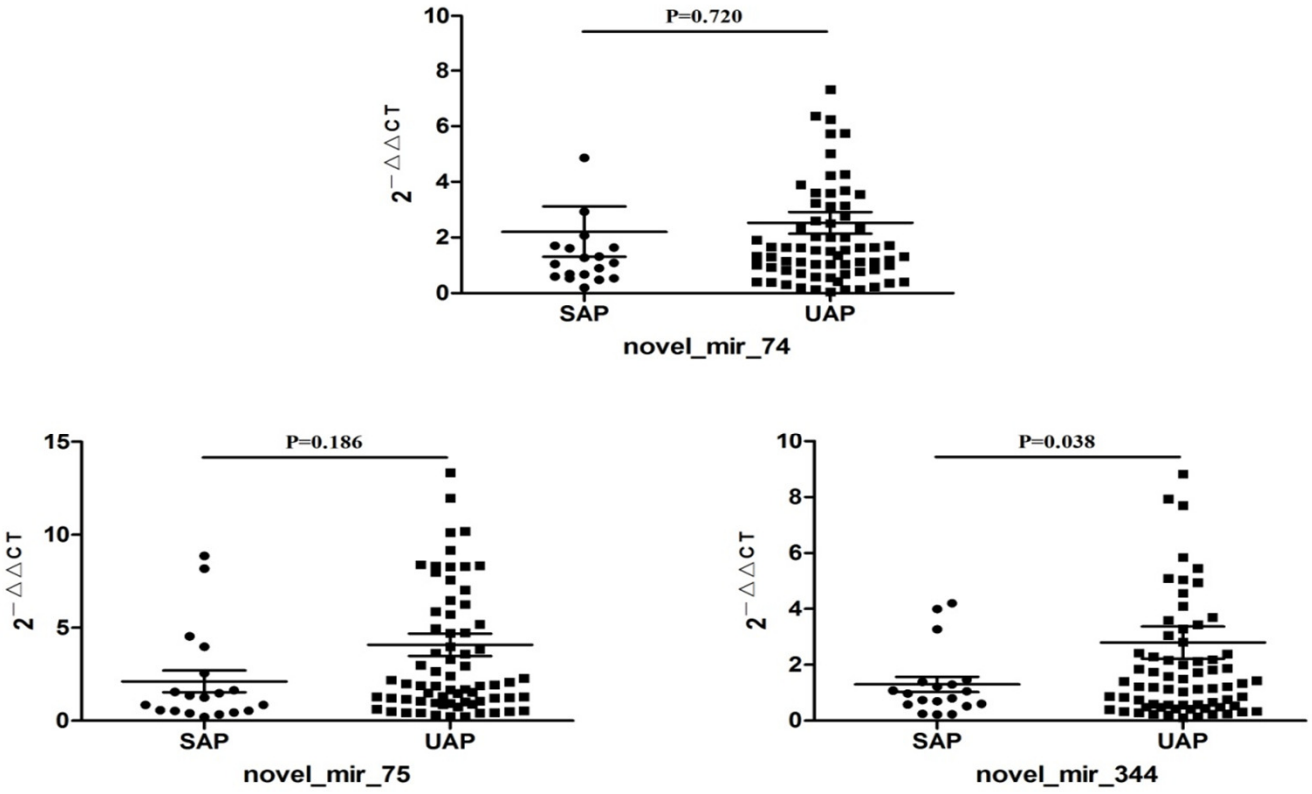
Serum microRNA in AS and control



Serum microRNA in UAP and control



Serum microRNA in AMI and control



Serum microRNA in UAP and SAP

Summary



- The blood circulating RNA in coronary artery atherosclerosis and UAP disease have been sequencing by Solexa.
- The peripheral blood circulating miRNA expression profileis have been established.
- Three new miRNAs have been found which related with coronary artery atherosclerosis and UAP disease . Estblishment and biological infor
- It provide some new clues for help the diagnosis of this diseases.


CITY RANGER
谢谢！

Thanks' for your kind attention!





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