DRG® Apolipoprotein(a) ELISA (EIA-1907)

Revised 2 Mar. 2011 rm (Vers. 7.1)

IVD

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

Lp(a) ELISA provides a method for the quantitative determination of human Lp(a) in serum or plasma.

2 SUMMARY AND EXPLANATION OF THE TEST

Apolipoprotein(a), Apo(a), is a glycoprotein linked by disulphide bridges to apolipoprotein B in the Lp(a) particle. Apo(a) is formed by three different structural domains. One of the domains, called kringle 4, type 2, is present in multiple copies, the number of which varies and is genetically determined, giving rise to different sizes of Apo(a). Depending on the method used, six to 23 different isoforms of Apo(a) ranging from about 300 to 900 kD have been identified (1,2,15,16). Most individuals have two Apo(a) isoforms, although in some subjects no Apo(a) band can be detected when analyzed in SDS-gel electrophoresis followed by immunoblotting (3). Recently, much interest has been focused on Lp(a) since there is a lot of evidence that circulating levels represents an independent risk factor for coronary vascular disease. The Lp(a) level has been found to be an inherited risk factor for ischaemic heart disease (4–8). High Lp(a) levels have been demonstrated in familial hypercholesterolemia and its measurement may be clinically useful for risk prediction in these patients (9,10). Results have also been published on Lp(a) as a strong indicator for cerebrovascular disease (11,12). Apo(a) is homologous to the protease zymogen plasminogen (13,14). Lp(a) inhibits plasminogen activation and recent studies have shown that Apo(a) compete with plasminogen for binding to the plasminogen receptor. These properties of Apo(a) may explain the association of high Lp(a) concentrations with myocardial infarction.

3 PRINCIPLE OF THE PROCEDURE

Lp(a) ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the Apo(a) molecule. During incubation Apo(a) in the sample react with per-oxidase-conjugated anti-Apo(a) antibodies and anti-Apo(a) antibodies bound to microtitration well. A simple washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine. The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

4 WARNINGS AND PRECAUTION

- For in vitro diagnostic use.
- Not for internal or external use in humans or animals.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop Solution in this kit contains 0.5 M H2SO4. Follow routine precautions for handling hazardous chemicals.
- All patient specimens should be handled as if capable of transmitting infections.

Warning! This kit contains reagents that may be infectious!

This kit contains reagents manufactured from human blood components. The source of material have been tested by immunoassay for hepatitis B surface antigen, antibodies for Hepatitis C virus and antibodies for HIV virus and found to be negative. Nevertheless, all recommended precautions for the handling of blood derivates should be observed. Please refer to HHS Publication no. (CDC) 88-8395 or corresponding local/national guide-lines on laboratory saftey procedures.

5 MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25 µL, 50 µL, 200 µL, 500 µL and 5 mL (repeat pipettes preferred for addition of enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water
- Test tubes, 5 mL
- Microplate reader with 450 nm filter
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device
- Magnetic stirrer

6 REAGENTS

Each Lp(a) ELISA kit contains reagents for 96 wells, sufficient for 43 samples and one Calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label.

The recommended storage temperature is 2–8°C.

Coated Plate	1 plate	96 wells	Ready for use
Mouse monclonal anti-Apo(a)	8-well strips		
For unused microplate wells completely reseal the bag by using adhesive tape. Store at 2-8°C, use within 8 weeks.			
Calibrators 1, 2, 3, 4	4 vials	500 µL	Lyophilized
Human Lp(a)			Add 500 µL redist. water per vial.
Concentration indicated on vial label. Color coded yellow For storage of reconstituted Calibrators for more than one week, store at –20°C.			
Calibrator 0	1 vial	500 µL	Ready for use
Color coded yellow			
Enzyme Conjugate 11X	1 vial	700 µL	Preparation,
Peroxidase conjugated mouse monoclonal anti-Apo(a) see below.			
Enzyme Conjugate Buffer	1 vial	7 mL	Ready for use
Color coded blue			
Pretreatment Solution	1 vial	5 mL	Ready for use
Sample Buffer 5X	2 bottles	50 mL	

Color coded red
Dilute each bottle with 200 mL redistilled water to make sample buffer 1X solution.
Note! Precipitate may occur when stored at 2-8°C. Allow Sample Buffer 5X to reach room temperature. Mix until precipitate has dissolved.

Wash buffer 21X	1 bottle	50 mL	
Dilute with 1000 mL redistilled water to make wash buffer 1X solution. Storage after dilution: 2-8°C for 8 weeks			
Substrate TMB Colorless solution Note! Light sensitive!	1 vial	22 mL	Ready for use
Stop Solution 0.5 M H_2SO_4	1 vial	7 mL	Ready for use

6.1 Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by mixing Enzyme Conjugate 11X in Enzyme Conjugate Buffer (1+10) according to the table.

When preparing enzyme conjugate 1X solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently.

Store at 2-8°C. Use within 2 weeks.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12strips	1 vial	1 vial
6 strips	300 µL	3.0 mL
4 strips	200 µL	2.0 mL

7 SPECIMEN COLLECTION AND HANDLING

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation.
Specimen may be stored for 1 week at 2-8°C. For longer periods store samples at -20°C.
Avoid repeated freezing and thawing.

Plasma

Collect blood by venipuncture into tubes containing EDTA or heparin as anticoagulant, and separate the plasma fraction.
Specimen may be stored for 1 week at 2-8°C. For longer periods store samples at -20°C.
Avoid repeated freezing and thawing.

8 PREPARATION OF SAMPLES

All samples have to be pretreated as follows:

1. Sample	25 µL
2. Pretreatment Solution	25 µL
3. Mix and incubate for 1 hour at room temperature	
4. Add Sample Buffer and mix	5.0 mL

As a result of this procedure the samples will be diluted 1/202.
This dilution is stable for 1 week at 2–8°C.

If the concentration of Lp(a) in the sample is >1000 U/L, dilute the pretreated and diluted sample (1/202) further in Sample Buffer, e.g. 1/4 giving a final dilution of 1/808.

9 TEST PROCEDURE

Prepare enzyme conjugate 1X solution, wash buffer 1X solution and sample buffer 1X solution.

Perform each determination in duplicate for Calibrators and samples.

Prepare a calibrator curve for each assay run. Avoid pipetting solution onto the walls.

Add to anti-Apo(a) wells	Calibrators	Samples
1. Calibrators	25 µL	-
2. Pretreated samples	-	25 µL
3. Enzyme Conjugate 1X solution	50 µL	50 µL

4. Incubate on a shaker (700-900 rpm) for 1 hour at room temperature (18-25°C).
5. Wash 6 times with 700 µL wash buffer 1X solution per well using an automatic plate washer with overflow-wash function, after final wash, invert and tap the plate firmly against absorbent paper. Do not include soak step in washing procedure.
   Or manually,
   discard the reaction volume by inverting the microplate over a sink. Add 350 µL wash buffer 1X solution to each well. Discard the wash buffer 1X solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing procedure.
6. Add 200 µL Substrate TMB.
7. Incubate for 15 minutes
8. Add 50 µL Stop Solution
   Put the plate on the shaker for 5 seconds to ensure mixing of Substrate and Stop Solution.
9. Measure the absorbance at 450 nm and evaluate.
   Read within 30 minutes.

Note! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

10 INTERNAL QUALITY CONTROL

Internal plasma pools with low, intermediate and high Lp(a) concentration should routinely be assayed as samples, and results charted from day to day, it is good laboratory practice to record the following data for each assay: kit lot number; reconstitution dates of kit components; OD values for the blank and Calibrators.

11 CALCULATIONS OF RESULTS

11.1 Computerized calculations

The concentration of Lp(a) is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator 0, versus the Lp(a) concentration using cubic spline regression.

Multiply the concentration of the samples with the dilution factor (e.g. x 202)

11.2 Manual calculation

1. Plot the absorbance values obtained for the Calibrators, except for Calibrator 0, against the Lp(a) concentration on a lin-lin paper and construct a calibrator curve.
2. Read the concentration of the samples from the calibrator curve.
3. Multiply the concentration of the samples with the dilution factor (e.g. x 202).

11.3 Example of worksheet

Wells	Identity	A_{450}	Mean conc. U/L*
1A–B	Calibrator 0	0.061/0.064	
1C–D	Calibrator 1**	0.194/0.197	
1E–F	Calibrator 2**	0.535/0.537	
1G–H	Calibrator 3**	1.129/1.131	
2A–B	Calibrator 4**	1.835/1.837	
2C–D	Sample 1	0.286/0.286	104.5
2E–F	Sample 2	0.562/0.563	238.4
2G–H	Sample 3	1.070/1.073	525.4

* Result multiplied by dilution factor (x 202).

** Concentration indicated on vial label

11.4 Example of calibrator curve

A typical calibrator curve is shown below. Do not use this curve to determine actual assay results.

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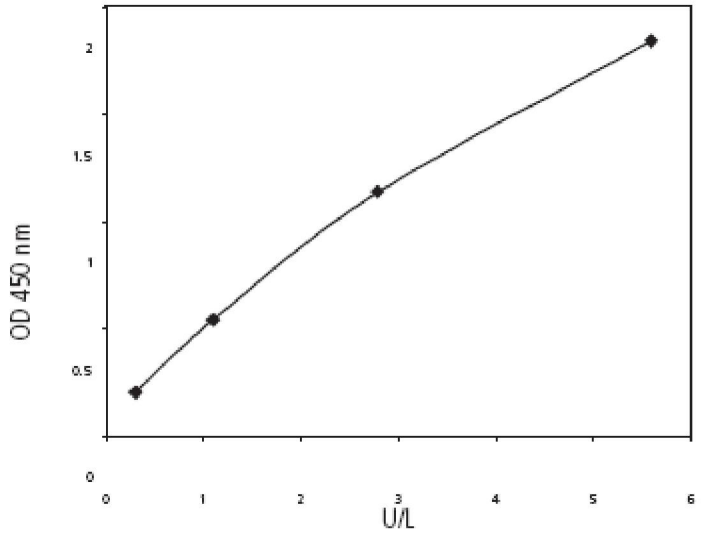


12 LIMITATIONS OF THE PROCEDURE

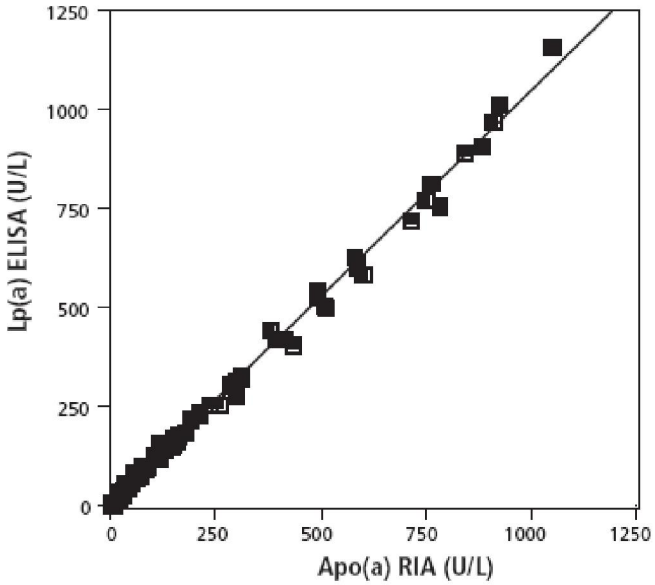
As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.

Grossly lipemic, icteric or hemolysed samples do not interfere in the assay.

13 COMPARISION WITH APO(A) RIA

Comparison studies between Lp(a) ELISA (EIA-1907) and Apo(a) RIA (RIA-2337) have been performed with 45 samples assayed in 2 replicates on 2 occasions. The values found, show a good correlation between the two techniques, r=1.00 (see figure).

Thus, the expected values for Apo(a) RIA can be used for Lp(a) ELISA as well.



14 EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values. The following results obtained with Apo(a) RIA (RIA-2337) may serve as a guide until the laboratory has gathered sufficient data of its own.

The Lp(a) levels has been studied in three different materials:

A. Normals, n=171, Sweden (Caucasian)

B. Normals, n=203, Canada (Caucasian-Asian, heterogenous)

C. Patients with familial hypercholesterolemia (FH), n=113, Canada (Caucasian-Asian, heterogeneous).

The group of normals were individuals chosen from the general population and with no apparent cardio- and/or cerebrovascular disease.

The distribution is shown in the following figures.

The three groups investigated did not show any age or sex differences in their Lp(a) levels.

No significant difference in Lp(a) levels was found between the group of normals from Sweden and the group of normals from Canada.

The group of FH patients had significantly higher Lp(a) levels than the group of normals from the same region ($p<0.001$, Wilcoxon rank sum test).

The following Lp(a) concentrations for median, 75th, 85th and 95th percentiles were obtained for the different groups.

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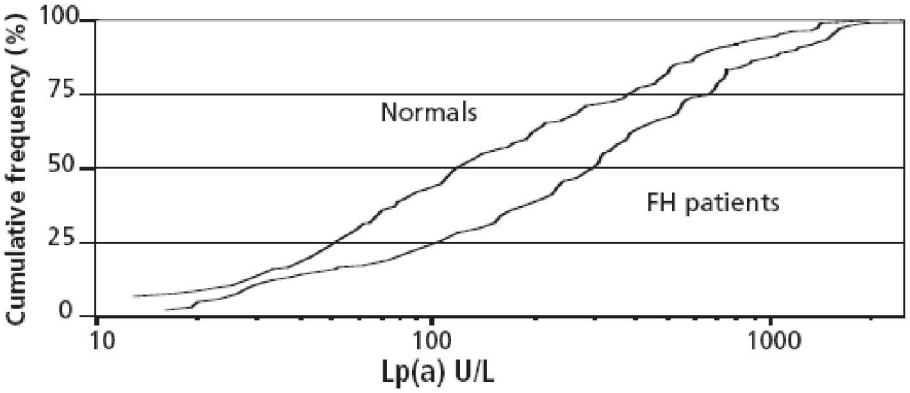
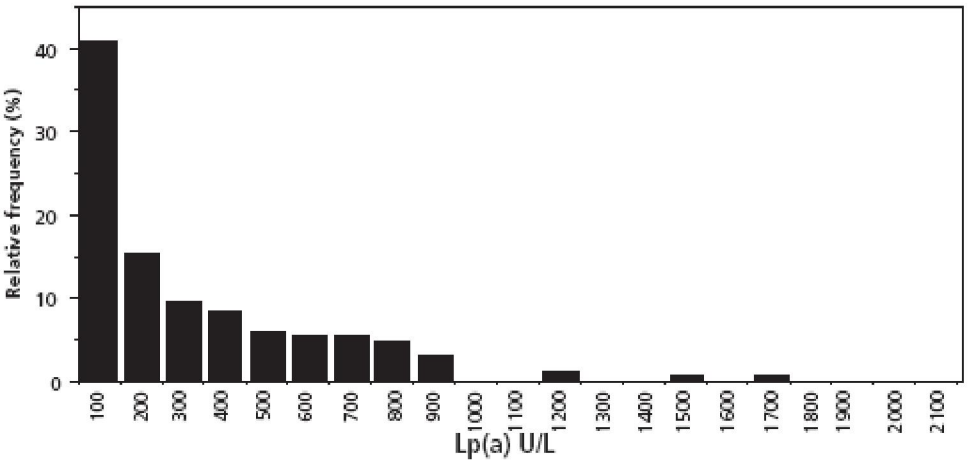
	Median U/L	75th perc. U/L	85th perc. U/L	95th perc. U/L
Normals, Sweden	131	448	612	795
Normals, Canada	117	379	525	1044
FH, Canada	294	660	863	1544

Apo(a) distribution in normals and FH patients (Canada)



Distribution of normals (Canada)



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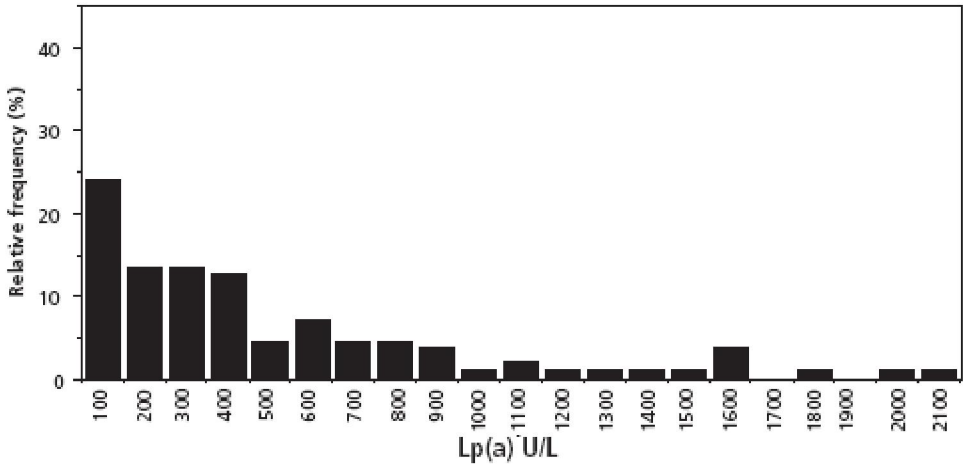
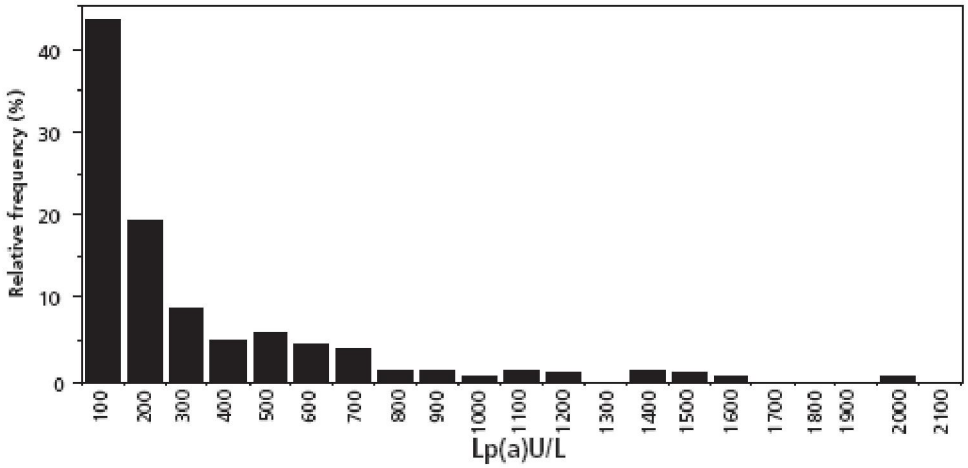
Distribution of normals (Sweden)



Distribution of FH patients (Canada)



15 PERFORMANCE CHARACTERISTICS

15.1 Detection limit

The detection limit is 0.05 U/L calculated as three standard deviations above the Calibrator 0.

This corresponds to a sample concentration of 10 U/L when the sample is diluted 1/202.

15.2 Recovery

Recovery upon addition is 96–111 % (mean 102 %).

15.3 Hook effect

Samples with a Lp(a) concentration of up to 9600 U/L can be measured without giving falsely low results if they are pretreated and diluted 1/202 as described above.

15.4 Precision

Samples pretreated and diluted 1/202 on one occasion and stored at –20°C until the assays were performed. Each sample was analyzed in 4 replicates on nine different occasions.

Sample	Obtained value U/L	Coefficient of variation %		
		Within assay	Between assay	Total assay
1	83	3.3	4.0	5.2
2	196	2.9	3.6	4.7
3	485	2.4	1.8	3.0

Samples pretreated and diluted 1/202 on each test occasion. Each sample was analyzed in 5 replicates on five different occasions.

Sample	Obtained value U/L	Coefficient of variation %		
		Within assay	Between assay	Total assay
1	103	3.1	4.2	5.2
2	251	3.6	3.7	5.2
3	744	2.4	5.2	5.7

15.5 Specificity

A concentration of up to 10 g/L of plasminogen gives no measurable cross-reactivity in the assay. (Clinical concentration of plasminogen is below 2.1 g/L)

Apolipoprotein B has no measurable cross-reactivity.

16 CALIBRATION

Lp(a) ELISA kit is calibrated against a highly purified, fully validated, commercial Lp(a) preparation.
The concentration of the Lp(a) ELISA is expressed in Units/L.

17 WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by DRG may affect the results, in which event DRG disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use. DRG and its authorised distributors, in such event, shall not be liable for damages indirect or consequential.

18 SUMMARY OF PROTOCOL SHEET

Add Calibrators and pretreated samples	25 µL
Add enzyme conjugate 1X solution	50 µL
Incubate	1 hour at 18–25°C on a shaker
Wash	6 times
Add Substrate TMB	200 µL
Incubate	15 minutes
Add Stop Solution	50 µL Shake for 5 sec to ensure mixing
Measure A_{450}	

1 REFERENCES / LITERATURE

1. Utermann G. (1989) The mysteries of lipoprotein (a).
Science 17 Nov:904–910
2. MBewu AD and Durrington PN (1990) Lipoprotein (a): structure, properties and possible involvement in trombogenesis and atherogenesis.
Atherosclerosis 85:1–14
3. Albers JJ, Marcovina SM and Lodge MS (1990) The unique lipoprotein(a): properties and immunochemical measurement.
Clin Chem 36: 2019–2026
4. Rosengren A, Wilhelmsen L, Eriksson E, Risberg B and Wendel H (1990) Lipoprotein(a) and coronary heart disease: a prospective case control study in a general population sample of middle aged men.
Br Med J 301:1248–1251
5. Rhoads GG, Dahlén G, Berg K, Morton NE and Danneberg AL (1986) Lp(a) Lipoprotein as a risk factor for myocardial infarction.
JAMA 256:2540–2544
6. Dahlen GH, Guyton JR, Attar M, Farmer JA, Kautz JA and Gotto AM Jr (1986) Association levels of lipoprotein Lp(a), plasma lipids and other lipoproteins with coronary artery disease documented by angiography.
Circulation 74:758–765
7. Dembinski T, Nixon P, Shen G, Mymin D and Choy PC. (2000) Evaluation of a new apolipoprotein(a) isoform-independent assay for serum Lipoprotein(a).
Mol Cell Biochem 207:149–155
8. Houlston R and Friedl W (1988) Biochemistry and clinical significance of lipoprotein (a).
Ann Clin Biochem 25:499–503
9. Wiklund O, Angelin B, Olofsson SO, Eriksson M, Fager G, Berglund L and Bondjers G (1990) Apolipoprotein (a) and ischaemic heart disease in familial hypercholesterolaemia.
Lancet 335:1360–1363
10. Seed M, Hoppichler F, Reaveley D, McCarthy S, Thopmson GR, Boerwinkel E and Utermann G (1990) Relation of serum lipoprotein(a) concentration and apolipoprotein(a) phenotype to coronary heart disease in patients with familial hypercholesterolemia.
New En J of Med 322:1494–1499
11. Zenker G, Költringer P, Boné G, Niederkorn K, Pfeiffer K and Jürgens G (1986) Lipoprotein (a) as a strong indicator for cerebrovascular disease.
Stroke 17:942–945
12. urai A, Miyahara T, Fujimoto N, Matsuda M and Kameyama M (1986) Lp(a) lipoprotein as a riskfactor for coronary heart disease and cerebral infarction.
Atherosclerosis 59:199–204
13. McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM and Lawn RM (1987) cDNA sequence of human apolipoprotein(a) is homologous to plasminogen.
Nature 330: 132–137

14. Eaton DL, Fless GM, Kohr WJ, McLean JW, Xu QT, Miller CG, Lawn RM and Scanu AM (1987) Partial amino acid sequence of apolipoprotein (a) shows that it is homologous to plasminogen
Biochemistry 84:3224–3228
15. Lackner C, Boerwinkle E, Leffert CC, Rahmig T and Hobbs HH (1991) Molecular basis of apolipoprotein(a) isoform size heterogenity as revealed by pulsed-field gel electrophoresis.
J Clin Invest 87:2153–2161
16. Kamboh MI, Ferrell RE and Kottke BA (1991) Expressed hypervariable polymorphism of apolipoprotein (a).
Am J Hum Genet 49:1063–1074
17. Solymoss BC, Marcil M, Wesolowska E, Gilfix BM, Lespérance J and Campeau L (1993) Relation of coronary artery disease in women <60 years of age to the combined elevation of serum lipoprotein(a) and total cholesterol to high-density cholestrolratio.
Am J Cardiol 72:1215–19

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