PATHOPHYSIOLOGY OF LIPOPROTEIN(A)
ATHEROSCLEROSIS, THROMBOSIS, AND MORE

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Structure of Lipoprotein (a)

Leibundgut et al JACC 2012 and JLR 2013, Rao ATVB 2015
Apo(a) isoform distribution – Dallas Heart Study

<table>
<thead>
<tr>
<th>Number of KIV repeats</th>
<th>Mean Lp(a) concentration (mg dL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11–16</td>
<td>60</td>
</tr>
<tr>
<td>17–19</td>
<td>49</td>
</tr>
<tr>
<td>20–22</td>
<td>52</td>
</tr>
<tr>
<td>23–25</td>
<td>19</td>
</tr>
<tr>
<td>26–28</td>
<td>15</td>
</tr>
<tr>
<td>29–31</td>
<td>14</td>
</tr>
<tr>
<td>32–34</td>
<td>12</td>
</tr>
<tr>
<td>35–37</td>
<td>7</td>
</tr>
<tr>
<td>&gt;37</td>
<td>5</td>
</tr>
</tbody>
</table>

Molecular weight of Apo(a): Low 19, 15, 14, 12; High 7, 5.

Prevalence of Elevated Lp(a) Levels

Based on Distribution of Lipoprotein(a) Levels in the Copenhagen General Population Study (6000 subjects)

<table>
<thead>
<tr>
<th>Lp(a) Level</th>
<th>≥50 mg/dL</th>
<th>≥100 mg/dL</th>
<th>≥150 mg/dL</th>
<th>≥200 mg/dL</th>
<th>≥250 mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td>20%</td>
<td>5%</td>
<td>1%</td>
<td>0.2%</td>
<td>0.02%</td>
</tr>
<tr>
<td>Number (US)</td>
<td>60,000,000</td>
<td>15,000,000</td>
<td>3,000,000</td>
<td>600,000</td>
<td>60,000</td>
</tr>
<tr>
<td>Number (EU)</td>
<td>100,000,000</td>
<td>25,000,000</td>
<td>5,000,000</td>
<td>1,000,000</td>
<td>100,000</td>
</tr>
<tr>
<td>Globally</td>
<td>1,400,000,000</td>
<td>350,000,000</td>
<td>70,000,000</td>
<td>14,000,000</td>
<td>1,400,000</td>
</tr>
</tbody>
</table>

Nordestgaard et al. European Heart Journal 2010:31;2844–2853
Prevalence of Elevated Lp(a) Levels in 629,858 Subjects from a Referral Laboratory Population in the US

- Health Diagnostic Laboratory referral dataset (2010-2014)
- Median age 56.
- Both genders (54% women)
- Mean±SD = 31±38 mg/dL
- Median (IQR) = 15 (7-43) mg/dL
- Range = 0-571 mg/dL.
- Lp(a) percentiles:
  - 75% >43 mg/dL
  - 80% >55 mg/dL
  - 90% >85 mg/dL
  - 95% >109 mg/dL
  - 99% >169 mg/dL
- Lp(a) >30 mg/dL = 32nd percentile
- Lp(a) >50 mg/dL = 22nd percentile
43% of subjects referred to UCSD for dyslipidemia had Lp(a) >50 mg/dL
Genetic Determinants of CAD

63,746 cases and 130,681 controls
Identified 46 loci and 104 independent variants for CAD
The most significant are genes involved in lipid metabolism and inflammation

CARDIoGRAMplusCAD Consortium  Nature Genetics Jan 2013
Lp(a) is Present in Humans, Apes and Monkeys, but the OxPL on Human Lp(a) is Unique to Humans Only

Human, Chimp, Bonobo, Gorilla, Orangutan - KIV and KV

Baboon, Cynomolgus, Rhesus - KIV

European hedgehog - KIII

Leibundgut et al JLR 2013

Only human Lp(a) has immunoreactive OxPL
What Makes the Relationship Between Lp(a) and OxPL Uniquely Human?

Determinants of binding of oxidized phospholipids on apolipoprotein (a) and lipoprotein (a)¹

<table>
<thead>
<tr>
<th>Species</th>
<th>Kringles</th>
<th>Attachment to LDL</th>
<th>Protease Domain</th>
<th>Fibrin binding</th>
<th>OxPL binding (EO8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>–</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bonobo</td>
<td>–</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Chimpanzee</td>
<td>– ✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>–</td>
</tr>
<tr>
<td>Gorilla</td>
<td>– ✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>–</td>
</tr>
<tr>
<td>Orangutan</td>
<td>– ✓</td>
<td>✓</td>
<td>✓</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Baboon</td>
<td>– ✓*</td>
<td>–</td>
<td>✓</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>– ✓</td>
<td>–</td>
<td>✓</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>– ✓</td>
<td>–</td>
<td>✓</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hedgehog</td>
<td>✓ –</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>–</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>✓ ✓ ✓</td>
<td>–</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

? = data not available in the literature
* = contains all 7 aminic acids in LBS but does not bind lysine-Sepharose
* = mutation in 1 aminic acid of LBS and does not bind lysine

<table>
<thead>
<tr>
<th>Lysine Binding Site</th>
<th>Key Amino Acid Differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasminogen KIV</td>
<td>Trp²²</td>
</tr>
<tr>
<td></td>
<td>Asp⁵⁵</td>
</tr>
<tr>
<td></td>
<td>Lys⁵⁵</td>
</tr>
<tr>
<td>Human apo(a) KIV₁₀</td>
<td>Lys³⁵</td>
</tr>
<tr>
<td>Cynomolgus &amp; Rhesus monkey apo(a) KIV₁₀</td>
<td>Trp⁷²</td>
</tr>
<tr>
<td>Rare human mutations</td>
<td></td>
</tr>
<tr>
<td>Chimpanzee &amp; Gorilla apo(a) KIV₁₀</td>
<td>Asp⁵⁷</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Aromatic</th>
<th>Anionic</th>
<th>Catonic</th>
</tr>
</thead>
</table>

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*Gregor Leitnundgut, Corey Scipione, Huiyong Yin, Matthias Schneider, Michael B. Hoffer, Simone Green, Xiaoyong Yang, Edward Dennis, Joseph L. Witzum, Marlys L. Koschinsky, and Sotirios Tsimplis.*
Apo(a) secreted in the media contains OxPL, but only if KIV-10 is intact
OxPL are present in purified human Lp(a) extracted with 2:1 chloroform:methanol

Leibundgut et al JLR 2013
Lp(a) and OxPL induce macrophage apoptosis

Seimon Cell Metabolism 2010
Elevated Lp(a) as CVD risk factor

Hazard ratios for incident cardiovascular events per SD increase in log-transformed Lp(a) levels in the Atherosclerosis Risk In Communities (ARIC) study. Data are shown for African American (solid line) and Caucasian (dotted line) populations separately.

CVD
- African American
- Caucasian

CHD
- African American
- Caucasian

Stroke
- African American
- Caucasian

Hazard ratio per standard deviation increase in log-transformed Lp(a) levels

Virani et al and Ridker et al Circulation 2012
Evidence Base for Lp(a) as an Independent, Causal, Genetic Risk Factor for CVD

Epi/Meta-analyses

Mendelian Randomization

Genome-wide Association

Adjustment for age and sex only
Nonfatal MI and coronary death (9318 cases)

Usual Lp(a), Geometric Mean, mg/dL

Risk ratio (95% CI)

Lp(a) mg/dL

>117

77-117

30-76

5-29

<5

Hazard Ratio (95% CI)

P<.002

Odds ratio for coronary disease

Lp(a) Lipoprotein (mg/dL)

0 variant alleles

1 variant allele

2 variant alleles

Erqou et al JAMA 2009:302:412-23

Kamstrup et al JAMA 2009:301:2331-9


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40% of patients reclassified to higher or lower risk category based on Lp(a) information
### Is Lp(a) a Risk Factor In Patients On Long-Term Statin Therapy?

<table>
<thead>
<tr>
<th>Study Name</th>
<th>N</th>
<th>Odds Ratio (95% CI)</th>
<th>P-Value</th>
<th>Lp(a)</th>
<th>Odds ratio (95% CI)</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIM HIGH - Placebo</td>
<td>1440</td>
<td>1.87 (1.32 - 2.66)</td>
<td>0.001</td>
<td>&gt;125 nmol/L (~50 mg/dL)</td>
<td></td>
<td>7.8%</td>
</tr>
<tr>
<td>AIM HIGH - Niacin</td>
<td>1427</td>
<td>1.90 (1.33 - 2.72)</td>
<td>0.001</td>
<td>&gt;125 nmol/L (~50 mg/dL)</td>
<td></td>
<td>7.7%</td>
</tr>
<tr>
<td>LIPID</td>
<td>7863</td>
<td>1.23 (1.09 - 1.40)</td>
<td>&lt;0.001</td>
<td>&gt;73.7 mg/dL (~183 nmol/dL)</td>
<td></td>
<td>42.6%</td>
</tr>
<tr>
<td>JUPITER</td>
<td>7730</td>
<td>1.61 (1.16 - 2.25)</td>
<td>0.005</td>
<td>&gt;50 nmol/L (~20 mg/dL)</td>
<td></td>
<td>41.8%</td>
</tr>
<tr>
<td><strong>OVERALL</strong></td>
<td>18470</td>
<td>1.65</td>
<td></td>
<td></td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

**Hazard ratio (95% CI) for CV Events**

Tsimikas CV Endo Diab Obes 2016
AIM-HIGH: Lp(a) remains a predictor of CVD events in patients with LDL-C of 54 mg/dl

Q4 of Lp(a): >125 nmol/L (50 mg/dL)

<table>
<thead>
<tr>
<th>Quartile</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>1 (--)-</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>1.19 (0.81, 1.75)</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>1.37 (0.94, 1.98)</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>1.87 (1.32, 2.66)</td>
</tr>
</tbody>
</table>

Statin + placebo

Statin plus niacin

Albers et al JACC 2013
Association of Lp(a) levels with incident T2D and CHD in the EPIC-Norfolk study

Lp(a): lipoprotein(a); CHD, coronary heart disease; T2D, type 2 diabetes; Confidence intervals (CIs) were calculated using a floating absolute risk technique; Hazard ratios were adjusted for age as underlying timescale, sex, BMI, alcohol, smoking status, systolic and diastolic blood pressure, physical activity, education level, and family history of diabetes (only for T2D) or family history of CHD (only for CHD);

Model for receptor-mediated catabolism of apo(a) and Lp(a)

Effect of Alirocumab on Lp(a) levels
150 mg Q2W on Lp(a)

All patients
Baseline Lp(a) >30 mg/dL
Baseline Lp(a) ≤30 mg/dL

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Median (% change from baseline) (Q1:Q3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>74</td>
<td>-30.3% (-50.0, -19.4)</td>
</tr>
<tr>
<td>Lp(a) &gt;30 mg/dL</td>
<td>31</td>
<td>-28.3% (-37.1, -19.4)</td>
</tr>
<tr>
<td>Lp(a) ≤30 mg/dL</td>
<td>43</td>
<td>-37.5% (-57.1, -7.4)</td>
</tr>
<tr>
<td>Baseline Lp(a) &gt;30 mg/dL</td>
<td>51</td>
<td>-4.4% (-15.3, 10.8)</td>
</tr>
<tr>
<td>Baseline Lp(a) ≤30 mg/dL</td>
<td>51</td>
<td>0.0% (-16.7, 17.2)</td>
</tr>
</tbody>
</table>

Median (Q1:Q3) % change from baseline, mITT population
Lp(a) change from baseline (%) vs. LDL-C change from baseline in alirocumab treatment group

R-square: 0.0463
Spearman's correlation coefficient: 0.2236
p=0.0298
Lp(a) change from baseline (%) vs. LDL-C change from baseline in alirocumab treatment group

R-square: 0.0463
Spearman’s correlation coefficient: 0.2236
p=0.0298
Reduction in Lipoprotein(a) With PCSK9 Monoclonal Antibody Evolocumab

<table>
<thead>
<tr>
<th>Evolocumab Q2W</th>
<th>Evolocumab Q4W</th>
</tr>
</thead>
<tbody>
<tr>
<td>70 mg</td>
<td>280 mg</td>
</tr>
<tr>
<td>105 mg</td>
<td>350 mg</td>
</tr>
<tr>
<td>140 mg</td>
<td>420 mg</td>
</tr>
</tbody>
</table>

- Lipoprotein (a) Percentage Change From Baseline

- 70 mg: -13.8%*
- 105 mg: -18.7%*
- 140 mg: -21.3%*
- 280 mg: -24.5%*
- 350 mg: -24.5%*
- 420 mg: -24.5%*
Correlation between Lp(a) and LDL-C changes induced by Evolocumab
Correlation between Lp(a) and LDL-C changes induced by Evolocumab

A

Observations: 1082
Spearman Correlation Coefficient: 0.5134

B

Observations: 1087
Spearman Correlation Coefficient: 0.5203
## CETP Inhibitor Anacetrapib

**Parameter** | **LS Mean Percent (95% CI) Placebo-Adjusted Change from Baseline** | **Week 24** | **Week 76**
--- | --- | --- | ---
Non-HDL-C | -31.7* (-33.6, -29.8) | -29.4* (-31.6, -27.3)
Apo B | -21.0* (-22.7, -19.3) | -18.3* (-20.2, -16.4)
Apo A-1 | 44.7* (42.8, 46.5) | 42.3* (40.5, 44.1)
TC | 13.7* (12.0, 15.3) | 15.6* (13.8, 17.3)
TG | -6.8 (-9.9, -3.9) | -5.3 (-8.9, -1.7)
Lp(a) | -36.4 (-40.7, -32.3) | -38.8 (-44.5, -33.9)
ApoE | 29.2* (24.7, 33.7) | 35.3* (30.6, 40.1)

*p<0.001; mean for all variables except for triglyceride and lipoprotein(a), for which median is shown*
**PCSK9 association with LDL in humans**

- **40%** of total plasma PCSK9 is found in association with LDL in plasma.
- PCSK9 is not associated with CM, VLDL, or remnant particles.

*References*


Kosenko T et al, JBC 2013.
Distribution of PCSK9 Forms in Plasma

- LDL-bound PCSK9 is mostly in the intact (62kDa) form.

*Tavori H, Fazio S et al. Unpublished*
Removal of LDL-associated PCSK9 by Lipoprotein Apheresis

- More than 50% of total plasma PCSK9 is lost during lipoprotein apheresis
- Most of the PCSK9 removed by the apheresis is LDL-bound

Association with LDL may protect PCSK9 from cleavage by Furin

Tavori H, Fazio S et al. Unpublished
LDL-bound PCSK9 is more efficient than apoB-free PCSK9 in binding to the EGF-A domain of the LDLR (ELISA Assay).

Tavori H, Fazio S et al. Unpublished
PCSK9 association with Lipoprotein(a)

- Natural gradient (iodixanol) ultracentrifugation to isolate LDL and Lp(a) fractions in patients with high Lp(a) (>30mg/dl)

Tavori H, Fazio S et al. Unpublished
PCSK9 association with Lipoprotein(a)

- PCSK9 is found in association with both Lp(a) and LDL isolated via natural gradient (Iodixanol) ultracentrifugation

*Tavori H, Fazio S et al. Unpublished*
PCSK9 association with Lipoprotein(a)

- Plasma PCSK9 associates with Lp(a) particles regardless of apo(a) size in humans.
- Plasma PCSK9 associates with human Lp(a) in transgenic mice.
- The ability of apo(a) to carry oxidized PL does not influence PCSK9 binding.
- Free apo(a) does not interact with PCSK9.

Tavori H, Fazio S et al. Unpublished
Characterization of PCSK9 association in a cohort of subjects with high Lp(a)

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Median</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error of Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>20</td>
<td>62</td>
<td>83</td>
<td>58.9</td>
<td>16.2</td>
<td>2.3</td>
</tr>
<tr>
<td>Lp(a) (mg/dl)</td>
<td>39</td>
<td>98</td>
<td>320</td>
<td>131.5</td>
<td>78.4</td>
<td>12.6</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>54</td>
<td>141</td>
<td>257</td>
<td>139.5</td>
<td>48.5</td>
<td>7.8</td>
</tr>
<tr>
<td>PCSK9 (ng/ml)</td>
<td>168</td>
<td>438</td>
<td>916</td>
<td>464.3</td>
<td>197</td>
<td>31.6</td>
</tr>
<tr>
<td>apoB (mg/dl)</td>
<td>52</td>
<td>173</td>
<td>313</td>
<td>182.7</td>
<td>70.5</td>
<td>11.3</td>
</tr>
<tr>
<td>Lp(a)-bound PCSK9</td>
<td>0</td>
<td>2.68</td>
<td>3.91</td>
<td>2.18</td>
<td>1.46</td>
<td>0.23</td>
</tr>
<tr>
<td>(RLU – log transformed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fold increase in PCSK9 on Lp(a)</td>
<td>0.2</td>
<td>1.7</td>
<td>9.2</td>
<td>2.4</td>
<td>2.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of High-Lp(a) subjects: Number of subjects: 39 (females = 25, on lipid-lowering medications = 25). RLU=Relative Light Units.

Tavori H, Fazio S et al. Unpublished
• Plasma PCSK9 levels do not correlate with LDL-C in patients taking statins or ezetimibe (red).

• Plasma PCSK9 levels correlate with Lp(a) levels regardless of lipid lowering medications.

Tavori H, Fazio S et al. Unpublished
Measuring the relative distribution of PCSK9 between LDL and Lp(a)

- LDL and Lp(a) fraction were isolated from all subjects and quantified using an apoB-PCSK9 ELISA.

Tavori H, Fazio S et al. Unpublished
Relative distribution of PCSK9 between LDL and Lp(a)

Relative distribution between apoB-PCSK9 levels in LDL and Lp(a) was studied separately in each subject.

Tavori H, Fazio S et al. Unpublished
CONCLUSIONS

- Lp(a) elevations are associated with and likely causative of CAD
- Lp(a) elevations are associated with and likely causative of calcified AS
- Lp(a) elevations are inversely associated with risk of T2DM
- Effect of PCSK9 agents on Lp(a) may suggest LDLR clearance
- Effect of CETP inhibitors on Lp(a) suggests that a different route is used
- Lp(a) is a reservoir for plasma PCSK9, and this may have something to do with Lp(a) changes under therapy