Lipoprotein(a): Science, evidence, management and emerging therapies

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SOTIRIOS TSIMIKAS, M.D.: Hello, everybody. This is Dr. Sotirios Tsimikas, the Director of Vascular Medicine and Professor of Medicine at the University of California, San Diego, and I am pleased to present this talk titled Lipoprotein (a) Science Evidence Management and Emerging Therapies on behalf of the Foundations of Cardiometabolic Health Certification course, and I hope you enjoy this next hour where you will learn everything there is to know about lipoprotein(a).

Lipoprotein(a): Prevalence, Pathophysiology, and Role in ASCVD Risk

Lipoprotein(a) is a highly prevalent and untreated risk factor currently for cardiovascular disease as well as aortic stenosis. As you see, the particle on the top left it is composed of three main components. One is the LDL-like particle that is typical of what you see in so-called LDL cholesterol and those particles are similar sized. There is an apolipoprotein(a) component that is covalently bound to the apo B of the LDL-like particle. Note that that particular apolipoprotein(a) does not have any lipid on it and therefore is not technically a lipoprotein, but the entire Lp(a)particle is a lipoprotein, and there are also oxidized phospholipids on both the LDL-like moiety as well as covalently-bound kringle IV 10 on the apo(a) moiety. The apo(a) moiety is quite interesting. It is evolved from the plasminogen gene. It has multiple kringle repeats that look like a Danish pastry and you see that there are 10 subtypes of which kringle IV type 2 is present, in this particular case, in four copies, but those copies can be present from one to more than 40 repeats. Therefore, this is a highly heterogeneous molecule among populations and individuals.

The cardiovascular phenotype is mainly two fold. One is cardiovascular arterial disease manifested in this case by coronary artery disease but it is also present diffusely systemically in the arterial circulation and small and medium size arteries. There also is an association with aortic stenosis and the calcification of the aortic valve leaflets that you see represented here on the bottom left. Now Lp(a) unlike some other lipid disorders is primarily genetically determined at birth and very few things influence it significantly although it can fluctuate by about 20% just through natural variability. It is main mechanism of atherogenesis are through the LDL moiety, through antifibronolytic effects of the apo(a) moiety that affects plasminogen activation and through proinflammatory effects of oxidized phospholipids. Currently, Lp(a) levels are seen as a major untreated risk factor, and there are no approved pharmacological therapies to reduce Lp(a) at the moment, although there are a lot of exciting new treatments on the way that we will review briefly.

Dr. Kare Berg discovered Lp(a) in 1963 when he was still a postdoc. This is paper that you see here. He initially determined that it was a genetic risk factor, and in the middle panel you see here that plasminogen - - kringles and as I mentioned earlier apo(a) has kringles 4 and 5 of plasminogen but has an inactive protease domain and therefore it inhibits plasminogen activation; however, through evolution it acquired multiple copies of the kringle IV that you see there in the yellow color and therefore each individual generally has two alleles but two different kinds of lipoprotein(a)that you see represented in these four examples. On the right side, you see the contribution of Lp(a) to various cardiovascular phenotypes and note peripheral arterial disease is prominent and so is

coronary heart disease, stroke, as well as aortic stenosis.

There are also secondary effects on heart failure which I believe are to be due from myocardial infarction. The contribution to chronic kidney disease is less clear although the kidney does affect plasma clearance of Lp(a). The metabolism of Lp(a) is primarily mediated through synthesis in the liver. The liver will make both apo(a) isoforms that you see represented here and here. These will bind to a newly-synthesized LDL through currently unclear processes and whether this is intracellular on the surface of the cell has not been fully determined but nonetheless the Lp(a) particles are secreted into the circulation, and note here this particular patient has two alleles. So he has two different sized particles, and so the plasma determinants of Lp(a) primarily through synthesis in the liver of apo(a) which mediates over 90% of the plasma levels. There are variety of receptors that you see here that can potentially clear a lipoprotein(a) CD36 through its oxidized phospholipid component SRA, these are scavenger receptors and also the LDL receptors LRP1 plasminogen receptors SR-B1 and the asialoglycoprotein receptor. The individual quantitative contribution of each one of these receptors is not fully determined yet.

Now in the plasma, the Lp(a) levels are determined by how much each chromosome induces protein synthesis of apo(a). So let me show you two examples here. This patient has 10 kringle IV type 2 repeats that you see here in the blue - - this patient's chromosome 2 is a much larger apo(a)particle. It has more gene. It has 25 repeats. So in each unit time you can make 2.5 times as many apo(a) molecules if you want to have 10 repeats versus if you have 25 because each one of these repeats is fairly large, and so for unit time in this particular example you can make much more of this particle in this one and so as you look now at the plasma concentration, the chromosome that has 10 repeats, kringle IV type 2 repeats, can make approximately 60 mg/dL of Lp(a). The chromosome that has 25 makes approximately 10, and so when this is measured in the clinical laboratory, the clinical laboratory will tell you that the Lp(a) level is 70, but the assays cannot differentiate how much each of the chromosomes it is making. So there is going to be some variability here between this number and this number depending on how many repeats are present in each allele. In general, this allele, the smaller one drives approximately 90% of the plasma levels in most patients; however, if these alleles are similar in size, it could be 50:50 or some other ratio. There are additional non-genetic influences and some other genetic influences on top of these isoform repeats. They can also contribute, and therefore this is not a one-to-one relationship that I am describing here. We also know that plasma levels of Lp(a) vary according to racial and ethnic groups at the population level. For example, if this is from a Dallas heart study, note here if you look at the number of kringles it is broad range and it is distributed in a Gaussian fashion in black patients; however, in Whites and Hispanics, it is more of a bimodal distribution. So some patients have small repeats and is another group that has large repeats. And Hispanics, they tend to have more the larger repeats because the larger repeats take a longer to make these tend to be associated with lower plasma levels. So now if you look at the Lp(a) levels here in nmol/L, note this is a log scale and note here black patients have the highest levels followed by whites followed by Hispanics and notice this is in some sense related to the isoforms but not completely. In black patients in particular, even though they tend to have smaller isoforms there is another factor that affects their

plasma levels so they tend to be higher approximately two to four fold higher than whites and Hispanics. If you now look at the relation between kringles, the number of kringles and the Lp(a) level notice here people that have small number of kringles, so 17, 18, 19, or less than that, tend to have high Lp(a) levels with the air bars being relatively small. If you now look at patients that have large number of kringles they tend to have low levels. Note here this is about 20 nmol/L. That will be about 7.5 mg/dL but notice the distribution is quite broad so that a patient with large isoform can have high levels but on average their levels will be quite low. Lp(a) is highly prevalent and likely underappreciated how prevalent it is. In fact one can argue it is more prevalent in elevated LDL and elevated triglycerides combined. This is data that we publish from a US laboratory database. First of all, notice the distribution of Lp(a) is not Gaussian. It is shifted to the right. Note here, most people have low or normal levels and the light blue is a border zone and then you have this tail of about 20% to 30% of the population they have levels over 50. So when you look at this numerically, most US laboratory use 30 mg/dL or less as normal and in this particular population, notice here 35% of the subjects had Lp(a) levels over 30 mg/dL. If this is extrapolated to the US, it is over 100 million people. If you extrapolate it globally, it is 2.45 billion people. If you use 60, which is twice normal that is about 20% of the population in this database and this is about 1.4 billion people. So I would estimate that maybe only 1% or 2% of these people know their Lp(a) level because it has been highly underappreciated as a risk factor and therefore in general not measured.

Now, there is a lot of determinants of cardiovascular disease. This is from the CARDIoGRAM plus CAD Consortium, and note here they list all of the alleles and genes that were associated with cardiovascular disease, and note here and these are the odds ratios for each one of those. Note here, this is PCSK9. This is the LDL receptor. This is 9p21. Note however, numerically, this is the Lp(a) gene. It actually came out to be the strongest single gene associated with cardiovascular disease. And most of these were in the lipid metabolism and inflammation area. So this is also underappreciated as a single contributor to risk. It is at the highest numerical level or you can argue these are small differences and nonetheless makes the point that this is a very important risk factor.

Now the potential causality for Lp(a) has been shown in three major settings, meta-analysis involving larger number of patients, Mendelian randomization studies, and genome-wide association studies. So when you look at meta-analysis, note here this is also a geometric scale that the risk is fairly flat until you get over 24 mg/dL then it tends to go up linearly with the modest effect on odds ratio of 1.5. This is now looking at Mendelian randomization where you have some genetic variable that predicts plasma levels, so this could be kringle repeats or could be SNPs. Note here, in these Mendelian randomization studies the risk is more linear, and the hazard ratio now is actually higher. It is up to 3 and people have levels over 117 mg/dL. Then finally you can look at genome-wide association studies by looking at variant alleles that affect plasma Lp(a) levels or associated with plasma. The difference is in plasma apo(a) levels. So this is from the PROCARDIS group with additional studies published by Robert Clark. Note here, if you have no variant alleles, your Lp(a) level is about 20, if you have one variant allele it is about 70. If you have two variant alleles, it is about 115. So these alleles are directly related to plasma levels, and then when you look at the odds ratio of CAD, note here there

is also a fairly linear relationship. So if you have two variants alleles with an Lp(a) level over 100, the odds ratio is over four compared to the lowest group.

So you see here there is a little bit of disparity between meta-analysis and genome-wide association studies with the genome-wide association study showing a more potent effect for the same Lp(a) level. One explanation for this is that there is a lot of issues related to meta-analysis. These are assays they are very different, studies are different, they are done different times. So there is a lot more heterogeneity in these types of studies then in genetic studies.

Now the question is if you are on a statin does it matter if your Lp(a) is elevated? The answer is yes. This is at almost 30,000 patient meta-analysis on patients on statins and note here despite being on a statin and note here again fairly flat risk until about 30 mg/dL and then the risk increases. In this particular example, it is also the odds ratio is a little bit over 1.4 similar to the prior meta-analysis studies.

Then finally in patients in studies that were PCSK9 inhibitor outcome trials. This is from ODYSSEY OUTCOMES. If you look at the placebo group and you look at the quartiles of Lp(a) note here the highest quartile is over approximately 60 mg/dL. Note here that the group that has the lowest levels, has a MACE incidence of about 8, and the group that has the highest levels, has a MACE incidence of about 12. So there is a about 25% higher risk whether you have low Lp(a) or high Lp(a).

So we talked a little bit about this and just to briefly summarize it based on looking at particle again, the proatherogenic effects are likely mediated by this LDL-like component and you can see here the potential mechanisms behind that typical what you seen before with LDL particularly foam/cell formation and necrotic core. You have proinflammatory effects from oxidized phospholipids. These oxidized phospholipids drive the expression of IL-8 other cytokines, chemotaxis, and MCP-1, and so these induced the recruitment of inflammatory cells into the lesion and those will cause plaque disruption. For this reason, Lp(a) is often associated with acute events rather than chronic cardiovascular disease. Finally, the apo(a) component because it has an active protease domain cannot be converted to plasmin with a tPA and therefore if you have high levels of this particular molecule, it will interfere with plasminogen activations through a competitive inhibition-type process and you can see all the different ways that can occur in this list on the right.

Now, I want to show the example of a patient of mine that we treated with stenting. This is a patient that had a saphenous vein graft to the obtuse marginal branch that you see here. This is a 12-year-old graft. You see a very bulky atheroma. This patient presented with unstable angina. We then treated this patient with a coronary stent and had a very nice result. However we used a distal protection device to capture some of the debris that may embolize, and you see here embolization of a large atheroma. So the question is how relevant is this to Lp(a) and particularly the oxidized phospholipid component. So we brought this atheroma back to our research lab and with collaborators we extracted it and then we actually looked for the presence of oxidized phospholipids. You see here this is the specific oxidized phospholipid called POVPC that you can see highly expressed within this atheroma.

Now in a different patient the unfortunately died of - - you see this very large fibroatheroma here with a significantly large necrotic core. The brown stain here is the Lp(a) presence. Note is both in the necrotic core and is particularly present in the subendothelial space and this brown stain is very diffuse. This is an oxidized LDL stain that looks to be primarily in the necrotic core but also a little bit in the subintimal space, and then this oxidized phospholipids which mirrors more of what is happening with Lp(a) and that is present really diffusely throughout. So the point of this is that and these lesions that we treat in the cath lab on a daily basis or in patients that have significant cardiovascular disease, there are lesions that really quite populated by both Lp(a) and oxidized LDL and oxidized phospholipids.

Now we have the capability to measure oxidized phospholipids in plasma now, and we can do this with an assay called OxPL-apoB and this captures OxPL on all apo B particles. Those include Lp(a), LDL, IDL, and VLDL.

This is a summary of nine studies that we published and note here that if you are in the highest level of OxPL, whether it is odds ratio or hazard ratio, and whether you have no prior CVD or prior CVD you have an odds ratio anywhere from 1.45 to 4.4 or having a prior cardiovascular risk then if you have low levels and this is adjusted for all known cardiovascular risk factors. Now in some cases this is independent of Lp(a). Some other cases it is not. It depends on the severity of illness. For the very severely ill patients, particularly those having acute MI or coronary syndromes, OxPL has to be a more stronger risk factor and independent risk factor of Lp(a) but in more a stable populations sometimes they give you the same information probably because they are integrally related in that they are both carried together and cause a simultaneous risk. So one could argue from these data the much of the risk prediction you get from Lp(a) is from its carriage of oxidized phospholipids.

Now in terms of aortic stenosis, we have published seven papers that show the relationship of Lp(a) and OxPL on aortic stenosis, but the Lp(a) by itself has been shown to be a causal genetic risk factor and genome-wide association studies are first shown by George - - and colleagues in New England Journal of Medicine in 2013. This is an evolving story and one link here maybe that oxidized phospholipid induce calcification and fibrosis and that maybe the mechanism through which Lp(a) triggers calcific aortic valve disease.

So here is some data from one of the studies. This is from the ASTRONOMER trial. They looked at patients with LDL of approximately 150 with mild-to-moderate aortic stenosis. They were randomized to rosuvastatin versus placebo. The study was overall negative, and we asked the question, “Well, was the right biomarker looked at in terms of aortic stenosis?” So we decided to measure Lp(a) and the oxidized phospholipids and notice here a fairly linear relationship of Lp(a) in terms of the progression rate which is now here quantified as the aortic valve velocity change in m/s per year. So note here you have got a doubling of the progression rate if you have an Lp(a) over 100. This is under 60. So this is a very clinically relevant because at 0.3 if you started at 3 m/s and basically through the year you are going to be 4 m/s and this is the level where generally surgery is considered to be needed particularly if the patient is symptomatic. Note here oxidized phospholipids on apo(a) here and the apo B, specifically on apo(a) and then on all apo B particles has a

fairly similar relationship to risk. Now does this matter? The answer is yes because if you have high Lp(a), you have high OxPL for apo B. Note here, if you are in that lowest levels, the need for valve replacement is minimal at least in this five-year follow-up, however, if you have high levels notice the valve replacement now about 40% of patients almost had need for valve replacement versus about 5%. So as a clinician if you measure Lp(a) and OxPL in a patient with mild-to-moderate aortic stenosis you might be able to pick a patient up in this yellow curve and target them through either further surveillance or more careful surveillance or you may be able to predict the need for aortic valve replacements more accurately.

Lp(a): Testing and Current Role in Guidelines and Risk Assessment

Okay. So let us switch gears now from the background to, "How to measure Lp(a)?" Traditionally, when the Lp(a) assay was set up in 1970s and 1980s, they were marked against the entire mass of the Lp(a) particle, and so when you order a mass assay which is mg/dL you do not just get the apo A component. You get the mass of apo(a), apo B, cholesterol, cholesteryl esters, triglycerides, phospholipids, and carbohydrates on the apo(a). This was never traceable to a reference system and so because of that, assays could not be harmonized with different manufacturers and different systems. So this has been a major problem with this. Unfortunately, in this field that we use in this particular metric and so now we have to train everybody to unthink this and use a better system and that better system is to use Lp(a) in molar concentration which detects only the apo(a) component. So now you get how many nanomoles of apo(a) per liter are in your sample. This is traceable to a IFCC/WHO reference material so that you can harmonize each of the laboratories at least to their standard. It does not mean the laboratories themselves are harmonized to each other but each laboratory can harmonize their values to a standard, and therefore you will not get a drift up and down of the values over time. There is an additional measurement called Lp(a) cholesterol. This measures the cholesterol in the entire particle. This is not fully validated clinically. There is work going on in these area right now but in general, this should not currently be used for clinical use to determine risk for your patients. Now there is no good way to convert one from the other accurately because you have to account for all these seven different particles in the mass. Unlike cholesterol if you can convert cholesterol from milligrams to nanomoles, cholesterol has one molecular weight. It is very easy to do that. You cannot do that with Lp(a) because it is seven different measurements; however, on average if you multiply mass by 2.5 you will get the molar concentration. Note here if you multiply 30 by 2.5 you get 75. Now this is not fully accurate. This is a study that we published recently. Note here, it depends on what level you start with. If you have low levels, this is in nanomoles, you do the conversion, your conversion factor is only 1.8 but if you have very high levels, is 3.64, so even though the average may be around 2.5, it really varies. So you have to be very careful how you interpret data when you try to do conversions and ideally what the field should do is move over to molar concentration and that will do away with conversions. Now one little issue here is that the FDA has not approved an assay in the US in nmol/L yet. All the assays are in mg/dL. So over the next five years, there is a movement underway to really try to get manufacturers to go to the FDA with better methods so that the whole field can be harmonized to one measurement across the board. Now even though Lp(a) levels are genetically determined there are certain things like increased

levels and certain things like decreased levels and those are due to couple of things, one is that the regulation of the Lp(a) gene can be affected by some of these processes or the assembly of the particles can be affected or the clearance of the particle. So you can get fluctuations. So things that affect the LDL receptor FH, familial defective apo B will increase the Lp(a) levels. Pregnancy, acute phase response, inflammatory mediators can activate apo(a) promoter through an IL-6 response element. Estrogens and testosterones across apo(a) synthesis, so when you go through menopause, those levels can rise because you lose the estrogen. Hypothyroidism can increase levels so can growth hormone. Chronic renal failure and nephrotic syndrome through reduced clearance. In contrast, there are certain things that can reduce Lp(a) levels. Hyperthyroidism, severe sepsis, both estrogen progesterone cross levels, bile acid in the liver suppress apo(a) synthesis, so biliary obstruction, severe liver disease through the lack of ability to no longer make Lp(a) through reduced synthetic capacity, abetalipoproteinemia. If you do not have apo B you cannot make an Lp(a) particle. LCAT deficiency is also associated with very low Lp(a) because the apo B particles can be lipidated into an LDL. And so you can see here several things can affect either up or down the Lp(a) levels and on top of genetically preset level from the liver synthesis.

Now why should I measure it? Does it make a difference in my patient? And the answer is yes. If you measure Lp(a) you can actually re-classify risk. This is a couple of papers that we published where you can actually show that if you measure Lp(a) you can affect four out of ten patients that were in intermediate risk category that you might now put either in a higher or lower risk category. To make this slide simple, just look at the color. This is from the Bruneck population. This is now adding risk to traditional risk assessment, so the risk here is 15 to 30%. This paper was published before some of the newer models were determined for risk prediction but nonetheless it makes a point that if your level is low, you get re-classified. If your level is high, you get re-classified and so about 4 out of 10 patients here change color and most people two-thirds go down around one-third goes up and the reason for that is we check Lp(a) you are much more likely to find a lower level than a higher level, so that might help you decide how aggressive you want to be on your patients depending on whether they have a high or low Lp(a).

Now what about guidelines? The guidelines are finally catching up to the data. They are now at least seven guidelines that recommend Lp(a) testing. The Canadian Cardiovascular Society and the ESC and European Society of Atherosclerosis both recommend every adult have an Lp(a) level checked at least once and so that is an important new advance because it really suggests that we should just be screening everybody for Lp(a) and the thing about is that make it very cost effective is it is about 70% to 80% who have normal levels, you never have to check it again. So you do a relatively inexpensive test once, it is especially a genetic test, if the level is low, it may fluctuate a little bit but it is never going to go into a different risk category. So I think this will allow much more testing going forward particularly in Europe and Canada and in the US right now it is called a risk modifier if it is over 50 mg/dL. I think eventually the whole field will eventually suggest that Lp(a) is checked at least once in everybody irrespective of baseline risks because it is so easy to do and in fact we suggest that in a paper recently from our Lp(a) clinic that this method of testing everybody should be cost effective and has the opportunity to pick up a lot of patients that otherwise would be missed. So we will see how this goes forward but

as new therapies come around there will be a lot more interest in getting patients evaluated for Lp(a), particularly if they have a family history, or if they have had an event when they were young. These are types of patients that we commonly have found to have high Lp(a). So I mentioned that ESC/EAS guidelines already and of course these are going to evolve over the next five years as more data comes through.

Now the other major advance that we have had which has really helped and will help more in the next decade or so is that we now have an ICD 10 code for Lp(a), and the Lp(a) foundation that Sandra Tremulous started really was instrumental in helping us get this. Now, you can actually diagnose a patient with high Lp(a) with a specific code and both the patient and the family, if there is family history and so this will allow hopefully better reimbursement by insurance companies that was felt to be appropriate and also can allow database studies and chart reviews to be able to track these patients and do more clinical research to ascertain risk in a variety of settings.

Current Treatment Options for Lp(a)

Now let us switch gears, what do we have currently available to reduce the levels based on the fact that epidemiology genetics have shown a strong association? What we lack is a randomized clinical trial showing that there is a clinical benefit from lowering Lp(a), and that really has never been done; however, this is what we have currently. We know that lipidapheresis can lower Lp(a) acutely by 70%; however, the liver makes it very quickly and so if you now two weeks for the patient to be back for the second session or even one week like they do in Germany, the levels will come back to baseline. So the time average reduction is only by half, about 35%. Apheresis removes Lp(a) but other lipoproteins and also reduces the viscosity. There has never been a randomized trial but the so called longitudinal prospective studies have all shown that you can reduce new events by about 80% as you go from no apheresis to yes apheresis. Now, you do not know how well a control group would have done in those cases. I am sure would not have been zero events, so we need a little more data on this but nonetheless this is an option for people having multiple recurrent events that continued to have that present to healthcare system, so you can get this approved in the US now for high Lp(a) assuming you have specific criteria and of course is done for high LDL cholesterol unresponsive to medical therapy. Nicotinic acid can lower Lp(a) 20% to 30%. This works through inhibiting the Lp(a) promoter via cyclic AMP. There has never been a randomized trial on high Lp(a) patients randomized in nicotinic acid or placebo; however, there had been several studies where the LDL has been normal and the Lp(a) on average has been normal and those studies have been negative. So the jury is out whether you would show a benefit with only 20% to 30% reduction, but we probably will never see that kind of study being done; however, we do have this available commercially in the US and maybe some other jurisdictions worldwide. PCSK9 inhibitors lower Lp(a) about 14% to 30%. The mechanism is not entirely known but likely has to do with decreased apo A secretion. I will go over a little bit of data on that but this is also a fairly modest effect relatively speaking. Mipomersen and lomitapide affect apo B they can reduce Lp(a). Statins on the other hand on an average tend to have an increase in Lp(a) and this may also have to do with interference of PCSK9 in the opposite direction as PCSK9 inhibitors. We do not know what is the clinical relevance of this is, however, as noted, fairly consistently now and several

studies. We do not have much data on ezetimibe/fibrates and bile acid sequestrants with Lp(a).

Now, let me give some examples of the statin effect. This is from 1989 and noted here is the title, HMG CoA Reductase Inhibitor Lower LDL Cholesterol Without Reducing Lp(a) Levels. This is technically correct but it is misleading because when you look at the effect of lovastatin on Lp(a) notice here there is a 23% to 33% increase in Lp(a). So this is noted right at the beginning of statin era but basically ignored for about 25 years.

This is from the ASTRONOMER trial that I mentioned earlier now what happened in ASTRONOMER the Lp(a) levels went up significantly about 20% from rosuvastatin and the lower LDL of course but Lp(a) went up and notice I showed you that the Lp(a) level was a predictor of outcome. So the oxidized phospholipid levels also went up in response to rosuvastatin because they carried by Lp(a), so the carrier went up and then this material was carried partially by Lp(a) increased along with it. So you can see here that this might be an explanation why this trial failed. Actually, the biomarker that probably was going to contribute most to progression of aortic stenosis actually went up, not down.

So this is a meta-analysis that we published recently. Notice here in the red are statin-treated patients, green is placebo. It is impossible to do placebo trials now with statins, so this is kind of old data but the nonetheless you can see the point here that on an average we have about a 30% increase in Lp(a), and this is a lot of patients, 5256 patients that were placed for the very first time on a statin versus placebo, and so it is a very clinging data and I think is believable in terms of the effect on statins on Lp(a).

Nicotinic acid I mentioned earlier beautifully affects all lipoproteins in the right direction but all very modest and of course has a lot of side effects, so this is an option for some patients but is not ideal at the current time.

Now PCSK9 inhibitors do not lower Lp(a) that much and this study in patients with high Lp(a) and ANITSCHKOW trial, so everybody here had Lp(a) level over 50 mg/dL. There was only a 14% reduction in Lp(a), and the other fascinating part about this trial is that notice the LDL cholesterol it was reduced by 61%, so that when you look at either carotid or aorta uptake of FDG-PET, there was actually no difference in the placebo over evolocumab. No suggestible difference. There was trend but not sure that can even called a trend because the air bars are large. But the point here is despite a 61% reduction in LDL, if you have very high Lp(a), this 14% reduction does not look like it translates to any kind of inflammatory change, so what would have been interesting if you lower Lp(a) 80% whether this would be different. That study has not been done yet. Now, despite all this, patients that are treated with PCSK9 inhibitors do seem to have a benefit if they have high Lp(a),and so this is ODYSSEY OUTCOMES. Note here this is the predicted four-year absolute risk reduction and notice here we have the Lp(a) and the corrected LDL and these are percentiles of Lp(a). So the lowest to highest, and so here people have low Lp(a) most of the benefit here in this MACE is due to the LDL change, okay? However, when you have very high Lp(a), you actually have about 25% of the benefit in MACE is related to the people that have high Lp(a). In other words, patients on high Lp(a), you derive benefit from reducing their Lp(a)and so that if you look at this overall MACE, three-fourths is from reducing their LDL and one-fourth is from reducing their Lp(a)even though

the Lp(a) was modest. So this suggests that when you just have a specific therapy just to reduce the Lp(a), it would translate to clinical benefit. The other way to look at this say well if you are in the fourth quartile of Lp(a) and you get a 20 mg/dL reduction absolute what does that translate to? You see here it translates to a hazard ratio about 0.9. So if you had an agent that is not PCSK9 inhibitors because it is not that powerful but if you lower the Lp(a) 40 mg/dL this would translate to a 20% relative risk reduction or hazard ratio of about 0.8. Keep in mind, if you reduce LDL 40 mg/dL you get the identical 20% relative risk reduction. So what this suggests if you can lower the Lp(a) 40 mg/dL, you should be able to get the exact same benefit if your patient has high Lp(a) level as if somebody's LDL was reduced the same amount.

Now what about aspirin? One study is suggesting that women who have high Lp(a) will benefit from aspirin therapy and these women have very high Lp(a) levels that were studied. You notice here if you happen to be on aspirin versus placebo, the hazard ratio is only 0.44, and this has not been replicated yet but it is possible that patients with high Lp(a) not only bleed less because Lp(a) is an antifibrinolytic risk factor but it may be the one group that is actually benefitting. So we will be able to test this in some of the new aspirin trials that have occurred recently and to update this information.

So this is the data from this particular study, note here in this curve that you see here, these are patients that were carriers that got placebo. So carriers of this one particular Lp(a) SNP. So if you are a carrier and got aspirin, notice here you are back in this group which is the same as in whether you are noncarrier that got placebo or aspirin. So basically, being a carrier of a SNP it predicts high Lp(a) levels on placebo which were the highest risk for having a myocardial infarction or stroke but if happen to be carrier on aspirin, you will not have baseline risk. So this really is quite interesting, it does suggests aspirin may have a benefit above and beyond affecting other pathways but it may affect the antifibrinolytic properties of apo (a) and may actually be mitigated by aspirin.

Emerging Therapeutic Options for Lp(a)

Okay, so let me switch now over to re-emerging areas, and I point to you this review on emerging RNA therapeutics that we published recently with Patrick Moriarty and Erik Stroes and this a field that is now growing rapidly and has potential to treat high Lp(a). And so antisense oligonucleotide is basically a single strand of modified DNA, there are - - modifications that allow it to become a drug. This is injected subcutaneously. It goes into the nucleus. It binds to the apo(a) mRNA that you see here so the duplex is formed. So the mRNA is in orange. The antisense oligonucleotide is in purple. This antisense oligonucleotide is only about 20 nucleic acids long, so the complex is here. A duplex such as this is seen as abnormal by every cell. There is an enzyme called ribonuclease H1 that will now cleave the sense strand which is the RNA strand that would make apo (a) protein. The antisense strand is not a cleave and combined to another one. Therefore this has very long half-life, and so this is a very elegant approach where you prevent the cell from secreting apo (A) and not having to worry about a new complex in the circulation, and so this field of RNA therapeutics is relatively new but it is now moving forward to treat diseases such as high Lp(a) that are very difficult to treat with small molecules. So this is the liver, it is making our two apo(a) alleles and molecules. These become Lp(a). The

antisense oligonucleotide will prevent both of these from forming. Liver will continue to make its pLDL and maybe large LDL particles, so there is no steatosis from this mechanism because it is specific to the apo(a) alone.

The other advance on this is you can put something called GalNAc which is this molecule right here. It is three galactosamine molecules complexed onto the antisense molecule. This allows the drug to go primarily to the liver because GalNAc targets the asialoglycoprotein receptor that is mostly present on hepatocytes. So it is a weak liver targeting drug and not have to go everywhere in the body and if you do that you can lower the dose you need by about 20% to 30% fold and so this is dose-response curve. In this example if you use non GalNAc molecule you need 122 mg of the drug to lower the Lp(a) 50%; however, if you put the GalNAc on the exact same molecule, you are now only 4 mg only. So basically what it does is it allows you to give a 30 fold lower dose to get the same effect. Of course it has many benefits particularly in side effect profile of the GalNAc molecules since the dose is so much lower.

Now in our laboratory at USCD in collaboration with Ionis Pharmaceuticals and Rosanne Crooke and her group, we combined forces and collaborated. We had the animal models that had the molecules. We did this work in our laboratory. Note here, if you inhibit apo B with an ASO, you can actually lower Lp(a) levels in this transgenic mice very nicely. You take them off the drug it comes back; however, these mice did make Lp(a) but because we did not affect the apo(a), they continued to make apo(a). So it was not elegant approach because you still had free apo(a) circulating in these mice and is not very clear what that means but it is best not to have any apo(a) at all. So Rosanne and her group went back to the drawing board to make an apo(a) antisense molecule and this way you can only inhibit the apo(a) component and that worked beautifully and these two papers I think were the proof of concept we needed to go and then do clinical development of these molecules for patients. So in the last 10 years or so, since those two papers were published we have had four papers published on antisense oligonucleotides that lower Lp(a) and note here there is quite a few studies quite a few doses and note here that the range of Lp(a) lowering can be anywhere from 35% to 92% depending on the dose and very large absolute reductions in Lp(a), as much as 188 nmol/L in some cases.

Let me just take you through some of the key findings from these studies. The drug is now called pelacarsen, and this is one study where it was given in patients with very high Lp(a) between 50 and 175 or greater than 175 mg/dL, so you have two groups and you have a placebo. The green shaded area is one that the drug was dosed and then light blue is one that off the drug. So you see a very nice response and it takes about three to four months for the Lp(a) levels to come back towards normal after you stop the drug here and that is because it has a long half-life in the hepatocytes. Notice oxidized phospholipids on apo B and apo(a) are reduced and notice here this is a transendothelial migration study, so the idea here is can we prevent monocytes from binding to the endothelial cells on a set up in the laboratory ex vivo, so we take monocytes from these patients, we add them to a layer of endothelial cells through a barrier or in front of a barrier and we see how easily the can crossover. The more they crossover, theoretically the worse it is because that is the first step in atherogenesis and monocytes get into the vessel wall through your endothelial cells. I noticed here when the Lp(a) was reduced in these patients, there was less

monocytes migration to this endothelial barrier and then note here that when we stopped the drug everything went back towards baseline. So this is a biological readout on top of the biomarker data that you see on this particular study. Now pelacarsen then was added to GalNAc - - and then the study was published just recently or about a year ago now in New England Journal of Medicine with different dose cohorts and doses and let me just take you through this. This was also placebo control six to twelve months of therapy. The primary endpoint was change in Lp(a) at week 25 to 27 depending on the regimen and this is now changed from baseline at that time point I mentioned in Lp(a) this is least square mean percent change, excuse me, note here not much change in placebo and there is a 80% reduction with the equivalent of 80 mg a month which will be the phase III dose. Now you might ask the question, "Well, how many patients actually got to a so called optimal level?" Which is under 50 mg/dL, and the answer is 98%. So these patients all had Lp(a) levels of approximately 200 to 250 nmoles/L, which is about a 100 mg/dL and so you see you can pretty much get almost everybody to goal with this approach, so it is going to be a beautiful test on the hypothesis because we have a potent drug that gets everybody into a normal range and then we can see the difference in the outcomes. There was significant reductions in OxPL for apo B and apo(a)and some modest reductions in LDL and apo B level which are likely related to enhanced clearance.

Now there has been several predictions about how much Lp(a) lowering do you need to get the same effect as you might get by lowering LDL 1 mmole or about 40 mg/dL. These numbers range anywhere from 40 to 101 mg/dL. Pelacarsen lowers Lp(a). In that study, it lowered Lp(a) 80 mg/dL. So it should be able to cover most of these predictions and test the hypothesis effectively.

The phase III trial is called Lp(a) HORIZON. It is a cardiovascular outcomes trial. It plans to enroll approximately 7680 patients that have established CVD with a prior event, MI, stroke, or PAD, and it is going to use a slightly high Lp(a) level that we used previously about 70 mg/dL or higher, and the patients have to be optimally controlled on cholesterol-lowering medication. Pelacarsen would be dosed at 80 mg monthly versus placebo, and it will be two co-primary endpoints, time to first occurrence of MACE and people with Lp(a) over 70 for about four years and then time to occurrence of MACE in people with Lp(a) over 90. This was anticipated to be the median level. It may be higher but both of these endpoints, if either one hits it will be considered a positive outcome. The trial is very robust. It is going to go to 4.5 years of median follow-up. Minimal, every single patient will have two and a half years and will be 993 events, and we anticipate this to be reading out sometimes in the middle of 2024 unless the timeline changes.

So what will happen in this trial? This is the frequency distribution of Lp(a). If we look at our crystal ball, all our patients in HORIZON will be in the red. The drug will lower almost everybody to the green here. So this is basically the test of hypothesis. You move people from the 70 and above to 50 and below and then you look to see if those patients have got pelacarsen had less MACE than the patients that got placebo.

Lp(a) Patient Case Studies

Okay. So that is the summary. I am going now show you a couple of case studies that impact another interesting aspect of Lp(a) biology which is how much cholesterol is

carried on and what does that mean? Let me show you a little bit of that work that we have done, so we have opened up an Lp(a) clinic at UCSD, and this has been going on since 2014. This particular clinic only sees patients with elevated Lp(a), so it is the ultimate in sub, sub, sub specialization. Nonetheless, it is a very popular clinic and the patients find it very useful to be able to have a place where they can have not only care for themselves but also the extended family members through cascade screening.

Now the LDL that is measured is actually a combination of LDL plus Lp(a) cholesterol. This is not as well known. We popularized this in 2015 but it has been known for a long time but can we bring in this to kind of more the forefront now for interest and let me show you why. When you do Friedewald calculations of LDL which most laboratories do it has the Lp(a) cholesterol in it. So in this example here, note that the density of LDL and Lp(a) are very similar and so when you get total cholesterol and you get your VLDL, and you subtract that HDL which can be measured you get the LDL but this LDL is really this here and there is no assay that can separate the two even direct LDL comes both and so does ultracentrifugation. So in a typical scenario where the patient has low Lp(a) this does not matter but if the patient a lot of these Lp(a) particles, much of the LDL cholesterol is going to be Lp(a). So here is an example of that and do not take the numbers as written in stone but I guess it makes the point that there should be an Lp(a) cholesterol in the total cholesterol equation like you see here, and so let me give you an example. Let us say the patient has an Lp(a) of zero and you measure their total cholesterol as 150, you get the breakdown like you see here; however, let us say the Lp(a) mass is 15 mg/dL, about 30% of that on average it changes from 20% to 45% but let us just say 50%, 30% is cholesterol. That means that 5 mg/dL of this going to be Lp(a) cholesterol. So this patient real LDL corrected is 65, the Lp(a) cholesterol is 5. You might say, "Well, this is clinically meaningless," which is probably true, but what about patient like this that has an Lp(a) over 150. It is possible 50 mg/dL of the LDL is actually Lp(a) cholesterol and the true LDL can be 20. So if you said, "Well, I want to lower this patient's LDL from 70 to 50," look what are you dealing and where the LDL is really 20. So this patient is not going to respond well to more LDL lowering therapies and an extreme example is possible in some patients may have mostly Lp(a) and they will be completely unresponsive to further LDL-lowering therapies.

Let me show you an example from my clinic. This is a patient who came with an acute coronary syndrome. You see she has a very complex lesion in the LAD and then she was successfully stented and did well, but note here when she came in she was only 49, mild obesity, father had an MI at 63 no other cardiovascular risk factors and note here that she did well with the procedure but let us look at her lipids. When she came in, you might wonder, "What is this woman doing in the ACS and the cath lab with a total cholesterol of 163 and an LDL of only 85?" So when she came in she did not have Lp(a) measured. She was placed in 80 of atorvastatin and the came back to see me six months later. We rechecked her lipids and lo and behold, her LDL is still 82. So you might say, "Well, she is either not taking her medications or this is statin resistance." Now, we measure Lp(a)level. It is 225. So if we make the assumption 30% of this is cholesterol and her Lp(a) cholesterol is 75 and her true LDL is only 7. So of course if you put a patient with an LDL of 7 on 80 mg of atorvastatin, it is not really going to change much so this in this particular case if you assume her Lp(a) cholesterol was 75, her initial LDL was 85. So it

basically went from an initial of 82 to 75 is a 7 mg/dL difference. And so she basically went from 10 to 7. So she had about a 30% reduction in her real LDL. The bottom line is she is resistant because most of her cholesterol is on Lp(a) and that is not responsive to statins. So we placed her on niacin which does respond in terms of lowering all the apo B containing lipoproteins and then when she came back she had actually a very dramatic reduction in her LDL as well as Lp(a). Now her Lp(a) came down as low as 75, so some of the 225 may have been related to the acute phase response. They may have been present even at six months but the point here is that these patients can be resistant but it is pseudo resistance because it is related to the fact that the LDL the lab is telling you thus can breakdown what is Lp(a) cholesterol and what is LDL and those do not respond the same way with statins versus niacin. So bottom line is for as a clinical pearl, the elevated Lp(a) can be present in a young patients particularly when there is a family history and if they do not respond well to statin therapy think that the Lp(a) might be elevated particularly if they do not respond to a statin therapy and they are compliant.

So I think the take-home message is from this talk are as follows. The Lp(a)'s clinical expression is manifested in arterial sites in the aortic valve. Lp(a) is a highly prevalent independent and genetic risk factor for CVD. It is risk after about 30 mg/dL or 75 nmoles/L is nearly linear to plasma levels. Measuring Lp(a) is now recommended in seven guidelines including two of the recommend measuring in every adult at least once. The patients with elevated Lp(a) have lower LDL cholesterol and it can be appreciated from the laboratory measurement it stands off an increase Lp(a). PCSK9 inhibitors are weakly effective in lowering Lp(a), but they actually may have a MACE benefit if the Lp(a) is elevated. So a highly effective therapy for Lp(a) has now finished stage II and the Lp(a) hypothesis will finally get his day in court from the phase III Lp(a) HORIZONS cardiovascular outcomes trial which is now ongoing.

Okay with that let us just do some multiple choice questions. Here is one, according to the 2018 ACC/AHA Cholesterol guidelines, which of the following is a risk enhancer? A: Lp(a) greater than 30 mg/dL. B: Apo B greater than 100 mg/dL. C: Triglycerides greater than 2 mmol/L. D: Ankle-brachial index.

This is not necessarily a trick question. It just questions your understanding of the numbers, and the answer actually is C, triglycerides greater than 2 mmol/L. Why is it not A? It is because the ACC/AHA uses greater than 50 mg/dL not 30. It uses apo B greater than 130 and it uses the ankle-brachial index less than 0.9. So all of these are part of that risk-enhancing profile but the numbers are 50, 130, 2, and 0.9.

Okay. Let us go to another question, the data for Lp(a) as a cardiovascular risk factor is stronger in A: Secondary prevention. B: Primary prevention. C: Genetic analysis. D: Acute coronary syndromes.

And the answer for this is C, genetic analyses, and the reason for that is that when you look at the overall body of work in genetic analyses, these are very, very consistent effects of looking at Lp(a) SNPs or small isoforms that then correlate with Lp(a) levels and then correlate with outcomes. So this triangle of a genetic marker that affects a quantitative trait in the plasma and is associated with cardiovascular phenotype is beautifully represented in this. The data next strong is for primary prevention and then

for secondary and acute coronary syndromes. Of course there is data on all of these but the question asked which one is stronger. All of this can be debated, I think, genetic analyses are really the best and most consistent data for Lp(a) as a cardiovascular risk factor.

Okay. Let us do three more questions. For which clinical phenotype does Lp(a) have the strongest association? A: Coronary artery disease. B: Acute myocardial infarction. C: Stroke/TIA. D: Aortic stenosis.

The answer is B, acute myocardial infarction. Most of the studies have linked an acute event like an acute myocardial infarction to risk and one potential reason is that Lp(a) does not have as much lipid on it if you look at all the mass and particles as LDL, so it could be associated with plaque burden but where it maybe more relevant is in plaque inflammation and antifibrinolytic affects tilting the balance of thrombosis. So it is associated with the aortic stenosis but in about a third of the patients with aortic stenosis and also associated with much higher levels looks like then with cardiovascular disease, maybe starting at 60 mg/dL versus 30 and then of course associated with CAD and stroke and TIA but those seem to have more modest odds ratio compared to the other two that I mentioned.

Okay, what is the best characterization for the pathophysiological role of developing cardiovascular disease? A: Atherosclerosis via the apo(a) component B: Cholesterol content of the apolipoprotein(a) component. C: Interplay of atherosclerosis inflammation and antifibrinolytic effects, and D: Apolipoprotein(a) component binding to the endothelium.

The answer is interplay of atherosclerosis inflammation and antifibrinolytic effects. We covered this in the earlier part of the talk. Why are the other answer is not correct? The apo(a) component is affecting only fibrinolysis. It does not have any lipid, so it is not directly affecting atherosclerosis. The apo(a) component also is not a lipoprotein, so even though it is called that, it actually has no cholesterol on it and apo(a) does bind to the endothelium but it is more potent effect is likely binding to the subendothelial matrix and accumulating in the plaque and causing its pathophysiology there.

Okay. Which biomarker or risk factor would you best measure in a 42-year-old thin male of South Asian ancestry with LDL cholesterol of 122, triglycerides 148, and whose father died suddenly at age 48 to best predict outcome? A: hsCRP. B: Lp(a). C: 9P21 gene SNP. D: Red cell fatty acid content.

Okay. So we know that South Asian individuals have a higher propensity to obesity and also metabolic syndrome for much lower BMI than other populations. However, we are told this patient is thin and so not even average weight, and so hsCRP tests correlate strongly with metabolic syndrome and obesity and it is less likely answer. The answer is B, Lp(a) and the reason for this is that in South Asia, the Lp(a) levels on average tend to be second highest relative to the rest of the world, the highest being patients from African descent. So a patient with a little bit of LDL elevation, a little bit of triglyceride elevation with a family history which is very important here because his Lp(a) is genetic is most likely to have also elevated lipoprotein(a). 9P21 is a reasonable answer but we do not

know how relevant that is in South Asian populations, and we also do not know what this particular SNP does because it is in the gene desert and so far no clear pathophysiologic association has been documented. Red cell fatty content maybe relevant in people that take fish oil supplements or on omega-3 fatty acids but in this particular patient we are not told he has that and although there could be some dietary influences there, this is likely not relevant to this particular patient.

Okay, and the final question is word association, which of these pairs go best together? A: Apolipoprotein(a)-fibrinogen. B: Lp(a) - directly pro-thrombotic. C: Small dense LDL - oxidized phospholipids. D: Antisense oligonucleotide - pelacarsen.

The answer is D. Pelacarsen is the antisense oligonucleotide being tested in the HORIZON trial. Why are the other ones go together less well? Apo(a) inhibits plasminogen not fibrinogen. Lp(a) is not directly pro-thrombotic in other words if you add Lp(a) to a cell or a system to induce thrombosis it does not actually occur any more than LDL does. It is antifibrinolytic. In other words, when a thrombus is forming it can actually allow it to enlarge but you will not necessarily initiate it, and then small dense can be associated with oxidized phospholipids but oxidized phospholipids are more strongly associated with lipoprotein(a).

So that is the end of the talk. I want to thank you for listening, and I hope you found this a rewarding one hour and you learned a lot in this session. Thank you.