State of a multicentric study on Lp(a)

MIGHTY MEDIC PROJECT

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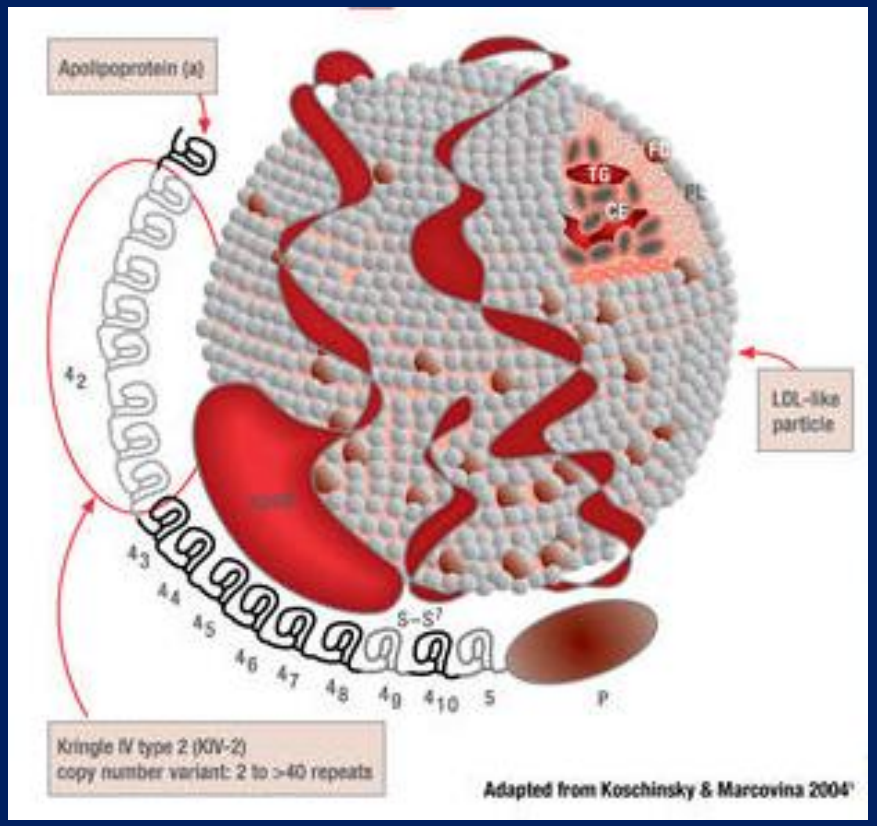
Structure of Lipoprotein(a)



A characteristic feature of apo(a) is the presence of loop-like structures called **kringles**.

Kringle domains are triple loop structures stabilized by three internal disulfide bonds

The linker domain between kringles is glycosylated in apo(a)

Plasma levels of Lipoprotein(a)

Plasma concentrations of Lp(a) show remarkable variation between individuals.
Such variation exists also between different human populations and has been observed in non-human primates, too

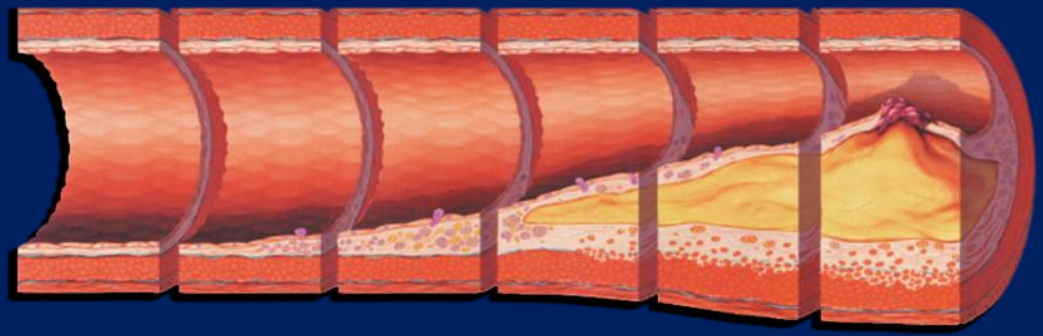
RANGE: from <0.1 to >200 mg/dl

Plasma levels of Lp(a) are genetically controlled for 70-90%

Lp(a) and atherosclerotic cardiovascular risk

Structural omology with LDL

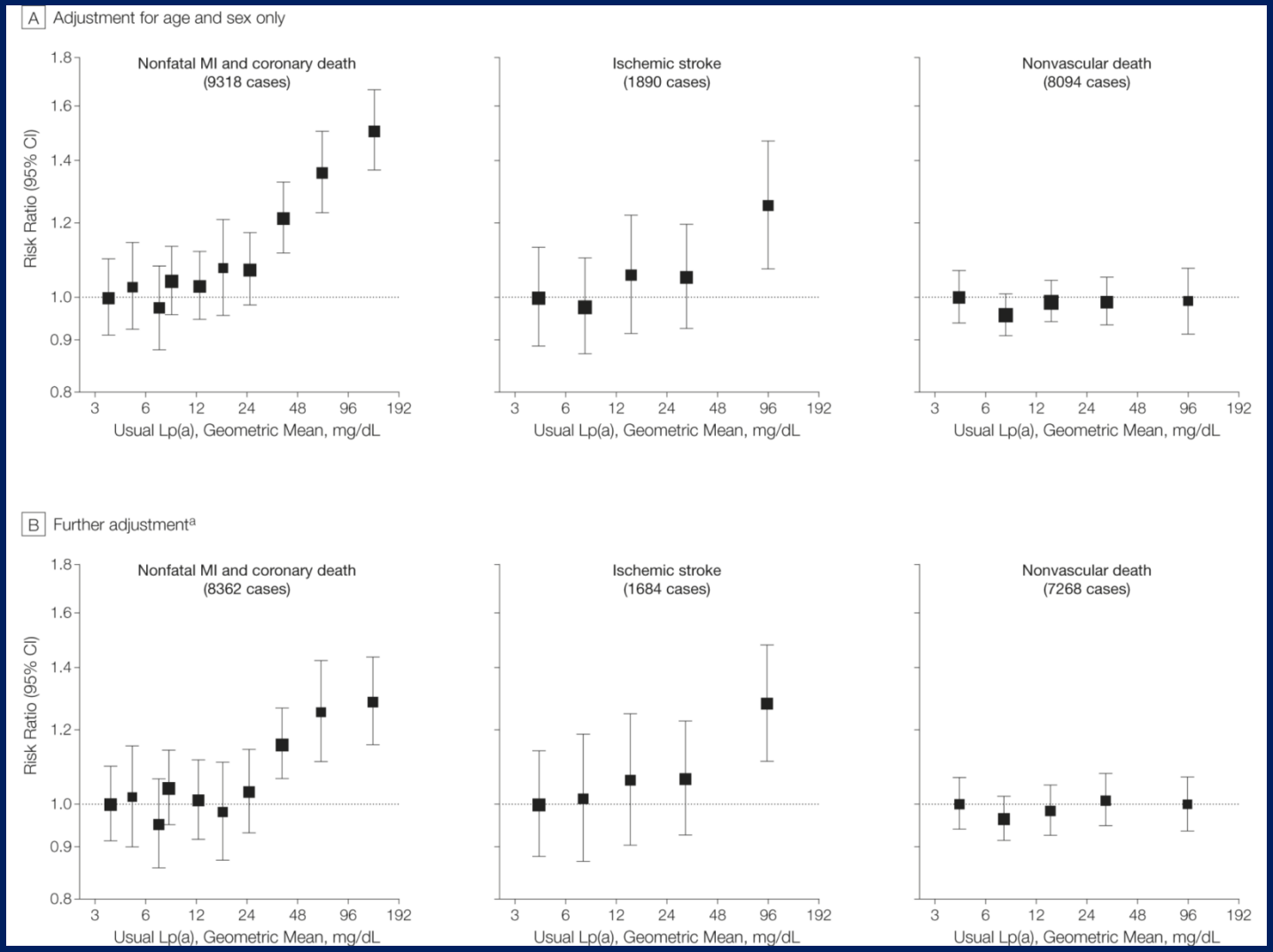
Structural omology with plasminogen



atherosclerosis

thrombosis

Lipoprotein(a) Concentration and the Risk of Coronary Heart Disease, Stroke, and Nonvascular Mortality

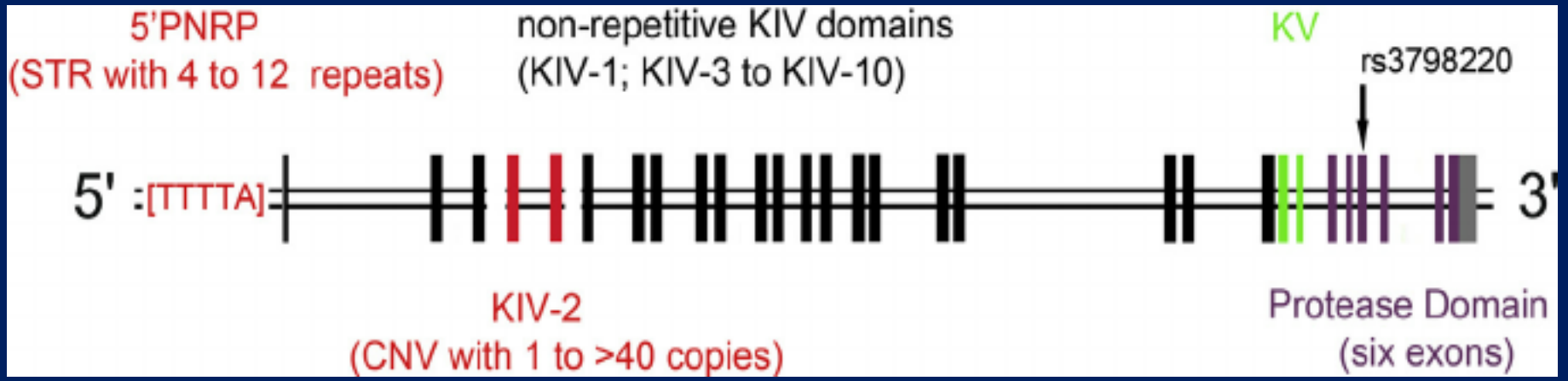


[a]Further adjustment for usual levels of systolic blood pressure, smoking status, history of diabetes, body mass index, and total cholesterol.

The JAMA Network

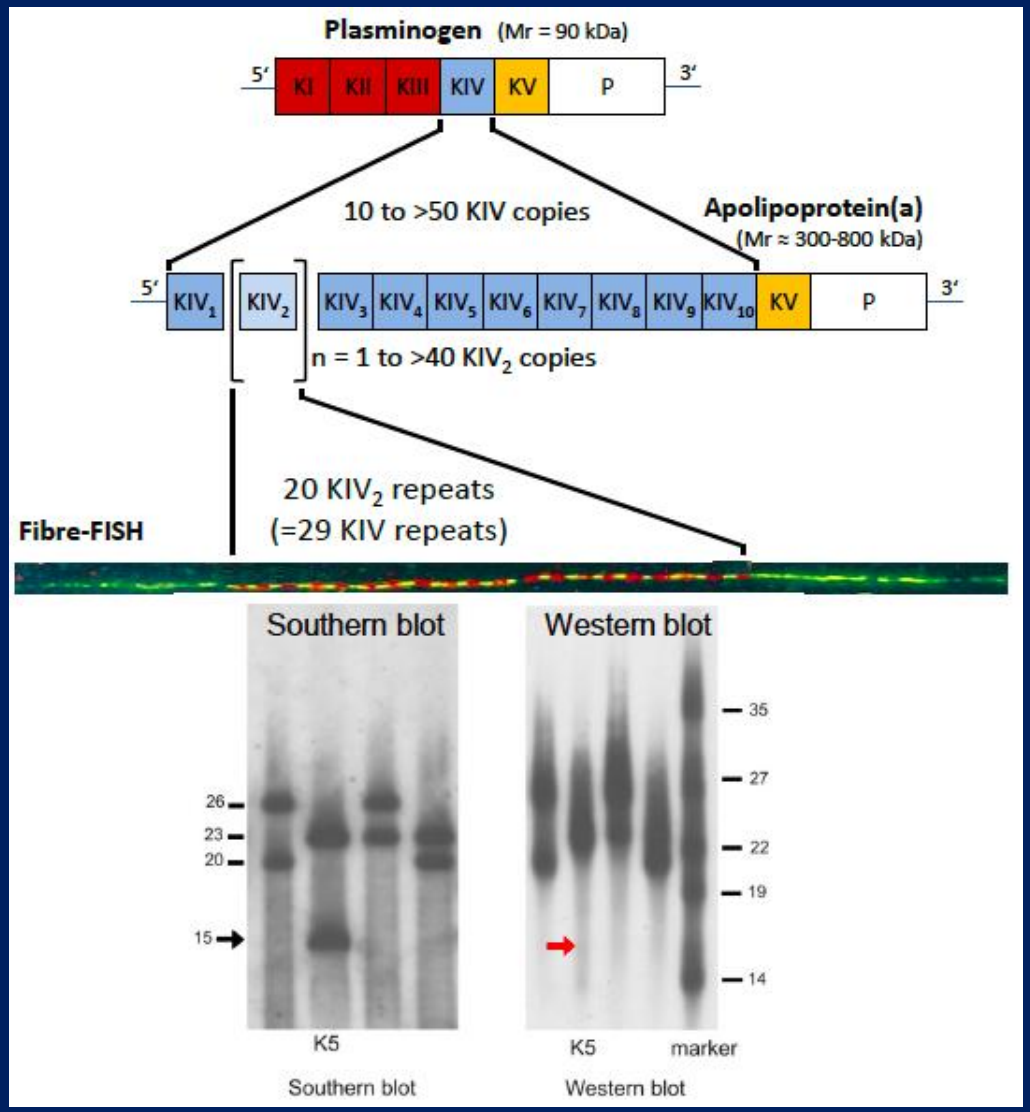
JAMA. 2009;302(4):412-423.

Gene LPA chr 6q27



The strong role of genetics in determine Lp(a) plasma levels, is responsible of the particular distribution in the population, not normal but very asimmetric

Structure of LPA gene

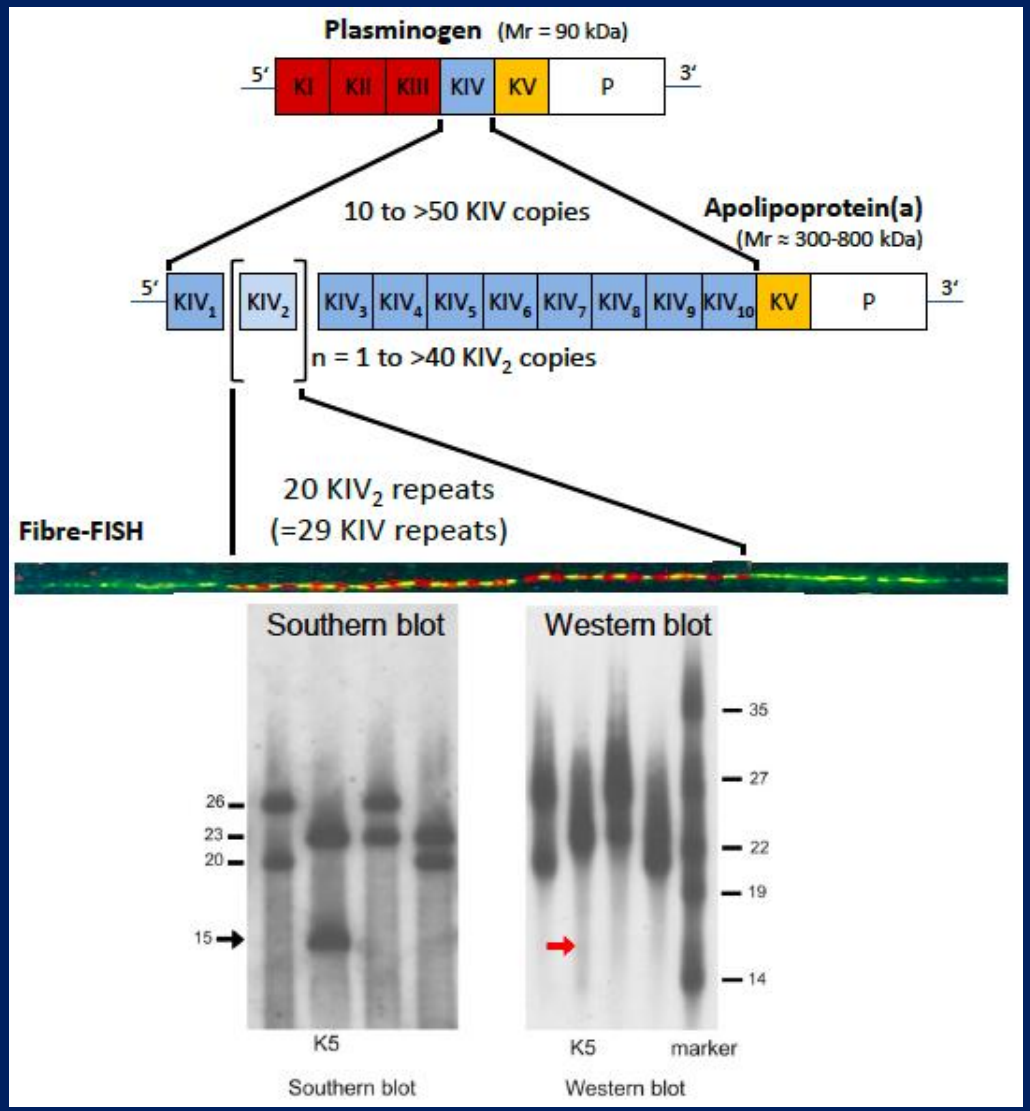


Schmidt et al. Jour of Lipid Res 2016

Human *LPA* shares a high sequence homology (78% to 100%) to human *PLG* in both the un-translated and coding regions:

- Kringles I to III were lost by deletion
- an expansion and differentiation of the Kringle IV domain in *LPA* resulted in ten different types of KIV domains, all specific in their amino acid composition

Structure of LPA gene



Schmidt et al. Jour of Lipid Res 2016

Further expansion of one of the KIV domains (Kringle IV type 2, KIV-2) resulted in the multiallelic (1 to >40 copies) intragenic copy number variation (CNV) known as the KIV-2 CNV

The other Kringle IV encoding domains (KIV-1 and KIV-3 to KIV-10) are present only as single copies

KIV-2 CNV and Lp(a) plasma levels

An inverse correlation of CNV length with Lp(a) levels has been demonstrated in almost all analyzed populations

This inverse correlation is explained by the processing of apo(a) isoforms during transit through the secretory pathway from the hepatocytes: shorter apo(a) isoforms are

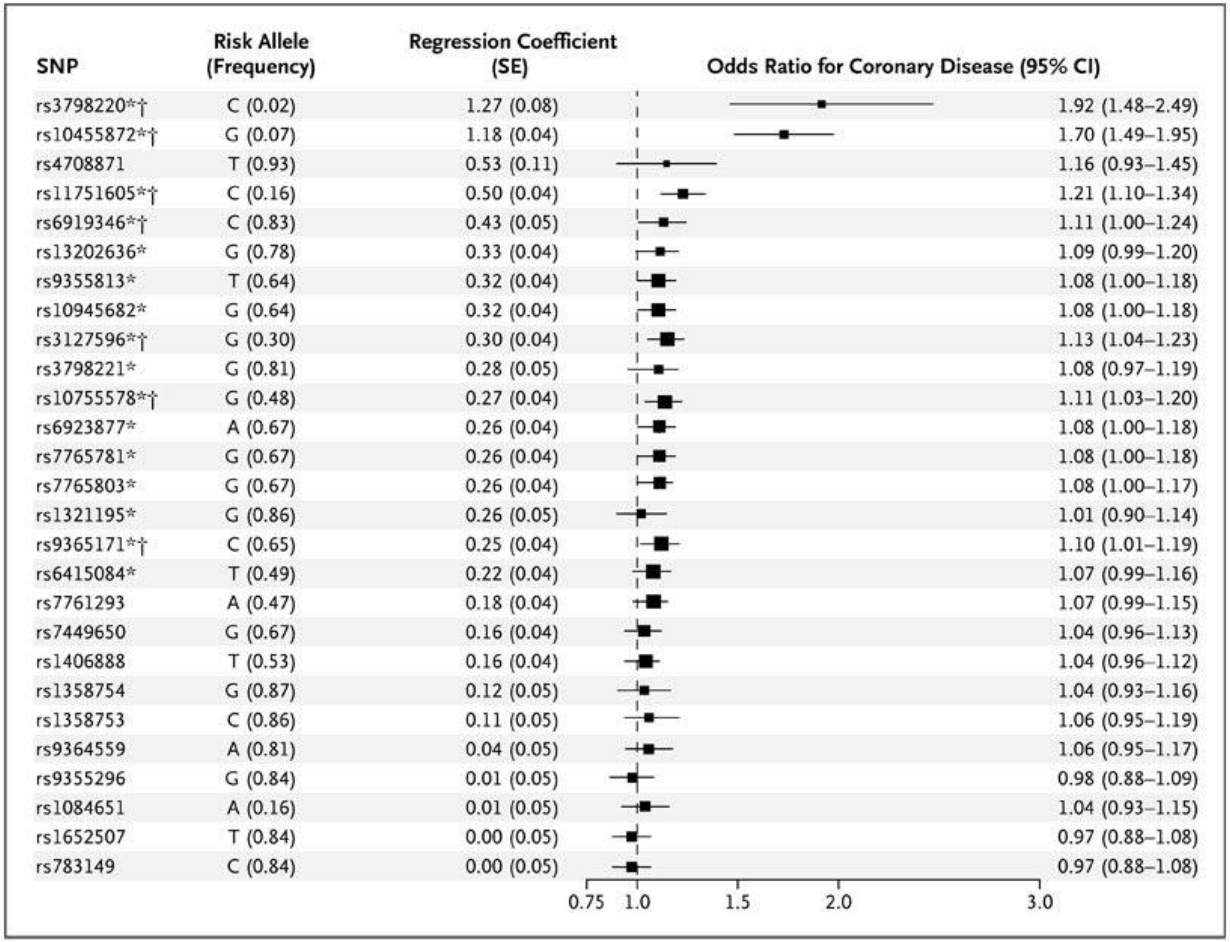
Apo(a) size polymorphism is a major predictor of Lp(a) levels contributing between 40% to 70% of the variation in Lp(a) concentrations

Recent Studies that Have Investigated Apo(a) Size and Non-Size Polymorphisms

Authors [Ref]	*Year*	*Major polymorphisms*	*LPA gene location/function*	*Population*	*Association with Lp(a)*
Rubin et al. [19]	2006	a C/T variation	promoter region	CA and AA	T allele was less and small PNR allele was more common in AA than CA. A stepwise decrease in Lp(a) level with increasing PNR number >8 was observed in CA, but not in AA.
		a pentanucleotide repeat (TTTTAn)	1 kb upstream		
Chretien et al. [23]	2006	G-21A	increases promoter activity	AA and CA	Lp(a)-increasing SNP G-21A was common in AA, whereas Lp(a)-lowering SNPs T3888P and G+ 1/inKIV-8A were common in CA. All 3 SNPs contributed to higher Lp(a) levels in AA.
		T3888P and G+ 1/ inKIV-8A	inhibits Lp(a) assembly		
Luke et al. [24]	2007	I4399M/rs3798220	protease-like domain	CA	Risk allele-carriers had 5-fold higher median Lp(a) level and significantly smaller apo(a) isoform (17 K4 vs. 22 K4) vs. non-carriers.
Clarke et al. [20]	2009	rs10455872	maps to intron 25	CA	16 SNPs had significant effects on Lp(a) level. The two SNPs had the strongest associations with an elevated Lp(a) level and a reduced copy number of K4 repeat and explained 36% of the variance in Lp(a) level.
		rs3798220	protease-like domain		
Ober et al. [30]	2009	rs6919346	maps to intron 37	Hutterites and CA	In Hutterites, both SNPs were significantly associated with an elevated Lp(a) level, independent of the apo(a) size and had a combined effect size of 4% on Lp(a) level. In CA, rs6919346 was associated with Lp(a) level.
		rs1853021 (+93C/T)	5′ untranslated region		
Lanktree et al. [27]	2010	rs10455872	maps to intron 25	South Asians, Chinese, CA	Prevalent only in CA and was associated with both Lp(a) level and K4 repeat number.
		rs6415084	the same haplotype block as the K4 type 2 variation		Prevalent in all 3 ethnicities and was associated with both Lp(a) level and K4 repeat number. SNPs and apo(a) size polymorphism together explained 36% of variation in Lp(a) levels in CA, 27% in Chinese and 21% in South Asians.
Ronald et al. [28]	2011	rs3798220	protease-like domain	CA	9 SNPs were predictive of Lp(a) level and accounted for 30% of Lp(a) variance. The two SNPs were associated with Lp(a) level after accounting for K4 repeat number and explained 22% of Lp(a) variance. SNPs and apo(a) size polymorphism together explained 60% Lp(a) variance.
		rs10455872	maps to intron 25		
Deo et al. [33]	2011	rs9457951	intronic	AA	A number of common SNPs were associated with Lp(a) level accounting for up to 7% of the variation, as well as >70% of the African-Caucasian interethnic difference in Lp(a) level. SNP rs9457951 expressed the strongest association and alone explained 5% of Lp(a) level variance.
		rs6930542	intronic		
		rs10455872	maps to intron 25		
		rs6922216	intronic		
		rs1801693	exonic, K4 type 9		
		T3888P	inhibits Lp(a)		
		G+ 1/inKIV-8A	assembly		

METABOLIC SYNDROME AND RELATED DISORDERS
Volume 9, Number 6, 2011

Associations of Single-Nucleotide Polymorphisms (SNPs) in *LPA* with the Lp(a) Lipoprotein Level and the Risk of Coronary Disease in the PROCARDIS Cohort.



SNP	Risk Allele (Frequency)	Regression Coefficient (SE)	Odds Ratio for Coronary Disease (95% CI)
rs3798220*†	C (0.02)	1.27 (0.08)	1.92 (1.48–2.49)
rs10455872*†	G (0.07)	1.18 (0.04)	1.70 (1.49–1.95)
rs4708871	T (0.93)	0.53 (0.11)	1.16 (0.93–1.45)
rs11751605*†	C (0.16)	0.50 (0.04)	1.21 (1.10–1.34)
rs6919346*†	C (0.83)	0.43 (0.05)	1.11 (1.00–1.24)
rs13202636*	G (0.78)	0.33 (0.04)	1.09 (0.99–1.20)
rs9355813*	T (0.64)	0.32 (0.04)	1.08 (1.00–1.18)
rs10945682*	G (0.64)	0.32 (0.04)	1.08 (1.00–1.18)
rs3127596*†	G (0.30)	0.30 (0.04)	1.13 (1.04–1.23)
rs3798221*	G (0.81)	0.28 (0.05)	1.08 (0.97–1.19)
rs10755578*†	G (0.48)	0.27 (0.04)	1.11 (1.03–1.20)
rs6923877*	A (0.67)	0.26 (0.04)	1.08 (1.00–1.18)
rs7765781*	G (0.67)	0.26 (0.04)	1.08 (1.00–1.18)
rs7765803*	G (0.67)	0.26 (0.04)	1.08 (1.00–1.17)
rs1321195*	G (0.86)	0.26 (0.05)	1.01 (0.90–1.14)
rs9365171*†	C (0.65)	0.25 (0.04)	1.10 (1.01–1.19)
rs6415084*	T (0.49)	0.22 (0.04)	1.07 (0.99–1.16)
rs7761293	A (0.47)	0.18 (0.04)	1.07 (0.99–1.15)
rs7449650	G (0.67)	0.16 (0.04)	1.04 (0.96–1.13)
rs1406888	T (0.53)	0.16 (0.04)	1.04 (0.96–1.12)
rs1358754	G (0.87)	0.12 (0.05)	1.04 (0.93–1.16)
rs1358753	C (0.86)	0.11 (0.05)	1.06 (0.95–1.19)
rs9364559	A (0.81)	0.04 (0.05)	1.06 (0.95–1.17)
rs9355296	G (0.84)	0.01 (0.05)	0.98 (0.88–1.09)
rs1084651	A (0.16)	0.01 (0.05)	1.04 (0.93–1.15)
rs1652507	T (0.84)	0.00 (0.05)	0.97 (0.88–1.08)
rs783149	C (0.84)	0.00 (0.05)	0.97 (0.88–1.08)

Clarke R et al. N Engl J Med 2009;361:2518-2528.

The NEW ENGLAND JOURNAL of MEDICINE

Mighty Medic multicentric project
SELECTION OF PATIENTS:

Inclusion criteria: isolated or combined+iperLp(a) +CVD or/and ATS in lipoprotein apheresis therapy

FROM ROME (prof.Claudia Stefanutti): 24 patients

FROM VERONA (prof. Maria Grazia Zenti): 5 patients

Parma LAB (dott. Federica Vacondio)

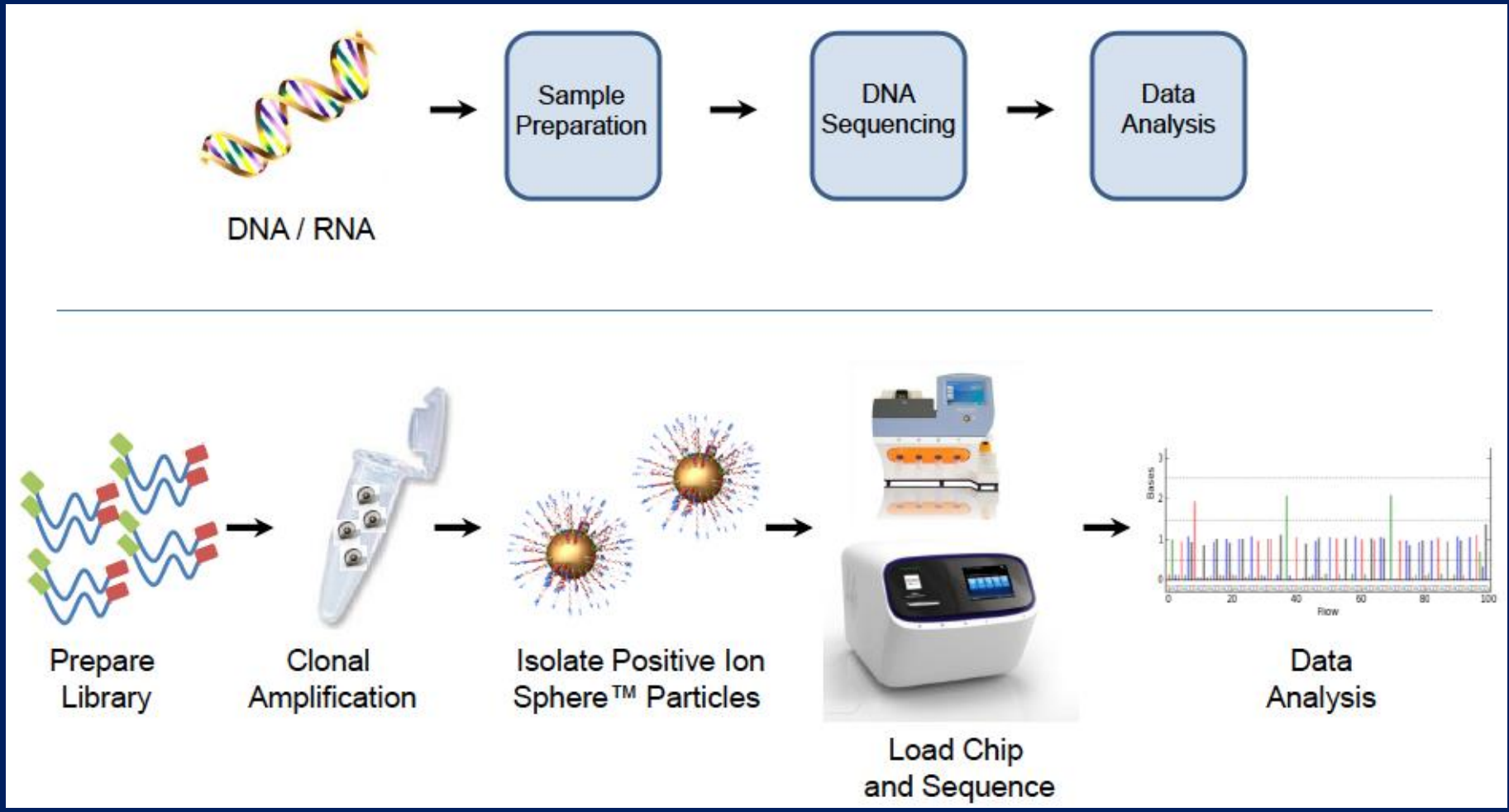
- Liquid chromatography/mass spectrometry method capable of measuring apolipoprotein(a) concentration while simultaneously determining the number of kringles present per protein will be set up.

Parma LAB (prof. Elda Favari)

- Cholesterol Loading Capacity (CLC) of sera containing Lp(a) will be performed in order to understand possible correlation to number/size of kringles determined by chromatography/mass spectrometry.

These analysis will be done in sera from patients before and after Lipoprotein Apheresis.

Genoa LAB: NGS sequencing



Next-generation sequencing (NGS) based on a custom AmpliSeq™ panel designed for sequencing of human genes related to the lipoprotein metabolism will be performed with Ion PGM™ Sequencer:

Genes in the panel:

LIPA, GBA, PCSK9, LDLRAP1, ANGPTL3,GALNT2, CH25H, APOA1.APOA5, APOC3, HNF1A, SCARB 1, GPD1, LIPC, LCAT, LMF1, CETP, SREBF1, LIPG, ANGPTL4, LDLR, APOC2, APOE, CREB3L3, APOC1, APOB, GCKR, ABCG5, ABCG8, PLTP, HNF4A, SCAP, STAP1, MTP, MYLIP, **LPA**, NPC1L1, CYP7A1, LPL, GPIHBP1, ABCA1

PRELIMINARY RESULTs in 9 patients from Rome (prof. Claudia Stefanutti)

R155 AF **Lp(a) 82 mg/dl**

MTTP Ex 6 Val168Ile c.502 G>A
HZ S=0.22 P=0.98 non common

LPA Ex 40 Arg 2016 Cys c.6046 C>T OZ S=0.01 P=1 pathogenic common variant

LPA rs 10755578 OZ gen G/G ref. C

R157 DLG **Lp(a) 96 mg/dl** **3V-CAD**

ABCA1 Ex25 Ala1182Thr c.3544 G>A
HZ non common
APOAV Ex3 Ser19Trp c.56 C>G HZ
Pathogenic common

APOE EX 4 GENOTYPE E3 E4

LPA Ex 40 Arg 2016 Cys c.6046 C>T HZ S=0.01 P=1 pathogenic common variant

LPA rs 10755578 HZ gen C/G ref. C

R158 AS **Lp(a) 134 mg/dl**

ANGPTL4 Ex 3 +1 G>A
HZ non common

APOE GENOTYPE E3 E4

SCAP Ex 17 Pro852Ser c.2554 C>T
HZ non common

LPA rs 10755578 HZ gen C/G ref. C

R159 CD **Lp(a) 104 mg/dl** **3V-CAD**

LPA Ex 40 Arg 2016 Cys c.6046 C>T HZ S=0.01 P=1 pathogenic common variant

LPA rs 10755578 OZ gen G/G ref. C

R 160 PP Lp(a) 244 mg/dl 3V-CAD

APOAV Ex3 Ser19Trp c.56 C>G HZ Pathogenic common

LPA Ex 40 Arg 2016 Cys c.6046 C>T HZ S=0.01 P=1 pathogenic common variant

LPA rs 10755578 OZ gen G/G ref. C

R161 AB Lp(a) 118 mg/dl 2-V CAD

APOE GENOTYPE E3 E4

NPC1L1 Ex 2 Leu272Leu c.816 C>G HZ common variant

LPA Ex 40 Arg 2016 Cys c.6046 C>T OZ S=0.01 P=1 pathogenic common variant

LPA Ex 27 Thr1339Pro c.4195 A>C HZ S=0.08 P=1 pathogenic common variant

LPA rs 7765803 HZ gen G/C ref. G

LPA rs 7765781 HZ gen G/C ref. G

LPA rs 10755578 OZ gen G/G ref. C

R162 EA Lp(a) 138 mg/dl 1V-CAD

ABCG8 Ex10 Tyr479 Cys c.1436 A>C
HZ S=0.18 P= 0.931 non common variant

LPL Ex2 Asp36Asn c.106 G>A
HZ S=0.05 P=0.074 pathogenic common variant

LPA Ex 40 Arg 2016 Cys c.6046 C>T
HZ S=0.01 P=1 pathogenic common variant

LPA Ex 21 Pro1090Arg c.3268 C>G
HZ S=0.0 P=1.0 pathogenic non common variant

LPA rs 10755578 HZ gen C/G ref. C

R 163 NC Lp(a) 169 mg/dl 2V-CAD

LPA Ex 27 Thr1339Pro c.4195 A>C
HZ S=0.08 P=1 pathogenic common variant

LPA rs 7765803 HZ gen G/C ref. G

LPA rs 7765781 HZ gen G/C ref. G

LPA rs 10755578 HZ gen C/G ref. C

R 164 DM Lp(a) 30 mg/dl

ABCG8 Ex1 Asp19His c.55 G>C HZ S=0.0 P=0.796 Common

APOE GENOTYPE E2 E3

LDL-R EX7 Cys352(331)Trp c.1056 C>G OZ S=0.0 P=1.0 (FH-AVELLINO-1)

NPC1L1 Ex 2 Leu272Leu c.816 C>G HZ Common

LMF1 Ex8 Arg364Gln c.1091 G>A HZ S=0.3 P=0.997 Common

PCSK9 Ex 1 p.Leu15 Dup c.65_66 INS GCT HZ Common

LPA EX 40 Arg2016 CYS c.6046 C>T HZ S=0.01 P=1.0 C

LPA rs 10755578 HZ gen C/G ref. C

CLINICAL DATA OF PATIENTS FROM VERONA (dott. Maria Grazia Zenti)

	sex	age	BMI	Lpa	CHD	ATS	therapy	HELP start
BL	M	56	25,7	120	2V-CHD	Carotid stent	alirocumab	2014
PV	M	60	27,8	122	3V-CHD	Popliteal bilateral Endoarterectomy	St+ Eze	2015
TM	M	61	28,4	130	2V-CHD	Carotid bilateral endoarterectomy	St+ Eze	1995
TE	M	75	22,9	108	1V-CHD	Carotid stenosis 30%	Nutraceutical	2015
MD	F	65	29,9	435	2V-CHD	Carotid stenosis 35-40%	St+ Eze	2005

Conclusions

Preliminary data demonstrated that LPA gene is extremely polymorphic

Studying the correlation between genotype and phenotype we will confirm rhe role of genetic variant of LPA gene and cardiovascular risk

We will analyze the Cholesterol Loading Capacity of sera in relation to number of kringles repeat and lipoprotein apheresis to investigate new mechanisms increasing cardiovascular risk and the therapeutical effect of apheretic therapy

Thank you to Kaneka for the support

Thank you for your attention!