A comprehensive map of the variability in the Lipoprotein(a) KIV-2 repeat region and follow-up of the KIV-2 Arg20Ter mutation in 11,000 individuals.

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Background

- The LPA gene locus controls >90% of Lipoprotein(a) [Lp(a)] variance¹.
- The KIV-2 repeat (5.6 kb) explains 40- 70 % of Lp(a) variance.
- However, individual Lp(a) levels still vary by up to 1000-fold and a sizeable proportion of Lp(a) variance is still unexplained. Moreover, null alleles (i.e. isoforms not expressed in plasma) are common.
- The KIV-2 repeat region has never been screened for mutations on a large scale^{3,5,6}, although it contains up to 70% of the protein coding sequence. It thus represents a natural candidate region for novel variants affecting Lp(a) concentrations.

Project summary

We developed a dedicated NGS sequencing pipeline for the KIV-2 region, cataloged the variability in 123 Caucasian samples and 2,504 samples from 1000 Genomes and followed up the loss-of-function variant Arg20Ter in 11,152 individuals.

Methods

- Ultra-deep “batch NGS sequencing”:** All KIV-2 are amplified as a mixture using primer binding to all repeats (“batch sequencing”)²⁻⁴ and collectively ultra-deep sequenced (Illumina MiSeq v2, TrueSeq Nano, average depth: 24,418X). 123 Caucasian samples from Austria and Germany were sequenced.
- Data analysis:** We created a dedicated low level variant caller for the KIV-2 region⁵, which takes into account several sequence peculiarities of this region. All reads are mapped to one KIV-2 repeat (hg19 chr6:161,033,785-161,038,888). Variants ≥1% mutational level were called (see Ref). Additionally, the public sequence data from the 1000 Genomes project (26 populations worldwide) has been reanalyzed (n=2,504 exomes, n=150 for intronic regions)
- Follow up of Arg20Ter:** Carrier status for the Arg20Ter⁶ loss-of-function was been assessed in 4 populations: GCKD (German Chronic Kidney Disease Study, n=4771), KORA F3 (n=3099) and F4 (n=3040; both: general populations from Southern Germany) using a high throughput, allele-specific TaqMan qPCR protocol. The assay showed sensitivity down to 0.5% mutation level in plasmid mixes.
- Association study:** Wilcoxon test, linear regression (unadjusted and isoform-adjusted) and fixed-effect meta-analysis. Lp(a) levels were determined by ELISA, apo(a) isoforms by Western Blotting.

GitHub

The variant calling pipeline for the LPA KIV-2 region is freely available at <https://github.com/genepi/lpa-pipeline>

The KIV-2 repeat contains hundreds of variants

Table 1. Number of variants found

	1000 Genomes n=2,504 (introns: n=150)	Discovery set n=123	Overlap
Nonsense	9	2	1
Missense & nonsense	115	12	11
Synonymous	56	11	8
Splice site	3	0	0
Non-coding	937	488	278

Ref.: Coassin S., Schoenherr S. et al, *A comprehensive map of single-base polymorphisms in the hypervariable LPA kringle IV type 2 copy number variation region*. J Lipid Res. 60: 186-199, 2019

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Some positions are variable in every individual

Figure 1: Number of discovery samples (n=123) with a variant at a given position. The KIV-2 Drall polymorphism (Mancini et al, 1995) was identified as a 2106 G>A. Black: non-coding; blue: splice region (±25 bp), orange: synonymous, red: missense and nonsense.

Figure 2: Overlapping coding variation between the 1000 Genomes populations. A consistent portion of variants are private to single populations.

Only variants observed at least twice shown. AFR, African; AMR, Ad-mixed American; EAS, East Asian; EUR, European; SAS, South Asian.

KIV-2 Arg20Ter decreases Lp(a) by 11.7 mg/dL

Table 2: Association results for Arg20Ter⁶ and plasma Lp(a).

* Original scale (mg/dL), † Inverse normal scale

Population	carriers	total n	β [CI] *	SE *	p †	R ² †
Age and sex adjusted model for Lp(a) plasma concentrations						
GCKD	74	4,771	-13.0 [-20.0, -6.0]	3.58	4.3e-12	0.0114
KORA F3	56	3,099	-12.6 [-19.5, -5.7]	3.51	6.5e-11	0.0132
KORA F4	63	3,040	-9.9 [-16.0, -3.8]	3.12	4.0e-12	0.0153
Meta analysis	211	10,910	-11.7 [-15.5, -7.8]	1.96	1.1e-32	-

- No SNP in perfect LD found among our recent GWAS hits, but all Arg20Ter carriers carry also the frequent KIV-8 splice site mutation rs41272114^{7,8} (D′=0.957, R²=0.275). Not applicable vice versa.

Figure 3: Box plot of the Lp(a) levels observed in the various combinations of R21X carrier status (WT or positive) and rs41272114 genotypes.

The p value between group 3 and 4 is p=0.47 (Wilcoxon test). The two variants do not have an additive effect, their effects cannot be separated.

Main findings

- Ultra-deep sequencing allows detection of variants in the KIV-2.
- The KIV-2 region is highly variable containing hundreds of variants.
- Arg20Ter is moderately frequent in Caucasians and associated with decreased Lp(a) (-11.7 mg/dL), explaining 1.1-1.5% of Lp(a) variance.
- Virtually all Arg20Ter carriers carry also rs41272114. The effects cannot be separated. Arg20Ter alleles represent “double null alleles”.