The kringle IV type 2 domain variant 4925G>A causes the elusive association signal of the LPA pentanucleotide repeat

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Abstract Lipoprotein(a) [Lp(a)] concentrations are regulated by the LPA gene mainly via the large kringle IV-type 2 (KIV-2) copy number variation and multiple causal variants. Early studies suggested an effect of long pentanucleotide repeat (PNR) alleles (10 and 11 repeats, PNR10 and PNR11) in the LPA promoter on gene transcription and found an association with lower Lp(a). Subsequent in vitro studies showed no effects on mRNA transcription, but the association with strongly decreased Lp(a) remained consistent. We investigated the isolated and combined effect of PNR10, PNR11, and the frequent splice site variant KIV-2 4925G on Lp(a) concentrations in the Cooperative Health Research in the Region of Augsburg F4 study by multiple quantile regression in single-SNP and joint models. Data on Lp(a), apolipoprotein(a) Western blot isoforms, and variant genotypes were available for 2,858 individuals. We found a considerable linkage disequilibrium between KIV-2 4925G>A and the alleles PNR10 and PNR11. In single-variant analysis adjusted for age, sex, and the shorter apo(a) isoform, we determined that both PNR alleles were associated with a highly significant Lp(a) decrease (PNR10: β = −14.43 mg/dl, 95% CI: −15.84, −13.02, P = 3.33e-84; PNR11: β = −17.21 mg/dl, 95% CI: −20.19, −14.23, P = 4.01e−29). However, a joint model, adjusting the PNR alleles additionally for 4925G>A, abolished the effect on Lp(a) (PNR10: β = +0.44 mg/dl, 95% CI: −1.73, 2.60, P = 0.69; PNR11: β = −1.52 mg/dl, 95% CI: −6.05, 3.00, P = 0.51). Collectively, we conclude that the previously reported Lp(a) decrease observed in pentanucleotide alleles PNR10 or PNR11 carriers results from a linkage disequilibrium with the frequent splicing mutation KIV-2 4925G>A.

Lipoprotein(a) [Lp(a)] is a highly atherogenic particle in the human plasma (1–6). Up to 90% of the variance in Lp(a) concentrations is determined by the LPA gene (1). LPA encodes for apolipoprotein(a) [apo(a)], which consists of 10 different kringle IV domains (KIV-1 to KIV-10). Notably, the KIV-2 is encoded by a 5.6 kb large coding copy number variation (KIV-2 repeat), which leads to >40 apo(a) protein isoforms in the population (1). The apo(a) isoforms are inversely correlated with Lp(a) plasma concentrations, with up to 10 times higher median Lp(a) concentrations observed in low molecular weight (LMW, 10–22 KIV repeats) isoform carriers than in high molecular weight (HMW, >22 KIV repeats) isoform carriers (1, 7). In heterozygous individuals, the smaller isoforms commonly determine the Lp(a) concentrations (albeit not always) (8). However, if stratifying individuals by their isoform, the individual Lp(a) concentrations within each group can nonetheless vary by up to 200-fold (9). This huge variance within each isoform size group cannot be explained by the sole isoform size (9), nor by the second isoform present in heterozygotes (10). For examples, while LMW isoforms are associated with up to 10 times higher median Lp(a) than HMW isoforms, at individual level, 30% of all LMW isoform carriers present Lp(a) <30 mg/dl (11). Such unexpected Lp(a) trait patterns have been termed...
recently discordant phenotypes (12) and have been shown to result from multiple functional variants modifying and partially overruling the impact of the isoform sizes (12, 13). Thus, a complete understanding of the complex Lp(a) trait requires a thorough understanding of the many functional SNPs that govern the trait additionally to the isoforms (recently reviewed in (7)). This promises to allow highly accurate prediction of Lp(a) concentrations from genetic data (13–16).

Several studies searched for causal genetic determinants of these peculiar phenotypes but causal variants have remained elusive until recently (12, 13, 17–20). The search for genetic variants regulating Lp(a) has been complicated by the fact that multiple genetic factors cooperate in a nonlinear manner in determining Lp(a) concentrations, such as isoform size, SNPs, and ancestry (7, 21). Interactions between these factors can mask true effects, and many SNPs indeed occur only in narrow isoform ranges (7).

Nonetheless, some early studies succeeded in identifying markers for these discordant phenotypes. One of these are long alleles of a short tandem repeat (STR) located 1.3 kb upstream of the first exon of LPA (hg19 chr6:161,086,617–161,086,663; hg38 chr6:160,665,587–160,665,631) in the LPA promoter (22–26). This STR has been named pentanucleotide repeat (PNR) and presents between about 5 and 12 repeats of a TTTTA motif (22, 27). Early studies showed that especially the alleles PNR10 (10 repeat units) and PNR12, compared to the most common allele in the population (PNR8), but this effect could not be replicated by subsequent studies (23, 29, 30). Surprisingly, epidemiological studies nevertheless observed a clear association between rs41272110 and PNR10 (24) led us to the hypothesis that the association of long PNR alleles with low Lp(a) concentrations also could be mediated by KIV-2 4925G>A. This would finally provide a solution to the long-standing riddle about the association of this nonfunctional promoter STR with lower Lp(a) concentrations.

MATERIAL AND METHODS

Study populations

The population-based study Cooperative Health Research in the Region of Augsburg (KORA) F4 (36) is a follow-up study of the previous KORA S4 study and includes 3,080 participants with German nationality aged 25–74 years, being recruited from 2006 to 2008. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Bavarian Medical Association (Bayrische Landesanztzekerammer). Lp(a) concentrations, apo(a) isoforms, and variant data (rs41272110, KIV-2 4925G>A, and PNR allele) were available for 2,858 participants and were measured at the Institute of Genetic Epidemiology at the Medical University of Innsbruck. Study population characteristics and carrier frequencies of the variants are given in Table 1.

Lp(a) phenotyping

Lp(a) concentrations were determined in mg/dl by ELISA with a polyclonal affinity-purified rabbit anti-human apo(a) antibody for coating and a horseradish peroxidase–conjugated monoclonal anti-apo(a) antibody 1A2 (37) for detection (38, 39). Absorbance was assessed on two dilutions (1:150 and 1:500), and measurements were applied to a 7-point standard curve.

Apo(a) isoforms were detected by Western blotting (38, 39). One hundred fifty nanograms of Lp(a) and a size standard containing apo(a) isoform 13, 19, 23, 27, and 35 KIV repeats (validated by fiber-FISH (40)) were separated on a 1.46% agarose gel with 0.08% SDS for 18 h at 0.04 A constant current. Semidry electrophotography was performed. The membrane was blocked with 1% BSA, 85 mM NaCl, 10 mM TRIS, and 0.2% Triton X-100 for 30 min at 37°C and incubated with

### TABLE 1. Study population characteristics

| Characteristics                  | KORA F4       |
|----------------------------------|---------------|
| N                                | 2,858         |
| Age, y                           | 56 (44, 67)   |
| Female sex                       | 1,482 (51.8%) |
| Lp(a), mg/dl                     | 117 (52, 303) |
| Total cholesterol, mg/dl         | 214 (188, 240)|
| HDL-C, mg/dl                    | 54 (45, 65)   |
| LDL-C, mg/dl                    | 134 (111, 158) |
| Triglycerides, mg/dl             | 105 (72, 151) |
| Type 2 diabetes mellitus         | 198 (6.9%)    |
| 4925G>A carrier                  | 634 (22.2%)   |
| MAF rs41272110 (genotype counts TT/TC/CC) | 14.2% (3,424/1,063/88) |

HDL-C, HDL-cholesterol; KORA, Cooperative Health Research in the Region of Augsburg; LDL-C, LDL-cholesterol; Lp(a), lipoprotein(a); PNR, pentanucleotide repeat.

PNR allele frequencies are given in Fig. 2. Values are provided as median (interquartile range) unless specified differently.

Recently we have identified a strong LD between rs41272110 and the frequent, strongly Lp(a) and coronary artery disease (CAD) risk-lowering splice variant 4925G>A in the KIV-2 region (35). We showed that the effect of rs41272110 on Lp(a) and on CAD risk depends on the KIV-2 4925G>A and that the Lp(a)-lowering effect previously attributed to rs41272110 is indeed caused by this splice variant (35). The reported LD
Low Lp(a) in carriers of long PNR is caused by KIV-2 4925G>A

RESULTS

Previous studies suggested an LD between alleles PNR10 and PNR11 and rs41272110 (24, 26). We have shown recently that the individual effect of rs41272110 on Lp(a) depends on the KIV-2 4925G>A carrier status and that the Lp(a) decrease previously attributed to rs41272110 is produced by a partial LD with 4925G>A (35). Given the occurrence of the alleles PNR10 and PNR11 in the same isoform range as 4925G>A and rs41272110, we hypothesized that a similar mechanism might also cause the association of PNR10 and PNR11 with low Lp(a) plasma concentrations (Fig. 1).

We observed eight different PNR alleles with 5–12 repeats in the analyzed European population sample. A range of 5–10 repeats was observed in the shorter PNR allele 1 and 8–12 repeats in the longer PNR allele 2. The frequency of the repeat numbers of both PNR alleles stratified by LMW and HMW isoforms is shown in Fig. 2, and the resulting PNR genotypes are shown in Fig. 3. The most frequent genotypes were PNR8/PNR8 (47.9%), PNR8/PNR9 (20.3%), and PNR8/PNR10 (19.2%). As the allele PNR11 was present only on PNR allele 2, all analyses were based on PNR allele 2. The PNR8 allele was the most frequent allele and used as a reference allele for all analyses.

In line with previous findings (23–26), in an age-, sex-, and isoform-adjusted quantile regression model, both the PNR10 and the PNR11 allele were associated with significantly lower Lp(a) plasma concentrations (PNR10: $\beta = -14.4$ mg/dl, $P = 3.35e-84$; PNR11: $\beta = -17.2$ mg/dl, $P = 4.01e-29$; confidence intervals for all regression models are shown in the respective tables; Table 2). Of note, this effect was noticeable only after isoform adjustment or stratification. In a non-isoform-adjusted model, the effect was very small in the total population (PNR10: $\beta = -3.1$ mg/dl, $P = 3.86E-05$; PNR11: $\beta = -2.2$ mg/dl, $P = 0.0604$) but was enhanced by one order of magnitude when the analysis was restricted to LMW samples (PNR10: $\beta = -24.9$ mg/dl, $P = 1.03E-16$; PNR11: $\beta = -40.2$ mg/dl, $P = 1.85E-11$; Table 2). Conversely, no relevant effects were observed in the HMW groups. Of note, also a minor association of PNR9 with lower Lp(a) was observed in the total population. Unlike PNR10 and PNR11, the overall effect was not modified by isoform adjustment, and despite a similar number of cases as for PNR10 and PNR11, it was no more significant when stratifying by LMW/HMW (Table 2). PNR12 was too rare for meaningful analysis ($n = 2$). Additional adjustment for the other PNR allele present (PNR allele 1) did not modify the results (supplemental Table S1) and was not applied to further regression models. PNR5 to PNR7 were observed only on the shorter PNR allele (PNR allele 1), PNR5 (n = 4) and PNR6 (n = 4) were too rare for analysis, and no association with Lp(a) was observed in carriers of PNR7 (n = 19).
In summary, the regression models revealed a strong isoform-specific effect of PNR10 and PNR11 in the total group, which stems from a very strong Lp(a)-lowering effect in the LMW group. Given the aforementioned coincidence of the strongly Lp(a)-lowering splice site mutation in the KIV-2 4925G>A in the same isoform range (12), we hypothesized a role of this mutation in establishing the observed Lp(a)-lowering effect of the PNR.

Indeed, Fig. 4 shows a very high 4925G>A carrier frequency in individuals with the PNR alleles PNR10

Fig. 1.  *LPA* gene structure with the LD statistics between the variants discussed.

Fig. 2. Distribution of the short and the long PNR allele (panel A: PNR allele 1, panel B: PNR allele 2) in the KORA F4 population, stratified by HMW and LMW carriers. Percentage is given per total individuals in LMW or HMW group.
(73.6%) and PNR11 (77.4%), compared to a carrier frequency of 22.2% in the entire population. Conversely, 4925G>A carrier frequency was very low in individuals with the PNR alleles PNR8 (1.4%) and PNR9 (1.2%). Accordingly, a high Lewontin’s D’ (and high to moderate R²) was observed between the two PNR alleles and 4925G>A (PNR10: D’ = 0.82, R² = 0.76; PNR11: D’ = 0.71, R² = 0.25). High D’ suggests a low likelihood of recombination between two loci and, accordingly, that both SNPs are most often inherited together. We have shown before that effects of correlated LPA SNPs on Lp(a) can be mediated also via the D’ structure instead of the R² structure (18) (reviewed in (7)).

Figure 3 shows the distribution of the PNR alleles across the different apo(a) isoform sizes and Lp(a) concentrations. It shows that a considerable number of individuals PNR allele 1 and PNR allele 2. Empty line intersections correspond to no carriers.

Conversely, adding subsequently also KIV-2 4925G>A (depicted in dark gray), leading to lower Lp(a) concentration. Numbers of individuals carrying 4925G>A separated for PNR allele: PNR8: n = 19; PNR9: n = 8; PNR10: n = 541, PNR11: n = 65, PNR12: n = 1.

We recently reported also a strong LD between 4925G>A and the well-known missense LPA variant Thr3888Pro (p.Thr1399Pro, rs41272110) (35). As others had suggested that the effect of the PNR on Lp(a) might derive from rs41272110 (24, 26), we investigated whether the effect of PNR10 and PNR11 allele could indeed be caused by an LD with rs41272110 instead of an LD with 4925G>A. In contrast to the regression models including 4925G>A, inclusion of rs41272110 into the regression models did not abolish the association between PNR10 and PNR11 alleles with low Lp(a) (both models including 4925G>A completely abolishes the effect of PNR10 and PNR11 alleles on Lp(a) concentration in both models (age- and sex-adjusted model: PNR10: ß = +1.03, P = 0.13; PNR11: ß = +1.84, P = 0.22; isoform-adjusted model: PNR10: ß = +0.44, P = 0.69; PNR11: ß = −1.52, P = 0.51; Table 3).

We investigated whether the effect of PNR10 and PNR11 allele could indeed be caused by an LD with rs41272110 instead of an LD with 4925G>A. In contrast to the regression models including 4925G>A, inclusion of rs41272110 into the regression models did not abolish the association between PNR10 and PNR11 alleles with low Lp(a) (isoform-adjusted model: PNR10: ß = −8.74, P = 3.89e-16; PNR11: ß = −11.52, P = 5.71e-13; supplemental Table S2). Conversely, adding subsequently also KIV-2 4925G>A to the regression model with rs41272110 showed a

![Fig. 3. PNR genotypes (PNR allele combinations) in the KORA F4 population (n = 2,858). Numbers in dots represent the individuals showing the PNR genotype resulting from the individual PNR allele 1 and PNR allele 2. Empty line intersections correspond to no carriers.](image)

![Fig. 4. Carrier frequency of KIV-2 4925G>A in each PNR allele. A large fraction of PNR10 and PNR11 carriers carries also KIV-2 4925G>A (depicted in dark gray), leading to lower Lp(a) concentration. Numbers of individuals carrying 4925G>A separated for PNR allele: PNR8: n = 19; PNR9: n = 8; PNR10: n = 541, PNR11: n = 65, PNR12: n = 1.](image)

| Group | Variant | Carrier N | Median Lp(a) Concentration (IQR) | Model 1: Adjusted for Age and Sex | Model 2: Adjusted for Age, Sex, and the Smaller apo(a) Isoform |
|-------|---------|-----------|-------------------------------|----------------------------------|---------------------------------------------------------------|
|       |         |           |                               | ß | 95% CI | P | ß | 95% CI | P |
| All   | PNR8    | 1,388     | 13.4 (4.8, 39.0) Reference     | Reference                        | Reference                                                      |
|       | PNR9    | 648       | 11.1 (4.6, 23.7)               | −2.07                            | −3.95, −0.19 | 0.0313 | −2.78 | −3.72, −1.83 | 1.05E-08 |
|       | PNR10   | 736       | 10.4 (5.2, 25.3)               | −3.10                            | −4.57, −1.63 | 3.80E-05 | −14.43 | −15.84, −13.02 | 3.33E-04 |
|       | PNR11   | 84        | 10.9 (5.5, 19.6)               | −2.33                            | −4.57, 0.10 | 0.0604 | −7.07 | −8.09, −6.05 | 4.01E-29 |
|       | PNR12   | 2         | NA                            | NA                              | NA | NA | NA | NA | NA | NA | NA |
| LMW   | PNR8    | 356       | 53.2 (40.3, 74.4) Reference    | Reference                        | Reference                                                      |
|       | PNR9    | 99        | 49.2 (31.4, 64.6)              | −5.61                            | −13.08, 1.86 | 0.0719 | −4.72 | −11.98, 2.53 | 0.9227 |
|       | PNR10   | 196       | 28.2 (10.8, 54.5)              | −24.92                           | −30.77, −19.07 | 1.03E-16 | −24.16 | −29.94, −18.39 | 1.1E-15 |
|       | PNR11   | 48        | 12.5 (5.7, 23.9)               | −40.20                           | −50.30, −30.11 | 1.85E-11 | −39.50 | −49.31, −29.69 | 1.3E-14 |
|       | PNR12   | 2         | NA                            | NA                              | NA | NA | NA | NA | NA | NA | NA |
| HMW   | PNR8    | 1,032     | 8.7 (3.6, 18.3) Reference      | Reference                        | Reference                                                      |
|       | PNR9    | 549       | 9.4 (3.8, 17.3)                | 0.54                             | −0.88, 1.96 | 0.4539 | 0.55 | −0.71, 1.81 | 0.3895 |
|       | PNR10   | 540       | 8.6 (5.3, 14.5)                | −0.24                            | −1.32, 0.84 | 0.6630 | −2.28 | −3.85, −1.91 | 7.13E-09 |
|       | PNR11   | 36        | 9.2 (4.2, 17.3)                | 0.91                             | −2.83, 4.65 | 0.6344 | −0.69 | −3.62, 2.24 | 0.6456 |
|       | PNR12   | 0         | NA                            | NA                              | NA | NA | NA | NA | NA | NA | NA |

HMW, high molecular weight; LMW, low molecular weight; Lp(a), lipoprotein(a); PNR, pentanucleotide repeat.

In all models PNR8 allele was used as reference allele. Median Lp(a) concentration and effect are given in mg/dl.
significant Lp(a)-decreasing effect of 4925G>A (\(\beta = -25.42, P = 3.05 \times 10^{-40}\)), a significant Lp(a) increase in carriers of rs41272110 (\(\beta = +4.49, P = 0.0018\)), which is in line with what we have shown previously (35), and confirm no effect of the PNR alleles PNR10 or PNR11 (PNR10: \(\beta = +0.24, P = 0.82\); PNR11: \(\beta = -1.84, P = 0.25\); Table 4). This further supports that the effect of the PNR alleles PNR10 and PNR11 allele on Lp(a) is, indeed, consistent with what we have shown previously.

### Fig. 5.

Distribution of the PNR alleles across the different apo(a) isoform sizes and Lp(a) concentration. The first row shows the total population, while the second and third rows show noncarriers and carriers of 4925G>A, respectively. A large proportion of the PNR10 and PNR11 carriers with low Lp(a) concentrations carry KIV-2 4925G>A as well. The remaining variance in Lp(a) concentration in carriers of 4925G>A results mostly from the second unaffected allele.

### Table 3. Quantile regression analysis of PNR allele 2 on Lp(a) concentration adjusted for 4925G>A (joint model)

| Group | Variant | Carrier [N] | Median Lp(a) Concentration (IQR) | Model 1: Adjusted for Age and Sex | Model 2: Adjusted for Age, Sex, and the Smaller apo(a) Isoform |
|-------|---------|-------------|-----------------------------------|-----------------------------------|-------------------------------------------------------------|
|       |         |             | \(\beta\) 95% CI \(P\) | \(\beta\) 95% CI \(P\) | \(\beta\) 95% CI \(P\) |
| All   | PNR8    | 1,388       | 13.4 (4.8, 39.0) | Reference | -1.98 | -3.68, -0.28 | 0.0299 | -2.97 | -4.1, -1.85 | 2.32E-07 |
|       | PNR9    | 648         | 11.1 (4.6, 23.7) |                   | 1.03 | -0.29, 2.34 | 0.1270 | 0.44 | -1.73, 2.60 | 0.6927 |
|       | PNR10   | 736         | 10.4 (6.2, 23.1) |                   | 1.84 | -1.07, 4.74 | 0.2154 | 1.52 | -6.05, 3.00 | 0.5102 |
|       | PNR11   | 84          | 10.9 (5.5, 19.6) |                   | NA  | NA          | NA     | NA  | NA          | NA     |
|       | PNR12   | 2           | NA          |                   | NA  | NA          | NA     | NA  | NA          | NA     |
|       | 4925G>A | 634         | 9.7 (6.3, 17.6) | -4.73 | -6.16, -3.31 | 8.65E-11 | -20.98 | -23.46, -18.51 | 2.03E-59 |
| LMW   | PNR8    | 356         | 53.2 (40.3, 74.4) | Reference | NA  | NA          | NA     | NA  | NA          | NA     |
|       | PNR9    | 99          | 492 (31.4, 64.6) | -5.58 | -11.96, 0.80 | 0.0871 | -5.04 | -11.42, 1.34 | 0.1220 |
|       | PNR10   | 196         | 28.2 (10.8, 54.5) | -2.84 | -10.25, 4.56 | 0.4520 | 1.90 | -5.56, 9.37 | 0.6174 |
|       | PNR11   | 48          | 12.5 (5.7, 23.9) | -10.82 | -21.62, -0.01 | 0.0502 | -8.80 | -19.61, 2.01 | 0.1111 |
|       | PNR12   | 2           | NA          |                   | NA  | NA          | NA     | NA  | NA          | NA     |
|       | 4925G>A | 196         | 15.1 (8.4, 39.6) | -32.85 | -23.46, -18.51 | 1.90E-16 | -35.02 | -42.65, -27.38 | 2.31E-18 |
| HMW   | PNR8    | 1,032       | 8.7 (3.6, 18.3) | Reference | NA  | NA          | NA     | NA  | NA          | NA     |
|       | PNR9    | 549         | 9.4 (3.8, 17.3) | 0.47 | -0.76, 1.71 | 0.4539 | 0.43 | -0.75, 1.62 | 0.4735 |
|       | PNR10   | 540         | 8.6 (5.3, 14.5) | 0.32 | -1.05, 1.70 | 0.6434 | 0.95 | -1.09, 2.98 | 0.5625 |
|       | PNR11   | 36          | 9.2 (4.2, 17.3) | 1.58 | -1.42, 4.58 | 0.3030 | 2.40 | -1.35, 6.15 | 0.2998 |
|       | PNR12   | 0           | NA          |                   | NA  | NA          | NA     | NA  | NA          | NA     |
|       | 4925G>A | 438         | 8.6 (6.0, 13.2) | -0.65 | -1.82, 5.53 | 0.2812 | -5.55 | -7.82, -3.28 | 1.71E-06 |

HMW, high molecular weight; LMW, low molecular weight; Lp(a), lipoprotein(a); PNR, pentanucleotide repeat.

In all models PNR8 allele was used as reference allele. Median Lp(a) concentration and effect are given in mg/dl.
caused by KIV-2 4925G>A and not by rs41272110. Additionally, we also stratified our data for rs10455872 and rs3798220 (known proxy SNPs for LMW isoforms (46) and high Lp(a)) to investigate the interplay between these two variants and KIV-2 4925G>A, but no overlap between these variants was evident (supplemental Figs. S1–S3 and supplemental Table S3).

As Lp(a) levels and also carrier frequencies of variants in the LPA gene differ across populations, we additionally investigated the LD between the PNR alleles and KIV-2 4925G>A on the 1000G Project whole-genome sequencing dataset (n = 2,504). This showed different carrier frequencies of KIV-2 4925G>A and of the PNR alleles across the different populations (supplemental Figs. S4 and S5; supplemental Table S4), as previously shown in (12, 23, 29, 31). Interestingly, we detected PNR4 alleles in 18% (n = 9) of the East Asian population (supplemental Fig. S4). Alleles with such low repeat numbers had been observed in East Asians also in another earlier study (47). LD statistics for the different populations are reported in supplemental Table S4. In Non-Finnish Europeans, the LD between PNR10 and PNR11 and 4925G>A (PNR10: D’ = 0.84, R² = 0.76; PNR11: D’ = 0.71, R² = 0.26) was indeed very similar to our observations in KORA F4 (PNR10: D’ = 0.82, R² = 0.76; PNR11: D’ = 0.71, R² = 0.25). Globally, the LD between KIV-2 4925G>A and the PNR10 and PNR11 alleles was highest in Europeans and South Asians (supplemental Table S5).

### DISCUSSION

Several studies investigated the role of the LPA promoter PNR in the regulation of plasma Lp(a) concentrations (22–24, 27–31, 33, 34). Different PNR alleles reproducibly presented a strong and highly significant association with Lp(a) concentrations (24, 26, 28, 31–34), despite the PNR has no direct impact on promoter activity (23, 29, 30). The relevance of the PNR and the reason for its association with Lp(a) concentrations is, therefore, still unknown (7). We robustly replicated the association of long PNR alleles with strongly lowered Lp(a), especially in LMW isoforms, and found that the association with low Lp(a) is indeed caused by a strong LD between PNR10 and PNR11 and the splice site variant 4925G>A (Fig. 1). KIV-2 4925G>A is located within the repetitive KIV-2 region. The variant reduces splicing efficiency by activating a cryptic intronic splice site (12). This results in an intron retention creating a premature stop codon and leads to reduced protein production (12), which in turn is associated with decreased CAD risk (35, 48). LMW and HMW carriers present rather different median Lp(a) concentrations (22–24, 27–31, 33, 34).

| Group | Variant | Carrier [N] | Median Lp(a) Concentration (IQR) | β (95% CI) | p |
|-------|---------|-------------|----------------------------------|------------|---|
| All   | PNR8    | 1,388       | 13.4 (4.8, 30.0)                 | Reference  | -2.68 (-4.42, -0.94) | 0.0025 |
|       | PNR9    | 648         | 11.1 (4.6, 23.7)                 | -0.78 (-1.32, 0.89) | 0.4600 |
|       | PNR10   | 736         | 10.4 (6.2, 23.1)                 | 0.064 (-2.38, 3.35) | 0.6783 |
|       | PNR11   | 84          | 10.9 (5.5, 19.6)                 | NA         | NA |
|       | PNR12   | 2           | 9.7 (6.3, 17.6)                  | NA         | NA |
|       | rs41272110 | 434       | 11.1 (6.8, 25.0)                 | 10.18 (6.04, 14.92) | 1.49E-06 |
|       | 4925G>A | 744         | 11.1 (6.8, 25.0)                 | NA         | NA |
|       |         |             |                                  | NA         | NA |
|       |         |             |                                  | NA         | NA |
|       |         |             |                                  | NA         | NA |
|       |         |             |                                  | NA         | NA |
|       |         |             |                                  | NA         | NA |
|       |         |             |                                  | NA         | NA |
|       |         |             |                                  | NA         | NA |
| LMW   | PNR8    | 356         | 5.2 (40.3, 74.4)                 | Reference  | -1.59 (-19.04, -10.94) | 0.78E-12 |
|       | PNR9    | 99          | 492 (31.4, 64.6)                 | -4.78 (-11.13, 1.58) | 0.1415 |
|       | PNR10   | 196         | 292 (10.8, 54.5)                 | -3.83 (-12.32, 4.86) | 0.3881 |
|       | PNR11   | 48          | 125 (5.7, 23.9)                  | -11.99 (-23.84, -0.14) | 0.0477 |
|       | PNR12   | 2           | NA                              | NA         | NA |
|       | rs41272110 | 259       | 21.1 (9.3, 15.4)                 | 4.48 (-3.63, 12.59) | 0.2790 |
|       | 4925G>A | 196         | 15.1 (8.3, 29.6)                 | -37.45 (-46.35, -28.55) | 8.08E-16 |
| HMW   | PNR8    | 1,032       | 8.7 (3.6, 18.3)                  | Reference  | -0.18 (-1.46, 1.10) | 0.7849 |
|       | PNR9    | 549         | 9.4 (3.8, 17.3)                  | -0.28 (-1.43, 1.21) | 0.7475 |
|       | PNR10   | 540         | 8.6 (5.3, 14.5)                  | 0.029 (-1.97, 5.88) | 0.3295 |
|       | PNR11   | 36          | 9.2 (4.2, 17.5)                  | 4.85 (-3.63, 12.59) | 0.2790 |
|       | PNR12   | 0           | NA                              | NA         | NA |
|       | rs41272110 | 505       | 9.4 (6.2, 16.5)                  | 7.52 (6.56, 8.69) | 1.27E-35 |
|       | 4925G>A | 438         | 8.6 (6.0, 13.2)                  | -8.33 (-10.30, -6.35) | 2.41E-16 |

| Model 1: Adjusted for Age and Sex | Model 2: Adjusted for Age, Sex, and the Smaller apo(a) Isoform |
|-----------------------------------|---------------------------------------------------------------|
| β (95% CI) | p | β (95% CI) | p |
| Reference | -2.68 (-4.42, -0.94) | 0.0025 | -3.37 (-4.50, -2.24) | 5.66E-09 |
| PNR10     | 0.78 (-1.32, 0.89) | 0.4600 | 0.24 (-1.80, 2.28) | 0.8154 |
| PNR11     | 0.64 (-2.38, 3.35) | 0.6783 | -1.84 (-4.96, 1.28) | 0.2473 |
| rs41272110 | 10.18 (6.04, 14.92) | 1.49E-06 | -25.42 (-29.21, -21.73) | 3.05E-40 |
| 4925G>A   | NA | NA | NA | NA |

HMW, high molecular weight; LMW, low molecular weight; Lp(a), lipoprotein(a); PNR, pentanucleotide repeat.

The model uses PNR8 allele as reference allele. Median Lp(a) concentration and effect are given in mg/dl.
predominantly located in the region of KIV-8 to KIV-10 (24–26), but no variant markedly accounted for the effect of any PNR allele (26). This supports the hypothesis that the association of the PNR with Lp(a) is mediated by a yet unidentified functional variant (23). Recently, we have shown that rs41272110 and the strongly Lp(a)-lowering KIV-2 splice site variant 4925G>A are in strong LD and that the Lp(a)-lowering effect previously attributed to rs41272110 is indeed caused by 4925G>A (35). This remained undetected until now because 4925G>A was hidden in the complex KIV-2 repeat region and became accessible to genetic-epidemiological studies only recently (12, 49). Also the PNR has been neglected by recent large-scale genetic studies because, unlike SNPs, STRs cannot be inexpensively determined in large populations in an automated manner. Thus, both polymorphisms had escaped detailed attention so far. Having both variants available from previous efforts in a large population-based study (n = 2,858) enabled us to investigate the interplay of these two elusive variants.

Individuals carrying both the PNR10 allele and KIV-2 4925G>A encompass a very large proportion of the PNR10 allele carriers with low Lp(a) concentrations (Fig. 5). Some small remaining clusters of individuals with low Lp(a) despite small isoforms might be explained by other Lp(a) decreasing variants. Indeed, Mukamel et al. (13) have recently defined 23 mostly loss-of-function variants that are associated with significantly lower Lp(a) across the complete minor allele frequency spectrum. Additionally, several studies describe SNP haplotypes within same-sized isoforms (7). Said et al. (20) for example describe an interplay between a rare missense variant and rs10455872 that lowers the median Lp(a) concentration in carriers of both variants. Conversely, we observed that carriers of rs10455872 and rs3798220 are located on a different haplotype than KIV-2 4925G>A, despite being both predominantly found in LMW isoforms.

Interestingly, Trommsdorff et al. (29) showed that there is no correlation between PNR and lower Lp(a) levels in African American individuals. Our data from the 1000G Project dataset suggest that this is due to the low carrier frequency of KIV-2 4925G>A (1.8%) in African individuals. The same observation could be translated also to the East Asian population. Due to this low frequency, the relevance of the splicing variant at an individual level in these populations is lower than in Europeans. However, such frequency differences of mainly Lp(a)-lowering variants may cooperate in establishing the observed differences in Lp(a) between ancestries. Mukamel et al. (13) indicated that the paucity of 4925G>A carriers in African populations possibly explains the higher median Lp(a) concentration in this population than in European individuals. Investigations on the LD between the apo(a) isoform and the PNR genotype in different populations are sparse. Various studies report data, but with a high heterogeneity between the studies (23, 29, 50).

Previous authors proposed rs41272110 as another possible causal SNP behind the association of PNR10 with low Lp(a). Very recently, we reported a high LD (I^2 = 0.84–0.86; D' = 0.98–0.99) between KIV-2 4925G>A and rs41272110 and showed that the decreasing effect in carriers of rs41272110 is caused by the splice variant. Indeed, we could show that addition of rs41272110 to the regression model does not abolish the association, while additional inclusion of 4925G>A does abolish the association with low Lp(a) of both the PNR and of rs41272110 (35). As shown previously, adjustment for 4925G>A also reveals a hidden Lp(a)-increasing effect of rs41272110 (35). This underscores the frequent splice site variant KIV-2 4925G>A as the major contributor to the association of the PNR with Lp(a).

The huge variance observed in the Lp(a) trait and even within each isoform group has puzzled Lp(a) research for a long time as causal variants have been elusive. Only very recently, multiple variants were identified (12, 13, 17–19, 48) and shed light on the genetic basis of this variance. Our study adds to these recent works and clarifies the causal basis of a further LPA polymorphism that had been linked with discordant phenotypes. It remains to be seen how many independent causal entities exist in the LPA gene, but elucidating all of them now appears to come into reach. For example, Mukamel et al. (13) proposed a manageable set of 23 variants that, together with the apo(a) isoforms, promise to explain a large portion (>80%) of Lp(a) variance. This puts even accurate genetic prediction of Lp(a) concentrations from genomic data within reach and may allow accurate stratification of individuals even without available Lp(a) measurements. It has been proposed recently that this could be used, for example, to identify individuals who most likely qualify for a given trial or therapy within available cohorts (15). In light of these advancements, elucidation of variants associated with discordant phenotypes has proven very important to complete our understanding of the Lp(a) trait (7, 13). Our work shows that the PNR is not an independent variant and most likely does not need to be taken into account. This might be a reassuring message for the community as microsatellites are still poorly captured by current high-throughput genomic technologies.

**Strengths and limitations**

Both the KIV-2 4925G>A splice site variant and the PNR are elusive LPA variants that are not commonly available in any large epidemiological study. Encompassing more than 2,800 individuals from a population-based cohort, our study is to the best of our knowledge the currently largest study combining data of the PNR, LPA SNPs, KIV-2 SNPs, and Lp(a) concentrations and directly determined apo(a) isoforms. This put us in the unique position to address the interplay of these two discussed variants.
We are aware that this study focuses on two specific variants. The functionality of the KIV-2 4925G>A variant has been shown by previous studies (12), and it has been included in a highly curated set of very likely functional LPA SNPs (13), but, clearly, we cannot definitely rule out the role of another yet unidentified polymorphism. Our observations highlight the need of scaling up such analyses up to locus- or genome-wide approaches in future studies. We acknowledge that we could not perform analyses at genotype level because the high polymorphic rate of the PNR still results in a lack of power for the single genotypes.

CONCLUSION

We found that PNRI0 and PNRII do not contribute to the variance of Lp(a) concentrations. Their apparent effect on Lp(a) is solely mediated by an LD with the KIV-2 splice site mutation 4925G>A. This study highlights the complexity of the genetic regulation of Lp(a) and how the effect of an LPA polymorphism on Lp(a) concentration can derive from other, hidden variants in partial LD. Elucidating these complex interactions will bring us closer to a comprehensive understanding of the high variance of the Lp(a) trait and its genetic regulation.

Data availability

Data from the KORA F4 study were used under license for the respective study and are not publicly available. Data access requires formal application to the steering committees. However, data supporting the findings are available from the corresponding author upon reasonable request and with permission from the respective steering committees. Requests to access the dataset can be made using the digital tool KORAPASST in accordance with the informed consent given by the study participants (https://www.helmholtz-munich.de/epi/research/cohorts/kora-cohort/data-use-and-access-via-korapasst). The 1000G data and all analysis tools are available at the locations provided in the Supplementary Methods section.

Supplemental data

This article contains supplemental data (12, 44, 51–59).

Author contributions

R. G., S. D. M., G. S., and F. K. investigation; R. G., H. W., S. S., and S. C. formal analysis; R. G., H. W., C. L., S. S., L. F., and F. K. data curation; R. G., H. W., and S. S. writing - original draft; R. G. visualization; H. W., S. S., and L. F. software; C. L., S. D. M., G. S., F. K., and S. C. methodology; C. L., L. F., S. D. M., G. S., A. P., C. G., and F. K. writing - review & editing; A. P., C. G., and F. K. resources; F. K. and S. C. funding acquisition; F. K. and S. C. project administration.

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Conflict of interest

F. K. has served on the advisory boards and has received lecture fees from Novartis, Amgen, and Kaneka. S. C., G. L., and L. F. have received lecture fees from Novartis. The other authors disclose no conflicts of interest.

Abbreviations

Apo(a), apolipoprotein(a); HMW, high-molecular-weight; LD, linkage disequilibrium; LMW, low-molecular-weight; Lp(a), lipoprotein(a); KIV, kringle IV; KORA, Cooperative Health Research in the Region of Augsburg; PNR, pentanucleotide repeat; STR, short tandem repeat.

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Low Lp(a) in carriers of long PNR is caused by KIV-2 4925G>A