Two-locus Linkage Analysis Applied to Putative Quantitative Trait Loci for Lipoprotein(a) Levels

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Plasma levels of lipoprotein(a) — Lp(a) — are associated with cardiovascular risk (Danesh et al., 2000) and were long believed to be influenced by the *LPA* locus on chromosome 6q27 only. However, a recent report of Broeckel et al. (2002) suggested the presence of a second quantitative trait locus on chromosome 1 influencing Lp(a) levels. Using a two-locus model, we found no evidence for an additional Lp(a) locus on chromosome 1 in a linkage study among 483 dizygotic twin pairs.

Levels of Lp(a) are highly heritable. More than 90% of the variance is attributable to genetic factors (Rainwater, 1996; Snieder et al., 1997). Variation in the gene encoding apolipoprotein(a) (*LPA*) on chromosome 6q27 accounts for more than 85% of the variation in Lp(a) plasma levels (Boerwinkle et al., 1992; Boomsma et al., 1993; DeMeester et al., 1995). Recently, Broeckel et al. (2002) reported a second chromosomal region linked with Lp(a) levels on chromosome 1 at 170 cM from pter, which explained 16% of the variance in Lp(a), in a linkage study of 513 Western European families with 2 or more cases of premature myocardial infarction (on average 2.7 subjects per family). To test whether this putative quantitative trait locus (QTL) also affects Lp(a) levels in the general population, we performed a linkage study in four samples of dizygotic (DZ) and monozygotic (MZ) twin pairs recruited regardless of their health status.

Materials and Methods

Plasma levels of Lp(a) were obtained of an adolescent Dutch sample (90 DZ, 70 MZ, mean age = 17 years) and three adult samples from the Netherlands (119 DZ, 94 MZ, mean age = 44 years), Sweden (87 DZ, 69 MZ, mean age = 65 years) and Australia (669 DZ, 734 MZ, mean age = 44 years) recruited regardless of their health status (Beekman et al., 2002). In these samples, heritability estimates of Lp(a) levels ranged from 90 to 96%. Genotypes for the linkage study were obtained for DZ pairs for whom DNA was available, and not for MZ twins, since these are genetically identical, sharing two alleles identical-by-descent (IBD) at all loci. Markers from the Marshfield Screening Set 8 were genotyped on chromosome 1 and 6 with an average spacing of 18 cM (Beekman et al., 2001). To adjust for non-normality, Lp(a) levels were transformed by the natural logarithm. Variance components linkage analyses were carried out, including data from the 483 genotyped DZ twins as well as the phenotypical data from the 967 untyped MZ twin pairs. The inclusion of MZ twins provides a more accurate estimate of non-shared environmental effects (MZ correlations are < 1), so that an upper limit for the estimation of genetic effects is obtained and overestimation is reduced. IBD status for the DZ pairs was estimated separately for each of the four samples with Genehunter 2.1 (Kruglyak et al., 1996) using population specific allele frequencies. All analyses were performed using Mx 1.52d (Neale et al., 1999) in a 4-sample simultaneous analysis. Mean Lp(a) levels, background genetic and non-shared environmental effects were estimated for each sample separately. The total variance was modeled as A + E + Q, where A represents additive genetic background

factors, E non-shared environmental factors and Q the QTL effect. The covariance equals A + Q for MZ twins and $^1/_2$A + $\hat{\pi}$Q for DZ twins, where $\hat{\pi}$ is estimated as $^1/_2$P(IBD = 1) + P(IBD = 2). To test for linkage, the fit of the AE model was compared to the fit of the AEQ model. For the two-locus models, the equations for the total variance, covariance for MZ twins and covariance for DZ twins were A + E + Q_1 + Q_6, A + Q_1 + Q_6 and $^1/_2$A + $\hat{\pi}_1Q_1$ + $\hat{\pi}_6Q_6$, respectively, where Q_1 and Q_6 refer to the putative QTLs on chromosomes 1 and 6, respectively. The proportion of the variance explained by the QTL was constrained to be equal over the samples. The power to replicate the putative Lp(a) QTL on chromosome 1 (QTL effect, 0.16; residual shared variance, 0.74) in 483 sib pairs at a significance level of .00074 (LOD-score of 2.2 constituting suggestive linkage according to Lander-Kruglyak critera) and assuming incomplete marker informativeness (theta = 0.10) is 0.51, as calculated using the Genetic Power Calculator (http://statgen.iop.kcl.ac.uk/gpc/) (Sham et al., 2000).
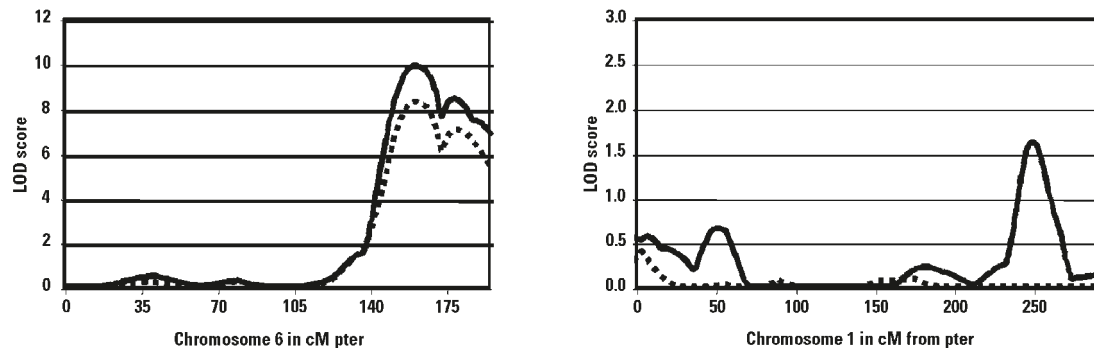
Results

As expected, we obtained highly significant evidence for linkage at the *LPA* locus on chromosome 6 (maximum LOD score [MLS] = 9.8, Figure 1; MLS of 1.7, 4.4, 1.8 and 5.1, in the adolescent Dutch and adult Dutch, Swedish and Australian samples, respectively). This QTL explained 82% of the total variance in Lp(a) levels (Table 1). Next, chromosome 1 was analyzed and a MLS of 1.6 was obtained at 251 cM from pter (Figure 1; MLS of 0.0, 0.7, 2.2 and 1.8, in the adolescent Dutch and adult Dutch, Swedish and Australian samples, respectively).

This QTL explained 44% of the total variance in Lp(a) levels (Table 1). The two putative QTLs on chromosome 6 and 1 together explained 126% of the variation in Lp(a) levels, which likely results from overestimation of effect sizes, a common phenomenon in genome-wide linkage analyses (Göring et al., 2001). We, therefore, analyzed the effect of both QTLs simultaneously in a two-locus model (Figure 1). In this two-locus model, evidence for linkage on chromosome 1 completely disappeared (LOD = 0; Figure 1). Moreover, the chromosome 1 locus now explained none of the variation in Lp(a) plasma levels, while the estimates for the *LPA* locus remained unaffected (Table 1).

Conclusion

Although our first analysis indicated linkage with Lp(a) on chromosome 1, disappearance of this linkage in a two-locus model suggests that, besides a possible power problem to detect an additional QTL with a small effect, our initial finding may have been a false positive result. Our analyses thus indicate that multi-locus models as employed here, may be a useful tool to distinguish true linkage results from spurious ones. In a recent study, Broeckel et al. (2002) reported a second QTL on chromosome 1 influencing Lp(a) levels in premature MI patients. Our study in twin pair samples provides no support for a Lp(a) QTL on chromosome 1 acting in the general population.

Figure 1

Linkage analysis of chromosome 6 and 1 with plasma levels of Lp(a) in four twin samples simultaneously. — shows the result of the initial analysis of the chromosomes; --- shows the result of the two-locus analysis.

Table 1

Effect Size of Putative Lp(a) QTLs at Chromosome 6 and 1 at the Position of the Maximum LOD Scores, in Separate and Simultaneous Analyses

Chromosome analyzed	Maximum LOD score (position in cM from pter)		Proportion of the variance attributable to the QTL at chromosome 6	Proportion of the variance attributable to the QTL at chromosome 1
	Chr 6	Chr 1	(95% C.I)	(95% C.I.)
6	9.8 (163)	—	0.82 (0.66–0.91)	—
1	—	1.6 (251)	—	0.44 (0.13–0.68)
6 and 1	0.82 (163)	0.0 (251)	0.82 (0.65–0.91)	0.00 (0.00–0.14)

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