Genome-wide search for cardiovascular risk factors in three independent populations

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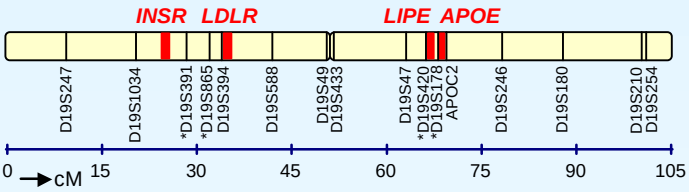
Background

The genetic basis of cardiovascular disease is highly complex. Focusing on less complex cardiovascular risk factors may contribute to the dissection of this genetic basis. The aim of our study is to map and identify genes with a major effect on these risk factors in the general population by performing a genome-wide search in three independent twin samples. Here, the analysis of chromosome 19 is presented with an emphasis on highly heritable lipid levels (table 1).

Methods

The search is performed in 194 Dutch dizygotic twin pairs (DZTs), 51 Swedish DZTs and 242 Australian DZTs from the general population with data on lipid levels. These pairs did not use lipid lowering drugs (8 Dutch and 10 Australian pairs removed from original sample) and their relationship was confirmed by analysing genome scan data with the software Graphical Relationship Representation (in the original sample, 2 Dutch, 2 Swedish and 11 Australian pairs appeared to be unrelated (representing laboratory errors) or monozygotic). Multiplex PCRs were designed so that finished chromosomes would become available for statistical analysis while the search is progressing. By regularly retyping 10% of the samples the genotyping error rate was established to be <1%. In addition, the software SIBMED was used to identify unlikely double recombinants. For chromosome 19 (>6000 genotypings), this revealed 5 genotyping errors. In the Dutch sample, 4 extra markers were typed on chromosome 19 resulting in a total of 16 markers (figure 1). These 4 markers were also typed in 194 parents and 81 additional sibs to improve identical-by-descent (IBD) estimation. Multipoint linkage was tested by variance components analysis using the software Mx. This approach has superior power because it not only takes into account pair differences but also absolute trait values. Adjustment for age and sex was implemented and MZT-data were incorporated. The proportion of alleles shared IBD, as estimated with Genehunter 2.0, was used (pi-hat approach).

Figure 1. Chromosome 19 with markers and candidate genes.



Average spacing: 8 cM in Australian and Swedish samples and 6 cM in Dutch sample in which 4 extra markers were genotyped (indicated with asterisk). *INSR*=insulin receptor, *LDLR*=LDL receptor, *LIPE*=hormone-sensitive lipase, *APOE*=apolipoprotein E.

Table 1. Heritability estimates of plasma levels of lipids and apolipoproteins in populations studied in genome scan.

Phenotype		Netherlands Mean 16 y	Netherlands Mean 44 y	Sweden Mean 65 y	Australia Mean 45 y
LDL	M	0.83	0.77	0.66	0.66
	F	0.83	0.77	0.66	0.60
Total chol.	M	0.87	0.77	0.67	0.62
	F	0.79	0.77	0.67	0.55
ApoB	M	0.82	0.79	0.75	0.62
	F	0.82	0.79	0.75	0.62
HDL	M	0.74	0.72	0.64	0.62
	F	0.77	0.72	0.64	0.62
ApoAI	M	0.85	0.72	0.61	0.37
	F	0.74	0.72	0.61	0.54
Triglyc.	M	0.70	0.71	0.48	0.50
	F	0.70	0.56	0.48	0.50
ApoE	M	0.86	0.87	-	0.48
	F	0.86	0.87	-	0.60

Heritabilities were estimated with a model allowing for additive genetic and unique environmental effects.

Results and conclusions

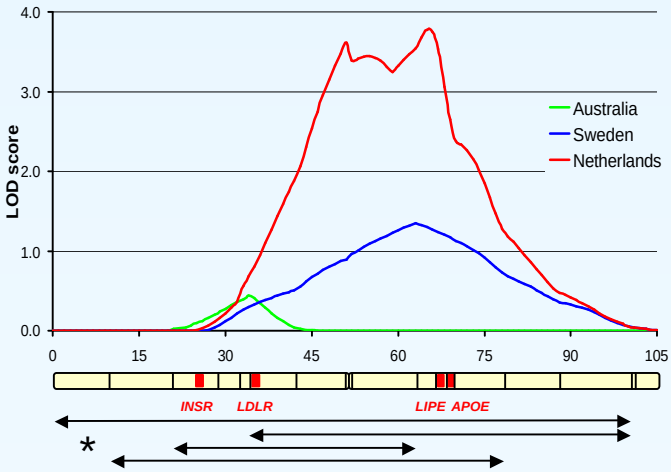
- Genetic factors determine 50-80% of the variation in the levels of lipids and apolipoproteins in plasma in the Dutch, the Swedish as well as the Australian population (table 1).
- Our study indicates the presence of a LDL quantitative trait locus on chromosome 19 in the Dutch population (maximum LOD score = 3.78, $p=3.0\times10^{-5}$) (table 2).
- Suggestive linkage with LDL levels was found in the same chromosomal region in a Swedish (maximum LOD score= 1.35, p=0.013), but not an Australian population (table 2 and figure 2).
- Analysis of the IBD-status of the Dutch pairs that mainly determined the linkage result indicated that in addition to novel genes, the genes encoding the LDL-receptor and apolipoprotein E could be involved (figure 2).

Table 2. Maximum LOD scores (MLS) on chromosome 19 for lipid traits in Dutch, Swedish and Australian twins.

Phenotype	Netherlands DZ pairs = 194		Sweden DZ pairs = 51		Australia DZ pairs = 242	
	Pos	MLS	Pos	MLS	Pos	MLS
LDL	65	3.78	63	1.35	34	0.44
Total chol.	51	2.60	69	1.19	57	0.07
ApoB	51	1.42	70	1.60	-	0.00
HDL	32	0.80	33	0.09	54	0.92
ApoAI	34	0.23	28	1.31	51	1.53
Triglyc.	48	1.01	51	0.10	63	0.37
ApoE	63	0.27	-	-	64	0.59

LOD scores were estimated by variance components analysis adjusting for age and sex and incorporating monozygotic twin data using Mx. Multipoint identical-by-descent (IBD) probabilities were estimated using Genehunter 2.0. DZ=dizygotic twin pair, Pos=chromosomal position in cM, MLS=maximum LOD score.

Figure 2. Result of variance components linkage analysis for LDL cholesterol level on chromosome 19.



*Arrows indicate the identical-by-descent status of the four Dutch pairs that mainly determined the linkage result. For pairs discordant for LDL level it indicates the region where IBD=0 (first 3 arrows) and for the concordant pair it indicates the region where IBD=2, i.e. the region where the putative gene influencing LDL cholesterol would be located.