Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Clinica Chimica Acta 374 (2006) 87 – 92
CCA
www.elsevier.com/locate/clinchim

Genome-wide linkage scan for loci influencing plasma triglycerides

Rita P. Middelberg *, Nicholas G. Martin, Grant W. Montgomery, John B. Whitfield

Genetic Epidemiology Unit, Queensland Institute of Medical Research, Brisbane, Queensland, Australia

Received 1 March 2006; received in revised form 26 May 2006; accepted 26 May 2006
Available online 3 June 2006

Abstract

Background: Plasma triglyceride concentration is known to be a significant risk factor for cardiovascular disease (CVD). Previous studies have found that the level of triglycerides is strongly influenced by genetic factors.
Methods: To identify quantitative trait loci influencing triglycerides, we conducted a genome-wide linkage scan on data from 485 Australian adult dizygotic twin pairs. Prior to linkage analysis, triglyceride values were adjusted for the effects of covariates including age, sex, time since last meal, time of blood collection (CT) and time to plasma separation.
Results: The heritability estimate for ln(triglyceride) adjusted for all above fixed effects was 0.49. The highest multipoint LOD score observed was 2.94 (genome-wide $p=0.049$) on chromosome 7 (at 65 cM). This 7p region contains several candidate genes. Two other regions with suggestive multipoint LOD scores were also identified on chromosome 4 (LOD score = 2.26 at 62 cM) and chromosome X (LOD score = 2.01 at 81 cM).
Conclusions: The linkage peaks found represent newly identified regions for more detailed study, in particular the significant linkage observed on chromosome 7p13.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Triglycerides; Cardiovascular disease; Genetics; Quantitative loci; Genome scan

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in most developed countries. In Australia, it accounts for 38% of all deaths [1]. Along with HDL and LDL cholesterol and apolipoprotein, plasma triglyceride concentrations are associated with the incidence of atherosclerotic heart disease [2,3]. Therefore the sources of individual differences in plasma triglyceride have been the subject of many twin, family, candidate gene association and linkage studies.

A recent comparison of twin study results has found that the heritability estimate of triglyceride levels ranged from 48% to 71% in samples from different countries [4]. A review by Snieder et al. [5] showed most studies gave a heritability estimate for triglycerides greater than 50%. These findings suggest that triglyceride levels are strongly influenced by genetic factors. Multiple genome scans have been conducted to map quantitative trait loci (QTLs) for triglycerides and several have previously been identified. For example, linkage analyses have identified regions located on chromosomes 2q in the Hutterite community [6], 10p in Finnish families [7], 15q in Mexican Americans [8] and 19q in Utah Caucasian families [9]. Further findings include loci on chromosome 3 among Caucasian families in the Genetics of NIDDM (GENNID) study [10], 10q in hypertensive non-Hispanic Whites [11], 8p21 in Turkish families with atherogenic dyslipidemia [12] and the 7q36 region (near marker D7S3058) in families selected for obesity [13–15]. Although many positive findings have emerged, few have been consistently replicated. One region which has shown positive results in multiple studies is chromosome 7. Several studies have provided consistent evidence for a QTL in region 107–176 cM [13–18] on chromosome 7. Other regions, for example 9 cM [8] , 21 cM [8,19] and 84 cM [20], have also been found to be linked to loci influencing triglycerides. It is notable that some of these studies have reported linkage for triglyceride alone, and others for triglyceride/HDL ratio.

Turning to association studies, the most consistent result is related to the APOA1/C3/A4 gene cluster on chromosome 11q23. The APOC3 *Sst*I polymorphism has been linked to hypertriglyceridemia in different ethnic groups [21–25]. Recently, a new apolipoprotein gene, APOA5, has been identified and shown to be associated with triglycerides in several

* Corresponding author. Genetic Epidemiology Unit, Queensland Institute of Medical Research, PO Royal Brisbane Hospital, Brisbane, Queensland, 4029, Australia. Tel.: +61 7 3362 0331; fax: +61 7 3362 0101.
E-mail address: ritaM@qimr.edu.au (R.P. Middelberg).

0009-8981/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2006.05.033

populations of different ethnicities, including Chinese, Japanese, African-Americans, Hispanics and Caucasians [26,27]. Associations between variants of APOA5 (−1131C, 19W and 56G alleles) and plasma triglyceride levels have been consistently shown. Two studies have found carriers of the APOA5 −1131C allele had a significantly higher concentration of triglycerides [27,28]. In both men and women, triglyceride concentration was significantly higher ($p<0.001$) in 19W carriers compared to 19S homozygotes. APOA5 gene polymorphisms or haplotypes could also influence myocardial infarction [28]. A study in the Chinese population revealed the −1131C allele frequency was significantly higher in coronary artery disease patients (39.9% vs. 33.3%, $p=0.02$) than in a control group [29].

Identifying other genes involved in determining triglyceride levels might provide valuable insights into the biological pathways that contribute to the development of CVD. Although many chromosomal regions have been provisionally identified by linkage studies, few have been replicated in subsequent studies. We have performed genome-wide linkage scans using data from 485 Australian twin pairs to identify quantitative trait loci (QTLs) influencing triglyceride and to determine whether previous findings are replicated in this population.

2. Materials and methods

2.1. Subjects and phenotype data

The twin data come from a study investigating genetic influences on biological, psychological and social manifestations of alcoholism and related disorders (SSAGA) [30] which started in 1993. Data were collected between 1993 and 1996 when twins were aged 28 to 92. There were 5999 respondents. Overall, 3347 individual twins provided blood samples for the study; all participants gave consent to the questionnaire, interview and blood collection. The study was approved by appropriate Ethics Committees. Height and weight were measured by self-report and body mass index (BMI) was determined by weight (kg)/height squared (m^2). The time of the blood collection was recorded and the twins reported the time of their last meal. The majority of the twins who gave blood attended blood collection sessions as they lived in or close to Adelaide, Melbourne, Brisbane or Sydney. About 24% of the blood samples were collected in more remote sites, and in these cases blood was delivered to the laboratory within 24 hours. Serum was separated and stored at −70 °C until analysis. Triglycerides were measured on a Hitachi 747 analyzer with Boehringer reagents by standard enzymatic methods [31]. Twin pair zygosity was determined from responses to a questionnaire with standard questions about physical similarity and the degree to which others could tell them apart, and blood group information supplemented by genome-wide microsatellite marker results for dizygotic twins in the linkage study.

2.2. Genotype data

Genotype data in this study come from a compilation of four smaller genome scans performed for other studies at the Queensland Institute of Medical Research. Genotyping was done at four facilities: Gemini Genomics (426 microsatellite markers), Sequana Therapeutics (519 markers), the Centre for Mammalian Genotyping Service, Marshfield, USA (776 markers), and the University of Leiden (435 markers).

Several steps were taken to clean the combined genotype data. First, relationship misspecifications and potential sample mix-ups were investigated. Pedigree relationships were checked using Graphic Representation of Relationships (GRR) [32] and RELPAIR [33,34]. The second step involved detecting genotypes that give rise to Mendelian inconsistencies. Mendelian errors were detected using SIB-PAIR [35] to correct autosomal data. The third step was to merge marker data from the four genome scans mentioned above. There were 458 unique markers that were typed in two or more genome scans. Marker inconsistency was assessed by cross-tabulating allele calls between different scans. Markers with genotype data inconsistent between different genome scans were removed using PEDSTATS [36] and MERLIN version 0.10.1 [37]. GENEHUNTER [38] and MENDEL [39] was used to identify potential map errors. Locations of the markers were taken from an integrated genetic map (http://www2.qimr.edu.au/davidD/). The map locations were estimated via locally weighted linear regression using NCBI Build 34.3 physical map positions and published Decode and Marshfield genetic map positions [40]. Positions from Kosambi centiMorgans (cM) were converted to Haldane cM. Where there were inconsistencies between genetic map distance and recombination fractions, the primer sequences for all markers in the region were BLASTed against the entire human genome sequence (http://www.ensembl.org, NCBI Build 34.3). A revised map which included the updated physical positions of all markers in problematic regions with the new map distance interpolated was hence obtained. The revised map was used to clean the unlikely genotype in the original genotype data. More details are provided elsewhere [41–43].

The combined genome scan included up to 1376 unique markers where a total 5160 individuals from 1587 families were typed. Triglyceride measurements were available for 1601 families/twin pairs (968 pairs monozygotic (MZ), 633 dizygotic (DZ)). Of these 633 DZ families, 28 families were not typed and only one twin individual participated in 120 families. Only 485 families had both dizygotic twins genotyped and measured for triglycerides. These pairs, on average had 595 markers (range = 0–1209) shared by both twin individuals within a family. Intermarker distances were estimated for each twin pair and then averaged across the 485 pairs. For autosomes, the mean intermarker distance was 5.0 cM (S.D. = 2.8 cM). The average marker heterozygosity was 75% for autosomal markers and 72% for markers on the X chromosome.

2.3. Statistical analysis

Before adjusting for covariates, the triglyceride variable was transformed by natural logarithm as the distribution was skewed. Twins who were taking lipid lowering medication and outliers (greater than 3 standard deviations from the mean) were identified and a total of 30 individual twins were excluded from the analysis

Table 1
General characteristics of the base population and sample available for genome-scan

	Data available	Base population
No. of families	485	3274
No. of individuals (no. of males)	970 (360)	5999 (2089)
Age at blood collection (years)	44.1 ± 10.8 (29−84)	45.5 ± 11.5 (29−92)
BMI (kg/m^2)	25.4 ± 4.3	25.2 ± 4.2 ₣
Alcohol-dependence (%) (males, females)	26, 8	24, 6
Proportion of current smokers (%)	21	21
Proportion of university graduates (%)	21	18
No religion (%)	17	15
Weekly church attendance (%)	22	24
Triglyceride (mmol/l)	1.73 ± 1.34	1.75 ± 1.24 ₣

Values are given as mean ± S.D.; BMI, body mass index.
₣ Values provided are based on 3347 twin individuals who provided blood samples.

on this basis. The time of blood collection was categorized as follows: blood collected before 10 a.m. (category 1), between 10 a.m. and 12 noon (category 2), between 12 noon and 2 p.m. (category 3), between 2 p.m. and 4 p.m. (category 4), between 4 p.m. and 6 p.m. (category 5) and after 6 p.m. (category 6). The following variables were tested for significance: age, squared age, sex, sex × age, sex × age^2, time since last meal (TM), squared TM, time of blood collection (CT) and time to plasma separation. Of these variables, only age, sex, time since last meal, CT and time to plasma separation were significantly associated with triglyceride results for all the significant covariates except BMI; the same procedure was also followed with the inclusion of BMI as a covariate. Residuals were saved using the “residuals” option in STATA [44] and genetic model-fitting and linkage analysis were then performed using these residuals.

Models containing additive and dominance genetic variation and shared and non-shared environment variations were fitted to the triglyceride data. The most parsimonious model that did not significantly worsen the fit was chosen. Quantitative trait linkage analysis was conducted using an inverse regression method, MERLIN-regress statistics, which regresses the estimated proportion of alleles shared identity by descent (IBD) on the squared sum and squared differences of trait values of relative pairs. MERLIN-regress was used as it is faster to run compared to variance components (VC) analysis and is less sensitive to the assumption of multivariate normality [45]. The method was performed using the software MERLIN. For X-chromosome analysis, MINX (Merlin-In-X), a modified version of MERLIN [37], was used.

The following procedure was used to obtain the empirical significance level of an observed LOD score and the suggestive and significant linkage thresholds. First, 1000 simulated genome scan data sets were generated using MERLIN. Each simulated set was generated under the assumption that there are no susceptibility loci using the pedigree structures in the data set and the observed patterns of genotype data, including any missing genotypes. Each simulated data set was analyzed in the same way as the observed data. The highest peak for each chromosome was recorded. The empirical significance level of an observed LOD score was estimated by counting the number of peaks in the entire genome that had a LOD score greater than or equal to the observed score across the 1000 replicates (i.e. the proportion of genome scans containing one or more peaks of observed score size). The cutoff for suggestive linkage was calculated as the LOD score that was observed on average once per genome scan (i.e. 1000th highest LOD score out of 23,000 LOD scores) [46]. The cutoff for significant linkage was defined as the LOD score of probability 0.05 (i.e. the 50th highest LOD score out of 23,000 LOD scores). A nominal p value of 0.01 was

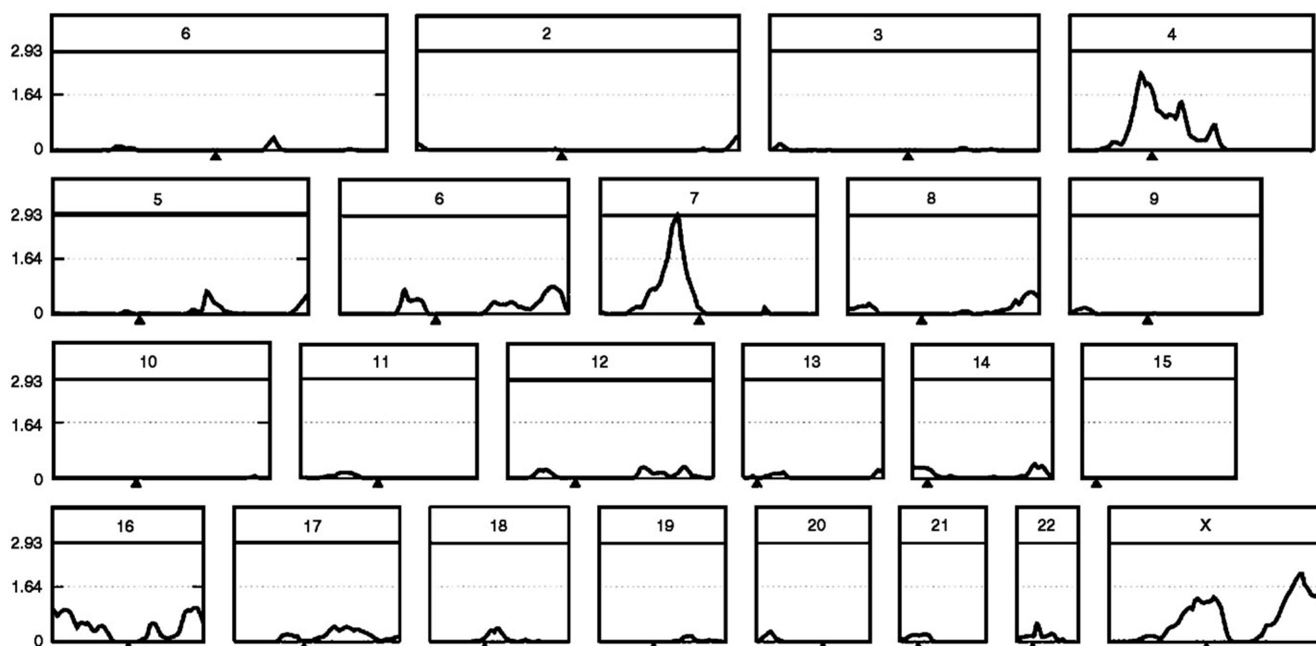

Fig. 1. Multipoint linkage analysis of ln(triglyceride) for autosomal and X chromosomes. x-axis shows the genetic map of each chromosome and y-axis shows the LOD score (LS). Thresholds for significant (LS = 2.93) and suggestive (LS = 1.64) genome wide linkage are shown.

Table 2
Regions with multipoint LOD score ≥ 1 in genome scan

Chromosome	Position of peak (cM)	MERLIN-regress LOD	Nearest marker
4	62	2.26	D4S405
7	65	2.94	TAT028
16	124	1.02	GATA11c06
X	81	2.01	DXS8043

cM, centiMorgans; LOD, logarithm of odds.

used to check for confirmation of previously reported significant linkage findings [46].

3. Results

The general characteristics of the base population and the 485 dizygotic twin pairs available in this study are shown in Table 1. In this study sample, approximately 15% of the twin individuals had been alcohol-dependent at some time in their lives, 21% were current smokers and 21% were university graduates (versus 12% alcohol-dependence, 21% and 18% graduates in the base population). Other socio-demographic variables that have been found to be associated with alcoholism risk, such as “at least weekly church attendance” and religious affiliation [30], are also compared in Table 1. The age at blood collection ranged from 29 to 84 years with a mean of 44.1 years (S.D. = 10.9 years). The average time between blood collection and processing was 0.75 h (S.D. = 1.21 h). The median time blood was collected was between 10 a.m. and 12 noon. The effects on triglyceride concentration of age, sex, time since last meal, time of blood collection (CT), and time to blood processing were found to be significant at 5% level when tested in the regression model.

Before embarking the linkage analysis, we first used the larger phenotyped sample of 968 MZ and 633 DZ pairs to estimate the proportion of genetic and environmental variance in adjusted ln(TG). The MZ correlations (r_{MZ}) were greater than the DZ (r_{DZ}) correlations ($r_{MZ}=0.44$; $r_{DZ}=0.27$), suggesting an important additive genetic influence. The estimate of the common environment variance component was 0.02 with 95% CI = (0.00, 0.05), and could be dropped from the model without a significant worsening of fit ($\Delta\chi_1^2=0.57$; $p=0.45$). With a model indicating only genetic and unique environmental factors influencing triglyceride levels in adults, the heritability estimate for triglycerides was 49%, after adjusting for the covariates.

The results from MERLIN-regress multipoint LOD scores calculated at 1-cM increments are given in Fig. 1. A number of peaks with LOD score greater than 1 are observed, including on chromosomes 4, 7, 16 and X. The highest peak (Table 2) was located on chromosome 7 with a LOD score of 2.94. The nearest marker to this peak was TAT028. The 1-LOD support interval surrounding the QTL peak (1-LOD unit reduction from the peak) is between markers GATA026 and GATA24D12, which are 15 cM apart. As shown in Fig. 1, several other regions exceeded the 1 LOD score threshold: chromosome 4 at 62 cM (LOD = 2.26), X chromosome at 81 cM (LOD = 2.01) and chromosome 16 at 124 cM (LOD = 1.02).

Simulation analysis estimated that the thresholds for significant and suggestive linkage were 2.93 and 1.64, respectively. The peak found on chromosome 7 surpasses the threshold for significant linkage. The simulation results indicated that there were 49 out of 1000 peaks ≥ 2.94 observed, giving a genome wide p value of 0.049 which surpasses our threshold for significant linkage. The peaks on chromosomes 4 and X surpassed our suggestive thresholds, whereas chromosome 16 peak fell short of the suggestive threshold.

4. Discussion

We performed a genome scan to map loci influencing plasma triglycerides concentration in 485 pairs of dizygotic Australian twins. The sample used for linkage analysis was drawn from a larger community study of adult twins and did not differ from the sample in respect to BMI, alcohol-dependent, smoking and other socio-demographic characteristics. Evidence of a significant

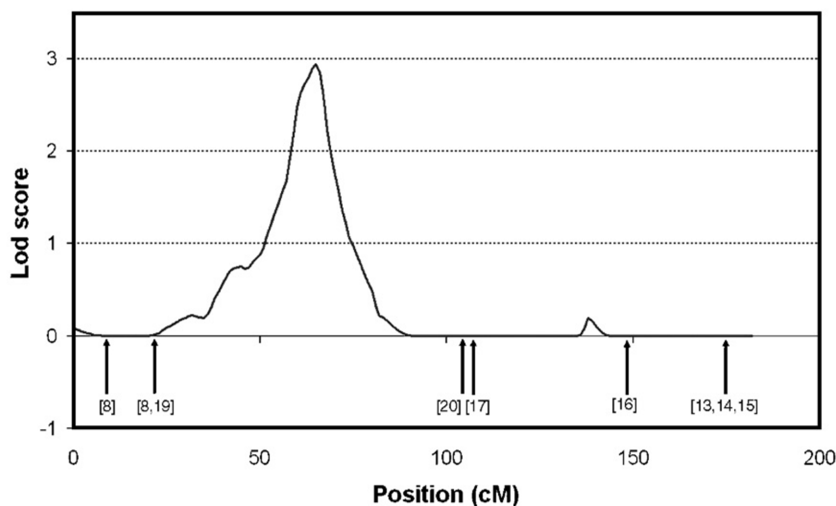

Fig. 2. Multipoint LOD scores on chromosome 7 from genome-wide scan of ln(triglyceride) adjusted for covariates. Linkage peaks reported in other studies are indicated by arrows with reference numbers.

linkage result (MLS higher than 2.93) was found on chromosome region 7p13 (at 65 cM).

There are several regions on chromosome 7 that have been suggestively linked to susceptibility loci influencing triglyceride levels [13–20]. There is strong evidence for a QTL on chromosome 7q36 (at 176 cM near marker FATA148A08) that influences the variation of TGs in extremely obese individuals [15], in the Framingham population [13] and in Caucasian families [14]. The peak found in these previous studies is quite some distance from the peak identified here (Fig. 2). However, it is known that there is considerable variation in the location of linkage peaks in samples of moderate size – including ours and others cited here [47]. Previous peak nearest to our 1-LOD support interval is one found by Lehman et al. [20] (7q11.23, 103–109 cM). There are no obvious known candidate genes for hypertriglyceridaemia in this region of chromosome 7. A possible potential candidate gene related to cholesterol in this chromosomal region could be Niemann-Pick C1 Like 1 (NPC1L1) (MIM 608010), which maps to chromosome 7p13. This gene has been suggested to be involved in the subcellular cholesterol trafficking [48] and thus could conceivably have a role in modulating or affecting triglyceride levels. Another possible gene, GSBS located on chromosome 7p15 (MIM 604088) has been associated with significantly higher plasma total cholesterol in people who carry the T allele of the −1323T-C polymorphism [49]. The candidate genes mentioned are purely speculative and hence further investigation including high-density SNP mapping will be needed to confirm the involvement of triglycerides with the candidate genes mentioned above.

Some studies have found suggestive linkage evidence on chromosome 7 for triglyceride/HDL ratio [16,18]. To investigate further, we performed a genome-wide scan for ln(triglyceride/HDL) ratio and found a similar linkage region (at 60 cM) on chromosome 7, although the peak for ln(triglyceride/HDL) ratio was lower (LOD = 2.65) compared to ln(triglyceride). In addition, we also performed a genome-wide analysis for ln(triglycerides) adjusted for all above mentioned covariates and also for BMI. We then found a LOD score of 2.66 in the similar linkage region (chromosome 7 at 64 cM). This is consistent with the known association between BMI and plasma triglyceride and suggests that this chromosomal region contains genes with effects on both phenotypes.

Results of our linkage analyses suggest the presence of a number of potential chromosomal regions which may harbour genes influencing ln(triglyceride) levels. As CVD is a multifactorial disease, it is the cumulative contribution of susceptibility genes that influences the disease, in combination with exposure to certain environmental factors.

There are several potential limitations of this study. The age range of the sample is wide (between 29 and 84 years of age) and different genes (and hence linkage peak) may operate across this age spectrum. A further limitation is that the participants had not been fasting before their blood samples were collected. Nevertheless, time since last meal has been recorded and it has been used as a covariate to adjust readings. Our data came from a volunteer panel rather than a register of twins systematically ascertained from birth records. There is consequently a slight over-representation of well-educated individuals [30], and this factor was not corrected for in the analysis.

In conclusion, on the basis of our genome-wide scan analysis, we found significant evidence for a susceptibility locus on chromosome 7p13 influencing triglyceride levels in an Australian population. This region has not been identified previously. In order to strengthen the evidence of this QTL, it will be necessary to conduct replication studies. In addition, high-density mapping with single nucleotide polymorphism and linkage disequilibrium mapping techniques will be required to identify functional variants.

Acknowledgements

We would like to thank Dixie Statham and Alison MacKenzie for project coordination, Megan Campbell and Anjali Engan for managing sample processing and preparation, David Smyth, Harry Beeby, Olivia Zheng and Scott Gordon for data management and their work in integrating the genome scans data, the twins and their families for their generous participation in the studies, and Sarah Medland for her valuable advice. For genome scans of twins and siblings, we acknowledge and thank the Mammalian Genotyping Service, Marshfield WI (Director: Dr James Weber) for genotyping under a grant to Dr Daniel T. O'Connor; Drs Eline Slagboom, Bastiaan Heijmans and Dorret Boomsma for the Leiden genome scan; Dr Peter Reed for the Gemini genome scan; and Dr Jeff Hall for the Sequana genome scan. We also gratefully acknowledge the valuable comments of the reviewers. Financial support was provided by grants from NIH (AA007535, AA014041), National Health and Medical Research Council of Australia (389891) and European Union (contract number QLRT-2001-01254). R.P.S.M. is supported by a Dora Lush NHMRC Scholarship (Grant ID 339484).

References

[1] National Heart Foundation of Australia. The shifting burden of cardiovascular disease in Australia. Australia: Access Economics Pty Limited; 2005.

[2] Forrester JS. Triglycerides: risk factor or fellow traveler? Curr Opin Cardiol 2001;16:261–4.

[3] Malloy MJ, Kane JP. A risk factor for atherosclerosis: triglyceride-rich lipoproteins. Adv Intern Med 2001;47:111–36.

[4] Beekman M, Heijmans BT, Martin NG, et al. Heritabilities of apolipoprotein and lipid levels in three countries. Twin Res 2002;5:87–97.

[5] Snieder H, van Doornen LJ, Boomsma DI. Dissecting the genetic architecture of lipids, lipoproteins, and apolipoproteins: lessons from twin studies. Arterioscler Thromb Vasc Biol 1999;19:2826–34.

[6] Newman DL, Abney M, Dytch H, Parry R, McPeek MS, Ober C. Major loci influencing serum triglyceride levels on 2q14 and 9p21 localized by homozygosity-by-descent mapping in a large Hutterite pedigree. Hum Mol Genet 2003;12:137–44.

[7] Pajukanta P, Terwilliger JD, Perola M, et al. Genomewide scan for familial combined hyperlipidemia genes in Finnish families, suggesting multiple susceptibility loci influencing triglyceride, cholesterol, and apolipoprotein B levels. Am J Hum Genet 1999;64:1453–63.

[8] Duggirala R, Blangero J, Almasy L, et al. A major susceptibility locus influencing plasma triglyceride concentrations is located on chromosome 15q in Mexican Americans. Am J Hum Genet 2000;66:1237–45.

[9] Elbein SC, Chu W, Ren Q, Wang H, Hemphill C, Hasstedt SJ. Evaluation of apolipoprotein A-II as a positional candidate gene for familial type II diabetes, altered lipid concentrations, and insulin resistance. Diabetologia 2002;45:1026–33.

[10] Malhotra A, Wolford JK. Analysis of Quantitative Lipid Traits in the Genetics of NIDDM (GENNID) Study. Diabetes 2005;54:3007–14.
[11] Kullo IJ, Turner ST, Boerwinkle E, Kardia SL, de Andrade M. A novel quantitative trait locus on chromosome 1 with pleiotropic effects on HDL-cholesterol and LDL particle size in hypertensive sibships. Am J Hypertens 2005;18:1084–90.
[12] Yu Y, Wyszynski DF, Waterworth DM, et al. Multiple QTLs influencing triglyceride and HDL and total cholesterol levels identified in families with atherogenic dyslipidemia. J Lipid Res 2005;46:2202–13.
[13] Lin JP. Genome-wide scan on plasma triglyceride and high density lipoprotein cholesterol levels, accounting for the effects of correlated quantitative phenotypes. BMC Genet 2003;4(Suppl 1):S47.
[14] Sonnenberg GE, Krakower GR, Martin LJ, et al. Genetic determinants of obesity-related lipid traits. J Lipid Res 2004;45:610–5.
[15] Li WD, Dong C, Li D, Garrigan C, Price RA. A genome scan for serum triglyceride in obese nuclear families. J Lipid Res 2005;46:432–8.
[16] Horne BD, Malhotra A, Camp NJ. Comparison of linkage analysis methods for genome-wide scanning of extended pedigrees, with application to the TG/HDL-C ratio in the Framingham Heart Study. BMC Genet 2003;4(Suppl 1):S93.
[17] Arya R, Blangero J, Williams K, et al. Factors of insulin resistance syndrome-related phenotypes are linked to genetic locations on chromosomes 6 and 7 in nondiabetic Mexican-Americans. Diabetes 2002;51:841–7.
[18] Shearman AM, Ordovas JM, Cupples LA, et al. Evidence for a gene influencing the TG/HDL-C ratio on chromosome 7q32.3-qter: a genome-wide scan in the Framingham study. Hum Mol Genet 2000;9:1315–20.
[19] Adeyemo AA, Johnson T, Acheampong J, et al. A genome wide quantitative trait linkage analysis for serum lipids in type 2 diabetes in an African population. Atherosclerosis 2005;181:389–97.
[20] Lehman DM, Arya R, Blangero J, et al. Bivariate linkage analysis of the insulin resistance syndrome phenotypes on chromosome 7q. Biol Hum 2005;77:231–46.
[21] Shoulders CC, Grantham TT, North JD, et al. Hypertriglyceridemia and the apolipoprotein CIII gene locus: lack of association with the variant insulin response element in Italian school children. Hum Genet 1996;98:557–66.
[22] Chhabra S, Narang R, Krishnan LR, et al. Apolipoprotein C3 *Sst*I polymorphism and triglyceride levels in Asian Indians. BMC Genet 2002;3:9.
[23] Russo GT, Meigs JB, Cupples LA, et al. Association of the *Sst*-I polymorphism at the APOC3 gene locus with variations in lipid levels, lipoprotein subclass profiles and coronary heart disease risk: the Framingham offspring study. Atherosclerosis 2001;158:173–81.
[24] Zeng Q, Dammerman M, Takada Y, Matsunaga A, Breslow JL, Sasaki J. An apolipoprotein CIII marker associated with hypertriglyceridemia in Caucasians also confers increased risk in a west Japanese population. Hum Genet 1995;95:371–5.
[25] Ahn YI, Valdez R, Reddy AP, Cole SA, Weiss KM, Ferrell RE. DNA polymorphisms of the apolipoprotein AI/CIII/AIV gene cluster influence plasma cholesterol and triglyceride levels in the Mayans of the Yucatan Peninsula, Mexico. Hum Hered 1991;41:281–9.
[26] Pennacchio LA, Olivier M, Hubacek JA, Krauss RM, Rubin EM, Cohen JC. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. Hum Mol Genet 2002;11:3031–8.
[27] Lee KW, Ayyobi AF, Frohlich JJ, Hill JS. APOA5 gene polymorphism modulates levels of triglyceride, HDL cholesterol and FERHDL but is not a risk factor for coronary artery disease. Atherosclerosis 2004;176:165–72.
[28] Hubacek JA, Skodova Z, Adamkova V, Lanska V, Poledne R. The influence of APOAV polymorphism (T-1131>C and S19>W) on plasma triglyceride levels and risk of myocardial infarction. Clin Genet 2004;65:126–30.
[29] Bi N, Yan S-K, Li G-P, et al. A single nucleotide polymorphism-113T>C in the apolipoprotein A5 gene is associated with an increased risk of coronary artery disease and alters triglyceride metabolism in Chinese. Mol Genet Metab 2004;83:280–6.
[30] Heath AC, Bucholz KK, Madden PA, et al. Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. Psychol Med 1997;27:1381–96.
[31] Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973;19:476–82.
[32] Abecasis GR, Cherny SS, Cookson WO, Cardon LR. GRR: graphical representation of relationship errors. Bioinformatics 2001;17:742–3.
[33] Epstein MP, Duren WL, Boehnke M. Improved inference of relationship for pairs of individuals. Am J Hum Genet 2000;67:1219–31.
[34] Duren, W.L., Epstein, M.P., Li, M., Boehnke, M. RELPAIR: a program that infers the relationships of pairs of individuals based on marker data, version 20 (http://csg.sph.umich.edu/boehnke/relpair.php). 2003.
[35] Duffy, D.L. SIB-PAIR version 0.99.9, Brisbane, Australia, Queensland Institute of Medical Research, 2002.
[36] Wigginton JE, Abecasis GR. PEDSTATS: descriptive statistics, graphics and quality assessment for gene mapping data. Bioinformatics 2005;21:3445–7.
[37] Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 2002;30:97–101.
[38] Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 1996;58:1347–63.
[39] Lange K, Weeks D, Boehnke M. Programs for pedigree analysis: MENDEL, FISHER, and dGENE. Genet Epidemiol 1988;5:471–2.
[40] Kong X, Murphy K, Raj T, He C, White PS, Matise TC. A combined linkage–physical map of the human genome. Am J Hum Genet 2004;75:1143–8.
[41] Zhu G, Evans DM, Duffy DL, et al. A genome scan for eye color in 502 twin families: most variation is due to a QTL on chromosome 15q. Twin Res 2004;7:197–210.
[42] Nyholt DR, Morley KI, Ferreira MA, et al. Genomewide significant linkage to migrainous headache on chromosome 5q21. Am J Hum Genet 2005;77:500–12.
[43] Cornes BK, Medland SE, Ferrerira MAR, et al. Sex-limited genome-wide linkage scan for body-mass index in an unselected sample of 933 Australian twin families. Twin Res 2005;8:616–32.
[44] StataCorp. Stata statistical software: release 7.0. College Station, Tex: StataCorp; 1997.
[45] Sham PC, Purcell S, Cherny SS, Abecasis GR. Powerful regression-based quantitative-trait linkage analysis of general pedigrees. Am J Hum Genet 2002;71:238–53.
[46] Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995;11:241–7.
[47] Roberts SB, MacLean CJ, Neale MC, Eaves LJ, Kendler KS. Replication of linkage studies of complex traits: an examination of variation in location estimates. Am J Hum Genet 1999;65:876–84.
[48] Davies JP, Levy B, Ioannou YA. Evidence for a Niemann-pick C (NPC) gene family: identification and characterization of NPC1L1. Genomics 2000;65:137–45.
[49] Ono S, Ezura Y, Emi M, et al. A promoter SNP (−1323T>C) in G-substrate gene (GSBS) correlates with hypercholesterolemia. J Hum Genet 2003;48:447–50.