ELSEVIER

Atherosclerosis 144 (1999) 393–401

ATHEROSCLEROSIS

Evidence of a major locus for lipoprotein lipase (LPL) activity in addition to a pleiotropic locus for both LPL and fasting insulin: results from the HERITAGE Family Study

Yuling Hong [a,*], Treva Rice [a], Jean-Pierre Després [b,c], Jacques Gagnon [b], André Nadeau [d], Jean Bergeron [c], Louis Pérusse [b], Claude Bouchard [b], Arthur S. Leon [e], James S. Skinner [f], Jack H. Wilmore [g], D.C. Rao [a,h]

[a] *Division of Biostatistics, Washington University School of Medicine, St. Louis, MO 63110, USA*
[b] *Physical Activity Sciences Laboratory, Laval University, Québec, Canada*
[c] *Lipid Research Center, Laval University, Québec, Canada*
[d] *Diabetes Research Unit, Laval University, Québec, Canada*
[e] *School of Kinesiology and Leisure Studies, University of Minnesota, Minneapolis, MN, USA*
[f] *Department of Kinesiology, Indiana University, Bloomington, IN, USA*
[g] *Department of Health and Kinesiology, Texas A&M University, College Station, TX, USA*
[h] *Departments of Genetics and Psychiatry, Washington University School of Medicine, St. Louis, MO, USA*

Received 11 June 1998; received in revised form 16 November 1998; accepted 1 December 1998

Abstract

A major gene hypothesis for heparin releasable plasma lipoprotein lipase (PH-LPL) activity was assessed using segregation analyses of data on 495 members in 98 normolipidemic sedentary families of Caucasian descent who participated in the HERITAGE Family Study. Segregation analyses were performed on PH-LPL adjusted for age, and on PH-LPL activity adjusted for age and fasting insulin. Prior to adjustment for insulin, neither a major gene effect nor a multifactorial component could be rejected, and support for a major gene was equivocal i.e. neither the Mendelian transmission nor the no transmission (equal τs) models were rejected. However, after adjusting for the effects of insulin, a major gene effect on PH-LPL activity was unambiguous. The putative locus accounted for 60% of the total phenotypic variance, and the homozygous recessive form affected 10% (q^2)of the sample (i.e. gene frequency (q) = 0.31), and led to a low PH-LPL value. The lack of a significant multifactorial effect suggested that the familial etiology of PH-LPL activity adjusted for insulin was likely to be primarily a function of the major locus. In conclusion, the present study is the first to report segregation analyses on PH-LPL activity prior to and after adjusting for insulin, and suggests that there is an indication of a pleiotropic genetic effect on PH-LPL activity and insulin, in addition to a major gene effect on PH-LPL activity alone. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Segregation; Heritability; Sedentary; Insulin resistance

1. Introduction

Lipoprotein lipase (LPL), a 60-kDa glycoprotein, is the major enzyme responsible for the hydrolysis of triglyceride molecules in triglyceride-rich circulating lipoproteins [1,2]. In addition to its hydrolytic activity, LPL might function as a ligand for lipoprotein receptors thereby contributing to cellular uptake of lipoprotein particles [3]. LPL is abundantly present in the endothelium of adipose tissue and skeletal muscle capillaries, and has been thought to play a role in the development of obesity [4,5].

* Corresponding author. Tel.: + 1-314-362-3635; fax: + 1-314-362-2693.

E-mail address: yuling@wubios.wustl.edu (Y. Hong)

0021-9150/99 $ - see front matter © 1999 Elsevier Science Ireland Ltd. All rights reserved.
PII: S0021-9150(98)00324-4

Mutations in the structural LPL gene, and fasting insulin levels or insulin sensitivity [6–13], sex steroids [14,15], catecholamines [16], nutritional factors [17,18], exercise [19,20], and gene–environment interactions [21] have been reported to be involved in regulating plasma LPL activity levels. Mutations in the LPL gene in homozygous or compound heterozygous state lead to rare forms of dyslipidemia such as chylomicronemia [22]. In the heterozygous form both rare and common form mutations have been associated with high triglycerides, low HDL cholesterol, or both [23–28]. However, studies on the genetic contributions to LPL activity in normolipidemic populations are few. Our previous study indicated that after adjustment for age, up to 50% of the variance of plasma post-heparin LPL (PH-LPL) activity is accounted for by genetic factors in an adult normolipidemic population [29]. However, this genetic effect could be complex and potentially accounted for by a single major gene with a large effect, or by a few genes (oligogenic) with relatively smaller effects, or by polygenic factors, i.e. a number of genes each having a small effect on the phenotype, or by a combination of these genetic mechanisms.

Among non-genetic factors, insulin has emerged as one of the most important regulators of LPL. In adipose tissue, insulin infusion is associated with increased LPL activity levels [7,8,10,13]. In skeletal muscle, however, LPL activity is usually inhibited by insulin [8,11]. The ability of insulin to modulate LPL activity is defective in insulin resistance which results in a negative association between LPL activity and fasting blood insulin levels [9,11,12]. Transcriptional, translational or post-translational mechanisms are all apparently involved in the regulation of LPL activity levels by insulin [1,30]. Since the fasting insulin level, as an index of insulin resistance, is in part under genetic control [31,32], the genetic effects on insulin could also influence the LPL activity levels.

In order to assess the nature of the genetic influences on plasma PH-LPL activity levels, univariate segregation analysis was applied to 495 subjects from 98 normolipidemic sedentary White families who participated in the HERITAGE Family Study. Univariate segregation analyses after adjustment for the effects of a correlated variable (insulin in this case) can also be used to evaluate whether there is a pleiotropic effect, although indirectly. Here, the major gene effect on plasma PH-LPL activity levels was investigated both prior to and after removing the effects due to fasting plasma insulin levels. If there is a major gene for plasma insulin which also affects PH-LPL activity, then the segregation patterns for PH-LPL activity will differ depending on whether or not it is adjusted for insulin levels. A difference in pattern could suggest how these two traits interact with each other from a genetic perspective.

2. Methods and study subjects

2.1. Study subjects

The HERITAGE Family Study is a multicenter exercise study involving families. The main objective of the study is to assess the role of genetic factors in the cardiovascular, metabolic, and hormonal responses to aerobic exercise training in sedentary families. The HERITAGE sampling procedure and the inclusion and exclusion criteria have been described in detail elsewhere [33].

In brief, several criteria were used to screen subjects for participation. First, children were required to be between the ages of 17 and 40 years, and parents were required to be 65 years or less, in order to minimize the effects associated with subjects undergoing growth (low end) and aging (high end) complications. Second, families were required to be sedentary, defined at baseline as no regular physical activity over the previous 6 months. Individuals involved in any activity lasting 30 min or more and involving an energy expenditure of at least 7 METS (1 MET equals resting metabolic rate or about 3.5 ml O_2 uptake per kg body wt. per min) in individuals ≥ 50 years or 8 METS for younger individuals, and occurring more than once a week were excluded. Families with some nonsedentary members were included only after they had remained inactive for at least 6 months. Third, individuals with a body mass index (BMI) greater than 40 kg/m^2 were usually excluded unless they could exercise on a cycle ergometer without difficulties. Fourth, individuals with blood pressures greater than 159 mmHg systolic and/or 99 mmHg diastolic were also excluded. Finally, individuals with any life-threatening condition or disease or a condition that could be aggravated by cycle exercise were excluded (e.g. a malignancy, uncontrolled endocrine and metabolic disorders, including diabetes, definite or possible coronary heart disease, chronic or recurrent respiratory problems). Finally, individuals taking lipid-lowering or antihypertensive drugs were excluded.

In all, 98 nuclear families of Caucasian descent, each with both biological parents and at least two biological children completed the protocol. Families of African–American descent were also recruited but their results are not reported here.

2.2. Measures

All participants underwent a series of tests both prior to and after completing the 20-week standardized exercise-training program. Results from the baseline (pre-exercise training) tests are reported in the present study.

2.2.1. Plasma PH-LPL activity

Blood samples for PH-LPL activity measurement were taken from subjects after 12-h overnight fasting and 10 min after an intravenous injection of heparin (60 IU/kg body wt.). The post-heparin plasma lipase activities were measured using a modification of the method of Nilsson-Ehle and Ekman [34] as previously described [14]. The PH-LPL activities were expressed as nmol of oleic acid released per ml of plasma per min. Determinations of the PH-LPL activities were highly reproducible, with intraclass correlation coefficients for repeated assays of 0.95. Non-fasting subjects were not included in the present study.

2.2.2. Fasting plasma insulin

Plasma insulin levels were also measured in a fasting condition using the radioimmunoassay [35]. Insulin levels were treated as missing for three individuals with insulin antibodies interfering in the insulin assay, four individuals considered diabetic on the basis of a fasting glucose level ≥ 7.0 mmol/l and one individual with both conditions.

2.3. Data adjustment

The PH-LPL activity was analyzed under two different adjustment schemes. First, PH-LPL activity was adjusted for a polynomial in age using stepwise multiple regression (denoted LPL). Second, PH-LPL activity was adjusted for the linear effects of fasting insulin in addition to the polynomial effects of age (denoted LPL-ins). All the data adjustments were carried out separately in the four sex by generation groups (fathers, mothers, sons, and daughters) since significant group differences in the means were noted. The significance level for retaining the terms in the stepwise regression analysis was 5%.

2.4. Commingling analysis

Commingling analysis characterizes the distribution of a quantitative trait as a single distribution, or as a mixture of two or three normal components, and allows for residual skewness [36]. A description of the model parameters and methods is given in Appendix A.

2.5. Segregation analysis

An admixture of distributions is consistent with a major gene hypothesis. However, commingling can arise from other sources, including environmental, and can also be induced by pooling potentially heterogeneous groups. In order to protect against type I errors (i.e. false inference of a major gene), segregation analysis with tests on the transmission probabilities (Appendix B) is required, which involves testing the hypotheses of Mendelian transmission and equal transmission probabilities [37,38].

The basic idea behind segregation analysis is an evaluation of whether the type of familial aggregation seen in the sample supports the existence of a gene with a relatively large effect (major gene). A more detailed explanation of the segregation analysis method is given in Appendix B. The overall mean (μ) and variance (V), heritable multifactorial effects in offspring (H) and parents (HZ), and parameters of the major gene component (single biallelic locus) are estimated. The major component is represented by three parameters: q which determines the relative proportion (q^2) of the component distribution with the highest (homozygous recessive) mean; t which is the displacement between the two extreme (homozygote) component means; and d which is the relative position of the middle (heterozygote) component mean (i.e. $d = 0$ is recessive and $d = 1$ is dominant). Three transmission probabilities (τ_1, τ_2, and τ_3 are estimated in order to test whether the major effect is transmitted in families according to Mendelian expectations (Appendix B). The percentage of variance explained by the major gene is a derived parameter that is calculated in the POINTER program from the major gene parameter (d, t, q) and the overall mean and variance, using the standard equations.

3. Results

3.1. Descriptive results

Table 1 presents the means and S.D.s of age, the unadjusted levels of PH-LPL activity, and fasting insulin in the four sex by generation groups. Based on a comparison of standard errors, parents have higher

Table 1
Characteristics of plasma variables by sex and generation groups

Variable	Group	N	Mean	S.D.
Age (years)				
	Fathers	94	53.5	5.2
	Mothers	86	52.2	5.1
	Sons	153	25.2	5.9
	Daughters	162	25.1	6.0
PH-LPL[a] (nmol/ml per min)				
	Fathers	94	52.51	26.46
	Mothers	86	72.93	37.33
	Sons	153	47.80	26.54
	Daughters	162	57.84	27.29
Fasting insulin (pmol/l)				
	Fathers	89	78.11	59.35
	Mothers	84	62.25	30.02
	Sons	149	67.24	40.92
	Daughters	160	59.16	26.63

[a] PH-LPL indicates post-heparin lipoprotein lipase activity.

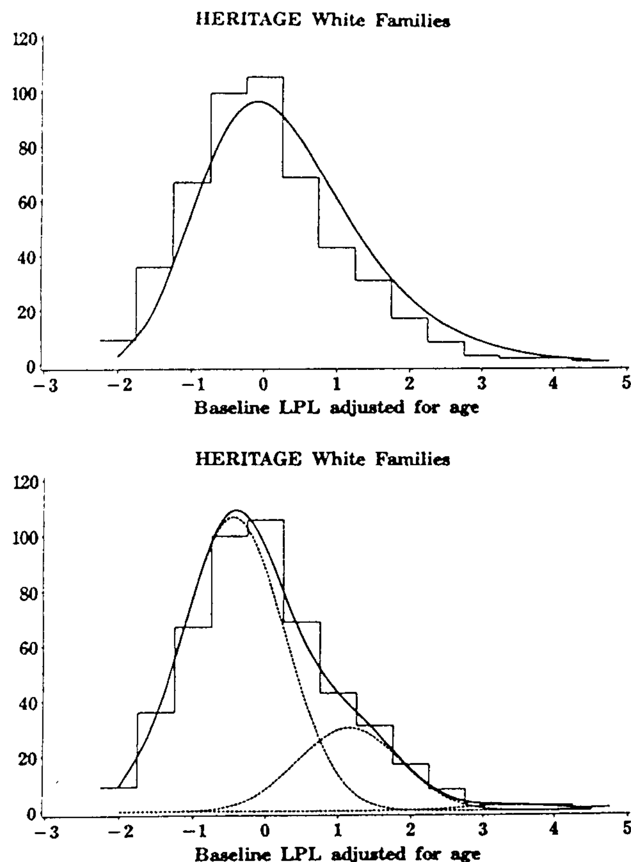

Fig. 1. (a) Frequency distribution of the PH-LPL activity adjusted for age, with one skewed distribution. (b) Frequency distribution of the PH-LPL activity adjusted for age, with three normal distributions. Upper dashed line, homozygous recessive distribution; middle dashed line, heterozygous distribution; lower dashed line, homozygous dominant distribution.

levels of PH-LPL activity and fasting insulin than offspring, and males have lower levels of PH-LPL activity than females in both generations, while males have higher levels of fasting insulin than females in both generations.

3.2. Commingling results

Commingling analysis was performed before segregation analysis. For age-adjusted PH-LPL activity, skewness was significant under one ($\chi^2 = 54.26$, $P < 0.01$) and two distributions ($\chi^2 = 17.48$, $P < 0.01$), but not significant in three distributions ($\chi^2 = 3.35$, $P = 0.07$). Two normal distributions fit better than one normal ($\chi^2 = 38.17$, $P < 0.01$), but two skewed distributions do not fit better than one skewed ($\chi^2 = 1.39$, $P = 0.50$). Finally, three normal distributions fit better than two normal distributions ($\chi^2 = 14.13$, $P < 0.01$), but three skewed distributions do not fit better than two skewed ($\chi^2 = 0$, $P = 1.00$). Thus, either one skewed or three normal distributions fit the data. Fig. 1 shows the frequency distribution, with one skewed (Fig. 1a) and three normal distributions (Fig. 1b) superimposed.

3.3. Segregation analysis

Segregation analysis results are given in Table 2 for LPL activity and in Table 3 for LPL-ins. For LPL, the test for a major effect only (model 2 vs. model 1) was rejected ($P < 0.01$), as was the test for a multifactorial effect only (model 3 vs. model 1; $P < 0.01$), thus suggesting that both of the components is necessary. No generation differences in the multifactorial component were found ($Z = 1$: model 5 vs. model 1; $P = 0.15$). In a model that included both components, the multifactorial component accounted for 47% of PH-LPL activity variance (model 5).

Under the general Mendelian model with no generation differences in the multifactorial component, a recessive mode of inheritance for the major effect could not be rejected ($d = 0$: model 6 vs. model 5; $P = 0.43$), while neither additive ($d = 0.5$: model 7 vs. model 5; $P < 0.01$) nor dominant modes ($d = 1$: model 8 vs. model 5; $P < 0.01$) fit the data. Finally, tests on the transmission probabilities suggest that neither Mendelian τs (model 9 vs. model 10; $P = 0.12$) nor equal τs (model 11 vs. model 10; $P = 0.74$) can be rejected. These results suggest that if there is a major gene for PH-LPL activity, its effect is obscured by other factors. Under the most parsimonious model, the major non-Mendelian effect (equal τs) accounts for 31% of the variance and the multifactorial component accounts for an additional 50% of the variance.

Results of segregation analyses for LPL-ins are given in Table 3. Both the test of no familial effects at all (model 4 vs. model 1; $P < 0.01$) and the test of no major effect (multifactorial effect only) (model 3 vs. model 1; $P < 0.01$) were rejected, while the test of no multifactorial effect (model 2 vs. model 1; $P = 0.85$) was not rejected, suggesting that there is only a major effect. Under the major effect only model, all of the recessive (model 5 vs. model 2; $P < 0.01$), additive (model 6 vs. model 2; $P < 0.01$), and dominant (model 7 vs. model 2; $P < 0.01$) modes of transmission are rejected. Tests of the transmission probabilities suggest that Mendelian τs cannot be rejected (model 8 vs. model 9; $P = 0.11$), while equal τs are rejected (model 10 vs. model 9; $P < 0.01$). Thus, for LPL-ins, all three conditions needed to infer a putative major locus are satisfied – rejection of the no major effect model, nonrejection of Mendelian transmission, and rejection of no transmission of the major effect (equal τs). The putative locus that leads to low PH-LPL activity accounts for 60% for the phenotypic variance, and the homozygous form affects 10% (q^2) of the sample (i.e. gene frequency ($q = 0.31$)). Since the multifactorial component is not significant, the familial basis of LPL-ins is presumed to be primarily a function of this putative major locus.

Table 2
Segregation analysis of PH-LPL[a] activity adjusted for age

Model	d	t	q	H	Z	$-2 \ln L+c$	Model comparison	χ^2	df	P-value
1. General model	0.00	2.65	0.39	0.36	1.24	3.14				
2. Major effect only	0.36	2.70	0.34	[0]	[0]	19.51	vs. model 1	16.37	2	<0.01
3. Multifactorial effect only	[0]	[0]	[0]	0.35	1.78	39.86	vs. model 1	36.72	3	<0.01
4. No familial effect	[0]	[0]	[0]	[0]	[0]	82.31	vs. model 1	79.17	5	<0.01
5. No generation difference in multifactorial effect	0.00	2.65	0.37	0.47	[1]	5.20	vs. model 1	2.06	1	0.15
6. Recessive	[0]	2.67	0.36	0.46	[1]	5.82	vs. model 5	0.62	1	0.43
7. Additive	[1/2]	0.002	0.26	0.45	[1]	40.79	vs. model 5	35.59	1	<0.01
8. Dominant	[1]	1.44	0.18	0.10	[1]	28.89	vs. model 5	23.69	1	<0.01
9. Parsimonious Mendelian*	[0]	2.67	0.36	0.46	[1]	5.82	vs. model 10	5.82	3	0.12
10. Free τs**	[0]	2.33	0.19	0.56	[1]	0.00				
11. Equal τs***	[0]	2.36	0.26	0.50	[1]	1.25	vs. model 10	1.25	3	0.74

[a] PH-LPL indicates post-heparin lipoprotein lipase activity.
* $V=1.73$, $\mu=0.22$, under parsimonious Mendelian model.
** $\tau_1=0.82$, $\tau_2=0.54$, $\tau_3=0.64$.
*** $V=1.11$, $\mu=0.04$, the percent of variance due to the major effect is 31% under the equal τs model; $\tau_1=\tau_2=\tau_3=0.74$.

4. Discussion

The present segregation analysis was performed to assess whether there is a major gene effect on PH-LPL activity and whether oligogenic-pleiotropic mechanisms are involved in the relationship between PH-LPL activity and insulin in an adult normolipidemic sedentary population by comparing the results for PH-LPL activity prior to and after adjustment for insulin. Prior to adjustment for insulin levels, the results for PH-LPL activity were inconclusive. Both a major effect and a multifactorial familial effect were individually significant. For the major effect, neither the Mendelian transmission model nor the equal τs model could be rejected, and the equal τs model fit the data better than the Mendelian model. It is, however, interesting to note that after adjusting for insulin, there is clear evidence of a putative major locus for the residual PH-LPL activity. This suggests that there is a major gene effect on PH-LPL activity alone. This also suggests that there likely is a pleiotropic effect on PH-LPL activity of an additional gene for insulin level.

To the best of our knowledge, the present investigation is the first segregation analysis of PH-LPL activity and the first to explore the hypothesis of a pleiotropic effect on PH-LPL activity and insulin level. In the past, segregation analyses have mainly focused on triglycerides, a substrate of LPL. A major gene effect for blood triglyceride levels has been suggested in some studies [23,39] but not in others [40]. The LPL gene located on the short arm of chromosome 8 (8p22) is a gene linked to the increased levels of triglycerides in familial hypertriglyceridemia [41]. Mutations in the LPL gene have been reported to be associated with increased levels of triglycerides, decreased levels of HDL cholesterol, or both [23–28]. There is only one study which has investigated the overall heritability for plasma PH-LPL activity levels in the same normolipidemic population [29], and no segregation analysis of plasma LPL activity levels has been reported to date. The results from the current investigation suggest that a gene for insulin may also affect blood PH-LPL activity levels in addition to the major gene for PH-LPL activity alone, although the evidence is not compelling. In fact, our results suggest that the evidence for the LPL activity locus is not clear unless the putative insulin locus is first taken into account.

The observation of hypertriglyceridemia in patients with diabetes and impaired glucose tolerance has suggested that insulin plays a role in the regulation of LPL activity levels. The association between exogenous insulin infusion and LPL activity has been examined both in vivo and in vitro. After insulin infusion, an increased LPL activity has been observed in adipose tissue [7,8,10,13] while a decreased LPL activity has been seen in skeletal muscle [8,11]. Although there is a positive association between insulin and increased levels of LPL activity in adipose tissue, plasma levels of insulin have been consistently reported to be associated with decreased levels of PH-LPL activity [6,9,11,12]. The reason could be that there is no association between adipose tissue LPL activity and plasma PH-LPL activity [42], and that adipose tissue LPL activity and mass contribute relatively little to the amount of LPL released into plasma in response to heparin [13]. It has been further proposed that it is the impaired ability of insulin to stimulate the transcription of the LPL gene in insulin resistant individuals which contributes to the decreased levels of plasma PH-LPL activity [13]. Indeed, PH-LPL activity is decreased in untreated dia-

Table 3
Segregation analysis of PH-LPL[a] activity adjusted for age and fasting insulin

Model	d	t	q	H	Z	$-2 \ln L+c$	Model comparison	χ^2	df	P-value
1. General Mendelian	0.34	2.82	0.32	0.04	0.001	5.65				
2. Major effect only	0.34	2.80	0.31	[0]	[0]	5.98	vs. model 1	0.33	2	0.85
3. Multifactorial effect only	[0]	[0]	[0]	0.34	1.67	30.93	vs. model 1	25.28	3	<0.01
4. No familial effect	[0]	[0]	[0]	[0]	[0]	66.55	vs. model 1	60.90	5	<0.01
5. Recessive	[0]	1.57	0.51	[0]	[0]	24.26	vs. model 2	18.28	1	<0.01
6. Additive	[1/2]	2.33	0.27	[0]	[0]	16.87	vs. model 2	10.89	1	<0.01
7. Dominant	[1]	1.42	0.18	[0]	[0]	21.32	vs. model 2	15.34	1	<0.01
8. Parsimonious Mendelian*	0.34	2.80	0.31	[0]	[0]	5.98	vs. model 9	5.98	3	0.11
9. Free τs**	0.38	2.84	0.44	[0]	[0]	0.00				
10. Equal τs***	0.00	2.09	0.35	[0]	[0]	38.63	vs. model 9	38.63	3	<0.01

[a] PH-LPL indicates post-heparin lipoprotein lipase activity.
* $V=1.15$, $\mu=0.07$, and 60% of variance due to the major locus under parsimonious Mendelian model.
** $\tau_1=1.00$, $\tau_2=0.59$, $\tau_3=0.30$.
*** $\tau_1=\tau_2=\tau_3=0.65$.

betic individuals, but it returns to normal after glycemic levels have been controlled [43]. As suggested, low levels of plasma PH-LPL activity as well as a low ratio of LPL to hepatic lipase should be considered among the features of the metabolic or insulin resistance syndrome [12].

Fig. 2 illustrates the paths accounting for the pleiotropic evidence found in the present segregation analysis studies. Two alternative mechanisms are illustrated. First, there may be a direct pleiotropic effect (i.e. G_p directly impacts on both insulin level and PH-LPL activity). This is noted as path ***a*** in Fig. 2. Second, there may be an indirect effect of the gene, one that directly influences plasma insulin levels, but with insulin levels then influencing PH-LPL activity. This is illustrated as path ***b*** in Fig. 2. Since a number of observational and experimental studies have indicated that insulin is involved in the regulation of PH-LPL activity [6–13], the evidence for an indirect effect of a gene (path *b*) for insulin on PH-LPL activity is likely supported. Whether there is an indirect plasma insulin effect on PH-LPL activity, or a direct pleiotropic effect,

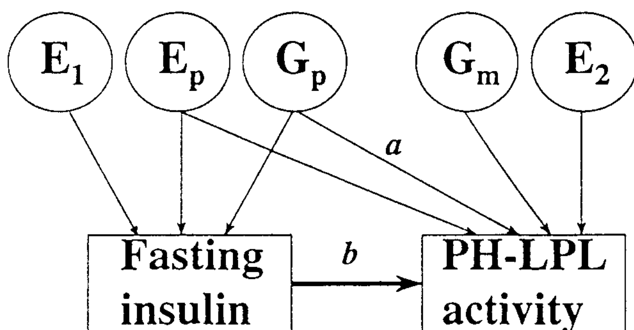

Fig. 2. Genetic and environmental influences on PH-LPL activity. G_p represents pleiotropic genes; G_m, a major gene for PH-LPL; E_p, pleiotropic environment; E_1, environmental factors for fasting insulin; E_2, environmental factors for PH-LPL only.

or both is still to be determined. Linkage analysis may be useful to clarify such associations when the genotypes become available. The structural LPL gene is the most likely candidate gene for the major gene effect on LPL activity alone (noted G_m in Fig. 2). However, we cannot rule out the possibility that other genes may also play important roles in regulating plasma heparin-releasable LPL activity.

Potential factors that might confound the present findings need to be addressed. Among these factors, BMI is a notable one since BMI is highly correlated with fasting insulin and PH-LPL as well. Although it is likely that BMI may have a role in the phenotypic association between PH-LPL and fasting insulin, it is unclear whether BMI can also influence the genetic association between PH-LPL and fasting insulin. Additional segregation analyses for PH-LPL after adjustment for BMI (results not presented) did not show a clear major gene effect on PH-LPL, suggesting that the influence of BMI on the genetic association between PH-LPL and fasting insulin found in the present investigation is minor, if there is any. Another possible confounding factor is hormone replacement therapy (HRT) since HRT can improve insulin sensitivity [44,45] and can also change the levels of serum PH-LPL and adipose LPL [46,47]. However, other studies did not find any changes of plasma PH-LPL levels after HRT [48]. In the present investigation, the levels of PH-LPL did not differ between postmenopausal women who used HRT and those who did not (data not shown). Thus, it is unlikely that HRT affected our present findings. An additional issue is the generalizability of the present findings. Since our study subjects were selected from an inactive Caucasian population who was otherwise relatively healthy, caution must be exercised when results are generalized to all populations. Moreover, among the present Caucasian subjects, approximately one-third are French Canadians. Cana-

dians of French descent with normal lipid levels have a higher frequency of LPL mutations than other Caucasians in North America [28,49,50] although the difference is not as striking as that in hypertriglyceridemic patients [51]. It is also important to note that the effects of most of these mutations on PH-LPL activities are modest and are modulated by the localization of the mutation and other associated factors such as body mass index, diabetes, and family history of hyperlipidemia. Based on above-mentioned information and because our HERITAGE study subjects are normal in lipid levels and generally in good health, the possible effects of these mutations in our study subjects are probably non-significant and did not affect our current findings.

In conclusion, there is evidence of a pleiotropic effect on PH-LPL activity of a gene for insulin in addition to a major gene for PH-LPL activity alone. The genes or the mutations responsible for these genetic effects remain to be identified.

Acknowledgements

The HERITAGE Family Study is supported by the NHLBI through the following grants: HL45670 (C. Bouchard, PI), HL47323 (A.S. Leon, PI), HL47317 (D.C. Rao, PI), HL47327 (J.S. Skinner, PI), and HL47321 (J.H. Wilmore, PI). A.S. Leon is also support by NIH-funded University of Minnesota's Clinical Research Center (M01 RR 00400) and partially by the Henry L. Taylor Professorship. C. Bouchard is partially funded by the MRC and Roche Canada Donald B. Brown Research Chair on Obesity. Thanks are expressed to all the co-principal investigators, investigators, co-investigators, local project coordinators, research assistants, laboratory technicians, and secretaries who are contributing to the study.

Appendix A. Commingling analysis

The maximum likelihood method described by MacLean et al. [36], and implemented in the computer program SKUMIX [36] was used. The parameters of the model include E, the common variance in each component; u, the overall mean; d, the relative position of the mean of the middle component; t, the displacement between the two extreme component means; q, which determines the relative proportion of the component distribution with the highest mean (q^2) under the assumption of Hardy–Weinberg proportions; and P, the power transformation parameter which eliminates skewness.

Hypotheses for nested models (e.g. comparing one vs. two normal distributions) are tested using the likelihood ratio test [the difference between (minus twice) the log-likelihoods ($-2 \ln L$) under two nested models], which is distributed asymptotically as a χ^2 with degrees of freedom equal to the difference in the number of parameters in the two models.

Appendix B. Segregation analysis

Segregation analysis was carried out using the unified mixed model [37] as implemented in the computer program POINTER [38]. The general model assumes that a phenotype is composed of the independent and additive contributions from a major gene effect, a heritable multifactorial background, and a unique environmental component (residual). The major effect is assumed to result from the segregation at a single locus having two alleles (i.e. A and a). The A allele is defined as decreasing the quantitative phenotypes. See text for a definition of the seven parameters in the model (V, μ, q, t, d, H, and Z).

The transmission pattern from parents to offspring can be tested in the unified mixed model to verify that the gene is segregating according to Mendelian expectations. The transmission pattern is characterized by three parameters: τ_1 is the probability that an AA individual transmits allele A to the offspring; τ_2 is the probability that Aa transmits A; and τ_3 is the probability that aa transmits A. Under Mendelian expectations, $\tau_1 = 1$, $\tau_2 = 0.5$, $\tau_3 = 0$, and no transmission of the major effect is obtained when the three τs are equal. To infer a major gene, three conditions are usually required: (i) rejection of the no major effect hypothesis ($d = t = q = 0$); (ii) failure to reject Mendelian transmission; and (iii) rejection of the no transmission model (equal τs model). Finally, maximum likelihood methods were used for fitting the models to data and likelihood ratio tests were used for evaluating hypotheses.

References

[1] Eckel RH. Lipoprotein lipase: A multifunctional enzyme relevant to common metabolic disease. New Engl J Med 1989;320:1060–8.

[2] Zechner R. The tissue-specific expression of lipoprotein lipase: implications for energy and lipoprotein metabolism. Curr Opin Lipidol 1997;8:77–88.

[3] Beisiegel U. New aspect on the role of plasma lipase in lipoprotein catabolism and atherosclerosis. Atherosclerosis 1996;124:1–8.

[4] Greenwood MRC. The relationship of enzyme activity to feeding behavior in rats: lipoprotein lipase as the metabolic gatekeeper. Int J Obes 1985;9(Suppl 1):67–70.

[5] Jemaa R, Tuzet S, Portos C, Betoulle D, Apfelbaum M, Fumeron F. Lipoprotein lipase gene polymorphisms: associations with hypertriglyceridemia and body mass index in obese people. Int J Obes 1995;19:270–4.

[6] Taylor KG, Galton DJ, Holdsworth G. Insulin independent diabetes: a defect in the activity of lipoprotein lipase in adipose tissue. Diabetologia 1979;6:313–7.

[7] Sadur CN, Eckel RH. Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique. J Clin Invest 1982;69:1119–25.

[8] Farese RV Jr, Yost TJ, Eckel RH. Tissue-specific regulation of lipoprotein lipase activity by insulin/glucose in normal-weight humans. Metabolism 1991;40:214–6.

[9] Pollare T, Vessby B, Lithell H. Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. Arterioscler Thromb 1991;11:1192–203.

[10] Fried SK, Russell CD, Grauso NL, Brolin RE. Lipoprotein lipase regulation by insulin and glucocorticoid in subcutaneous and omental adipose tissues of obese women and men. J Clin Invest 1993;92:2191–8.

[11] Eckel RH, Yost TJ, Jensen DR. Alternations in lipoprotein lipase in insulin resistance. Int J Obes 1995;19(Suppl 1):S16–21.

[12] Knudsen P, Eriksson J, Lahdenperä S, Kahri J, Groop L, Taskinen MR and the Botnia Study Group. Changes of lipolytic enzymes cluster with insulin resistance syndrome. Diabetologia 1995;38:344–350.

[13] Maheux P, Azhar S, Kern PA, Chen YDI, Reaven GM. Relationship between insulin-mediated glucose disposal and regulation of plasma and adipose tissue lipoprotein lipase. Diabetologia 1997;40:850–8.

[14] St-Amand J, Despres J-P, Lemieux S, Lamarche B, Moorjani S, Prud'homme D, Bouchard C, Lupien PJ. Does lipoprotein or hepatic lipase activity explain the protective lipoprotein profile of premenopausal women? Metabolism 1995;44:491–8.

[15] Ramirez ME, McMurry MP, Wiebke GA, Felten KJ, Ren K, Meikle W, Iverius P-H. Evidence for sex steroid inhibition of lipoprotein lipase in men: comparison of abdominal and femoral adipose tissue. Metabolism 1997;46:179–85.

[16] Eckel RH, Jensen DR, Schlaepfer IR, Yost TJ. Tissue-specific regulation of lipoprotein lipase by isoproterenol in normal-weight humans. Am J Physiol 1996;271:R1280–6.

[17] Enerback S, Gimble JM. Lipoprotein lipase gene expression: physiological regulators at the transcriptional and post-transcriptional level. Biochim Biophys Acta 1993;1169:107–25.

[18] Olivecrona T, Bergo M, Hultin M, Olivecrona G. Nutritional regulation of lipoprotein lipase. Can J Cardiol 1995;11:73–8.

[19] Savard R, Bouchard C. Genetic effects in the response of adipose tissue lipoprotein lipase activity to prolonged exercise: a twin study. Int J Obes 1990;14:771–7.

[20] Seip RL, Angelopoulous TJ, Semenkovich CF. Exercise induces human lipoprotein lipase gene expression in skeletal muscle but not adipose tissue. Am J Physiol 1995;268:E229–36.

[21] Ma Y, Liu MS, Ginzinger D, Frohlich J, Brunzell JD, Hayden MR. Gene-environment interaction in the conversion of a mild-to-severe phenotype in a patient homozygous for a ser172-to-cys mutation in the lipoprotein lipase gene. J Clin Invest 1993;91:1953–8.

[22] Benlian P, De Gennes JL, Foubert L, Zhang H, Gagne SE, Hayden M. Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. New Engl J Med 1996;335:848–54.

[23] Cullen P, Farren B, Scott J, Farrall M. Complex segregation analysis provides evidence for a major gene acting on blood triglyceride levels in 55 British families with familial combined hyperlipidemia. Arterioscler Thromb 1994;14:1233–49.

[24] Georges J-L, Regis-Bailly A, Salah D, Rakotovao R, Siest G, Visvikis S, Tiret L. Family study of lipoprotein lipase gene polymorphism and plasma triglyceride levels. Genet Epidemiol 1996;13:179–92.

[25] Heiba IM, DeMeester CA, Xia YR, Diep A, George VT, Amos CI, Srinivasan SR, Berenson GS, Elston RC, Lusis AJ. Genetic contributions to quantitative lipoprotein traits associated with coronary artery disease: analysis of a large pedigree from the Bogalusa Heart Study. Am J Med Genet 1993;47:875–83.

[26] Gerdes C, Fisher RM, Nicaud V, Boer J, Humphries SE, Talmud PJ, Faergeman O. Lipoprotein lipase variants D9N and N291S are associated with increased plasma triglyceride and lower high-density lipoprotein cholesterol concentrations—Studies in the fasting and postprandial states: The European Atherosclerosis Research Studies. Circulation 1997;96:733–40.

[27] Hokanson JE. Lipoprotein lipase gene variants and risk of coronary disease—a quantitative analysis of population-based studies. Int J Clin Lab Res 1997;27:24–34.

[28] Nordestgaard BG, Abildgaard S, Wittrup HH, Steffensen R, Jensen G, Tybjærg-Hansen A. Heterozygous lipoprotein lipase deficiency—Frequency in the general population, effect on plasma lipid levels, and risk of ischemic heart disease. Circulation 1997;96:1737–44.

[29] Perusse L, Rice T, Despres JP, Bergeron J, Province MA, Gagnon J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C. Familial resemblance of plasma lipids, lipoproteins and post-heparin lipoprotein and hepatic lipases in the HERITAGE Family Study. Arterioscler Thromb Vasc Biol 1997;17:3263–9.

[30] Semenkovich CF, Wims M, Noe L, Etienne J, Chan L. Insulin regulation of lipoprotein lipase activity in 3T2-L1 adipocytes is mediated at posttranscriptional and posttranslational levels. J Biol Chem 1989;264:9030–8.

[31] Hong Y, Pedersen NL, Brismar K, Hall K, de Faire U. Quantitative genetic analyses of insulin-like growth factor I (IGF-I), IGF-binding protein-1, and insulin levels in middle-aged and elderly twins. J Clin Endocrinol Metab 1996;81:1791–7.

[32] Mayer EJ, Newman B, Austin MA, Zhang D, Quesenberry CP Jr, Edwards K, Selby JV. Genetic and environmental influences on insulin levels and the insulin resistance syndrome: an analysis of woman twins. Am J Epidemiol 1996;143:323–32.

[33] Bouchard C, Leon AS, Rao DC, Skinner JS, Wilmore JH, Gagnon J. The HERITAGE family study: aims, design, and measurement protocol. Med Sci Sports Exerc 1995;27:721–9.

[34] Nilsson-Ehle P, Ekman R. Specific assays for lipoprotein lipase activities of post-heparin plasma. In: Peters H, editor. Protides of Biological Fluids. Oxford: Pergamon, 1978:243–6.

[35] Desbuquois B, Aurbach GD. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. J Clin Endocrinol Metab 1971;33:732–8.

[36] Maclean CJ, Morton NE, Elston RC, Yee S. Skewness in commingled distributions. Biometrics 1976;32:695–9.

[37] Lalouel JM, Morton NE. Complex segregation analysis with pointers. Hum Hered 1981;31:312–21.

[38] Lalouel JM, Rao DC, Morton NE, Elston RC. A unified model for complex segregation analysis. Am J Hum Genet 1983;35:816–26.

[39] Friedlander Y, Kark JD. Complex segregation analysis of plasma lipid and lipoprotein variables in a Jerusalem sample of nuclear families. Hum Hered 1987;37:7–19.

[40] Iselius L, Carlson LA, Morton NE, Efendic S, Lindsten J, Luft R. Genetic and environmental determinants for lipoprotein concentrations in blood. Acta Med Scand 1985;217:161–70.

[41] Sparkes RS, Zollman S, Klisak I, Kirchgessner TG, Komaromy MC, Mohandas T, Schotz MC, Lusis AJ. Human genes involved in lipolysis of plasma lipoproteins: mapping of loci for lipoprotein lipase to 8p22 and hepatic lipase to 15q21. Genomics 1987;1:138–44.

[42] Coppack SW, Yost TJ, Fisher RM, Eckel RH, Miles JM. Periprandial systemic and regional lipase activity in normal humans. Am J Physiol 1996;270:E718–22.

[43] Simsolo RB, Ong JM, Saffari B, Kern PA. Effect of improved diabetes control on the expression of lipoprotein lipase in human adipose tissue. J Lipid Res 1992;33:89–95.

[44] Lindheim SR, Buchanan TA, Duffy DM, Vijod MA, Kojima T, Stanczyk FZ, Lobo. Comparison of estimates of insulin sensitivity in pre- and post-menopausal women using the insulin tolerance test and the frequently sampled intravenous glucose tolerance test. J Soc Gynecol Invest 1994;1:150–4.

[45] Foster RH, Balfour JA. Estradiol and dydrogesterone—a review of their combined use as hormone replacement therapy in post-menopausal women. Drugs Aging 1997;11:309–32.

[46] Jaccoby S, Pinchasov Y, Snapir N, Robinzon B. Hypothalamic obese, functionally castrated hens are hypersensitive to estrogenic modulation of lipid metabolism. Physiol Behav 1996;60:913–8.

[47] Price TM, O'Brien SN, Welter BH, George R, Anandjiwala J, Kilgore M. Estrogen regulation of adipose tissue lipoprotein lipase—possible mechanism of body fat distribution. Am J Obstet Gynecol 1998;178:101–7.

[48] Urabe M, Yamamoto T, Kashiwagi T, Okubo T, Tsuchiya H, Iwasa K, Kikuchi N, Yokoto K, Hosokawa K, Honjo H. Effects of estrogen replacement therapy on hepatic triglyceride lipase, lipoprotein lipase and lipids including apolipoprotein E in climacteric and elderly women. Endocr J 1996;43:737–42.

[49] Murthy V, Julien P, Gagné C. Molecular pathobiology of the human lipoprotein lipase gene. Pharmacol Ther 1996;70:101–35.

[50] Minnich A, Baloukas J, Roederer G, Lussier-Cacan S, Davignon J, Genest J Jr. Lipoprotein lipase gene mutations in coronary artery disease. Can J Cardiol 1998;14:711–6.

[51] Julien P, Gagné C, Murthy MRV, Cantin B, Cadelis F, Moorjani S, Lupien PJ. Mutations of the lipoprotein lipase gene as a cause of dyslipidemia in the Quebec population. Can J Cardiol 1994;10:54–60.