Ann. Hum. Genet., Lond. (1974), **38**, 47
Printed in Great Britain

The genetics of the Lp Antigen

II. A family study and proposed models of genetic control

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INTRODUCTION

In 1963 Berg first described the Lp polymorphism of human β lipoproteins distinguished by heteroimmune antibody in Ouchterlony immunodiffusion tests (Berg, 1973). Although a number of surveys of Lp(a) frequencies in various populations, as well as several family studies, have already been published, a genetic study using both family and population data was initiated for several reasons. Evidence has been accumulating that variation of the Lp trait is quantitative rather than qualitative (Harvie & Schultz, 1970; Utermann & Wiegandt, 1970; Rittner & Wichmann, 1967; Ehnholm *et al.* 1972, 1971). Furthermore, the preceding paper (Schultz *et al.* 1974) shows that the quantitative variation of serum Lp level in a Caucasian population is bimodal with division between positive and negative sera near the trough of the distribution. This quantitative variation combined with variability in antiserum titre, may provide a basis for discrepancies in Lp(a) frequencies found by different investigators for similar populations (see Table 1) and variation in strength of reaction found with different reagents.

Any simple model of autosomal control of the antigen to explain the bimodality must be consistent with the quantitative variation in antigen levels and the observed overlap between modes. Therefore, further studies of the genetics of this antigen were indicated to establish what proportion of the quantitative variability is due to a major gene and what proportion is due to polygenic modification, environmental variability, and measurement error. An interesting and useful Caucasian sample became available from the population under surveillance by the Tecumseh Community Health Study (described by Napier, 1962; Epstein *et al.* 1965; Napier, Johnson & Epstein, 1970). Data on family structure, incidences of diseases, and frequencies of known blood group antigens were available for this population which had been previously untested for Lp(a) frequency. Several possible models for genetic control of this trait are considered utilizing these family data and the quantitative distribution obtained from the data in publication 'I' of this series.

MATERIALS AND METHODS

Lp Classification

The Lp classification method by Ouchterlony immunodiffusion employed in the population survey was identical to the method described elsewhere. All reactions were scored as positive, weak positive, or negative by the criteria already described (Schultz, Shreffler & Harvie, 1968; Schultz & Shreffler, 1973).

Antisera

The immunization schedule and absorption protocols employed in antiserum preparation have been described elsewhere (Schultz *et al.* 1968, 1974).

Table 1. *Lp(a) phenotype frequencies for a number of populations*

Population	No. tested	Phenotype Frequency [% Lp(a+)]	References
Norwegian	1109	35·2	Berg (1966*a*)
Frankfort/Main	321	51·4 (20 % weak)	Seidl *et al.* (1966)
Greece	218	26·7 (9·7 % weak)	Walter & Yannissis (1967)
Peulh (Africa)	78	51·2 (25·6 % weak)	Wendt *et al.* (1967)
Marka (Upper Volta)	112	81·2 (49·1 % weak)	Wendt *et al.* (1967)
Dresden	679	38·7 (9·42 % weak)	Thomas & Büttger (1966)
Berlin	377	41·4 (9·8 % weak)	Speiser & Pausch (1965)
U.S. Whites	126	38·9	Berg (1966*b*)
U.S. Negroes (Washington, D.C.)	242	34·3	Berg (1966*b*)
Marburg	301	32·6	Berg (1966*b*)
Vienna	310	31·3	Berg (1966*b*)
Brazilians	104	30·8	Berg (1966*b*)
Easter Islanders	106	8·5	Berg (1966*b*)
Labrador Indians	234	1·7	Berg (1966*b*)
Hungarians (Bodrokoz)	533	24·6	Walter & Nemeskeri (1967)
Göttingen	1030	29·8	Jögensen (1966)
Berlin	135	34·8	Jögensen (1966)
Venezuela			
Mestizos–Caracas	904	43·1 (Lp(x)?)	Gallango & Arends (1966)
Negroes–Tapipa	71	74·6	Gallango & Arends (1966)

Specimens

Blood samples were obtained from a portion of the population sampled as part of the Tecumseh Community Health Study. Donors were almost entirely Caucasian of West European ancestry. Fresh sera were obtained by vena puncture. The sera were stored at 4° C. and tested within 72 hr. of bleeding. They were never frozen. Sera from 2418 donors were tested.

RESULTS

I. *Analysis of genetic ratios in the Tecumseh Population*

The Tecumseh population studied in this report includes a large body of family data and offers an opportunity for documentation or refutation of the autosomal dominant mode of inheritance of the Lp trait reported previously (Berg, 1963; Berg, 1973; Rittner, 1970). The available family data are summarized in Table 2. A strong familial effect can be noted; the percentage negative offspring increases from 31 % in Lp(a+) strong × Lp(a+) strong matings to 90 % in Lp(a−) × Lp(a−) matings. However, the occurrence of Lp(a+) offspring from Lp(a−) × Lp(a−) matings, as well as the high frequency of negative offspring from Lp(a−) × Lp(a−) (31 % when no simple genetic hypothesis allows for more than 25 % negatives from a positive by positive mating), is inconsistent with the simple dominant mode of inheritance previously proposed. Direct tests of different genetic hypotheses (e.g. multiple allele at one locus *v.* the effects of 2, 3 or more loci) which might explain the results are precluded, however, by two confounding effects: quantitative variation and an influence of age on Lp levels.

First, the serum level of the Lp antigen in Caucasians appears to follow a continuous frequency distribution rather than a discrete frequency distribution (Schultz *et al.* 1974). The qualitative precipitation test employed in the family studies detects a threshold level of antigen and therefore the strength of antiserum used for analysis would have a strong effect on the resulting

Table 2. *Lp(a) mating types and phenotypes of offspring for a representative set of families from the Tecumseh population*

Mating types	No. of families	Progeny† (a+)s	(a+)w	Total (a+)	(a−)	Total	Negatives (%)
(a+)s × (a+)s	45	53	11	64	29	93	31·2
(a+)s × (a+)w	20	19	5	24	13	37	35·1
(a+)s × (a−)	67	60	21	81	69	150	46·0
(a+)w × (a+)w	8	5	4	9	9	18	50·0
(a+)w × (a−)	23	9	6	15	25	40	62·5
(a−) × (a−)	41	3	5	8	74	82	90·2
Totals	204	149	52	201	219	420	

† (a+)s = Lp(a+) strong, (a+)w = Lp(a+) weak, (a−) = Lp(a−).

Table 3. *Lp(a) frequencies in a representative sample from the Tecumseh population*

	Number	Frequency
Lp(a+) strong	989	0·41
Lp(a+) weak	363	0·15
Total Lp(a+)	1352	0·56
Lp(a−)	1066	0·44
Total Sample	2418	

segregation of Lp types within families. In Table 3 are summarized the Lp(a) frequency results for a representative sample of 2418 individuals from the Tecumseh population.

Second, an inspection of the family data in Table 2 showed an Lp(a+) phenotype frequency of 0·58 for parents and 0·48 for children. Although previous reports (Berg, 1966*a*, *b*; Mohr, 1966; Berg, 1973) have stated that there is no age effect on Lp(a+) frequency, other lipoprotein species have been shown to be quantitatively influenced by age (Nichols, 1969; Frederickson, Levy & Rees, 1967). The present data indicate that a strong age effect on Lp(a+) frequency is operating in this population. An age by decade analysis of Lp(a) frequencies in the Tecumseh population is shown in Table 4. With the exception of the 60–69 age-group, a consistently increasing trend in Lp(a+) frequencies with age is noted. A contingency χ^2 for homogeneity of the Lp distribution by age was 31·4 with 14 degrees of freedom. The significance for the hypothesis that the frequencies of the three classes are homogeneous across age groups is less than 0·01. The linear regression of Lp(a+) on age ($+0{\cdot}0276 \pm 0{\cdot}0048$) was calculated by least squares, weighting proportions by sample size according to the formula presented by Snedecor & Cochran (1967). The significance level for the null hypothesis that this regression is zero is less than 0·001. The lower Lp(a+) phenotype frequency found by other investigators for Caucasian samples (see Table 1) may be partially attributable to samples from younger age-groups. The average blood bank donor ranges in age from 20 to 40, spanning two decades of moderately low Lp(a+) frequency (see Table 4). Many of the population samples reported consisted largely of blood bank donors. Individuals in the same age range (20–40) dominate the sample used for study of the quantitative Lp frequency distribution (Schultz *et al.* 1974).

The confounding factors of age and quantitative variation raise the question of the validity

Table 4. *Distribution of Lp(a) by age for a representative sample of 2418 individuals of the Tecumseh population*

Lp Type	Age by decade							
	0–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79
Lp(a+) strong	35 (0·30)	165 (0·40)	90 (0·33)	155 (0·41)	244 (0·43)	162 (0·46)	83 (0·42)	55 (0·50)
Lp(a+) weak	16 (0·14)	44 (0·10)	61 (0·23)	51 (0·13)	89 (0·15)	61 (0·17)	28 (0·14)	13 (0·12)
Total Lp(a+)	51 (0·44)	209 (0·50)	151 (0·56)	206 (0·54)	333 (0·58)	223 (0·63)	111 (0·56)	68 (0·62)
Lp(a−)	64 (0·56)	208 (0·50)	120 (0·44)	177 (0·46)	239 (0·42)	130 (0·37)	86 (0·44)	42 (0·38)
Total Tested	115	417	271	383	572	353	197	110

Table 5. *Family data on Lp(a) types in the Tecumseh population assuming incomplete dominant inheritance*

Mating type†	No. of families	No. of progeny				Total
		(a+)s†	(a+)w†	Total (a+)	(a−)†	
(a+)s × (a+)s	45	53 (93)‡	11 (0)	64 (93)	29 (0)	93
(a+)s × (a+)w	20	19 (18·5)	5 (18·5)	24 (37)	13 (0)	37
(a+)s × (a−)	67	60 (0)	21 (150)	81 (150)	69 (0)	150
(a+)w × (a+)w	8	5 (4·5)	4 (9·0)	9 (13·5)	9 (4·5)	18
(a+)w × (a−)	23	9 (0)	6 (20)	15 (20)	25 (20)	40
(a−) × (a−)	41	3 (0)	5 (0)	8 (0)	74 (82)	82
Totals	204	149	52	201	219	420

† (a+)s = Lp(a+) strong, (a+)w = Lp(a+) weak, (a−) = Lp(a−).
‡ Figures in parentheses are expected values assuming autosomal co-dominant inheritance (see text).

Table 6. *Family data for Tecumseh population (weak positives included in negative group)*

Mating type	No. of families	No. of progeny: (a+)	(a−)	Total
(a+) × (a+)	45 (87·31)†	53 (80·259)‡	40 (12·741)	93
(a+) × (a−)	87 (92·208)	79 (117·997)	108 (69·003)	187
(a−) × (a−)	72 (24·422)	17 (0)	123 (140)	140
Total	204	149	271	420

† Figures in parentheses are expected values.
‡ Expected values based on gene frequencies Lp = 0·7524 and Lp^a = 0·2475 calculated from the population.

Table 7. *Family data for the Tecumseh population (weak positives included in positive group)*

Mating type	No. of families	No. of progeny (a+)	(a−)	Total
(a+) × (a+)	73 (131·815)†	97 (133·94)‡	51 (14·06)	148
(a+) × (a−)	90 (15·190)	96 (130·53)	94 (59·47)	190
(a−) × (a−)	41 (8·037)	8 (0)	74 (82)	82
Total	204	201	219	420

† Figures in parentheses are expected values.
‡ Expected values are based on gene frequencies Lp = 0·6477 and Lp^a = 0·3522 calculated from the entire population.

of a more detailed analysis of the data based only on qualitative results. However, because of the excellent fit of the data collected by previous investigators to an autosomal dominant hypothesis, the present qualitative data on families were subjected to further analysis to test this hypothesis. A sample totalling 204 families with 420 offspring (Table 2) was available for such an analysis. An added variable in this study was the classification of the weak positive category. This was dealt with in the analysis in three different ways: (1) as the heterozygous class under the hypothesis of incomplete dominance, (2) as belonging to the negative class, and (3) as belonging to the positive class. Analyses of these alternatives which reflect the possible roles of the weak positive class are summarized in Tables 5–7. Inspection of these tables shows that there are numerous cells in which a number of progeny are found, even though none are expected. In other cells the observed number of progeny is as much as four times the expected number, as for example in the case of negative offspring from strong positive by strong positive matings. Thus, for these data at least, we reject the hypothesis of simple autosomal inheritance.

II. *Genetic analysis of Lp family data as a continuously distributed trait*

The analysis of genetic ratios was not consistent with any simple autosomal mode of inheritance. Each possibility was rejected because of an excessively large number of offspring of one particular phenotype in one or more mating types. We therefore continued our analysis with an attempt to fit a model for genetic control of the Lp trait which is consistent with both the

Table 8. *Summary statistics for the distribution of $\log_e$ quantitative Lp values*

	Pooled data	Qualitatively† negative class	Qualitatively positive class‡
Mean	−1·62	−2·56	−0·89
Standard deviation	0·99	0·44	0·62
χ^2 (D.F.)§	60·18 (18)*	18·06 (11)	15·99 (9)
N	146	64	82

* Significant at the 0·01 level of probability.

† Quantitative values associated with individuals identified as negative on the qualitative test.

‡ Quantitative values associated with individuals identified as positive on the qualitative test.

§ Goodness-of-fit to a normal probability density function with the estimated means and standard deviations as indicated. Degrees of freedom taken to be the number of equally qualitative classes minus 3. See Kendall & Stuart (1969) for a discussion of degrees of freedom for this application of χ^2.

bi-modal frequency distribution of quantitative levels of the Lp antigen and the available qualitative family data.

A summary of the quantitative Lp measurements reported in the preceding paper of this series (Schultz *et al.* 1974) is given in Table 8. These data were used to establish the nature of the statistical frequency distribution of the quantitative Lp levels among the parents and among the offspring of the family sample. These quantitative data strongly indicate a symmetrical dispersion about each of two modes. The goodness of fit of these quantitative data to two distributions, one identified by the positive and one by the negative qualitative designation is measured by the χ^2 in Table 8. The improvement of fit of these data to two normal distributions over the fit to one normal distribution is significant at the 0·001 level of probability ($\chi^2 = 26{\cdot}13$). The means for these two overlapping frequency distributions are given in Table 8. An analysis of variance of the quantitative data estimates that 87 % of the variation in the quantitative level of Lp antigen is attributable to the difference between the mean values of individuals with the positive and negative qualitative phenotypes (Table 9). This difference is highly significant ($F = 347{\cdot}55$). The residual variability (13 %) among individuals within classes may be the consequence of the modifying effects of environmental variations and/or polygenes, each with a small effect on the level of Lp antigen in the serum.

In order to apply the information from these quantitative data to the analysis of the qualitative family data, it is necessary (1) to establish for the family data the degree of overlap of the quantitative phenotypic distributions of the two classes of genotypes under a hypothesis of a single Lp locus with a major effect and (2) to establish the truncation point on the quantitative scale between the positive and negative qualitative phenotypes in the family data. We assume that the qualitatively negative class of phenotypes is determined by a recessive homozygous genotype and that the qualitatively positive phenotype class consists of individuals carrying at least one allele determining the positive response. The overlap of the two quantitative distributions established above can provide a basis for predicting in the families how many recessive homozygous genotypes we expect to test positively and how many genotypes which carry a positive allele we expect to test negatively on the qualitative test. We know that the truncation point on the quantitative scale between the positive and negative qualitative phenotypes may vary among laboratories or antisera. However, regardless of the position of the

Table 9. *Analysis of variability among and within the positive and negative classes*

Source	D.F.	SS	MS	F ratio
Between classes	1	100·79	100·79	347·55
Within classes	144	41·32	0·29	

$\hat{\sigma}^2_{\text{total}} = 1{\cdot}69$, 100%. $\hat{\sigma}^2_{\text{between}} = 1{\cdot}40$, 87%. $\hat{\sigma}^2_{\text{within}} = 0{\cdot}29$, 13%.

Table 10. *Fit of two Gaussian distributions to the quantitative data*

Estimate	Distribution in negative genotypes	Distribution in positive genotypes
Mean	−2·54	−0·79
Standard deviation	0·47	0·47
Proportion	0·47	0·52

Table 11. *Fit of family data to the model*†

Parents					Positive children		
Mating phenotype	No.	Obs. freq.	Exp. freq.	No. children	Observed no.	Exp. no.	Exp. no.‡ age adjusted
+ × +	73	0·36	0·33	148	97	116·6	96·5
+ × −	90	0·44	0·49	190	96	111·4	92·2
− × −	41	0·20	0·18	82	8	8·5	7·0
Total	204			420	201	236·5	195·7
					χ^2_3 D.F.	20·71*	1·46

* Significant at the 0·01 level of probability.
† With f(Lp^a) = 0·6481, f(Lp^a/Lp^a) = 0·42, and the truncation on the quantitative scale to be −1·50.
‡ Expected positive children = 0·83 × expected positive when they reach adulthood.

truncation point, we assume that the means of the two quantitative distributions are the same in the sample of qualitatively evaluated family data as in the sample of quantitative measurements reported by Schultz *et al.* (1974).

To obtain estimates of the means and a common standard deviation of the two phenotype distributions independently of the qualitative assay, we simultaneously fit two Gaussian distributions to the quantitative data. The results of the method-of-moments estimation procedure (Rao, 1952) are given in Table 10. The estimate of the common standard deviation of 0·47 may be compared with the values of 0·62 for qualitatively positives and 0·44 for qualitatively negatives computed directly from the data. The estimate of the proportion of qualitatively negative genotypes at the major locus was 0·48. The estimated mean of the negative class of genotypes is −2·54 compared to −2·56 computed directly from the qualitatively negative phenotypes. The estimate of the mean of the positive genotypes is larger (−0·79) than the mean for the qualitatively positive phenotypes (−0·89). Taking the arbitrary value of −1·89 to be the truncation point (Schultz *et al.* 1974), and considering the overlap of the two estimated Gaussian curves, we would expect 8·0% of individuals of the homozygous negative genotype to fall above the truncation point (react qualitatively as positives). Conversely 1·0% of individuals of the positive genotype are expected to react negatively in the qualitative test.

Because we do not know the true truncation point, we must obtain an independent estimate

for this parameter. We will assume that the mean quantitative values and the standard deviation of each genotype class are the same in the family data as those estimated from the quantitative data (Table 10). By varying the frequency of the negative class of genotypes and the truncation point, variable degrees of overlap between the two genotype classes were computed using the normal Z variable tabled in Fisher & Yates (1938). For each combination of genotype frequency and truncation point the probability of a positive child from the jth mating (j = 1, 2, 3) is taken to be

$$\sum_{i=1}^{9} [\text{Pr (mating '}i\text{' genotypes/}j\text{th mating phenotype)} \times \text{Pr (positive child/}i\text{th mating genotypes)}].$$

A frequency of negative genotypes in the parents of 0·42 (frequency of negative allele = 0·6481) and truncation point of $-1·50$ gave the minimum χ^2 goodness of fit of the data to expected computed values (Table 11). The minimum χ^2 was statistically significant when the age difference discussed above is not considered. However, the fit to age adjusted expectations is excellent ($\chi^2_{3\,\text{D.F.}} = 1·46$).

The difference in the estimate of the frequency of a negative genotype class between the quantitative data and the parents of the family data is not statistically significant at the 0·05 level of probability. Using the truncation point of $-1·50$ results in an estimate of 1·25 % of individuals who have the negative genotype at the major locus to react qualitatively as positives and 6·55 % of individuals of the positive genotype class to be negative in the qualitative test.

DISCUSSION

According to the analysis presented above, the simple autosomal dominant control of the Lp antigen which has been previously postulated (Berg, 1963; Rittner, 1970) has been modified to take into account several confounding factors which have become evident during family and quantitative studies of the trait. The influence of age, the variable amount of antigen in both positive qualitative and negative qualitative reactors, and the actual presence of antigen in all negative individuals doubtless have all contributed to the vastly different phenotype frequencies of Lp(a+) individuals in samples from Caucasian populations reported from different centres (see Table 1).

It has, however, been possible to fit a *reasonable* model genetic system to the relatively large sample of data available in this study. The fit of the family data to a single locus model is appropriate only when one allows for a relative contribution of 13 % of the quantitative variability to be due to age, environmental and polygenic factors. The overlap of the quantitative frequency distributions of Lp antigen level for the two genotype classes confounds any simple interpretation of a qualitative assay. However, the most likely mode of genetic control would appear to be a single autosomal locus with a major effect. At least two alleles of this gene are indicated and several (or many) modifying factors affect the expression of these alleles. Such an interpretation is consistent with that originally given to the genetics of the Lp system by Dr K. Berg and several other investigators.

One may question whether such an analysis of genetic data might not show that other human serum proteins exhibit similar quantitative patterns and are likewise influenced by one or more factors such as age or environment. This type of interpretation may serve to delineate the reasons

for deviations from simple genetic control which may have been observed from time to time. Such a model may account for the complexity of the analysis of the genetics of disease syndromes, such as diabetes, which have appeared to follow simple autosomal inheritance, but which frequently have become increasingly confounded by 'exceptional cases' as more is known of the disease. Variable expression of a disease may follow a pattern similar to the variable qualitative expression of the presence of Lp antigen.

It may become evident that analyses such as the one above more closely approach the real world of quantitative biochemical genetics than the more simplified interpretations which have been largely applied up to the present time.

SUMMARY

Several genetic models were considered to explain the distribution of qualitatively positive and negative children in 204 Caucasian families. A model which best describes the inheritance of the Lp antigenic expression involves a major genetic locus which distinguishes two overlapping continuously distributed modes of quantitative activity.

We are indebted to Barbara Eggleston for excellent technical assistance and to Dr J. V. Neel and Dr N. R. Harvie for helpful suggestions.

This study was supported by U.S. Atomic Energy Commission Contract AT(11-1)-1552 and U.S.P.H.S. Research Career Development Award K3-HE-24980 to D. C. Shreffler and National Institute of Health Predoctoral Traineeship 2T01-GM-00071-10 to J. S. Schultz.

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