Am. J. Hum. Genet. 40:267–277, 1987

Does the PI Polymorphism Alone Control Alpha-1-Antitrypsin Expression?

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SUMMARY

Whether genetic factors other than the protease-inhibitor (PI) polymorphism itself contribute to variation in alpha-1-antitrypsin is of both theoretical and practical interest. We have measured the quantity of alpha-1-antitrypsin (by an immunoturbidometric assay) and its activity (by assaying elastase inhibitory capacity [EIC]) in 583 individuals from 114 twin kinships who were also typed for PI by isoelectric focusing. Models of variation were fitted directly to the raw observations by a maximum-likelihood method. Specification of phenotypic means led to highly significant improvements in fit over models including only individual environment variance and additive genetic variance. The 29 phenotype means could also be described as the appropriate additive combinations of the 12 allelic effects. Only small improvements in fit could then be obtained by addition of polygenic components of variance. We conclude that nearly all genetic variation in alpha-1-antitrypsin quantity and activity can be explained by detectable variation at the PI locus and that this variance is largely additive. Bivariate analysis of alpha-1-antitrypsin and EIC revealed marginal evidence for differences in specific activities of molecules coded by different PI alleles. The correlation between environmental deviations for the two measures was only .63, which may reflect, in part, the rather low reliability of the assays and account for the modest heritabilities (<.5) of the two measures. An intriguing finding was the presence of significant differences in E_1 variance for different PI types, suggesting that different phenotypes have differing capacities to react to environmental challenges.

Received September 2, 1986; revision received December 5, 1986.

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INTRODUCTION

The monomeric protein alpha-1-antitrypsin is coded for by the protease-inhibitor (PI) locus on chromosome 14. The relatively common Z (1%) and S (4%) alleles (and several others) are associated with deficiency of this antiprotease, which, in turn, predisposes to a number of diseases, particularly respiratory ones (Fagerhol and Cox 1981; Gibson et al. 1983; Martin et al. 1983). Alpha-1-antitrypsin deficiency has also been associated with increased fertility, particularly as manifested in dizygotic (DZ) twinning (Clark and Martin 1982), and it has been postulated that the PI polymorphism is maintained by the counterbalancing selective forces of increased morbidity and greater fertility associated with low alpha-1-antitrypsin levels (Martin and Oakeshott 1983).

Whether genetic factors other than the PI polymorphism itself contribute to variation in alpha-1-antitrypsin is thus of both theoretical and practical interest. Oakeshott et al. (1985) investigated the causes of variation for alpha-1-antitrypsin levels in 1,688 healthy blood donors. They found that only 23% of variance in alpha-1-antitrypsin activity and 26% of variance in alpha-1-antitrypsin quantity could be accounted for by differences between PI phenotypes. Alpha-1-antitrypsin is an acute-phase reactant and levels rise rapidly in response to infection or other exogenous stimuli, so we might expect to find a large environmental component of variation. We wished to know whether all the residual variance is environmental in origin or whether background polygenic variation also affects alpha-1-antitrypsin levels and therefore interacts with or modifies the selective forces postulated to be working on the PI polymorphism. To find the answer it is necessary to measure alpha-1-antitrypsin levels and to type the PI polymorphism in kinships.

We have measured alpha-1-antitrypsin *quantity* (AAT) by an immunological assay and alpha-1-antitrypsin *activity* as elastase inhibitory capacity (EIC; see Bieth et al. 1974; Ofulue and King, in press) in a series of genetically informative individuals who were also typed for PI by isoelectric focusing. We find that nearly all genetic variation in AAT and EIC can be explained by detectable variation at the PI locus and that the allelic effects are largely additive.

METHODS

Sample

The sample comprised 583 subjects in 114 kinships containing from two to 11 individuals. About half of these kinships comprised adolescent monozygotic (MZ) or DZ twins and their parents; the remainder were from kinships comprising the spouses and offspring of (mainly MZ) twins. All subjects were white Americans and ranged from 2 to 62 years of age with a mean of 26 years.

Serum samples were stored at −70 C, and, on thawing, PI typing and assays were performed on the same day. Most samples had been collected within a year of analysis, but some had been stored for as long as 6 years and had been thawed and refrozen on previous occasions. Obvious deterioration of samples could be detected during isoelectric focusing, and any such aliquots were rejected.

PI Typing

PI was typed by isoelectric focusing on 0.5 mm-thick acrylamide gradient gels (pH 4.2–5.2) for 18 h at an ambient temperature of 8 C, a maximum electromagnetic force of 2,500 V, and a maximum permitted current of 2 mA (LKB Power Supply 2197 and the LKB "Ultrophor" equipment). Gels were then stained with Coomassie blue R250 (Sigma Chemical; Clark 1985).

Assays

In addition to EIC, AAT was assayed (by an immunoturbidometric technique; Ofulue and King, in press). Units for EIC are international units per milliliter and for AAT are milligrams per 100 milliliters. Assays were performed in 25 batches of up to 25 samples each, and all members of a given kinship were assayed in the same batch.

Statistical Methods

Models of variation were fitted directly to the raw observations by a maximum-likelihood method (Lange et al. 1976; Eaves et al. 1978; Hopper and Mathews 1982), making use of the Numerical Algorithms Group optimization routine E04JAF (Numerical Algorithms Group 1984). This powerful routine allows one to fix or bound parameter estimates, although in most cases we find it more informative to leave parameters unbounded so that we can see the effects of covariation between estimates. Many of our models have a large number of parameters, and, to ensure that they are identified and that the routine is arriving at an optimum, in a number of cases the same models were fitted with widely differing starting values. No problems were noted.

The Lange algorithm (Lange et al. 1976) allows one to specify the expected value of an individual in terms of the fixed effects of sex, age, assay batch, and PI phenotype. Variance about these means and covariance between relatives can then be specified in terms of environmental and genetic components of variance. Provided that models are properly nested, improvement or deterioration in fit due to addition or deletion of parameters can be judged by likelihood-ratio test. Twice the difference between the log likelihoods of a given model and a more elaborate model is distributed approximately as χ^2 with df equal to the number of additional parameters estimated in the more elaborate model.

RESULTS

Twelve different alleles were detected in 29 distinct PI phenotypes. The first three subtypes of M have been used, as agreed to by the International PI Typing Committee (Cox et al. 1980), M1 being the most anodal, followed by M3 and M2. Additional subtypes have since been identified by more sensitive isoelectric-focusing methods, and M4 and M5 are now recognized, the former being between M2 and M3 and the latter between M1 and M3. The designations M4 and M5 have been used in this work simply to denote their positions relative to M1, M3, and M2 and do not imply any identity with previously

described alleles (Constans et al. 1980; Klasen et al. 1982; Weidinger et al. 1982, 1985).

M null is a rare state in which no detectable AAT is produced by the allele (Talamo et al. 1973; Muench et al. 1986). In the present work a null allele has been inferred because there is an apparent incompatibility in PI types between the mother and her MZ twin sons. The father was not tested, but the mother was apparently M1M1 and the twins were apparently M3M3. The physical and historical evidence suggested that the twins were the offspring of this mother. AAT and EIC levels of the mother and both twins were half or less than half those expected from individuals with the above genotypes.

Since all members of a family were assayed in the same batch, we carried out preliminary analyses of variance and found that 34% of variance in EIC occurred between batches, 16% between families within batches, and 50% between individuals within families. The corresponding figures for AAT were 9%, 20%, and 71%. This indicates that there were considerable differences between the means of assay batches, particularly for EIC. However, since batches were heterogeneous in both sex and genotype composition, it was not possible to judge the extent to which these effects were responsible for the differences in batch means. In specifying the model for mean effects, it was therefore necessary to include parameters to identify the batch, as well as the sex and genotype, of each observation.

Results of fitting models to data for EIC and AAT are shown in table 1. The most basic model (model 1) specifies only male and female means and environmental variance (E_1) about these means. To take account of differences between batch means, we next specified that the sex means were those for batch 1 and added to our model deviations from these means for batches 2–25. This model (model 2) produced a large increase in likelihood for both EIC (χ^2_{24} = 299) and AAT (χ^2_{24} = 114). Batch effects could be added to genetically more elaborate models and still produce similar large increases in likelihood.

When polygenic additive variance (V_A) was added to the model (model 3), significant improvements were obtained for both EIC (χ^2_1 = 36) and AAT (χ^2_1 = 24), indicating, not surprisingly, significant genetic variation for both variables. Estimates of E_1 and V_A from model 3 were 15.5 and 13.4 for EIC and 642 and 333 for AAT, representing heritabilities of .46 for EIC and .34 for EIC. We wished to know whether all this genetic variance could be accounted for by differences between the means of PI phenotypes. We therefore specified that the male and female means were those for the M1M1 phenotype in batch 1 and added parameters for the deviations from these grand means of all 28 other phenotypes found in our sample. These phenotypic deviations were added to the E_1 and $E_1 + V_A$ models in models 4 and 7, respectively. It can be seen that specification of phenotypic mean effects in model 7 produced a dramatic improvement over the simpler $E_1 + V_A$ model (model 3) for both EIC (χ^2_{28} = 213) and AAT (χ^2_{28} = 193). Furthermore, comparison of models 4 and 7 shows that adding polygenic variance to a model already specifying phenotypic effects made no significant difference to the likelihood for EIC (χ^2_1 = 1.08) or AAT (χ^2_1 = 0.06). Estimates of E_1 and V_A from model 7 are 16.8 and 1.6 for EIC and

TABLE 1

RESULTS OF UNIVARIATE MODEL FITTING TO EIC AND AAT

Model	$\bar{X}_m$, $\bar{X}_f$	Batch Means 1–25	Phenotype/ Allele Effects	Age[a]	Age Squared[b]	E_I	V_A	V_D	EIC −LL	AAT −LL
1	+					+			1414.00	2352.83
2	+	+				+			1264.37	2295.76
3	+	+				+	+		1246.11	2283.63
4	+	+	P			+			1140.22	2187.23
5	+	+	P			+[c]			1134.63	2170.33
6	+	+	P			+[d]			1134.54	2167.61
4(log)	+	+	P			+			−544.00	−909.08
5(log)	+	+	P			+[c]			−550.78	−915.21
6(log)	+	+	P			+[d]			−550.78	−917.33
7	+	+	P			+	+		1139.63	2187.20
8	+	+	A			+			1154.41	2198.76
9	+	+	A	+		+			1143.68	2198.23
10	+	+	A		+	+			1142.84	2198.52
11	+	+	A	+	+	+			1142.80	2197.17
12	+	+	A			+	+		1152.43	2198.41
13	+	+	A	+		+	+		1140.86	2197.95
14	+	+	A		+	+	+		1139.93	2198.23
15	+	+	A	+	+	+	+		1139.88	2196.77
16	+	+	A			+	+	+	1151.32	2196.29
17	+	+	A	+		+	+	+	1140.41	2195.89
18	+	+	A		+	+	+	+	1139.55	2196.13
19	+	+	A	+	+	+	+	+	1139.52	2194.93

NOTE.—A plus sign indicates that the effect is included in the model. $\bar{X}_m$ = male mean; $\bar{X}_f$ = female mean. P = 29 phenotypic means included; A = 12 allelic means specified; −LL = minus the log likelihood; and (log) = fitted to a logarithmic transformation of the raw data.

[a] Linear regression of mean effect on age allowed.

[b] Quadratic regression of mean effect allowed.

[c] MM phenotypes allowed a different variance from all other phenotypes.

[d] MM homozygotes and MM heterozygotes each allowed different variances from all other phenotypes.

677 and −10 for AAT. From this and several other comparisons, we tentatively conclude that most, if not all, genetic variation in EIC and AAT can be explained by differences between the means of PI phenotypes. Estimates of male and female means and phenotypic deviations from model 4 are shown in table 2. Because of the number of parameters estimated, it was not practicable to estimate SEs.

Next we tested whether phenotypic means could more simply be accounted for as the sum of their corresponding two allelic deviations. Once again, we specified the male and female means as those representing the value of the M1 allele in batch 1 and specified deviations for the 11 other alleles that we detected rather than for the 28 phenotypic deviations. This model (model 8) can be compared directly with model 4, and it can be seen that specification of allelic rather than phenotypic deviations caused a worsening of fit of $\chi^2_{17} = 28.38$ for EIC and $\chi^2_{17} = 23.06$ for AAT. This worsening is just significant at the 5% level for EIC but is not for AAT ($.10 < P < .20$). Estimates of the allelic effects from model 8 are shown in table 3.

TABLE 2

ESTIMATES OF EFFECTS OF PI PHENOTYPES ON EIC AND AAT

	N	EIC	AAT
		A. Sex Deviations	
$\bar{X}_m$	272	19.0[a]	210[a]
$\bar{X}_f$	311	21.0[a]	221[a]
		B. Phenotype Deviations	
M1M1	270	0[b]	0[b]
M2M2	6	+0.1	+6
M3M3	10	+5.4	+34
M1M2	81	+1.2	+7
M1M3	101	−0.3	−3
M2M3	9	+1.5	−5
MxM3[c]	2	−6.6	−10
M3M5	2	+1.6	+10
M1M4	1	+2.4	+20
M1M5	8	−3.3	−18
L M1	2	−3.3	+14
M2M5	1	−2.4	−18
M1S	32	−3.5	−28
M2S	9	−4.1	−23
M3S	8	−5.3	−38
M5S	1	−7.3	−44
I M1	2	−4.8	−32
I M3	1	+0.3	−15
F M1	3	−1.0	+4
F M3	1	−3.1	−17
M1Z	16	−10.4	−62
M2Z	3	−16.3	−65
M3Z	2	−13.3	−64
M5Z	1	−9.1	−78
M1Mnull	1	−17.5	−76
M3Mnull	2	−13.7	−84
S S	4	−5.6	−58
S Z	2	−11.2	−108
I S	2	−8.9	−80
	583		

NOTE.—Estimates are from model 4 (see Results). Abbreviations are as in table 1.

[a] $\bar{X}_m$ and $\bar{X}_f$ are those for M1M1 in batch 1. Deviations for batches 2–25 are not listed.

[b] Fixed to zero.

[c] Mx is a previously unreported PI allele that is situated just anodal to M1 and occurs in a pair of MZ twins. It has not been investigated sufficiently to be recognized by the International PI Typing Committee and is called Mx for the purpose of this work only.

We proceeded on the assumption that allelic effects at the PI locus are additive for both EIC and AAT. In models 9–19 we first added V_A to the model and then polygenic dominance variance (V_D), with all possible combinations of linear and quadratic regression of mean levels on age. This was done because failure to accurately describe regressions of the mean on age can sometimes result in patterns of familial similarity also predicted by V_D (Corey et al. 1986).

TABLE 3

ESTIMATES OF EFFECTS OF PI ALLELES ON EIC AND AAT

	EIC	AAT
A. Sex Deviations		
$\bar{X}_m$	18.9[a]	208[a]
$\bar{X}_f$	20.9[a]	220[a]
B. Allelic Deviations		
M1	0[b]	0[b]
M2	+0.7	+5
M3	+0.5	+2
M4	+2.5	+21
M5	−2.1	−13
Mnull	−15.8	−86
Mx	−6.7	−9
S	−3.7	−30
Z	−11.0	−64
F	−2.1	−4
I	−4.2	−36
L	−3.4	+14

NOTE.—Estimates are from model 8 (see Results).

[a] Male and female means are those for M1M1 in batch 1. Deviations for batches 2–25 are not listed.

[b] Fixed to zero.

Inspection of the log likelihoods of these models reveals a highly significant (quadratic) regression of EIC on age and evidence for a small and only marginally significant amount of residual V_A. Estimates from model 14 for EIC are $E_1 = 15.0$, $V_A = 3.7$, and a regression coefficient for age squared of $-.0011$, indicating a decline in mean EIC level with advancing age—by ~4 units over the age range of our sample. By contrast, there is no evidence for age affecting AAT levels, nor is there evidence for residual V_A, but there is a just-significant indication of nonadditive (dominance) residual polygenic variance. Estimates from model 16 for AAT are $E_1 = 535$, $V_A = -31$, and $V_D = 189$. Of course, variance components cannot be negative, and the fact that their estimates are reflects the large negative correlation between the estimates of V_A and V_D. Estimates of V_D are biased by other nonadditive genetic effects, including epistasis (Eaves, in press) and genotype × age interaction (Eaves et al. 1978), so it cannot necessarily be concluded that V_D is a major cause of variation in AAT levels.

Beckman and Beckman (1980) reported higher mean AAT levels and smaller variances for MM heterozygotes than for MM homozygotes, and others have found some evidence to support this finding (Rantala et al. 1982; Oakeshott et al. 1985). To test the hypothesis about heterogeneity in AAT means between MM subtypes, we constrained the means for M1M1, M1M2, M1M3, M2M2, M2M3, and M3M3 to be equal, which resulted in a worsening of fit of $\chi^2_5 = 16.48$ ($P < .01$). Constraining the means for the MM homozygotes (M1M1,

M2M2, and M3M3) to be equal and the MM heterozygotes (M1M2, M1M3, and M2M3) to be equal has an effect that is almost as bad ($\chi^2_4 = 16.40$). We conclude that there is heterogeneity between MM subtype means but that this does not reside in a difference between the groups of MM homozygotes and MM heterozygotes.

To test hypotheses about the heterogeneity of E_1 values between different PI phenotypes, we fitted two modifications of model 4. In model 5 we allowed MM phenotypes to have an E_1 value different from that of all other phenotypes and in model 6 we allowed both MM homozygotes and MM heterozygotes to have their own E_1 values different from each other and from that of all other phenotypes. It can be seen from table 1 that, for EIC, model 5 was a significant improvement on model 4 ($\chi^2_1 = 11.18$) but that allowing further heterogeneity of E_1 between MM homozygotes and heterozygotes (model 6) produced no further improvement. Model 5 estimated an E_1 of 20.1 for MM phenotypes and 11.0 for all other phenotypes. Even larger increases in likelihood were seen when heterogeneity of E_1 values for AAT was allowed. The fit improved by $\chi^2_1 = 33.8$ when a different MM variance was allowed (model 5), and a further improvement of $\chi^2_1 = 5.44$ was obtained by allowing MM homozygotes and heterozygotes to have different E_1 values. Model 6 estimated E_1 values of 861 for MM homozygotes, 619 for MM heterozygotes, and 265 for all other phenotypes.

Lest this heteroscedasticity be purely a result of relationship between mean and variance, models 4–6 were fitted again to logarithmically transformed raw data. The results are shown in table 1 and reveal exactly the same pattern of log likelihoods as the raw data. However, for log(EIC) we now find that the estimated E_1 of MM phenotypes is *smaller* (0.050) than that for all other phenotypes. This suggests that it might be possible to find a transformation of EIC values in which there will be no heteroscedasticity. For AAT, on the other hand, the same pattern of E_1 values is found as for the raw scale, with E_1 for MM homozygotes estimated as 0.198, for MM heterozygotes as 0.148, and for all other phenotypes as 0.097. It seems, then, that heterogeneity of E_1 for AAT between different PI phenotypes will not readily be removed by scale transformation and may represent a biologically interesting example of genotype × environment interaction. This, in turn, could provide an explanation for the significant evidence for V_D for AAT (see above).

Finally, we wished to estimate the environmental correlation between EIC and AAT measures, given that all the genetic covariance could be accounted for by the additive effects of the PI alleles. We therefore used the bivariate extension of the Lange algorithm (Eaves 1980; Lange and Boehnke 1983) to fit a model simultaneously to both EIC and AAT measures and to their covariance. Each measurement requires a male and female mean, 24 batch deviations, 11 allelic deviations, and an estimate of E_1. Additionally, we require the environmental correlation between the two measures, so the model contains 77 parameters in all. Parameter estimates for this model were very close to those from the respective univariate analyses.

The environmental correlation between EIC and AAT was only .63. Since we

should expect EIC and AAT to change in concert in response to environmental challenges such as pathogens, the low environmental correlation may simply reflect analytic variation of the two assays. This, in turn, would help to account for the low heritabilities.

The unweighted correlation between the allelic deviations for AAT and EIC (as shown in table 3) for the 11 alleles whose effects were not fixed was .90. The bivariate model also allowed us to test the hypothesis that the specific activity (activity per unit protein) of each allelic form is the same. We did this by fitting a reduced form of the model in which we specified only one set of allelic deviations (for EIC) and allowed the corresponding deviations for AAT to be a constant multiple of these. The estimate for this constant was 6.3, and the reduced model worsened the fit by $\chi^2_{10} = 17.51$ ($.05 < P < .10$), so we could not reject it.

DISCUSSION

The most striking finding of our study is that, once differences between phenotype means have been allowed for, there is little if any genetic variance left for either AAT or EIC. The small residual V_A for EIC may reflect the action of other gene products that perform some of the same functions as alpha-1-antitrypsin (Fagerhol and Cox 1981, p. 26).

Since, in our unbalanced design, the effects of genotype, sex, and batch were confounded, and since the number of families assayed in a batch is small (≤ 7), it is possible that removal of batch effects caused underestimation of the genuine differences between families, so that, in turn, polygenic components of variance are underestimated. If batch effects are important (as they appear to be in this study), there is no way around this problem short of complete randomization of all individuals in the study across assay batches.

We found that phenotype means could be summarized more economically as the sum of contributions of the respective alleles. However, because 23 of the 29 phenotypes that we observed were found in fewer than 10 individuals, it is possible that there are some strong nonadditive interactions between alleles, interactions that our study does not have the power to detect. This may explain the just-significant evidence of dominance for AAT after allelic effects have been allowed for, although the fit of the allelic-deviations model was more marginally acceptable for AAT than for EIC where no evidence of V_D was detected.

Another explanation for the detection of genetic nonadditivity for AAT is the large amount of nonscalar genotype $\times$ environment interaction. Whether measured on a logarithmic or the raw scale, it seems that MM homozygotes have greater environmental variance than MM heterozygotes, which, in turn, have greater environmental sensitivity than all other phenotypes. Similar differences between PI phenotypes in environmental sensitivity were found for EIC when it was measured on the raw scale, but the order of sensitivities was reversed when the scale was logarithmically transformed.

Alpha-1-antitrypsin is an acute-phase reactant and is produced in large quantities by the liver in response to exogenous stimuli such as infection. Previous

results (Beckman and Beckman 1980; Rantala et al. 1982; Oakeshott et al. 1985) have all supported the notion that different PI types have different capacities for response to environmental challenges. We also find evidence for genotype × environment interaction, but its size and direction depend on the scale of measurement of AAT and EIC. What the appropriate scales are must await much more detailed biochemical, physiological, and evolutionary studies (Mather and Jinks 1982, chap. 3). It would not be surprising, however, to find that responsiveness of different PI types to environmental sensitivity is a function of specific activity of the protein. Our bivariate analysis has found no evidence for significant differences between the specific activities of alpha-1-antitrypsin proteins produced by different alleles. If this is so, then it is difficult to explain the apparent differences in scale-sensitivity of the genotype × environment interaction detected for the two measures. Once again, we have to take into account the small size of our sample and consider the possibility that there really are significant differences between the specific activities of alpha-1-antitrypsin molecules produced by different alleles but that our study has insufficient power to detect them.

Differences in specific activities and in environmental sensitivities of different PI types may partly explain the low correlation (.63) between environmental deviations for AAT and EIC in our sample. Errors in the two assays would also lower this correlation.

ACKNOWLEDGMENTS

We thank Marsha Blanchard and Anita Cook for collating the samples and Dr. R. Scheiken for access to samples from his study. This work was supported by National Institutes of Health grants HD15838, HL31010, GM30250, GM32782, AA06781, AG04954, and DE05139.

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