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Association between Alpha-1-Antitrypsin Types and the Common Cold

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Abstract. Associations have been found between self-reported cold symptoms and *Pi* type in 84 pairs of identical twins. The risk of cold symptoms relative to MM phenotypes was 1.2 times greater in MS and 2.5 times greater in MZ individuals. Up to 9% of variance in cold symptoms could be attributed to *Pi* type, representing at least 20% of the total genetical variance.

Alpha-1-antitrypsin (α_1-AT) is the major inhibitor of protease activity in human serum. It is encoded by the protease inhibitor (*Pi*) locus which is polymorphic in European populations for five relatively common alleles, *M1*, *M2*, *M3* (the *M* subtypes), *S* and *Z*, at approximate frequencies of 0.75, 0.15, 0.04, 0.04 and 0.01, respectively. Of the eight most common phenotypes, MS and MZ have low α_1-AT levels, the heterozygotes M1M2, M1M3 and M2M3 have high levels and the homozygotes M1M1, M2M2 and M3M3 have intermediate means but large variances [*Cook*, 1974; *Beckman and Beckman*, 1980]. The MS and MZ phenotypes have been associated with several clinically defined respiratory disorders, including emphysema and chronic obstructive lung disease, but no differences in susceptibility have been found among the six MM genotypes [*Beckman* et al., 1980]. In this paper we compare *Pi* genotypes for susceptibility to the common cold.

Sample and Methods

Our sample is 84 pairs of monozygotic twins who took part in a co-twin control study of the effect of vitamin C on susceptibility to the common cold, and who were also typed for *Pi*. The twins were all volunteers from the Australian National Health and Medical Research Council Twin Registry, aged 16–64 and living in the Sydney metropolitan area. The design of the study, the collection and analysis of cold data and the finding of no significant effect of vitamin C on cold susceptibility are described in detail in *Carr* et al. [1982].

The trial lasted 100 days during the winter of 1980 and was double-blind so that neither the experimen-

Table I. Number of identical twin pairs of each genotype and percent expected to have α_1-antitrypsin levels > 160 mg/100 ml

Genotype	M_1M_1	M_1M_2	M_1M_3	M_2M_2	M_2M_3	M_1S	M_1Z
Pairs, n	50	15	5	2	2	7	3
Percent > 160 mg/100 ml	97.5	100	100	95.6	100	87.5	60.3

Table II. Correlations (× 100) between cold variables

		2	3	4	5	6	7	8	9	10	11	12
1	Total duration	81	92	65	62	75	67	80	57	36	38	46
2	Incidence		73	57	58	55	47	58	58	23	35	40
3	Total severity			65	66	76	73	81	69	44	54	58
4	Sore throat				29	38	37	44	55	19	26	26
5	Sneezing					60	43	46	38	24	24	34
6	Runny nose						55	60	31	03	28	45
7	Blocked nose							45	36	22	31	33
8	Coughing								46	38	34	41
9	Headache									44	56	41
10	Feverish										18	14
11	Tiredness											65
12	Muscle ache											

$r_{0.05} > 0.14$; $r_{0.01} > 0.18$; $r_{0.001} > 0.23$ (2 tail).

ters nor the subjects knew which twin was receiving the 1 g dose of vitamin C and which the well matched placebo. Each day of the trial, subjects were asked to rate (0 = absent, 1 = mild, 2 = moderate, 3 = severe) the nine cold symptoms: sore throat, sneezing, runny nose, blocked nose, cough, headache, feverish, tiredness and muscle ache. From the symptom ratings, three summary variables are computed: total duration (number of 'cold days'), incidence (number of cold episodes) and total severity (sum of all symptom ratings within cold episodes). The total ratings for each of the nine symptoms are also reported here. Full definitions of a cold episode and the summary variables are given by *Carr* et al. [1982].

Of the 95 pairs of twins who completed the trial and had cold data suitable for analysis, *Pi* types are available for 84 pairs. The method of *Pi* typing is described in *Clark and Martin* [1982].

Results

The number of twin pairs of each *Pi* genotype is shown in table I and the correlations between the twelve cold variables in table II. Although most of the correlations are substantial, the individual symptoms are sufficiently independent (average r = 0.37) to be measuring different effects of the common cold.

Our initial hypothesis is that there is a negative linear relationship between α_1-AT levels and cold symptoms so we regress the twin pair means for each variable on the α_1-AT levels for different gen-

Table III. Means and standard errors of cold variables for three *Pi* phenotypes and standardised regression coefficients (β) on percent > 160 mg/100 ml α_1-antitrypsin for all pairs and within M subtypes only

	β all pairs	β MM only	MM (72 pairs)	MS (7 pairs)	MZ (3 pairs)
Total duration	-0.20**	0.04	10.4 ± 1.0	14.1 ± 3.9	19.7 ± 10.4
Incidence	-0.18*	0.06	1.5 ± 0.1	2.0 ± 0.5	2.3 ± 0.6
Total severity	-0.20**	0.02	44.9 ± 4.5	48.8 ± 12.0	92.2 ± 53.0
Sore throat	0.05	-0.05	6.3 ± 0.8	7.1 ± 2.3	3.5 ± 1.7
Sneezing	-0.05	-0.02	5.2 ± 0.7	6.9 ± 3.7	6.0 ± 3.4
Runny nose	-0.26***	0.14	8.0 ± 1.0	11.2 ± 3.7	21.7 ± 13.9
Blocked nose	-0.30***	0.02	7.4 ± 1.0	4.6 ± 1.0	26.8 ± 13.6
Coughing	-0.21**	-0.05	7.9 ± 1.1	10.7 ± 4.6	18.8 ± 14.7
Headache	0.01	0.10	3.4 ± 0.5	2.1 ± 0.6	4.3 ± 1.6
Feverish	0.02	-0.05	1.6 ± 0.6	1.4 ± 0.7	1.0 ± 0.6
Tiredness	-0.05	0.07	2.6 ± 0.5	2.0 ± 1.4	4.3 ± 3.0
Muscle ache	-0.18*	0.11	1.1 ± 0.2	1.6 ± 1.0	3.3 ± 2.1

* $0.05 < p < 0.10$; ** $0.01 < p < 0.05$; *** $0.001 < p < 0.01$ (1-tail tests).

otypes reported by *Cook* [1974] and *Beckman and Beckman* [1980]. The resulting regression coefficients are nearly all negative and many are significant. However, even stronger negative relationships are obtained by regressing on the percentage of each genotype expected to have α_1-AT levels greater than 160 mg/100 ml, the threshold considered by *Beckman* et al. [1980] in their analysis of chronic obstructive lung disease. These percentages above the threshold are shown in table I and are a function of both the mean and variance in level of each genotype.

The standardised regression coefficients on these percentages and their significance are shown in table III. The unit of analysis is defined as the mean value for the two individuals of each twin pair. The rationale for this is the genetic identity of the co-twins. However, it is a conservative criterion because it ignores any differences in the co-twins' exposure to infection.

Of the twelve variables, seven regressions are negative and substantial, two of which are significant at the 10% level, three at the 5% level and two at the 1% level. If the regressions are performed on individual observations, rather than pair means, the magnitude of the β's is much the same but all seven are now significant at least at the 5% level.

It is possible that the regressions arise mainly from a contrast of the MM genotypes with the small groups of MS and MZ pairs. Consequently, we recalculate the regression on the MM genotypes omitting the other ten pairs. The results are shown in the second column of table III. It can be seen that there are no significant regressions of symptoms on percent > 160 mg/100 ml and only four out of twelve are even negative.

It is apparent therefore that the significant regressions obtained arise from the differences in mean susceptibilities of the

Table IV. Proportions of variance in cold variables showing significant regressions in table III (A) due to regression and (B) due to all genetical and shared environmental factors; the percentage A/B is a lower limit for the contribution of the *Pi* locus to genetic variance in the cold variable

	A	B	A/B%
Total duration	0.042	0.342	12.3
Incidence	0.031	0.366	8.5
Total severity	0.041	0.259	15.8
Runny nose	0.069	0.306	22.5
Blocked nose	0.090	0.422	21.3
Coughing	0.042	0.333	12.6
Muscle ache	0.033	0.138	23.9

MM, MS and MZ phenotypes and that differences within M subtypes make no contribution. We therefore show the means and standard errors for these three phenotypes in the remainder of table III.

Our data also present the opportunity to test for the presence of interaction between the vitamin C treatment and *Pi* genotypes. In the total sample of 95 pairs there was no significant difference between vitamin C and cold treatments in any of the variables considered here [*Carr* et al., 1982]. However, it is possible that this masks differences between genotypes in sensitivity to the treatment. Accordingly we compare the means for the twin on vitamin C and the co-twin on placebo for each cold variable in several groupings of *Pi* genotypes. Even in the most extreme comparison of MM vs. MS + MZ genotypes, six of the contrasts are positive, six are negative, and only one approaches significance.

Discussion

We have shown associations between *Pi* and seven of the twelve cold variables. All three summary variables (total duration, incidence and total severity) showed significant associations. Of the nine individual symptoms it is worth noting that three of the five which could be classed as respiratory (viz. runny nose, blocked nose and coughing) show the strongest associations while only one of the four non-respiratory symptoms (muscle ache) shows a significant association and this only at the 10% level.

The strongest associations between cold variables and *Pi* types were obtained not by regressing on to mean α_1-AT levels but by considering the proportion of each genotype expected to have levels greater than some threshold, in our case 160 mg/100 ml (2SD below the mean). *Martin and Oakeshott* [1983] have argued from the data on α_1-AT levels of *M* subtypes of *Beckman and Beckman* [1980] that it is the proportion above such a threshold which can be equated to fitness in order to predict a stable polymorphism of these alleles at the observed gene frequencies.

It seems highly likely that susceptibility to respiratory disorders of *S* and *Z* genotypes, of which our research provides only one example, is the principle component of such fitness differentials. If the seven variables with significant *Pi* associations are averaged, the risk for MS genotypes is 1.23 ± 0.11 times and for MZ is 2.45 ± 0.27 times that in MM individuals. These compare well with the relative risks of 2.6 for MS and 2.4 for MZ which *Beckman* et al. [1980] found for chronic obstructive lung disease. Like these authors, we also failed

to find any differences in risk among MM subtypes.

It is surprising that we have found such a strong association between the *Pi* locus and the common cold firstly because of the small numbers of *MS* and *MZ* pairs and secondly because the total genetical variation in the self-reported cold variables is only modest. The intraclass correlation coefficient of MZ twins is an upper limit to heritability and it can be seen from column B of table IV that this ranges from a maximum of 0.42 for blocked nose down to 0.14 for muscle ache. In column A of table IV are the proportions of variance due to the significant regressions on α_1-AT in table III. The proportion A/B is thus a lower limit for the contribution of the *Pi* locus to genetical variation in the cold variables. This ranges from 8.5% of the genetical variation in incidence of colds to over 20% for runny nose, blocked nose and muscle ache. With the exception of the *Hb* locus and malaria, we know of few identified loci which can account for as much genetical variation in such a common disease state.

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