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Co-twin Control Studies: Vitamin C and the Common Cold

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INTRODUCTION

Most investigations of the efficacy of drugs in humans begin by assigning subjects at random to control or treatment groups. This is less efficient than the matched pair design, which removes from consideration all the factors tending to make one pair different from another, so providing a more efficient test of the treatment effect. The increase in efficiency of the matched pair over the random group design is dependent upon the extent to which the matching has removed extraneous factors influencing the effect of the drug. The ultimate matched pair in human experimentation is a pair of monozygotic (MZ) twins who have been reared together. Not only is there a perfect match for age, sex, and family background but, perhaps most importantly, genetic constitution is identical, at least for nuclear genes.

The paired and unpaired designs are shown in Table I and have been compared extensively by Christian and Kang [1972]. The paired analysis allows the partition of variance between individuals (σ^2_{BI}) into that between pairs (σ^2_{BP}) and that within pairs (σ^2_{WP}). For sufficiently large N, if $\sigma^2_{BP} > 0$ (ie, if the between-pairs mean square is significantly greater than the pairs $\times$ treatments mean square), then the paired design will give a more powerful test of the between-treatments effect than the unpaired design. For the numbers we have here, it may be shown that $\sigma^2_{BI}/\sigma^2_{WP}$ is the multiplier to estimate how many more pairs of unrelated individuals would be needed to provide a test of the between-treatments effect of the same power as is provided by a given number of pairs of twins.

TABLE I. Comparison of Paired and Unpaired Analyses of Variance

Source	DF	Expected mean square
Unpaired design (N individuals in each group)		
Between treatments	1	$\sigma^2_{BI} + N\,\sigma^2_{BT}$
Between individuals	2(N − 1)	σ^2_{BI}
Paired design (N pairs of MZ twins)		
Between pairs	N − 1	$\sigma^2_{WP} + \sigma^2_{BP}$
Between treatments	1	$\sigma^2_{WP} + N\,\sigma^2_{BT}$
Pairs × treatments	N − 1	σ^2_{WP}

Relative efficiency of paired vs unpaired design: RE = $\sigma^2_{BI}/\sigma^2_{WP}$.

The co-twin control design was used to study the effect of vitamin C on cold symptoms in 44 school-aged MZ twins by Miller et al [1977]. They found no effects in the total sample but significantly shorter and less severe colds in the youngest group of girls and less severe colds in the youngest group of boys. We have replicated here their study of the effects of supposedly prophylactic doses on the common cold but with a larger sample of MZ twins in an older age group. By typing twins for the genetic marker *Pi*, which controls the level of serum α_1-antitrypsin, we were able to test for genotypes × environments interaction and to estimate the contribution of this polymorphism to genetic variation in cold susceptibility.

MATERIALS AND METHODS

A total of 125 pairs of twins believed to be monozygotic (MZ) born between 1916 and 1965 and living in the Sydney metropolitan area volunteered to begin the trial. Zygosity was checked by typing all twins with the following antisera (anti-A,A_1,B,M,N,S,s,C,c,D,E,e,K,k,Fy^a,Jk^a) and none was excluded as a dizygotic pair. One twin of a pair was assigned at random to the treatment group and the other to the control group. The experiment was "double-blind" in that neither the subjects nor the experimenters knew which group was which until the experiment and the analysis were completed.

The treatment group received 1 gm of ascorbic acid per day in the form of Redoxon® tablets (Roche Products) and the control group received a placebo with the same ingredients in different proportions but with lactose substituted for ascorbic acid. In a separate trial the placebo and active tablets were found to be well matched with respect to taste and appearance. Twins were asked to take their 1-gm tablet (active or placebo) and a supplied multivitamin capsule at the same time each day, for 100 days beginning June

2, 1980. They were asked not to take any other vitamin preparations during the course of the trial and to note each day any cold symptoms present. The nine cold symptoms listed were sore throat, sneezing, runny nose, blocked nose, cough, headache, feverishness, tiredness, and muscle ache. Subjects were asked to rate their symptoms 0 = absent, 1 = mild, 2 = moderate, 3 = severe.

Of the 125 pairs of twins who began the trial, we have analysed complete cold data for 95 pairs. A cold episode was defined by strict criteria, described with other details of the experiment in Carr et al, [1982]. In addition to the total severities for the nine individual symptoms, three summary variables were computed from each individual's cold data: *incidence* of colds—the number of defined cold episodes experienced over the trial period; *total duration* of colds—the total number of "cold days" experienced; *total severity*—the total of all severity points within cold episodes reported by each individual.

RESULTS

Treatment means for the three summary variables and nine individual symptoms are shown in Table II. It can be seen that there were no significant differences between vitamin C and placebo means for any variables in either males, females, or the total sample. A breakdown of the sample into three age groups revealed no heterogeneity in the ineffectiveness of vitamin C.

It has been claimed that large doses of vitamin C can significantly lower plasma lipid levels [Turley et al, 1976]. Therefore, we compared serum biochemistry results before and at the end of the 100-day trial (Table III). The only significant effects were for alanine aminotransferase (increase) and uric acid (decrease). No significant effects were observed on cholesterol or its fractions HDL and non-HDL cholesterol, nor on triglyceride. The power of our experiment was such that a change as small as 3% in serum cholesterol levels would have been detected at the 5% level in 95% of similar experiments.

The relative efficiency of the paired over unpaired design ranged from parity to 1.73 for the cold variables with a mean of 1.39 (Table IV). This is only a modest increase in efficacy, equivalent to two random groups each of 132 individuals to achieve equal power with the MZ co-twin design of 95 twin pairs.

However, for the serum biochemistry results, the relative efficiency ranged from 2.29 to 7.35, with a mean of 3.85 (Table V). This reflects the greater genetic control of these variables and the much greater potential for co-twin control studies on treatments thought to affect them [Christian and Kang, 1972; Carr et al, 1981].

TABLE II. Treatment Means for Cold Variables

	Males (38 pairs)		Females (57 pairs)		Total (95 pairs)	
	Active	Placebo	Active	Placebo	Active	Placebo
Incidence	1.3	1.3	1.8	1.6	1.6	1.5
Total duration	8.6	8.8	12.7	12.6	11.0	11.1
Total severity	34.4	37.8	56.7	53.3	47.8	47.1
Sore throat	5.0	6.8	7.6	7.5	6.5	7.2
Sneezing	4.1	2.7	6.6	5.7	5.6	4.5
Runny nose	6.1	7.4	10.1	10.1	8.5	9.0
Blocked nose	6.8	6.1	10.4	7.4	9.0	6.8
Coughing	6.1	6.3	9.2	11.2	8.0	9.2
Headache	1.8	2.7	4.4	3.9	3.4	3.4
Feverishness	0.6	1.1	2.8	1.2	1.9	1.2
Tiredness	1.5	2.5	2.9	3.1	2.3	2.9
Muscle ache	1.1	1.0	1.1	1.5	1.1	1.3

TABLE III. Serum Biochemistry Changes on Vitamin C and Placebo Regimes

	Vitamin C	Placebo	P	$\beta = 0.95$ ($\alpha = 0.05$,two-tail)
Alanine aminotransferase IU/liter	7.47	4.12	0.017	
Uric acid (mmol/liter)	−0.006	0.005	0.038	
Cholesterol (mmol/liter)	−0.29	−0.15	0.054	~3%
HDL cholesterol (mmol/liter)	−0.03	0.01	0.085	~5%
Non-HDL Cholesterol (mmol/liter)	−0.26	−0.16	0.068	
Triglyceride (mmol/liter)	−0.13	−0.02	0.160	~18%

Negative value indicates decrease from initial reading.

Of the 95 pairs of twins for whom we had cold data, 84 pairs were typed for the *Pi* polymorphism which controls the enzyme alpha-1-antitrypsin (α_1-AT). The *Z* and *S* alleles, which are associated with low levels of α_1-AT, have been repeatedly associated with clinical respiratory disorders including emphysema and chronic obstructive lung disease (see, for example, Beckman et al, [1980]).

TABLE IV. Relative Efficiency of Paired Over Unpaired Design, Cold Variables

	σ^2_{BI}	σ^2_{WP}	Relative efficiency
Incidence	1.50	0.95	1.58
Total duration	122	80	1.52
Total severity	2,650	1,960	1.35
Sore throat	93	72	1.33
Sneezing	55.55	38	1.47
Runny nose	137	95	1.44
Blocked nose	139	81	1.73
Coughing	163	107	1.50
Headache	26	22	1.18
Feverishness	45	46	0.98
Tiredness	26	19	1.41
Muscle ache	7	6	1.16
		Mean	**1.39**

Would need 264 individuals for equal power.

TABLE V. Relative Efficiency of Paired Over Unpaired Design, Serum Biochemistry

Alanine aminotransferase	2.89	Glucose	7.35
Albumin	2.48	HDL cholesterol	2.60
Alkaline phosphatase	5.84	Iron	3.13
Aspartate aminotransferase	2.29	Lactate dehydrogenase	3.83
Bicarbonate	5.44	Phosphate	4.00
Bilirubin	3.72	Potassium	5.66
Calcium	4.80	Protein	3.60
Chloride	4.14	Sodium	5.35
Cholesterol	2.88	Triglyceride	2.77
Creatine kinase	2.76	Urea	2.37
Creatinine	4.33	Uric acid	4.00
Gamma-glutamyl transferase	2.30	**Mean**	**3.85**

We tested whether *Pi* genotypes have different susceptibilities to colds by regressing each of the cold variables on to the proportion of each genotype expected to have α_1-AT levels greater than 160 mg/100 ml serum, ie, greater than 2 SD below the mean [Martin et al, 1982]. The number of pairs of each genotype and these proportions are shown in Table VI and the standardised

TABLE VI. Number of Identical Twin Pairs of Each *Pi* Genotype and Percentage 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
No. of pairs	50	15	5	2	2	7	3
Percentage of > 160 mg/100 ml	97.5	100	100	95.6	100	87.5	60.3

TABLE VII. Standardised Regression Coefficients of Cold Variables on Percentage Greater Than 160 mg/100 ml alpha-1-Antitrypsin

	All pairs	MM only
Total duration	−0.20**	0.04
Incidence	−0.18*	0.06
Total severity	−0.20**	0.02
Sore throat	0.05	−0.05
Sneezing	−0.05	−0.02
Runny nose	−0.26***	0.14
Blocked nose	−0.30***	0.02
Coughing	−0.21**	−0.05
Headache	0.01	0.10
Feverishness	0.02	−0.05
Tiredness	−0.05	0.07
Muscle ache	−0.18*	0.11

Probability: *P<0.10, **P<0.05, ***P<0.01.

regression coefficients in the first column of Table VII. It can be seen that the three summary cold variables and four of the nine symptoms regress negatively and significantly on α_1-AT levels.

We suspected that these regressions were due only to the contrast of the various MM subtypes with the low-activity MS and MZ phenotypes. Accordingly, we repeated the analysis using only MM subtypes and excluding MS and MZ. As can be seen from the second column of Table VII, no significant regressions remained. We therefore show the means for only MS, MZ, and the pooled MM phenotypes in Table VIII. The average risk for the seven significant symptoms relative to the pooled MM mean is 1.2 for MS and 2.5 for MZ.

TABLE VIII. Cold Variable Means for Three Pi Phenotypes

	MM(72 pairs)	MS(7 pairs)	MZ(3 pairs)
Total duration	10.4	14.1	19.7
Incidence	1.5	2.0	2.3
Total severity	44.9	48.8	92.2
Runny nose	8.0	11.2	21.7
Blocked nose	7.4	4.6	26.8
Coughing	7.9	10.7	18.8
Muscle ache	1.1	1.6	3.3

TABLE IX. Contribution (%—lower limit) of *Pi* Locus to Genetic Variation in Cold Symptoms

	Pi contribution to variance (R^2)	Upper limit of heritability (H^2)	R^2/H^2
Total duration	4	34	12
Incidence	3	37	9
Total severity	4	26	16
Runny nose	7	31	23
Blocked nose	9	42	21
Coughing	4	33	13
Muscle ache	3	14	24

Because these calculations were performed on identical twins we may make a further calculation. In Table IX are shown the contributions of regression on α_1-AT levels to total cold variation (R^2), the intraclass correlation of MZ twins (H^2), and the ratio of these two. Because H^2 is an upper limit to heritability, the ratio R^2/H^2 is a lower limit to the proportion of genetic variation in cold susceptibility accounted for by the *Pi* locus. It can be seen that over 20% of the genetic variation in cold symptoms is due to the *Pi* locus.

Although there was no effect of vitamin C on colds in the total sample, it is possible that, within this total, *Pi* phenotypes with high susceptibility to colds reacted differently to vitamin C than those with lower susceptibility. In Table X are shown the (active-placebo) means for the 74 MM subtypes

TABLE X. Genotypes × Environments Interaction: Do *Pi* Genotypes Respond Differently to Vitamin C?

	Means for (active-placebo)		
	MM(N = 74)	MS + MZ(N = 10)	P
Total duration	0.3	−1.3	0.70
Incidence	0.1	0.2	0.87
Total severity	0.8	4.4	0.86
Sore throat	−1.0	−0.7	0.93
Sneezing	0.9	1.8	0.77
Runny nose	−0.6	1.1	0.80
Blocked nose	1.4	8.9	0.07
Coughing	−0.8	−2.3	0.83
Headache	0.2	−1.1	0.56
Feverishness	1.0	0.2	0.64
Tiredness	0.2	−3.4	0.08
Muscle ache	−0.1	−1.4	0.31

Negative value means vitamin C does better.

and the 10 MS + MZ phenotypes. It can be seen that there were no significant differences between phenotypes in response to vitamin C. There was not even any consistency in the direction of response so we may dismiss the possibility of interaction between vitamin C treatment and these *Pi* phenotypes.

DISCUSSION

From this analysis three distinct advantages of an MZ co-twin control study over a random groups design are apparent. First, the MZ co-twin design is more efficient. In comparing the effects of vitamin C and placebo treatments on cold symptoms, the increased efficiency was a relatively modest 40%. However, nearly four times as many randomly selected individuals as twins would be needed to achieve the same efficiency in the comparison of treatments on serum biochemical variables. In contrasting the effect of the treatments on serum cholesterol, a difference as small as 3% would have been detected with 95% power.

The second advantage is that the MZ intraclass correlation gives an upper limit to the heritability of a trait (it also includes any effect of shared environment). Thus, in investigating associations with genetic markers, including

those often used in zygosity diagnosis itself, one can readily estimate a lower limit to the contribution of a particular polymorphism to total genetic variance. We have seen that the contribution of the *Pi* polymorphism to genetic variability in cold symptoms may be at least 20%.

The third advantage of the MZ co-twin control study is that it provides an excellent test for genotypes × environments interaction. The general test in MZ twins for this was first developed by Jinks and Fulker [1970], but in the present analysis we have checked for interaction between a specific genetic polymorphism, *Pi*, and a specific environment, vitamin C treatment. The failure to find any such interaction, coupled with the absence of a main treatment effect, is further evidence against the efficacy of vitamin C in preventing or ameliorating the common cold.

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