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Protease inhibitor (Pi) locus, fertility and twinning

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Summary. In a sample of 160 Dutch twin pairs and their parents, we found that mothers of dizygotic twins had frequencies of the S and Z alleles at the protease inhibitor (Pi) locus that were 3 times higher than a control sample. Mothers of identical twins also had a higher frequency of S than controls. The S allele may thus both increase ovulation rate and enhance the success of multiple pregnancies. There was also an increased frequency of the S allele in fathers of dizygotic twins; however, this may be a secondary effect of assortative mating for family size (indicating by the number of siblings of the parents), for which a correlation of 0.2 was observed. Parents of dizygotic twins came from larger families than parents of monozygotic twins, but no effect of Pi type on family size was seen.

Introduction

Alpha-1-antitrypsin (AAT) is the major inhibitor of a variety of proteases (for a review, see Crystal et al. 1989). Numerous genetic variants (designated by letters), encoded by codominant alleles at the protease inhibitor (Pi) locus on the distal part of chromosome 14, have been described, the M alleles being most frequent. Some allele variants (S and Z) cause deficiencies in AAT measured in serum. Certain combinations of these deficiency alleles and the much rarer null alleles are associated with lung and liver pathology. Chromosomal aberrations, mainly nondisjunctions, have also been reported to be associated with S and Z (Jongbloet et al. 1981). It is therefore puzzling that the frequencies of S and Z alleles are relatively high in Caucasian populations, and it has been suggested that these alleles are associated with increased fertility. Specifically, it has been proposed that MS and MZ heterozygotes have higher fertility (Fagerhol and Gedde-Dahl 1969), and that this is manifested particularly in an increased rate of dizygotic twinning in females.

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Clark and Martin (1982) found that the frequenc of the S allele in mothers of dizygotic (DZ) twins (0.088) was double that in controls (0.044). The frequency of S in monozygotic (MZ) twin parents and in fathers of DZ twins was no higher than in controls. Normal frequencies were observed for the Z allele. The number of Z alleles observed in the study of Clark and Martin (1982) was very low, however, because the number of parents of twins of known zygosity was low (32 MZ twin and 51 DZ twin parents). No fertility indices other than twinning itself were available. To study relationships between Pi types, fertility and twinning in more detail, we present new data for 90 DZ and 70 MZ Dutch twin pairs and their parents, for whom information on family size is also available.

Subjects and methods

This study is part of a project in which cardiovascular risk factors are being studied in adolescent twins and their parents. Addresses of twins were obtained from city council population registries in The Netherlands. Three fathers of twins were born in South-Europe and Turkey and 11 in former Dutch colonies (Indonesia, Surinam and the Dutch Antilles); 8 mothers of twins and 4 twin pairs were also born in former colonies. However, some of these subjects are ethnic Dutch.

Twin zygosity was determined by analyzing the following polymorphisms: ABO, MNS, P, Rhesus, Lutheran, Kell, Duffy, Kidd, Gm, Am and Km. In addition, 36 pairs were also typed by DNA fingerprinting (Jeffreys et al. 1985). There were 70 MZ and 90 DZ pairs.

Fasting serum samples were stored at −20°C. Isolectric focusing was carried out in 1.0 mm flat-bed polyacrylamide gels (250 × 115 mm) using an LKB Multiphor apparatus at 4°C. The gels consisted of 10 ml acrylamide/bisacrylamide solution (T = 15%, C = 3%), 20 ml sucrose (36 g/100 ml), 0.5 mml ampholine pH 3.5–5.0, 1.0 ml ampholine pH 4.06–6.0. After de-aeration, polymerization was performed for 1–2 h after addition of 1 ml ammonium persulfate (1 g/100 ml). The serum samples (15 µl diluted 1:1 with 10 m*M* dithiothreitole) were applied to Whatman 3 mm filter paper pieces at a distance of 1 cm from the cathodal end of the gel. The electrode solutions were 1 *M* $H_3PO_4$1 (anode) and 1 *M* NaOH (cathode). The focusing conditions were defined by the following maxima:

1000 V, 25 mA and 15 W. Prefocusing was performed 1 h before sample application, followed by 2-h focusing. Gels were fixed and stained according to Vesterberg (1972).

The control group was compiled from all published Dutch population studies: 1474 subjects from an investigation of cardiorespiratory disease carried out by Hoffmann and Van den Broek (1976), 708 blood donors from a study by Klasen et al. (1977), and 131 subjects from a study by Frants and Eriksson (1978). The last two studies included Caucasians only (personal communication); the number of non-Dutch subjects in the first study is likely to be very small. It should be noted that this can only strengthen our conclusions as the frequency of non-M alleles is much lower in populations outside North-West-Europe.

Results

Phenotype counts

All non-M alleles were found in subjects born in The Netherlands. Table 1 gives the phenotype counts for fathers and mothers of DZ and MZ twins, for twins (MZ pairs are counted as 1 phenotype and DZ pairs as 2 phenotypes), and for controls. To compare twin parents with controls, Pi phenotypes were recoded into 3 groups (group 1 = MM, MF and MOther, group 2 = MS and SOther, group 3 = MZ and ZZ). The phenotpye distribution was significantly different in mothers of DZ twins versus controls ($\chi^2 = 26.4$, $df = 2$, $P < 0.000$), with groups 2 and 3 being more frequent in mothers of DZ twins. The same trend was apparent in mothers of MZ twins, but did not reach the 5% significance level ($\chi^2 = 5.5$, $df = 2$, $P = 0.062$). For fathers, there also was a significant difference in Pi type for DZ twin fathers ($\chi^2 = 6.9$, $df = 2$, $P = 0.031$), but not for MZ twin fathers ($\chi^2 = 3.1$, $df = 2$, $P = 0.216$).

Allele frequencies

Table 2 shows gene frequencies and their standard errors (Vogel and Motulsky 1986). For DZ twins, maximum likelihood estimates of gene frequencies and their standard errors are obtained as described in Martin (1975) using the sib genotype frequencies of Smith and Penrose (1955). For DZ pairs, the number of genes sampled lies between $2n$ and $4n$, where n is the number of pairs. For the S allele, we find that the effective number of genes in 90 pairs of DZ twins is 237 or $2.63n$.

For parents and MZ twins, allele frequencies have been compared with controls by Fisher's exact test on allele counts. The S allele is about 2.5 times more frequent in controls than in mothers of MZ twins ($P = 0.035$), about 3 times more frequent in mothers of DZ twins ($P = 0.002$), and 2.5 times more frequent in fathers of DZ twins ($P = 0.019$). Although the S allele is roughly twice as frequent in fathers of MZ twins and in MZ twins themselves, these differences were not significant. The Z allele is also more frequent in mothers of DZ twins ($P = 0.001$) than in controls. Allele frequencies in DZ twins have been compared with those of controls by pairwise c-tests. Both the S and Z alleles are about 2.5 times more frequent in DZ twins than in controls, but whereas this difference is significant for the S allele ($c = 2.19$, $P = 0.014$, 1 tail), it falls just short for the Z allele ($c = 1.49$, $P = 0.068$, 1 tail).

Fertility

Parents of twins answered questions about their number of offspring, number of (half) siblings, and number of (half) siblings of their parents. Table 3 lists these data (half-sibs were counted as 0.5 in the analyses) by zygos-

Table 1. Phenotype counts (percentages in parentheses; MZ twins counted as 1 phenotype and DZ twins as 2 phenotypes). F, S, M, Z, Alleles of Pi locus; Oth., the remaining Pi alleles

	MZmother	DZmother	MZfather	DZfather	MZ-twin	DZ-twin	Control
MM	60 (86%)	70 (78%)	63 (90%)	79 (88%)	61 (87%)	150 (83%)	2128 (92%)
MS	6 (9%)	10 (11%)	6 (9%)	9 (10%)	5 (7%)	21 (12%)	93 (4%)
MZ	2 (3%)	7 (8%)	1 (1%)	1 (1%)	3 (4%)	7 (4%)	46 (2%)
MF	–	2 (2%)	–	1 (1%)	1 (1%)	2 (1%)	25 (1%)
SZ	–	1 (1%)	–	–	–	–	2 (0.1%)
ZZ	–	–	–	–	–	–	1 (0.1%)
MOth.	1 (1%)	–	–	–	–	–	14 (0.6%)
SOth.	1 (1%)	–	–	–	–	–	4 (0.2%)
Total	70	90	70	90	70	180	2313

Table 2. Gene frequencies and standard errors (in parentheses)

	MZmother	DZmother	MZfather	DZfather	MZ-twin	DZ-twin	Control
M	0.9214 (0.0227)	0.8833 (0.0239)	0.9500 (0.0184)	0.9389 (0.0179)	0.9357 (0.0207)	0.9124 (0.0191)	0.9585 (0.0029)
S	0.0500 (0.0184)	0.0611 (0.0179)	0.0429 (0.0171)	0.0500 (0.0162)	0.0357 (0.0157)	0.0541 (0.0148)	0.0214 (0.0021)
Z	0.0143 (0.0100)	0.0444 (0.0154)	0.0071 (0.0071)	0.0056 (0.0055)	0.0214 (0.0122)	0.0257 (0.0099)	0.0108 (0.0015)
F	0.0071 (0.0071)	0.0111 (0.0078)	–	0.0056 (0.0055)	0.0071 (0.0071)	0.0074 (0.0053)	0.0054 (0.0011)
Oth.	0.0071 (0.0071)	–	–	–	–	–	0.0039 (0.0009)

Table 3. Number of own offspring, own siblings, and aunts and uncles of twin parents

Zygosity	Age	Own offspring	Own sibs	Sibs of mother	Sibs of father
MZ fathers	46.5	3.2	3.3	4.7	4.8
DZ fathers	49.4	3.5	4.1	5.2	5.1
MZ mothers	44.2	3.2	3.1	4.5	5.0
DZ mothers	46.7	3.5	4.2	4.4	4.7
Pi phenotype					
MM fathers	47.8	3.4	3.6	5.0	5.0
S/Z fathers	50.3	3.4	4.7	4.6	5.3
MM mothers	45.3	3.4	3.8	4.6	4.9
S/Z mothers	47.3	3.3	3.1	3.9	4.6

ity of the twin offspring and by Pi type. There were 26 parents with incomplete data, but there was no association between missing values and Pi type ($\chi^2 = 0.3$, $df = 1$, $P = 0.58$). DZ twin families seem to have a larger number of offspring than MZ twin families. It is possible, however, to explain this difference by the differences in age of MZ twin and DZ twin parents. DZ twin parents are older than MZ twin parents [$F(1,158) = 8.93$, $P = 0.003$ for fathers, $F(1,158) = 7.72$, $P = 0.006$ for mothers]. When age was used as a covariate, the number of offspring of MZ twin and DZ twin parents did not differ. Mothers of DZ twins had more siblings than mothers of MZ twins [$F(1,154) = 6.10$, $P = 0.015$] and, for fathers, this difference was almost significant [$F(1,150) = 3.07$, $P = 0.08$]. None of the differences between the Pi groups was significant, although the difference between the number of siblings of fathers is in the expected direction.

Assortative mating

It is possible that the increase of S alleles in DZ twin fathers is a secondary effect of assortative mating for a variable that is associated with Pi type. Parents of twins showed significant assortative mating for number of siblings, but not for number of aunts and uncles. The intraclass correlation between the number of siblings of father and mother was 0.2 ($P = 0.006$). No assortative mating was seen for Pi type, but the power of this test is very low given the low frequeny of non-M alleles.

Preferential transmission

There were 116 MMxMM marriages that produced only MM offspring. Of the other 44 marriages, 40 involved 1 or 2 parents carrying S or Z alleles. These gave no evidence for the preferential transmission of S or Z alleles.

Discussion

We have replicated the results of Clark and Martin (1982) in showing a large excess of the S allele in mothers of DZ twins compared with controls, and an intermediate frequency in DZ twins themselves. Unlike Clark and Martin (1982), we also find an increased frequency of S in fathers of DZ twins and in mothers of MZ twins. We show an increased frequency of the Z allele in mothers of DZ twins, but not in any other group. The pattern of gene frequencies from their study led Clark and Martin (1982) to postulate that the sole effect of S (and perhaps Z) on fertility was mediated through an increased ovulation rate in women who carried these alleles, the most obvious manifestation of this being that these women show a higher frequency of bearing DZ twins. In the present study, the most striking increase in the frequency of the S allele is also seen in mothers of DZ twins. The presence of AAT in ovarian tissue has indeed been established (Bagdasarian et al. 1981); indeed, proteolytic enzymes may play a role in the rupture of mature follicles. The increased frequency of S in mothers of MZ twins suggests that another mechanism is also at work, although it is also possible that MZ and DZ twinning are not independent phenomena (Parisi et al. 1983; Philippe 1985). The Dutch data are more in line with earlier data of Cook (1975) and Lieberman et al. (1979) who have found increased frequencies of S in both MZ and DZ twins. This suggests that the S (and perhaps the Z) allele confer an advantage in bringing a multiple pregnancy of either zygosity to term. The increased frequency of the S allele in fathers of DZ twins is puzzling. One can think of physiological mechanisms by which S-bearing spermatozoa may be at an advantage (e.g., greater acrosomal protease efficiency), but it is difficult to imagine that this would have any appreciable effect on the probability of DZ twinning, since the rate limiting step must surely be the number of ova. We suggest, therefore, that the increased frequency of S in fathers of DZ twins is a secondary effect of assortative mating for family size, an effect which in our data is appreciable. We have also found a relationship between family size and zygosity of offspring. Parents of DZ twins come from lager families than parents of MZ twins. In conclusion, there are clearly positive relationships between Pi type, twinning propensity and fertility in general.

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