TWINNING AND BLOOD GROUPS

II. Distribution of ABO, MN and Rh-Hr Phenotypes in a Twin Sample*

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The distribution of ABO, MN and Rh-Hr phenotypes has been examined in a sample of 688 twin pairs, with particular emphasis on the ABO distribution. It appears that MZ and DZ pairs exhibit opposing deviations from the expected distribution; such deviations tend to cancel out each other in the total sample, thus masking the phenomenon.
The results are briefly discussed, calling for an extension of the study to the twins' families, while forthcoming papers will present further analyses.

Immunological interactions between genetic polymorphisms are well known, and interaction between ABO and Rh-Hr is probably the best example (Levine 1958, De George 1969, Cohen 1970*a* and 1970*b*). Other interactions were recently investigated between ABO and haptoglobins (Kirk et al. 1970) or between ABO and placental alkaline phosphatase (Bottini 1975).

Interrelationships between mother and fetus are probably much more complex than hitherto realized, and additional complexity seems to be involved in multiple births.

Previous studies on interactions between genetic polymorphisms have generally ignored multiple births, much as those relatively few studies on twins and supertwins which have dealt with genetic polymorphisms seem to indicate that genetic equilibria are generally altered in multiple births (Osborne and DeGeorge 1957, Renkonen and Timonen 1967, DeGeorge 1969). This prompted us to undertake a series of studies on the subject.

In a previous study (Bolognesi and Milani-Comparetti 1970) conducted at Rome's Mendel Institute, it was shown that the distribution of the ABO phenotypes in a sample of 1190 twins differed significantly from the corresponding distribution found in a control group of 1454 non-twins. The largest contribution to the difference came from the low number of AB group twins. Several interpretations were discussed, as related to previous papers in the literature. Further exploration of the subject was clearly desirable, and in fact a research plan was undertaken with initial support from the Italian National Research Council (grant no. 71.00824.04). The plan was aimed at extending ABO, MN and Rh-Hr typing to include twins' parents and sibs, but this became impossible when the grant was discontinued; yet blood typing for twins only was continued, and a new study was undertaken, on the basis of computer analysis of ABO, MN and Rh-Hr phenotypes in a further sample of 688 twin pairs.

The study was conducted in cooperation between the Mendel Institute, the Institute of Biology and Genetics of the University of Ancona and the Chair of Biometry, School of Statistics, University of Rome. All available data were transferred onto punched cards, and a Fortran V program was written by one of us (S.D.) in order to sort out individuals and pairs in the various classes of blood group phenotypes and sex.

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Tables 1, 2 and 3 concern the respective distributions of ABO, MN and Rh-Hr phenotypes, by concordance and zygosity, in our sample. It must be pointed out that MZ pairs in our sample are 55.5%, as compared to an estimated percentage of about 30% in the total twin population in Italy. This is ascribed to the fact that blood typing tests are often intended for zygosity diagnosis, which is unnecessary in opposite-sexed pairs (the latter, in fact, are only 9.3% of our sample, as compared to an expected 35%). As a consequence, whenever MZ pairs differ from DZ ones in any distribution, the excess of MZ pairs in our sample is expected to alter accordingly the total distribution. We shall come back to this later.

Analyses reported in the present paper cover only the ABO system: the distributions within the MN and Rh-Hr systems, as well as sex, have been utilized only to ascertain zygosity in the analysis of the ABO distribution. Our tables do seem to indicate the action of selective pressures in our sample for the MN and Rh-Hr systems as well, but these will be analized in subsequent papers.

The distribution of ABO phenotypes was obtained for our total sample and for various sub-samples differing as to sex and

Table 1. *Distribution of ABO phenotypes in a sample of 688 twin pairs, by concordance and zygosity*

	Total	MZ	DZ	DZ conc.	DZ disc.
A-A	243	166	77	77	—
A-B	10	—	10	—	10
A-AB	13	—	13	—	13
A-O	61	—	61	—	61
B-B	59	42	17	17	—
B-AB	3	—	3	—	3
B-O	16	—	16	—	16
AB-AB	13	10	3	3	—
AB-O	4	—	4	—	4
O-O	266	164	102	102	—
Total	688	382	306	199	107

Table 2. *Distribution of MN phenotypes in a sample of 636 twin pairs, by concordance and zygosity*

	Total	MZ	DZ	DZ conc.	DZ disc.
M-M	177	131	46	46	—
M-MN	72	—	72	—	72
N-N	99	76	23	23	—
N-MN	36	—	36	—	36
MN-MN	237	175	62	62	—
M-N	15	—	15	—	15
Total	636	382	254	131	123

Table 3. *Distribution of Rh-Hr phenotypes in 664 twin pairs, by concordance and zygosity*

	1	3	6	7	8	9	12	18	Others	Total
1 Rh_1Rh_2	64									
3 Rh_1rh	15	190								
6 Rh_1Rh_1	7	31	132							
7 Rh_2rh	7	5	1	37						
8 Rh_2Rh_2	2	3	—	4	10					
9 Rh_0rh	—	6	2	2	—	6				
17 rh′rh	2	3	2	—	—	—	10			
18 rh rh	4	24	4	2	2	—	1	59		
Others	5	3	6	1	—	—	—	3	8	
Total	106	265	147	46	12	6	11	62	8	663
MZ	46	142	94	31	7	4	9	45	4	382
DZ	60	123	53	15	5	2	2	17	4	281
DZ conc.	18	48	38	6	3	2	1	14	4	134
DZ disc.	42	75	15	9	2	—	1	3	—	147

zygosity. Each such experimental distribution was matched against the corresponding expected distribution in three alternative hypotheses:

a) expected distribution in the hypothesis of genetic equilibrium (Hardy-Weinberg) based on gene frequencies derived, by the maximum likelihood method, from each sample or subsample;

b) expected distribution in the hypothesis that the non-twin control sample reported by Bolognesi and Milani-Comparetti (1970) would still be valid as representing the population from which our present twin sample was drawn;

c) expected distribution in the hypothesis that pooling the three population samples reported by Mourant et al. (1976) for the Rome area would better represent the population from which our twin sample was drawn.

Tables 4, 5 and 6 list the results of these comparisons in terms of levels of significance (chi square).

Table 4 indicates that only concordant pairs (i.e., mostly MZ pairs) deviate from genetic equilibrium, tending to a decrease in the observed frequency of group AB. Two notes are worth making: a) there is considerable difference between all-male and all-female pairs; b) estimated gene frequencies differ greatly between sub-samples: the gene frequency for I^o (r) varies between 0.60 and 0.70. This latter finding is not consistent with data from Italian populations, and in any case one could hardly believe that our MZ and DZ twins came from different (and so very different!) populations.

Since the parental phenotypes were unknown, it was decided to match our sample against two possible control groups as indicated above.

Table 5 indicates that:

— our twin sample differs significantly from our 1969 control group, the main difference being the decrease of group AB;

— the frequency of group AB individuals

Table 4. *Differences (χ^2 and main contributions thereof) as to distribution of ABO phenotypes between various twin subsamples and the corresponding expected values in the hypothesis of genetic equilibrium*

Subsample	χ^2_1%	Main contributions			
		A	B	AB	O
3 ABO concordant	0.2			—	
9 same-sexed ABO concordant	0.15			—	
13 all-female ABO concordant	0.13			—	

Table 5. *Differences (χ^2 and main contributions thereof) between various twin subsamples and the corresponding frequencies in the "1969" controls (see text)*

Subsample	χ^2_3%	Main contributions			
		A	B	AB	O
1) All pairs	5			(—)	
2) ABO discordant	1			+ +	
3) ABO concordant	0.1			— —	
5) Concordant as to all criteria	1			—	
7) All same-sexed pairs	5			—	
8) Same-sexed, ABO discordant	0.5			(+)	
9) Same-sexed, ABO concordant	0.1			— —	
11) All-female pairs	5			—	
13) All-female, ABO concordant	1			— —	
15) All-female, concordant as to all criteria	5			(—)	
17) All-male, ABO discordant	5			(+)	

Table 6. *Differences (χ^2 and main contributions thereof) between various twin subsamples and the corresponding frequencies in the "Rome area" controls (see text)*

Subsample	χ^2_3%	Main contributions			
		A	B	AB	O
1) All pairs	0.1	++			—
2) Discordant as to ABO	0.1			+++	—
3) Concordant as to ABO	0.1	++		—	(—)
5) Concordant as to all criteria	0.1	++			—
7) All same-sexed	0.1	++			—
8) Same-sexed, discordant as to ABO	0.1			++	(—)
9) Same-sexed, concordant as to ABO	0.1	++		—	(—)
11) All-female pairs	1	+			
13) All-female, concordant as to ABO	1	+		—	
15) All-female, concordant as to all criteria	1	+			
16) All-male pairs	5	+			(—)
17) All-male, discordant as to ABO	0.1			+	
18) All-male, concordant as to ABO	1	+			
20) All-male, concordant as to all criteria	1	+			(—)

tends to maximum decrease in same-sexed (especially female) concordant pairs, while it increases in pairs discordant as to ABO phenotype only.

Table 6 again indicates a significant difference between the frequencies observed in our sample and those expected in the hypothesis that the reference population corresponds to the data listed by Mourant for the Rome area. The main differences are:

— the decrease in the observed frequencies of group AB in concordant (i.e., mainly MZ) pairs is confirmed, with the additional consistent finding of an increase of group A in the same pairs;

— the tendency to increased observed frequencies of group AB in discordant (i.e., DZ) pairs is also confirmed;

— the frequency of group O tends to a general decrease;

— the frequency of group B shows no deviations;

— differences between male and female subsamples is confirmed.

At this point the need for the definition of the control group, and especially of the parental distribution, is again evidenced. Until such time as funding of the proposed plan is obtained, we can only try to verify whether one of the two hypotheses as to control distribution shows better fit. For this purpose we have estimated the ABO phenotype distribution in a theoretical twin sample composed of 30% MZ, 35% same-sexed DZ and 35% opposite-sexed DZ pairs, the respective distributions of ABO phenotypes being proportional to the corresponding experimental frequencies in our sample. In Table 7 we have matched this theoretical sample against each of the two already

Table 7. *Differences in distribution of ABO phenotypes between a "theoretical" twin sample (30% MZ, 35% opposite-sexed DZ, 35% same-sexed DZ) and: (1) the "1969" controls; (2) the "Rome area" controls (see text)*

	"Theoretical" twin sample	"1969" controls	χ^2_3	"Rome area" controls	χ^2_3
A	398	387	0.30	359	7.64
B	107	112	0.23	105	0.03
AB	43	46	0.20	35	1.48
O	452	455	0.01	501	5.31
Total	1000	1000		1000	
			0.74		14.46

mentioned "control" groups. No differences are found between our "theoretical" twin sample and our 1969 control group, while an increase of group A and a decrease of group O is found in twins when compared to the "Rome area" group.
In both cases the frequency of group AB is as expected, confirming that MZ and DZ pairs deviate in opposite directions which seem to compensate each other in the twin population.
Without going into great detail in discussing our findings, which certainly require further analysis but already give quite definite indications, we believe we can state that the equilibrium of immunologically relevant genetic polymorphisms is altered in multiple births in general (as evidenced in MZ pairs) and further, different alterations are found in DZ pairs, in which the multiplicity of feto-maternal and possibly feto-fetal incompatibilities add to the complexity of the situation. We believe that precious indications in this respect are bound to come as a result of further analysis of our twin material.

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