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Blood Groups of Chinese in New York City: Application to Problems of Disputed Parentage

In a previous article [*1*], the A-B-O blood groups and subgroups, M-N types, Kell types, and Rh-Hr types were presented for 400 Chinese living in New York City. After this report appeared an additional 546 Chinese persons were typed, and the purpose of the present article is to report the combined results for the entire series of 946 individuals, with special reference to certain aspects of the blood group findings which appear to be peculiar to Chinese and constitute a source of error in medicolegal tests for disputed parentage.

Materials and Methods

The individuals tested for the present investigation presented themselves for blood grouping tests because they were required to do so by the Department of Immigration in connection with their petitions for derivative citizenship. In most cases, only the parents or the children could be tested, because the remainder of the family was living in Hong Kong, though a moderate number of complete families were also tested. In order that the series of individuals constitute a random one, for the purpose of this report only the parents have been included in the analysis, or, where the parents were in Hong Kong and the children in New York City, only the oldest child of the family has been included in the series. The great majority of the individuals tested spoke Cantonese and came originally from Southern China.

All the blood specimens were drawn by venipuncture performed by the author, and each was tested for its A-B-O group and subgroup, M-N type, Rh-Hr type, and the Kell factor. All tests were done in duplicate, and repeated on a subsequent day again in duplicate, so that every test was done at least four times. In the very rare instances where there were discrepancies in the findings, further tests were carried out until the reason for the discrepancy was determined and cleared up. Furthermore, the A-B-O blood grouping results were verified by tests on the oxalated plasma against known cells of groups O, A_1, A_2, and B. The A-B-O blood grouping tests were carried out on well slides, using high-titered anti-A and anti-B sera[2] from stimulated donors, and the A_1-A_2 subgrouping tests were also carried out on well slides, using absorbed human group B

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[2] To avoid ambiguity, boldface is used for symbols representing serological specificities (blood factors), *italics* for symbols representing genes and genotypes, and regular type for symbols representing agglutinogens, phenotypes, and blood group systems.

serum and anti-A_1 lectin (*Dolichos biflorus*). All the blood specimens were also tested by the tube method with anti-H lectin (*Ulex europeus*) [2].

The M-N tests were carried out with anti-M and anti-N reagents prepared from rabbit immune sera, and the N tests were confirmed with anti-N lectin (*Vicia graminea* and/or *Vicia unijuga*) [3]. The well-slide method was also used from the M-N tests, except that the tests with *Vicia graminea* lectin were done in tubes at body temperature.

For the Rh-Hr tests human antisera of specificities anti-Rh_0, anti-rh′, anti-rh″, and anti-hr′ were used routinely, but only rh″-positive red cells were tested with anti-hr″ serum in order to conserve the supply of that reagent. Moreover, whenever the findings indicated the desirability of tests with anti-hr serum, these were carried out. Two different anti-rh′ reagents were used; both were actually blocked anti-Rh_0' sera, so that these tests were done by the saline agglutination method. Two anti-rh″ reagents were used; one was a blocked anti-Rh_0'' serum and the other a "pure" anti-rh″ reagent used by the ficinated cell method. Of the anti-Rh_0 reagents, one was a saline agglutinating serum while the others had to be used by the ficin method. All blood specimens giving negative reactions for Rh_0 in those tests were further tested by the antiglobulin method.

Full details concerning the saline agglutination method, ficinated red cell method, and the antiglobulin test used in this investigation can be found in the recent book of Erskine [4].

Results

A-B-O Blood Groups

In Table 1 are given the A-B-O blood groups of the 946 New York City Chinese, in comparison with the blood groups of 4648 Southern Chinese tested by Mackay et al [5] in Hong Kong. All the group A and group AB Chinese tested by the present author were subgroup A_1; no Chinese of subgroups A_2 or A_2B were encountered among the 946 Chinese tested. In the series of Mackay et al, tests for the subgroups of A were not carried out.

TABLE 1—*Comparison of the distributions of the A-B-O blood groups in Chinese from New York City and from Hong Kong.*

Series	O	A	B	AB	Totals
New York City					
Previously reported [1]	172	108	101	19	400
New series	223	145	139	39	546
Totals	395	253	240	58	946
Total Percent of Group	41.75	26.74	25.37	6.13	
Hong Kong					
Mackay et al [5]	2025	1127	1233	263	4648
Total Percent of Group	43.57	24.25	26.53	5.66	

The gene frequencies for the series of 946 Chinese from New York City were calculated by using the usual square root formulae [6]. Each of these first estimates of the gene frequencies was then divided by the sum of those first estimates, and the estimated gene frequencies were thus corrected so as to add up to 100 percent. These calculations,

as well as all the other calculations of chi squares, etc, were greatly facilitated by the use of the Texas Instruments SR-10 pocket computer. No attempt was made to determine the "maximum likelihood" estimates of the gene frequencies, because this would have entailed much more effort and for reasons given elsewhere [7]. The adjusted gene frequencies determined from the square root formulae were: $p = 0.1813$, $q = 0.1730$, and $r = 0.6457$, where p, q, and r represent the frequencies of the alleles A^1, B, and O, respectively. When these estimates of the gene frequencies were then used to calculate the expected numbers of Chinese of groups O, A_1, B, and A_1B, the test for goodness of fit showed $\chi_{(1)}^2 = 0.0304$, so that $0.80 < P < 0.90$, indicating the presence of Hardy-Weinberg equilibrium in the series of Chinese tested by the present author.

In comparison, the estimated gene frequencies for the 4648 Chinese from Hong Kong tested by Mackay et al [5] proved to be $p = 0.1633$, $q = 0.1771$, and $r = 0.6595$, and $\chi_{(1)}^2 = 0.1372$, $0.80 < P < 0.90$, again indicating the presence of Hardy-Weinberg equilibrium. When the distribution of the four A-B-O blood groups in the 946 Chinese from New York City was compared with the distribution among 4648 Hong Kong Chinese, the chi square test showed $\chi_{(3)}^2 = 3.21$, so that $0.30 < P < 0.40$, indicating the absence of any statistically significant difference between the two distributions. While the Hong Kong series was not tested for the subgroups of A, there is no reasonable doubt that tests on that series also would have showed only subgroup A_1.

M-N Types

In Table 2 is shown the distribution of the M-N types in the series of 946 Chinese from New York City. The most interesting and important finding was the significant percentage of individuals having a weakly reactive agglutinogen N, namely, 1.79 percent Chinese of type MN_2. In the earlier report of Mackay et al [5] on 4648 Chinese in Hong Kong, no mention is made of the existence of type MN_2. One may therefore assume that because of the use of anti-N reagents which failed to agglutinate type MN_2 red cells, in the Hong Kong series all individuals of type MN_2 must have been classified as type M. If, similarly, the type MN_2 individuals found in the present study are classified instead as type M, and the distribution of the three M-N types resulting is compared with the distribution in the Hong Kong series, the differences prove to be not significant statistically: namely, $\chi_{(2)}^2 = 0.29$, so that $0.50 < P < 0.60$. Thus, as for the A-B-O blood groups so also for the M-N types—the distribution in the present series of Chinese from New York City proves to be virtually the same as for the Chinese tested by Mackay et al in Hong Kong.

TABLE 2—*Comparison of the distributions of the M-N types in Chinese from New York City and from Hong Kong.*

Series	M	N	MN_1	MN_2	Totals
New York City					
Previously reported [1]	141	58	192	9	400
New series	180	90	268	8	546
Totals	321	148	460	17	946
Total Percent of Group	33.93	15.64	48.62	1.79	
Hong Kong					
Mackay et al [5]	1651	760	2237	...	4648
Total Percent of Group	35.53	16.35	48.13	...	

Mistyping of type MN_2 blood as type M must be avoided, however, because it can give rise to injustice in forensic cases of disputed parentage, as almost occurred in the following example.

Case 1702—In this case the petitioner, a married Chinese woman living in New York City, wished to bring her father and mother, both living in Hong Kong, to New York City. However, her application for derivative citizenship on behalf of her parents was denied because the serologist who had typed her parents' bloods in Hong Kong reported them both to be type M, while the serologist in New York City reported the petitioner herself to be type MN, indicating that she was not the daughter of her reputed parents in Hong Kong. When the case were referred to the present author for solution, he arranged to have blood samples of the putative parents sent to him from Hong Kong, so that he could test them in parallel with the daughter's blood. The blood specimens were shipped by air freight and arrived within 48 hours in perfect condition, having been refrigerated in transit. Comparative tests on the blood samples from all three individuals were then carried out, with the following results.

Blood of	A-B-O	M-N	Rh-Hr
Putative father	B	MN_2	Rh_1rh
Putative mother	O	M	Rh_1Rh_2
Daughter	O	MN_2	Rh_1Rh_2

As can be seen, the results did not exclude parentage; instead, the finding of the relatively rare type MN_2 in both the putative father and the daughter provided strong circumstantial evidence that the couple in Hong Kong actually were the parents of the daughter living in New York City. When this report was submitted to the Department of Immigration, the petitioner's application on behalf of her parents in Hong Kong was finally approved.

Calculation of the gene frequencies for the series of 946 Chinese from New York City shows: $M = 0.5914$, $N^1 = 0.3940$, and $N^2 = 0.0146$. [The frequency of gene M was determined by direct count; the frequency of gene N^1 was taken equal to $(460/477)(1 - M)$, and the frequency of gene N^2 equal to $(17/477)(1 - \bar{M})$.] These estimates of the gene frequencies were used to calculate the expected numbers of individuals of each M-N type, and the results, $\chi_{(2)}^2 = 1.76$, $0.4 < P < 0.5$, indicated that the population was in Hardy-Weinberg equilibrium. For the Hong Kong population, $\chi_{(1)}^2 = 0.002$, $0.90 < P < 0.95$, which again indicated the presence of equilibrium.

Rh-Hr Types

In Table 3 are given the results of tests for the Rh-Hr types on the series of 946 Chinese from New York City. For comparison are also given the previously reported observations of Mackay et al [5] for 4648 Chinese from Hong Kong. Comparison of the two distributions gives $\chi_{(10)}^2 = 12.09$, so that $0.20 < P < 0.30$, indicating (as for A-B-O and M-N) a close similarity between the distributions of the Rh-Hr blood types in the two populations.

Inspection of Table 3 shows that the most striking differences between the Rh-Hr blood types of Chinese and Caucasians are the low frequency in Chinese of type rh (only about 1 among 1400 in Chinese as against 15 per 100 in Caucasians), the apparent absence of type rh″ in Chinese, and the much higher frequency in Chinese of the rare agglutinogen Rh_Z (and rh_y), namely, as many as 1 in 80 Chinese of type Rh_ZRh_1.

TABLE 3—*Comparison of the distribution of the Rh-Hr types in Chinese from New York City and from Hong Kong.*

Series	rh	rh′rh	rh_yrh'	Rh_o	Rh_1Rh_1	Rh_1rh	Rh_2Rh_2	Rh_2rh	Rh_zRh_o[a]	Rh_zRh_1	Rh_zRh_2	Totals
New York City												
Previously reported [1]	0	0	0	1	213	30	19	6	126	4	1	400
New series	2	1	0	2	293	40	23	15	158	11	1	546
Totals	2	1	0	3	506	70	42	21	284	15	2	946
Total Percent of Group	0.21	0.10	0	0.32	53.49	7.40	4.44	2.22	30.02	1.59	0.21	
Hong Kong												
Mackay et al [5]	2	7	1	11	2640	388	208	99	1224	65	3	4648
Total Percent of Group	0.04	0.15	0.02	0.23	56.80	8.35	4.48	2.13	26.33	1.40	0.06	

[a] Type Rh_zRh_o includes type Rh_1Rh_2 (hr negative) and type Rh_zrh (hr positive). Tests with anti-**hr** serum were not done routinely to subgroup blood of type Rh_zRh_o in order to conserve the supply of this rare and valuable reagent. Anti-**hr** was used only in cases where the constellation of Rh-Hr types in parents and children indicated that the test would be rewarding, bearing in mind that in Chinese as well as Causcasians almost all individuals of type Rh_zRh_o belong to subtype Rh_1Rh_2.

In a previous article [8] it was pointed out that many of the commercially available anti-rh′ sera are really of the specificity anti-rh$_i$ and that this could be a cause of errors in Rh-Hr typing, because with such anti-rh$_i$ reagents, type Rh_Zrh blood would be misclassified as type Rh_2rh, while type Rh_ZRh_2 blood would be misclassified as type Rh_2Rh_2. In fact this must have occurred in the series of Chinese reported from Hong Kong, because as shown in Table 3, only three type Rh_ZRh_2 were reported for that series whereas as many as 17 were to be expected, as estimated from the gene frequencies. In a previous article [8] a number of medicolegal cases of disputed paternity were presented in which mistakes in Rh-Hr typing of this nature gave rise to false exclusions of paternity, which would have led to miscarriage of justice had the errors not been detected in time. Another such case is described below which occurred only recently.

Case 4216—In this case, the mother and children were living in New York City, and the father in Hong Kong. The petition of the family to bring the father to New York City was denied on the basis of the report shown below.

Blood of	Anti-A	B	M	N	C	D	E	c	e	Blood formula
Putative mother	+	—	+	+	+	+	+	—	+	A MN CDE/CDe (R_ZR_1)
Daughter	—	—	+	—	—	+	+	+	+	O M cDE/cde (R_2r)
Putative father	—	—	+	+	—	—	—	+	+	O MN cde/cde (rr)

According to the pathologists in Hong Kong and New York City who submitted these reports, maternity was excluded because the mother was reported to be type CC and the daughter type cc. No notice appeared to have been taken of the fact that since the mother was type Rh_ZRh_1 she had to be a carrier of the rare gene R^z (or r^y), which she could transmit to her children, and which could be a source of error in typing. Thus, if serum of specificity anti-rh$_i$ was used instead of anti-rh′, the daughter, if she was really type Rh_Zrh, could have been misclassified as type Rh_2rh.

The family then consulted an attorney who referred them to the present author for help in solving their problem. When the mother and daughter reported for the blood tests, it was noticed that another young woman had accompanied them. Inquiry revealed that she was a younger daughter, so blood was drawn from her for testing as well as from the mother and daughter in question. The blood of the putative father, who was in Hong Kong, could not be retested, but as it turned out his blood was actually not needed to resolve the problem; in fact, the tests already made on his blood could be presumed to be correct. The findings, including for comparison the previously reported results for the putative father, proved to be as follows.

Blood of	A-B-O	M-N	Rh-Hr
Putative father	O	MN	rh
Putative mother	A_1	MN	Rh_ZRh_1
1st daughter	O	M	Rh_Zrh
2nd daughter	A_1	N	Rh_1rh

As can be seen, the daughter in question did prove to be type Rh_Zrh, so that the original report which gave her type as Rh_2rh was in error. Aside from its interest as an illustration of this important pitfall in the Rh-Hr typing of blood from Chinese, this case

is unique in that the father was type rh, an Rh-Hr type which is rare among Chinese, as shown in Table 3. Since the father is genotype *rr* and the mother genotype R^zR^1, half of the children would be expected to be type Rh_zrh and half type Rh_1rh, and indeed the older daughter was type Rh_zrh while the younger daughter was type Rh_1rh. Finally, mother and daughter were clearly both carriers of the rare gene R^z (or r^y), so that the blood findings, instead of excluding maternity, provided strong circumstantial evidence in support of maternity.

It is noteworthy that the pathologist who made the error in Rh-Hr typing in this case used the discredited, naive CDE notations. In a report [9] published in the *Journal of the American Medical Association* in 1957, it was recommended that in all medicolegal reports only the original, scientifically sound Rh-Hr nomenclature be used, to the exclusion of other "simpler" (actually naive) but fallacious notations, notably the CDE symbols. The continued use for as long as 17 years after the publication of this report of the pseudoscientific CDE notations is as reprehensible as would be the continued use of the Moss and/or Jansky Roman numerals for designating the A-B-O blood groups. Such naive notations not only render thorough mastery of the subject impossible, but also cause a lack of insight into the complexities of the subject which inevitably leads to mistakes, such as occurred in the case reported here.

With the aid of the square root formulae derived by Wiener [6], the frequencies of the various Rh-Hr alleles for the series of 946 Chinese from New York City were calculated, with the following results: $r = 0.0448$, $r' = 0.00063$, $r'' = 0$, $R^O = 0.0261$, $R^1 = 0.7068$, $R^2 = 0.2055$, and $R^z = 0.0157$. These estimates of gene frequencies were used to calculate the expected number of individuals of each Rh-Hr type, and then computation of goodness of fit gave $\chi_{(4)}^2 = 12.8$, and $0.01 < P < 0.02$. This poor fit proved to be due mainly to the difference between the expected number (95.7) and the observed number (70) of individuals of type Rh_1rh. This in turn proved to be due to the fact that the estimated frequencies for genes *r* and R^O were too high, due to the small number of individuals of types rh and Rh_0, the percentages of which were used to calculate the gene frequencies. Rather than resort to the maximum likelihood method of calculating gene frequencies, advantage was taken of the demonstration that there was no statistically significant difference between the distributions of the Rh-Hr types in the Chinese from New York City and from Hong Kong. Therefore, the two series of Chinese could be combined, and from the frequencies of the Rh-Hr types in the resulting combined series of 5594 individuals, the gene frequencies were estimated with the following results: $r = 0.0261$, $r' = 0.01908$, $r'' = 0$, $r^y = 0.0045$, $R^O = 0.0293$, $R^1 = 0.7108$, $R^2 = 0.2014$, and $R^z = 0.0089$. From these gene frequencies the expected number of individuals was calculated for the series of 946 Chinese from New York City, and the test for goodness of fit gave $\chi_{(4)}^2 = 5.05$, so that $0.2 < P < 0.3$. Thus, as to be expected, the series of 946 New York City Chinese was proved to be in Hardy-Weinberg equilibrium for the Rh-Hr types, as well as for the A-B-O groups and M-N types.

These observations and calculations do not reveal still another far more subtle pitfall in the application of the Rh-Hr types to problems of disputed parentage in Chinese. A case demonstrating this source of error has already been reported [10] and a second such case is presented below.

Case 4161—An attorney referred this immigration case because previous blood tests had indicated that the putative father in a Chinese family was not the actual father of his daughter. The tests were repeated by the present author, and when the father was found to be type Rh_1Rh_1 and his daughter type Rh_2Rh_2, this seemed at first to confirm the report of nonpaternity. The daughter was married and had four children. Because of the experience in the previous similar case [10], the author instructed her to return with

all her four children for typing. The combined results for the family proved to be as follows.

Blood of	A-B-O	M-N	Rh-Hr
Putative father	A_1	M	Rh_1Rh_1
Daughter	A_1	MN	Rh_2Rh_2
Daughter's children			
1st	A_1	M	Rh_1Rh_1
2nd	O	MN	Rh_1Rh_2
3rd	O	MN	Rh_1Rh_2
4th	A_1	MN	Rh_1Rh_2

If these findings were taken at their seeming face value, they could be interpreted as proving not only that the putative father was not the actual father of his daughter, but also that the daughter was not the mother of her own oldest child. Actually, the results provided the solution to the problem, since it now became apparent that instead of the usual genotype R^1R^1 the putative father belonged to the rare genotype $R^1\bar{\bar{R}}^O$. Moreover, the daughter's genotype was evidently $R^2\bar{\bar{R}}^O$ (instead of the usual genotype R^2R^2) while the daughter's oldest child evidently belonged to genotype $R^1\bar{\bar{R}}^O$ like the grandfather. Thus, the completed results of the family study, instead of excluding paternity, provided strong circumstantial evidence in favor of paternity, in view of the low frequency in the general population of the gene $\bar{\bar{R}}^O$ prevalent in this particular family.

Though individuals carrying the gene $\bar{\bar{R}}^O$ appear to occur with significant though low frequency among Chinese in New York City, this is not evident from the data on the distribution of the Rh-Hr blood types shown in Table 3. The reason for this is that only individuals of type $\bar{\bar{R}}h_O$ (that is, individuals homozygous for gene $\bar{\bar{R}}^O$) have blood that gives reactions clearly establishing the presence of the gene, without resort to family studies. Since gene $\bar{\bar{R}}^O$ appears to be even less common among Chinese than gene r, type Rh_O must be extremely rare. Heterozygous individuals of genotype $R^1\bar{\bar{R}}^O$ and $R^2\bar{\bar{R}}^O$ react in ordinary tests as type Rh_1Rh_1 and Rh_2Rh_2, respectively, and this can give rise to false exclusion of parentage as in the case just described.

Kell Types

In the previous series of 400 New York City Chinese there were only two Kell-positive individuals, while in the new series of 546 Chinese no additional Kell-positive persons were encountered. Thus, in the combined series of 946 Chinese, only 0.21 percent were Kell positive, yielding the gene frequencies $K = 0.0011$ and $k = 0.9989$.

No tests for Kell factor were carried out by Mackay et al on their series of Hong Kong Chinese, but presumably they would have obtained similar results.

Obviously, tests for Kell factor have hardly any value for problems of disputed parentage in Chinese, since only one among about 500 Chinese is Kell positive.

Summary

The distributions of the blood groups among 946 Chinese in New York City proved to be almost identical with the distributions previously reported for Chinese in Hong Kong. For the present series, the gene frequencies for the A-B-O blood groups were: $O = 0.6457$, $A^1 = 0.1813$, $A^2 = 0$, and $B = 0.1730$. For the M-N types the gene frequencies

were $M = 0.5914$, $N^1 = 0.3940$, and $N^2 = 0.0146$. For the Rh-Hr system the gene frequencies were: $r = 0.0261$, $r' = 0.0191$, $r'' = 0$, $r^y = 0.0045$, $R^o = 0.0293$, $R^1 = 0.7108$, $R^2 = 0.2014$, and $R^z = 0.0089$. For the Kell system the gene frequencies were $k = 0.9989$ and $K = 0.0011$. Chi square tests showed the New York City Chinese to be in Hardy-Weinberg equilibrium for the A-B-O, M-N, and Rh-Hr blood types. Comparison of the distributions of those three blood group systems showed no statistically significant difference between the New York City Chinese and a previously tested large series of Chinese from Hong Kong, in conformity with other evidence that both series of Chinese originated from the same area in Southern China.

The observations reported here demonstrate that the Chinese have a significant frequency of individuals carrying the genes N^2 and R^z (or r^y) both of which are much rarer among Caucasians. Illustrative cases are reported in which reports excluding parentage were shown to be in error because of mistakes in blood grouping caused by failure to take into account the possibility of occurrence of these rare genes. An even more subtle pitfall in parentage blood grouping tests in Chinese is shown to be due to the occurrence in Chinese of the rare gene $\bar{\bar{R}}^o$, so that individuals of phenotypes Rh_1Rh_1 and Rh_2Rh_2 who were presumed to be of the common genotypes R^1R^1 and R^2R^2 were shown by family studies actually to be of genotypes $R^1\bar{\bar{R}}^o$ and $R^2\bar{\bar{R}}^o$, respectively.

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