Frequency Distribution of Blood Groups ABO, MN and Rh Factor in Philippine Cosmopolitan, Regional, and the National Populations

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ABSTRACT

Frequency distribution of blood groups is important as it is used in modern medicine, genetic research, anthropology, and tracing ancestral relations of humans. Blood groups include the ABO, Rh and the MN red cell antigens. The frequency distribution of these three blood groups were obtained and assessed for differences from three populations: (1) a regional population from the town of Cabagan located in Isabela province; (2) a cosmopolitan population from the University of the Philippines' roster of students; and (3) the national population's data obtained from blood banks all over the Philippines. This study sought to determine the frequency distribution of ABO, MN, and Rh factor blood groups to establish whether there exist differences in distribution among the three population categories. Standard blood agglutination sampling was conducted in these populations to determine blood types. Chi-square tests on the genetic frequencies reveal that there is no significant difference in the distribution of blood groups ABO and Rh. The blood group MN, however, displayed twice as many M blood type in the regional population than in the cosmopolitan population. This suggests a localized segregation of alleles responsible for the MN blood type within distinct populations in the Philippines. Computation of the allelic frequencies also revealed that both populations are not at Hardy-Weinberg equilibrium based on the distribution of the different MN blood types.

Keywords: genetic frequencies, ABO, MN, Rh factor, Hardy-Weinberg equilibrium

INTRODUCTION

Among the various factors that contribute to a person's individuality are antigens attached to surface of red blood cells and naturally occurring antibodies that circulate in the serum. The ABO blood group and the Rhesus Factor or Rh blood group are two of the most notable type groups in humans due to their importance and association with blood transfusion (Khattak, et al. 2008). The mode of inheritance of the ABO blood group follows the multiple allelic mode of inheritance and is quite stable to be used to exclude paternity in paternity issues. The Rh antigen is named after the rhesus monkey, *Macaca mulatta* (Zimmerman) where it was initially detected. The mode of inheritance of these antigens is complex, and there are two

theoretical models that attempt to explain the pattern of inheritance. The Wiener system postulates a single gene locus with a series of at least ten multiple alleles. The Fisher system assumes the existence of at least three closely linked loci designated as C, D, and E. Both are currently in use and are still being studied. However, only the presence of the D antigen in the Fisher system serves as the basis for classification of the Rh blood group; this way, the mode of inheritance is simply single gene inheritance with accompanying dominance. The most notable medical importance of this blood group system is the occurrence of Rh incompatibility between mother and fetus, which is a major factor in the development of erythroblastosis fetalis or hemolytic disease of the newborn (Dennis et al., 1998). M and N blood group antigens are presented by glycophorins A (GPA) and B (GPB) of the erythrocyte membrane. GPA expresses M or N blood group antigen depending on the allelic gene (GPAm gene or GPAn gene), while GPB expresses only the N antigen. M or N blood group is specified by the first and fifth NH2-terminal amino acid residues in the mature proteins that are encoded by the second exon of these genes (Kudo & Fukuda, 1994). The agglutinogens M and N are inherited as a single pair of allelomorphic genes (Hyman, 1942), and the M and N alleles are codominant to one another. This allows determination of the allelic frequency of the M and N alleles with relative ease. Many studies of human genetics have used the MN system because it is possible to distinguish the heterozygote MN from both homozygotes MM and NN. Additionally, there does not appear to be any selection pressure against either allele. Thus, the MN system is a good test of the Hardy-Weinberg Law.

These three blood groups have their genetic components confined to different regions. The ABO locus is located on chromosome 9, specifically, in the segment 9q34.1-q34.2 (Narahara et al. 1986). The human blood group Rh polypeptide has been used to map the Rh locus, by in situ hybridization, to the region p34.3-p36.1 of chromosome 1 (Cherif-Zahar, 1991). Lastly, the locus of the gene responsible for the MN antigen activity is confined to chromosome 4, in the 4q28.2–4q31.1 segment (Wakui, 1990). Since these genes are not linked, it is assumed that the behaviour of the genes responsible for the blood groups will not affect the other blood groups' expressivity in individuals.

The Hardy-Weinberg model describes a mathematical relationship that allows the prediction of the frequency of offspring genotypes based on parental allele frequencies. It also predicts that allele frequencies will not change from one generation to the next, indicative of non-evolution (Klug & Cummings, 2002; Mayo, 2008). For a population to be in Hardy-Weinberg equilibrium, the following assumptions are required to hold: random mating, no mutation, no migration, no stochastic effects or genetic drift due to small population size, and equal fertility for all genotype groups so that no selection is occurring (Minelli et al. 2008). Violation of any of these assumptions can result to evolutionary change in terms of allelic frequency distribution (Mayo, 2008). These conditions, however, seldom occur simultaneously, resulting to most populations not exhibiting Hardy-Weinberg equilibrium and are therefore evolving.

All human populations share the same blood group systems, differing only in the frequencies of specific types. The incidence of ABO, Rh snd MN groups varies in different parts of the world and in different races (Khattak et al. 2008, Thamaria et al. 1972). Assessing blood group frequency distribution is multipurpose, as beside their importance in evolution, their relation to disease and environment is being increasingly sought out in modern medicine (Khattak et al. 2008). Blood group antigens are not only crucial in the medical field, but can also be utilized in genetic research, anthropology, and tracing ancestral relation of human (Khan et al. 2004).

It is predicted that the inhabitants of a rural town with a regional population and considerably less human migration in and out of the area than a cosmopolitan location, would maintain significantly different genetic frequencies. To test this, we performed blood type tests for ABO, MN and Rh blood groups on individuals from a rural town and compared them with results taken from Metro Manila, capital city of the Philippines, as well as national data collected from blood banks across the country. The main objective of this paper was to identify frequency distribution differences in the ABO, Rh and MN blood groups among a local population, a "mixed" population and the collective

national data. Patterns of local distribution were then compared to the global data available.

MATERIALS AND METHODS

Sample Sites Description

Blood samples for a regional population were obtained from the rural town of Cabagan, located in the province of Isabela. Adjacent towns include Santa Maria, San Pablo and Tumauini. Cabagan is a relatively small town approximately 465 kilometres from Metro Manila with a population size of 43,562 individuals according to the 2007 government census. The population is comprised mostly of people belonging to the Ybanag ethnolinguistic minority. For the cosmopolitan population, undergraduate and graduate students in the University of the Philippines in Diliman were requested to donate blood samples. The working assumption is that University students come from different provinces, and are thus distributed in a random fashion contributing to a mixed cosmopolitan population. The collective national data on ABO and Rh blood group distribution was acquired through the Philippine National Red Cross Main Office located in Gen. Luna cor. Victoria St., Intramuros, Manila. Data were obtained from blood bank databases.

Blood Sample Collection

Sample collection was standardized to at least 50 donors in order to get statistically sound data. A total of 110 students from two participating universities were screened for their respective ABO, MN, and Rhesus (Rh) factor blood types. Fifty-one blood samples were taken from 31female and 20 male students of the University of the Philippines-Diliman (UPD). Fifty-nine blood samples were taken from 20 female and 49 male students of the Isabela State University (ISU), Garita Heights Campus, Cabagan, Isabela. To comply with the requirement that blood sample donors be genuine Ybanag, each student from ISU was interviewed for his/her ethnicity. The interview clarified questions of ethnicity by tracing the residence of the students' parents and grandparents.

Blood Typing

Five drops of blood were obtained from each donor by pricking the tip of the index finger with a sterile lancet. Each drop of blood was placed on a spot plate containing a blood typing anti serum. The following monoclonal antibodies were used: EryCloneTM Anti-A and Anti-B of Tulip Diagnostics Ltd. (India) for the ABO blood type; Epiclone-2 Anti-D of CSL-Australia for the Rh group; and Anti-M and Anti-N of Cypress Diagnostics (Belgium) for the MN blood group. Agglutination of the blood drop with the five test sera was then assessed by gently probing through the mixture using a lancet. Blood drops exhibiting a clotting reaction with the test sera were considered positive for that particular blood grouping reagent. Each reaction and corresponding blood types for each blood donor was recorded and subjected to pooling and statistical analysis.

Statistical analysis

To determine the degree of association, if present, among the different categories in each blood type, genotypic frequencies from the cosmopolitan (UPD), regional (ISU) and national (PNRC Intramuros Chapter) populations were subjected to Chi-square tests. All statistical tests were conducted using the Statistical Package for Social Sciences (SPSS) version 15.0 (Chicago, Il, USA). Significant differences were determined at $P \leq 0.05$. For the MN blood group, allelic frequencies were computed, and then factored in the Chi-square test that was used to verify whether the participating populations are in Hardy-Weinberg equilibrium. Likewise, P values less than 0.05 were considered statistically significant.

RESULTS

The blood group ABO is randomly and independently distributed in the cosmopolitan (UPD), regional (ISU) and national populations.

A common pattern on the distribution of blood types among the three populations is seen in Figure 1 wherein blood type O is most prominent, followed by blood type A, blood type B, then the least common blood type AB. Pearson chi-square values comparing the UPD and the ISU populations to that of the national confirmed no positive association between the frequency distribution of blood groups and the location of sampling.

However, verification by allelic frequencies for the blood types cannot be carried out, as it is not known whether blood types A and B are homozygous or heterozygous.

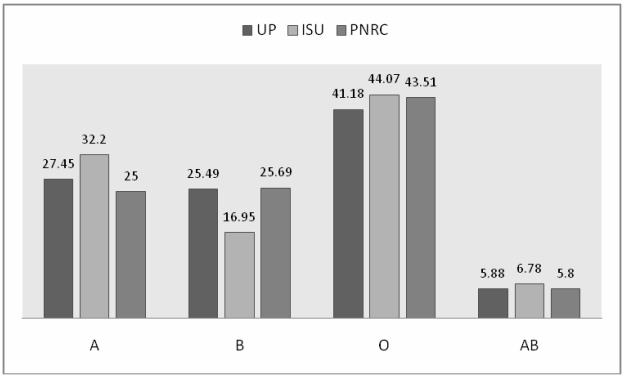

Figure 1. Percentage distribution of ABO blood group in three populations. UPD = University of the Philippines, Diliman; ISU = Isabela State University, Cabagan, Isabela; PNRC = Philippine National Red Cross national data. General trend for all three populations consist of blood type O having the highest frequency, followed by type A, then type B then lastly, type AB.

There is no significant difference in the frequency distribution of the blood group Rhesus factor among cosmopolitan (UPD), regional (ISU) and national populations.

A seen in Figure 2, the distribution of the Rh types in the three populations are all consistent, with Rh+ being the more dominant allele and Rh- expression being almost negligible. The two smaller populations (UPD and ISU) corroborate the pattern set by the national population in that Filipinos, in general, are Rh+.

There is a significant difference in the frequency distribution of blood group MN in cosmopolitan (UPD) and regional (ISU) populations.

The distribution of the MN blood groups is shown in Figure 3. Blood type MM is seen as the predominant blood type in the regional population (72.88%) as opposed to the cosmopolitan population (37.25%) wherein the distribution of the three blood types is more closely associated. Allelic frequencies were also computed; with the M allele occurring at 78.8% in the ISU population, far higher than 52.0% for the UPD population. Statistical analysis showed that, using the computed allelic frequencies, the cosmopolitan and the regional populations were both not in Hardy-Weinberg equilibrium with P values less than 0.05 and 0.01, respectively (Table 1).

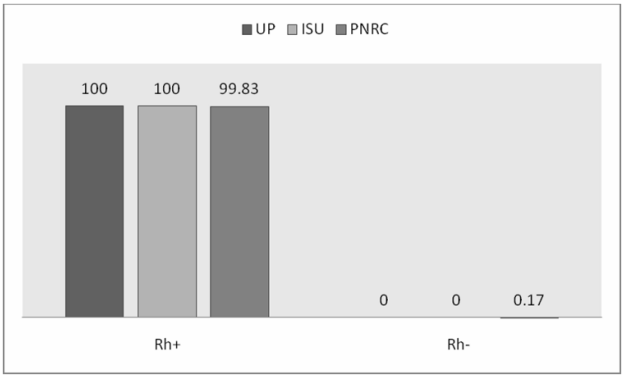

Figure 2. Percentage distribution of Rhesus factor blood group in cosmopolitan, regional and national populations. UPD = University of the Philippines, Diliman; ISU = Isabela State University, Cabagan, Isabela; PNRC = Philippine National Red Cross national data. Occurrence of type Rh- among the three population data is negligible.

DISCUSSION

The immune system is accountable for the success or failure of blood transfusions. It is of utmost importance that the donor blood cells match that of the recipient; otherwise, donor blood cells may be destroyed by antibodies present in the plasma of the recipient. The ABO system is the main blood group considered in such procedures (Adeyemo & Soboyejo, 2006). The results of this study present data indicative of similar, random segregation of the different blood types of this system in regional, cosmopolitan and the overall population. Chi-square testing confirmed no positive association of ABO genotypic frequency between populations in Metro Manila, Cabagan town and nationwide Philippine population. This was despite the fact that only 16% of subjects from Isabela were type B, as compared to 26% for the nationwide data. The O blood type has the highest percentages in the three data sets, followed by A and B types, and the AB type having the smallest proportion (Figure 1). Interestingly, the same trend of ABO blood group distribution is also seen in the American Indian population in Cherokee, USA, in the African American population in St. Louis, USA, and in the Chinese population in Hong Kong (Mourant, 1976). This trend, however, is not representative of the majority of populations surveyed (Americans, French Canadians, Japanese,

Palestinians, Jews and Eskimos), with blood type A having the greatest proportion followed by type O, type B, and type AB (Mourant, 1976).

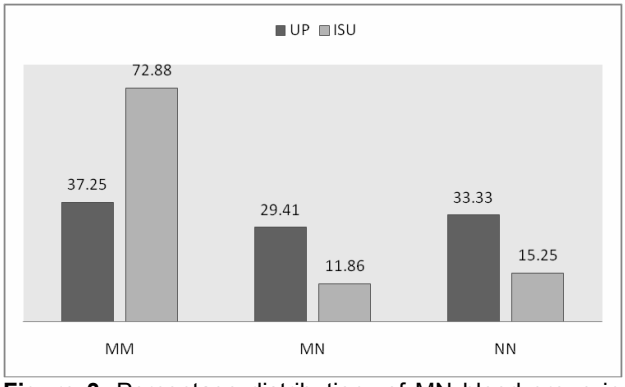

Figure 3. Percentage distribution of MN blood group in cosmopolitan (UPD) and regional (ISU) populations. UPD = University of the Philippines, Diliman; ISU = Isabela State University, Cabagan, Isabela; PNRC = Philippine National Red Cross national data. Frequency distribution of the MN blood group types differs significantly between the regional and cosmopolitan populations.

Most antigens from the blood group Rh are weak and do not really elicit antibody production. The exception is the D antigen, which is strong and is likely to cause transfusion problems. Thus, the classification of being Rh+ or Rh- is dependent on the presence of this particular antigen (Klug & Cummings, 2002). As such, this blood group is considered to be a case of single gene inheritance with dominance. The three sample populations yielded similar results; all subjects sampled in Cabagan and UP Diliman were Rh+ and the national data showed an insignificant percentage of Rh- individuals (Figure 2). These outcomes indicate that the recessive allele for the D antigen is very rare in the Philippine population. When compared to global data presented by Mourant (1976) and Khattak (2008), the Rh factor data from this study follows the same pattern found in all the populations surveyed, with a minor Rh- type representation with a range of 0% to 17%. These populations include American Indians, Arabs, Bengalis, Africans, Chinese, Eskimos, Mexicans, and Americans.

The MN blood group system is of little apparent importance in cases of transfusion reactions or maternal-fetal incompatibilities because few people produce anti-M or anti-N even after repeated exposures to the antigens (Weder et al. 1991). Data from the MN blood group illustrate different results from that found in ABO and Rh blood groups; the frequencies of the MN genotypes of the populations in Cabagan, Isabela (ISU) and Manila (UPD) were found to have positive association (Figure 3), suggesting that the two populations are distinct (Klug & Cummings, 2002). Moreover, comparing the calculated gene frequencies for the M and N alleles for both populations reinforce the positive association. The data taken from the ISU population, with the allelic frequency of M tripling that of the allelic frequency of N, is similar to the MN data obtained from the more isolated populations such as the Apache Indians in the USA and some groups in Mexico City and Iran (Mourant, 1976). The data obtained from the cosmopolitan setting of Manila produced similar proportions of allelic frequencies of M and N with respect to those of the African-Americans, Caucasians and Chinese populations in the USA (Mourant, 1976).

To assess the state of the population in terms of the Hardy-Weinberg Equilibrium, the M and N allelic frequencies of the regional and cosmopolitan populations were subjected to the Hardy-Weinberg Law. Computations indicate that both populations were not in equilibrium (Table 1). For the cosmopolitan population, it is postulated that this condition is accounted for by gene flow facilitated by migration. Residents of the cosmopolitan population tend to originate from different places in the country, giving the population the characteristic of an increased rate of migration that leads to hybridization. New genes are passed into the gene pool of the parental populations. This intogression results in gene flow, hence the violation of the Hardy-Weinberg Law (Minelli et al. 2008). The regional population, on the other hand, displays little migration. However, compared to the cosmopolitan population with a population of about 20 million, the regional population is much smaller in size. Hardy-Weinberg equilibrium might not have been achieved due to possible interbreeding in the members of the local population. The frequency distribution of M and N alleles, with the M allele being more dominant, further reinforces this idea of genetic drift, as stochastic effects usually leads to the frequency alleles drifting toward higher or lower values (Wang et al. 1998; Trikalinos et al. 2008). Both the cosmopolitan and regional populations' violation of the Hardy-Weinberg Law indicate

Table 1. Chi square tests for significant difference from the expected blood group distributions of populations at Hardy-Weinberg equilibrium (HWE). Both populations are not at HWE equilibrium.

	UPD (M=52.0%; N=48.0%)			ISU (M=78.8%; N=21.2%)		
	Observed (o)	**Expected (e)**	**(o-e)2/e**	**Observed (o)**	**Expected (e)**	**(o-e)2/e**
MM	19	13.79	1.97	43	36.64	1.11
MN	15	25.46	4.3	7	19.71	8.2
NN	17	11.75	2.35	9	2.65	15.2
Total	51		χ^2 = 8.6102	59		χ^2 = 24.5021
Conclusion	P<0.05; significantly different from expected HWE			P<<0.01; significantly different from expected HWE		

evolution at some level but is likely to be caused by different reasons.

Studies have shown some medical implications of the different blood types in the MN blood group system. The MN blood group is most commonly associated and is a good candidate for future studies on the genetic basis of human essential hypertension (Miller et al. 1979; Heise et al. 1987). Moreover, Weder et al. (1991) reported results that indicate the possible relationship of the MN blood group antigen to systolic blood pressures but with effects that are significantly different and oppositely directed in men and women. Lastly, the study by Delanghe et al. (1995) links the distribution of the MN phenotypes according to age with the diagnosis of essential hypertension, suggesting that the MN phenotype, indeed, is a genetic factor associated with early detection of essential hypertension.

We report in this paper the blood type frequencies for multiple blood groups of a regional population in Isabela province in Northern Luzon, Philippines. No significant differences were found in the ABO and Rh blood groups' frequency distribution compared to the cosmopolitan and national data. Based on data gathered for the MN blood group, however, we conclude that this population is distinct from the population in the capital city of Manila. Both populations showed departure from the Hardy Weinberg Equilibrium, which could be attributed to migrations for the UPD population and genetic drift for the ISU population.

It is recommended that further surveys on the distribution of the MN blood group system be conducted on the different populations to determine if there is a trend in the regional variation of the M and N alleles across the country in order to shed light on the behaviour of these genes at the population level. Data on hypertension and age of diagnosis should also be obtained from the corresponding populations to confirm or contradict the medical implication of the MN blood group.

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