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Evaluating the Prevalence of Five Genetic Traits of Simple Inheritance in Association with the Distribution Pattern of ABO and Rhesus Phenotypes among Families in Calabar, Nigeria

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Morphological and behavioral genetic traits of simple inheritance indicate ethnic variation and have been widely employed in population variation studies. Five selected genetic traits of simple inheritance, ABO and Rh (D) blood phenotype was investigated to establish the inheritance pattern, prevalence and to assess association between the traits, ABO rhesus phenotypes and gender of 45 unrelated families living in Calabar. Blood group O was the most prevalent (55.2%) followed by B (21.6%), A (18.8%) while the least was AB (4.4%). The majorities (91.6%) were Rh (D) positive and 8.4% were Rh (D) negative. The frequency of the 5 genetic traits were earlobe (69.2% free earlobe, 30.8% attached earlobe), hand-clasping (51.6% right hand-claspers, 48.4% left hand-claspers), dimples (21.2% had facial dimples, 78.8% had no dimples), mid-digital hair (presence in 92%, absence in 8%) and tongue rolling (48.4% rollers, 51.6% non-roller). There was association between earlobe and blood group, sex and hand clasping, sex and facial dimples and between tongue rolling and sex. Chi-square analysis of the inheritance pattern of these genetic traits provides strong evidence for a familial and probably a genetic component in the control of these traits. The pattern also showed that the traits are inherited in dominance versus recessive manner.

Key words: Genetic, traits, inheritance, rhesus (D), ABO, phenotypes

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INTRODUCTION

There are over 200 traits that are transmitted from generation to generation in humans. This interesting aspect of human genetics is known as hereditary traits and includes dominant and recessive traits in humans. The physical traits are observable characteristics determined by specific segments of DNA called genes which makes every individual an 'Individual' (Batul, 2010). Morphological and behavioral genetic traits indicate ethnic variation and based on somastoscopy, mankind is discerned into primary racial types. This diversity among humans provides a unique opportunity to study the morphogenetic variation among the endogamous populations living in different geographical and ecological conditions (Bhasin and Khanna, 1994). The existence of genetic variation in man is caused by many factors among which selection; migration, gene flow and genetic drift are the most important (Bhasin *et al*., 1992). Over years, blood groups have been projected as a useful marker in studying variations in family, linkage analysis and population study. Blood groups are inherited from both parents and approximately 300 different types of blood groups are identified so far, however, the Rh and ABO antigens are still the clinically most significant (Klein and Anstee, 2005) and genetically most polymorphic of all human blood groups (Blumenfeld and Patnaik, 2004). The ABO are carbohydrate antigens (Watkins, 1966) depending on the enzymatic activity and specificity of allelic glycosyl transferases (Yamamoto *et al*., 1990), whereas Rh antigens are protein motifs (Gahmberg, 1982; Moore *et al*., 1982) whose surface expression entails an interaction of two genetic loci (Huang *et al*., 2000; Le Van Kim *et al*., 2006).

The prevalence of ABO, rhesus phenotypes and morphometric traits pattern has been studies in different races (Lyko *et al*., 1992; Boskabady *et al*., 2005; Anees and Mirza, 2005; Hanania *et al*., 2007; Jaff, 2010; Abdullah, 2010; Hassan, 2010; Rai and Kumar, 2010; Saxena *et al*., 2015). Singh and Sengupta (2004) conducted a research on some morphogenetic and behavioral traits among the Assamese Sikhs. Their results revealed that the Assamese Sikhs has blood group O with the highest frequency of 37.5% followed by blood group A (29.81%), group B (24.04%) and group AB (8.65%), respectively. Out of the individuals assessed, 6.73% were Rh negative and 93.27% were Rh positive. The frequency of the free ear lobe was 83.65% and attached earlobe was 16.35%. The middle phalangeal hair (mid-digital hair) frequency was 44.23% affected and 55.77% non-affected. The right type of hand clasping frequency was 88.46% and the left type was 11.54%. Specifically in Nigeria, studies have shown that in the phenotypic distribution of ABO, the four phenotypes (A, B, AB and O) are always present in varying frequencies but with group O having the highest frequencies and group AB the lowest. Iyiola *et al*. (2012) studied the gene frequencies of ABO and Rh (D) blood group alleles in Lagos, South-West Nigeria and revealed the frequencies as follows: 23.1% A, 21.3% B, 2.7% AB and 52.9% O. They found that the trend of the ABO blood group was O>B>A>AB. With regards to the Rh blood group, they reported that individuals with Rh (D) positive (DD and Dd) were 69 and 28% while Rh (D) negative individual (dd) was 0.03 (3%). Similar results were also obtained in Ekpoma (Nwaopara *et al*., 2008), Kano (Chima *et al*., 2012), Benin (Enosolease and Bazuaye, 2008) all in Nigeria. However, there is currently paucity of information on the prevalence of ABO and morphometric traits of individual in Calabar. Thus, data generated from this study will be of immense benefits to geneticist, biologist, blood transfusion services, policy makers and clinicians as such information is currently not documented in Calabar. Although there are reports on the relationship between ABO phenotypes and the prevalence of some diseases such as duodenal ulcer (De Mattos *et al*., 2002; Martins *et al*., 2006; Keramati *et al*., 2012), Cholera (Glass *et al*., 1985), Cancer (Akhtar *et al*., 2010), Malaria (Cserti and Dzik, 2007; Rowe *et al*., 2007), regrettably, there are no available study on the association of ABO phenotypes with simple traits of inheritance. Therefore, the novelty of the current research is to determine if the prevalence of ABO phenotypes are associated with the prevalence of genetic traits of simple inheritance (earlobe, hand clasping, facial dimple, mid digital hair and tongue rolling) and gender.

MATERIALS AND METHODS

Study design, subjects and population: This study was conducted in Calabar South region of Calabar, Cross River State during a period of 1 month. The study populations was made up of a total of 45 families making up 250 individuals (120 male, 130 female) irrespective of age. Families with only one child was excluded from this study and within a family, individual with gross malformation of at least one phenotype in study and/or individuals that are mentally retarded were excluded from this study.

Blood collection: Blood samples were collected from the side of the thumb. The portions were wiped vigorously with cotton wool, which had been dipped in methylated spirit to sterilized and stimulate blood flow. The cleaned surface was then pricked with a fresh sterile blood lancet and the thumb or heel was then pressed gently to allow blood flow. The first few drops of blood were wiped off with cotton wool, after few drops of the blood were allowed to drop on the tile for blood grouping.

Determination of ABO blood group and Rh (D) blood phenotype: The principle of the ABO and Rh (D) blood grouping is based on the fact that when red blood cells are

Table 1: Agglutination reaction

Test			Inference
Anti-A plus blood sample	Anti-B plus blood sample	Anti-D plus blood sample	Blood group and Rh (D) confirmed
-	-	+	O+
-	-	-	O-
+	-	+	A+
+	-	-	A-
-	+	+	B+
-	+	-	B-
+	+	+	AB+
+	+	-	AB-

+: Agglutination or clumping of the anti-serum with the blood sample, -: Compatibility between the anti-serum and the blood sample

mixed separately with potent anti-sera (Anti-A, Anti-B and Anti-D), agglutination occurs with the corresponding antibody depending on whether A, B or D antigens are present. Agglutination is observed when cellular antigens react with its corresponding antibody at their antigen binding sites to form large antigen-antibody complex. The ABO blood grouping and Rh (D) blood grouping was carried out using the tile-technique. Three anti-sera: Anti-A, Anti-B and Anti-D were used for the blood grouping. The white tile was properly wiped with a clean white cloth. Three drops of blood sample were placed on three different places on the tile and the different anti-sera were dropped accordingly on the blood and with one end of a glass pistol, the blood samples and the anti-sera were mixed properly. After, the tile was rocked for some seconds and incubated for 2 min at room temperature for agglutination to take place which was examined macroscopically. However, the agglutination reaction was recorded for each sample as shown in Table 1.

Determination of genetic traits of simple inheritance: In recording and analyzing various morphogenetic traits, various standard techniques were employed in the course of the study. For tongue rolling, each individual was asked to perform the activity, however, a person was classified as positive (+) or negative (-) depending upon his ability to turn up the lateral edges of the tongue. Meanwhile, in the cases of earlobe, dimples, mid-digital hair and hand clasping, physical observation was carried out to check for the presence or absence of the phenotype and results recorded accordingly.

Ethical consideration and informed consent: Ethical approval to carry out this research was obtained from the Cross River State Health Research Ethics Committee. Meanwhile, informed consent was obtained from each participant before the commencement of the study.

Statistical analysis: Data generated from this study were expressed in simple percentages. However, chi-square analysis was used to test for statistically significant associations between different parameters at $p<0.05$.

RESULTS

Table 2 shows the distribution of the various ABO blood phenotypes; blood group O had the highest frequency of 55.2% (n = 138), followed by B 21.6% (n = 54), A 18.8% (n = 47) and AB 4.4% (n = 11). In rhesus phenotype, majority of the studied population (91.6%, n = 229) were typed Rh (D) positive while 8.4% (n = 21) were typed Rh (D) negative. Comparatively, there was a higher proportion of rhesus positive in females than in males and a higher proportion of rhesus negative in males than in females (Table 2). On the distribution of the population with presence or absence of free earlobe, it was observed that a higher percentage of the population (69.2%, n = 173) had free earlobe while 30% (n = 77) had attached earlobe as shown in Table 3. The result revealed a significant association between earlobe and ABO blood phenotype ($X^2 = 8.18$, df = 3, $p<0.05$) but no significant association with sex ($X^2 = 0.31$, df = 1, $p>0.05$). The prevalence of free earlobe in the blood group was O>A>B>AB while attached earlobe was O>B>A>AB (Table 4) for the different ABO phenotypes. With respect to hand clasping, 51.6% were found to be right hand claspers while 48.4% were left hand claspers. The result revealed differences between the percentage frequencies of right hand claspers and left hand claspers (51.6 and 48.4%, respectively) (Table 3). Chi-square analysis showed a non significant value and no influence of blood group on hand clasping ($X^2 =5.69$, df =3, $p>0.05$). Sex on the other hand had a significant association with hand clasping ($X^2 = 4.03$, df = 1, $p<0.05$). Females were more likely to be right hand claspers and the male left hand claspers.

The frequency distributions of facial dimples amongst males and females are shown in Table 3. Individuals with no facial dimples predominates individuals with dimples while sex and facial dimples were associated ($X^2 = 3.98$, df = 1, $p<0.05$). Facial dimples were found to be more in the females than in the males. Similar analysis of the influence of blood group phenotypes on facial dimples showed a non significant value ($X^2 = 4.49$, df = 3, $p<0.05$) implying that blood group had no influence on facial dimples. Mid-digital hair was present on 92% of the studied population while 8% lacked it.

Table 2: Frequency distribution of ABO/Rh blood group by gender in the studied population N = 250

	Male		Female		Total	
Blood groups	No.	%	No.	%	No.	%
A	24	9.6	23	9.2	47	18.8
B	29	11.6	25	10.0	54	21.6
AB	4	1.6	7	2.8	11	4.4
O	63	25.2	75	30.0	138	55.2
Rh (D) +	104	41.6	125	50.0	229	91.6
Rh (D) -	15	6.0	6	2.4	21	8.4

Table 3: Frequency of genetic traits of simple inheritance by gender in the studied population N = 250

	Male		Female		Total	
Traits	No.	%	No.	%	No.	%
Earlobe						
Free	81	32.4	92	36.8	173	69.2
Attached	39	15.6	38	15.2	77	30.8
Hand clasping						
Left	66	26.4	55	22.0	121	48.4
Right	54	21.6	75	30.0	129	51.6
Facial dimples						
Dimples	19	7.6	34	13.6	53	21.2
No dimples	101	40.4	96	38.4	197	78.8
Tongue rolling						
Roller	72	59.5	49	40.5	121	48.4
Non roller	48	37.3	81	62.7	129	51.6
Mid digital hair						
Presence	114	45.6	116	46.4	230	92.0
Absence	6	2.4	14	5.6	20	8.0

Table 4: Frequency of blood group with genetic traits

	Blood groups									
	A		B		AB		O		Total	
Traits	No.	%	No.	%	No.	%	No.	%	No.	%
Earlobe										
Free	31	12.4	30	12.0	7	2.8	105	42	173	69.2
Attached	16	6.4	24	9.6	4	1.6	33	13.2	77	30.8
Hand clasping										
Right	20	8.0	23	9.2	7	2.8	79	31.6	129	51.6
Left	27	10.8	31	12.4	4	1.6	59	23.6	121	48.4
Facial dimples										
Dimples	15	6.0	10	4.0	3	1.2	25	10.0	53	21.2
No dimples	32	12.8	44	17.6	8	3.2	113	45.2	197	78.8
Tongue rolling										
Roller	24	9.6	31	12.4	7	2.8	59	23.6	121	48.4
Non roller	23	9.2	23	9.2	4	1.6	79	31.6	129	51.6
Mid-digital hair										
Presence	43	17.2	49	19.6	10	4.0	128	51.2	230	92.0
Absence	4	1.6	5	2.0	1	0.4	10	4.0	20	8.0

Mid-digital hair had no associations with sex and ABO blood group phenotypes as well. Furthermore, the frequency distribution of tongue rolling showed a lower percentage of tongue rollers (48.4%) in the population and a higher percentage of non rollers of tongue (51.6%), although the difference was marginal (Table 3). Chi-square analysis revealed a significant association between sex and the ability or inability to roll the tongue ($X^2 = 12.43$, df = 1, $p<0.05$) with male having higher prevalence of tongue rollers. The same analysis showed that blood group phenotypes had no influence on tongue rolling ability ($X^2 = 4.67$, df = 3, $p>0.05$). With respect to earlobe and considering the dominant allele, Table 5 shows that 93.9% of the progeny inherited free earlobe from mating between F×F parents, 62.8% from F×f parents and 12.5% from f×f parents. A chi-square analysis of frequency of the inherited allele between these mating parents showed a significant differences between their inheritance frequency ($X^2 = 45$, df = 2, $p<0.05$). Table 6-9 show the inheritance pattern of hand clasping, facial dimples, tongue rolling and mid-digital hair. The difference in inheritance frequency within them were found to be significant: $X^2 = 23.07$, df = 2, $p<0.05$; $X^2 = 36.57$, df = 2, $p<0.05$;

Table 5: Inherited frequency of earlobe (n = 160)

	Progeny			
Parent	Free earlobe	Attached earlobe	Free (%)	Attached (%)
F×F	62	4	93.9	6.1
F×f	49	29	62.8	37.2
f×f	2	14	12.5	87.5

F: Free earlobe (dominant) and f: Attached earlobe (recessive)

Table 6: Inherited frequency of hand clasping (n = 160)

	Progeny			
Parent	Right	Left	Right (%)	Left (%)
L×L	15	26	36.6	63.4
L×l	37	45	45.1	54.9
l×l	32	5	86.5	13.5

L: Left hand clasping (dominant) and l: Right hand clasping (recessive)

Table 7: Inherited frequency of facial dimples (n = 160)

	Progeny			
Parent	Dimples	No dimples	Dimples (%)	No dimples
D×d	31	51	37.8	62.2
d×d	0	78	0.0	100.0

D: Presence of dimples (dominant) and d: Absence of dimples (recessive)

Table 8: Inherited frequency of tongue roller (n = 160)

	Progeny			
Parent	Roller	Non-roller	Roller (%)	Non-roller (%)
R×R	20	14	58	42
R×r	55	45	55	45
r×r	0	26	0	100

R: Rolling of tongue (dominant) and r: Inability of rolling tongue (recessive)

Table 9: Inherited frequency of mid-digital hair (n = 160)

	Progeny			
Parent	Presence	Absence	Presence (%)	Absence (%)
P×P	118	5	95.9	4.1
P×p	32	5	86.5	13.5

P: Presence of mid-digital hair (dominant) and p: Its absence (recessive)

X^2 = 27.54, df = 2, $p<0.05$) and X^2 = 4.31, df = 1, $p<0.05$, respectively. However, there was no data on mating between dominant parents with respect to facial dimples and mid-digital hair (Table 7 and 9).

DISCUSSION

The ABO blood phenotype frequency distribution in the present study generally followed the pattern seen in previous studies by Krishna *et al*. (2014) and Chima *et al*. (2012). Group O was found to have the highest occurrence and group AB the least. Blood group trend of O>B>A>AB corresponds with the typical Nigerian situation as reported by Erhabor *et al*. (2013) and Indian situation (Pandey *et al*., 2013). It is pertinent to note the advantage of the high prevalence of blood group O in this study since the previous studies revealed that blood group O had less sever malaria compare to group A, B and AB (Gupta and Chowdhuri, 1980; Tadesse and Tadesse, 2013). This could suggest that individuals within Calabar may have high resistance to malaria even in malaria endemic region such as Calabar. Also, there are reports that individuals with blood group A, B and AB are more susceptible to oral, pancreatic, ovarian, gastric, leukemia, rectal and cervical cancers (Greer *et al*., 2010; Wolpin *et al*., 2009; Amundadottir *et al*., 2009; Mortazavi *et al*., 2014; Jaleel and Nagarajappa, 2012). Thus, the decrease in the prevalence of blood group A, B and AB suggest that the prevalence of these disease conditions in Calabar could be low. The Rh (D) positive was more common with a frequency of 91.6 and 8.4% for the Rh (D) negative. The frequency was similar to 91.73 and 8.27% reported by Jaff (2010) in Parkistan; 91.78 and 8.22% reported by Rajshree and Raj (2013) in Western Rajasthan, India for Rh (D) positive and Rh (D) negative respectively. In the frequency distribution of the various ABO blood phenotype and Rh (D) phenotype of males relative to females, the females had the highest proportion of group O, AB and Rh (D) positive while the males had greater percentage in blood group A, B and Rh (D) negative, however these differences were not

statistically significant, implying that blood groups are inherited in autosomal pattern, thus, the frequencies are not difference in both sex.

The need for blood group prevalence studies is multipurpose as in addition to their importance in evolution, their relation to disease and environment is being increasingly sought in modern medicine. Though not included in this investigation, the ABO blood group has been reported to be associated with many diseases like cancer (Akhtar *et al*., 2010), Cholera (Glass *et al*., 1985), malaria (Cserti and Dzik, 2007) and chronic periodontitis (Al Ghamdi, 2009), though the explanation for the association between ABO blood group and disease is still unclear (Saxena *et al*., 2015). Association existed between earlobe and ABO blood group but not with sex with blood group A having more prevalence in free earlobe individuals while group B had more prevalence in attached earlobe. Blood group had no influence on hand clasping, facial dimples and tongue rolling. Meanwhile, sex, on the other hand had associations with tongue rolling, hand clasping and facial dimples. No associations were found between ABO blood group and mid-digital or between sex and mid-digital hair. With respect to genetic traits of simple inheritance, the present study in Calabar observed the frequency of tongue rollers similar to that reported by Bulliyya (2003) and Pandey *et al.* (2013). The earlobe frequency is similar to the reports of Singh and Sengupta (2004) and Pandey *et al*. (2013).

The familial data frequencies of inheritance of different traits from different mating combination of the parents were significantly different from each other considering each genetic trait separately. This high significance suggests strong evidence that the traits are being inherited in a dominant versus recessive pattern with the traits having variable degree of dominance, as well as penetrance. The inheritance pattern of the familial data also revealed the possibility of incomplete dominant between the alleles of the morphometric traits studied. For instance, when two free earlobe parents were crossed (F×F), not all the progeny had free earlobe. Similar observation was noticed in the inheritance pattern of hand clasping and mid digital hair.

CONCLUSION

From the findings of this study, it was revealed that people within Calabar have high prevalence of blood group O while AB blood group was lowest. Similarly, rhesus positive phenotype individuals were more prevalence than rhesus negative. There was association between blood group and prevalence of earlobe, sex and hand clasping, sex and facial dimples and between sex and tongue rolling.

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