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Short Communication

Transferrin Polymorphisms in Childhood Malarial Anaemia in the Gabonese Children

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Abstract

Severe malarial anaemia (SMA) is one of the most outstanding complications of malaria in African children. It is often associated with iron deficiency, but explorations of soluble transferrin revealed controversial data. Despite the implication of host genetics factors in malaria pathogenesis, nothing is known about the role of iron carried polymorphisms in this plague. Nevertheless, these polymorphisms have been associated with pathogenesis of diseases associated with iron deficiency.

We conducted a cross-sectional study including 59 children with SMA, 176 with mild malaria anaemia (MMA) and 92 with non-anaemia malaria (NAM). We investigated polymorphisms G258S, R300H, A477P, P570S from transferrin exons 7, 8, 12, 15 respectively and S142G from transferrin receptor1 (TfR1) exon 4 by PCR-RFLP. The mean age of children with SMA, MMA and NAM was 27.7 ± 8.8, 38.6 ± 10.2 and 47.3± 15.4 months respectively, confirming that SMA is associated with young age ($p<0.05$). Alleles of transferrin C2 (corresponding to P 570S) and C3 (corresponding to G258S) occurred in 13.8% and 1.2% of the children, respectively. Allele C3 was detected only in children with SMA (n=4, 6.8%). The frequency of allele C2 was significantly different between study groups: 1.7%, 11.4%, and 26.2% respectively for SMA, MMA, NAM; $p<0.0003$. Allele of transferrin C2 was associated with decreased risk in malarial anaemia (malarial anaemia [8.9%] versus NAM [26.2%], $p<0.01$). Transferrin polymorphisms R300H and A477P were not found. The frequency of TfR1 polymorphism S142G was 13.6%, 12.5%, 13.0% respectively for SMA, MMA, and NAM, suggesting that it had no influence on the risk of malarial anaemia. Data support the conclusion that transferrin polymorphisms influence the risk of SMA.

Key words: *P. falciparum*, malarial anaemia, transferrin polymorphisms, transferrin C2 allele, transferrin C3 allele.

INTRODUCTION

One of the most important causes of childhood mortality worldwide is the malaria parasite *Plasmodium falciparum*, which annually kills about 1 million children in Africa alone [1]. Malarial anaemia is one of the most prevalent symptoms of this infection. It is caused partly by the loss of red blood cells, both infected erythrocytes and uninfected erythrocytes, which are destroyed by hemolysis or phagocytosis [2]. Rigidification of the membranes of infected erythrocytes and uninfected erythrocytes during infection may be an important factor in the destruction of these cells during passage through the spleen [3]. Severe malarial anaemia (SMA) is the most frequent complication of ma-

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laria and it is often associated with a decrease of erythrocyte volume reflected by a reduced mean corpuscular volume (MCV) [4, 5]. One of the causes of this mycrocytosis is iron deficiency [6].

Iron deficiency during a malaria attack could be due to nutritional factors or to excessive loss and dismetabolism of iron. This dismetabolism could be the consequence of the iron-binding protein deficiencies, like transferrin and its receptors. Thus, they may be considered as factors involved in SMA pathogenesis.

Transferrin is the major circulating glycoprotein involved in iron metabolism [7]. It tightly binds two iron (III) ions per molecule, together with the synergistic anion carbonate, giving rise to a characteristic ternary complex. Modifications in its structure could therefore affect iron metabolism. A great number of transferrin variants have been reported in different human populations [8-12]. Polymorphisms of these proteins have been associated with the pathogenesis of certain diseases. Among these, several are involved in the pathogenesis of diseases related to iron disorders. For instance, transferrin variant C2 corresponding to C1815T transition in exon 15 (mutation P570S in protein) which is characterized by weak iron-binding capability is associated with rheumatoid arthritis [13]. The C3 variant characterised by mutation G258S (G876A transition in exon 7) increases the risk of developing anaemia associated with iron deficiency and the severity of Parkinson disease [14, 15]. Moreover, the A477P mutation has been identified as responsible for an atransferrinemia patient [16]. On the other hand, S142G mutation in transferrin receptor 1 (TfR1) combined with C282Y mutation in HFE gene has been associated with haemochromatosis [17].

Data describing the effects of soluble transferrin on the outcome of malaria remain controversial.

Since it has been shown that host genetic factors control blood infection levels and malaria pathogenesis [18-20], the aim of this study is to investigate the influence of the polymorphisms of transferrin and those of TfR1 on susceptibility to childhood SMA.

PATIENTS AND METHODS

This cross-sectional study was conducted in two hospitals in Libreville, Gabon. The Centre Hospitalier de Libreville, which is the largest public hospital in the country situated in the capital city of Libreville and in Centre Hospitalier Régional de Melen, which is a peripheral hospital of Libreville. Ethical clearance and national endorsement were received from the Gabonese Ministry of Health. Malaria diagnosis was based on blood smears according to the Lambaréné method [21] with *P. falciparum* parasitemia of more than 1000 parasites per microliter and fever (tympanic temperature $\geq$ 37.5 ℃). After obtaining informed consent from parents or guardians, 327 children diagnosed with malaria and ranging in age from 6 to 84 months were included in the study. They were classified according to previous reports [22] in 3 groups: 59 in the group of severe malaria anaemia (SMA) (Hb<5 g/dl) without neurological signs, 176 in the group of mild malaria anaemia (MMA) (5$\leq$ Hb $\leq$ 10 g/dl) and 92 in the group of non anaemia malaria NAM (Hb >10 g/dl). Microcytosis anaemia was defined as anaemia with a mean cell volume (MCV) less than 80 fL.

According to the recommendation of the National Program of Malaria Control of Gabon, each episode of illness was treated with artesunate-amodiaquine for three days. Children with severe malaria were treated with intravenous quinine and those presenting with SMA received blood transfusions in addition. At the end of the treatment, para-

Table 1. Sequences of primer sets and restriction enzymes used to characterize transferrin polymorphisms of transferrin and transferrin receptor 1

Gene	Primers	T ℃	Mutation, (AA^1)	Restriction enzyme	sizes*
Tf exon 7	5'-CTCACTCCAGACCTCTCAGCT - 3' 5'-CTGCTCTGTGGGGAGTCCCA-3'	63	A879G, (G258S)	*Bbv I*	324+134
Tf exon 8	5'-GCAGAGATTTCTTTTCTCTTCAGT - 3' 5'-GACTAACGCCATTCAGCATCAG - 3'	60	A1006G, (R300H)	*Acc II*	256+170
Tf exon 12	5'-CTGATTGAGGATATCTTTGCTT-3' 5'-ACAGCACCTTATGCCACACT-3'	60	G1428C, (A477P)	*Mva I*	150(x2)+50
Tf exon 15	5'-GCTGTGCCTTGATGGTACCAGGTAA -3' 5'-GGACGCAAGCTTCCTTATCT - 3'	58	C1815T, (P570S)	*Bst E II*	89+21
TfR1 exon 4	5'-ACGTCTCTGGCATCCTTCCCT - 3' 5' -GGTGAGCGCCCCGAGCCGCG-3'	62	A687G, (S142G)	*Ban I*	240+40

sizes* indicate the sizes of fragments generated after restriction enzyme digestions
AA^1= mutation on amino acid sequence
T ℃ = hybridization temperature during PCR programme

Table 2. Biological characteristics of patients

	SMA	MMA	NAM	p
No. of subjects	59	176	92	
Sexe Ratio (M/F)*	1.3	1.6	1.4	NS
Mean Age, (Months±SD	27.7 (±8.8)	38.6 (±10.2)	47.3 (±15.4)	0.01
Geo. Parasitemia, (P/µL)**	20728 (1860-33928)	18954 (2367-31235)	17356 (8131-32657)	NS
Means (m±SD)				
Hemoglobin, g/dl	3.7 (±0.5)	8.1 (±1.0)	10.7 (±0.3)	
MCV, fL	70 (±7.8)	69.2 (±6.3)	73.8 (±10.6)	NS
Leucocytes, X 1000/mL	11.4 (±7.5)	8.8 (±3.7)	8.2 (±1.5)	NS
Platelets, X 1000/mL	155.3 (±112.4)	158.5 (±83.4)	168.5 (±34.7)	NS
Frequencies of polymorphisms				
Transferrin				
G258=*C3 allele*, (%; n)	6.8 ; 4	0 ; 0	0 ; 0	0.05*
R300=*Dchi allele*, (%; n)	0 ; 0	0 ; 0	0 ; 0	ND
A477 (%; n)	0 ; 0	0 ; 0	0 ; 0	ND
P570=*C2 allele*, (%; n)	1.7 ; 1	11.4 ; 20	26.1 ; 24	0.00005
TfR1				
S142 (%; n)	13.6 ; 8	12.5 ; 22	13.0 ; 12	0.98

SD = Standard Deviation, MCV = Mean of Corpuscular Volume of Red Blood Cells, % = percentages
n = number
M/F*: Male/Female
Geo. Parasitemia (P**/µL) : Geometric means of parasitemia by Parasites/µL, m (minimal-maximal)
0.05*: Significant difference between SMA versus NAM and SMA versus MMA, but no difference between NAM and MMA
ND : Not determined
NS : Not significant

sitemia was checked by blood smears according to the Lambaréné method [21].

Routine haematological data were collected from all the children using an automate blood cell counter (STKS, Coulter Corporation), and malaria parasite densities were determined by the Lambaréné method [21]. After DNA extraction using the EZNA kit (Biofidal®, France) according to the manufacturer's instructions, transferrin and transferrin receptor 1 gene polymorphisms were investigated using PCR-RFLP. The sequences of primer sets and restriction enzymes used to genotype the samples are detailed in Table 1.

The chi-square test was used to compare categorical variables among groups. Student's *t* test, Pearson chi-square and Fischer's exact tests were used when appropriate for group comparisons. In all cases, *P* values of <0.05 were considered significant. We determined the odds ratios (OR) for SMA, MMA, and NAM by EpiInfo 2000 (CDC, Atlanta).

RESULTS

We examined 2018 children visiting the hospitals. Although 760 (37.7%) had a positive blood smear, 433 were excluded because of parasitemia<1000parasite/µL. Subsequently, 327 children (16.2 % of the total examined) with parasitemia more than 1000parasites/µL were included in this cross-sectional study. The mean age of children with NAM (47.3± 15.4 months) was significantly higher than that of children with SMA and MMA (27.4± 8.8, 38.6± 10.2 months respectively), p<0.0001, confirming that younger children are the most likely to develop SMA. The biological characteristics of the patients are summarised in Table 2.

The mean of temperatures was 38.7℃ ± 1.1 in the study population, with no significant difference observed among the study groups (38.4℃±1.0, 38.9℃ ± 1.1 and 38.5℃± 0.9 respectively for SMA, MMA and NAM). The mean level of parasitemia and level of leucocytes and platelets (Table 2) did not differ among study groups. These haematological parameters were not associated with the development of SMA in our study population. Microcytic anaemia, corresponding to a mean cell volume (MCV) less than 80 fL, accounted for 95.2% of all anaemia.

To analyse the influence of transferrin and its receptor TfR1 on the pathogenesis of SMA, we investigated four polymorphisms of transferrin (exons 7, 8, 12 and15) and S 142G polymorphism of TfR1 genes.

Transferrin variant C3 characterised by mutation G258 S (G876A transition in exon 7), which leads to the loss of a *BbV I* endonuclease restriction site [23], was also investigated. Only 4 children (1.2% of the study population) were heterozygous C3 (figure 1A). All of them were children with SMA corresponding to 6.8% of this group. This was

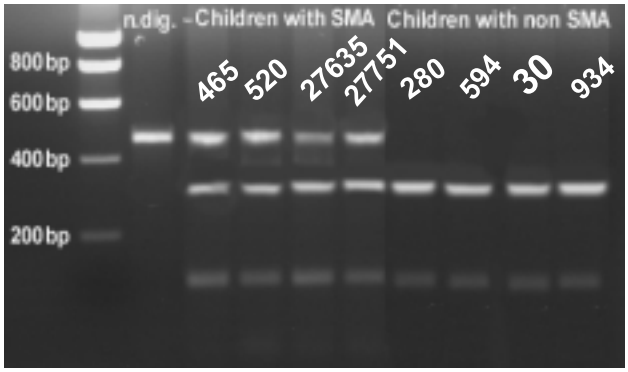

Figure 1A:
A. Polymorphism G258S in amino acid sequence leading variant C3 of transferrin Exon 7 was amplified by PCR with specific primers. PCR products were digested with *Bbv I* to investigate transition G879A. The variant C3 of transferrin is characterised by the lost of *BbV I* restriction site. Digested products were migrated in 2% agarose gel. Only children 465, 520, 27635, and 27751 included in the SMA group were C3 genotyped, 280 and 594 included in UM as well as 30 and 934 included in the MMA group were examples of the profile of children who do not carried variant C3 of transferrin. n. dig corresponds to the non-digested PCR fragment of exon 7.

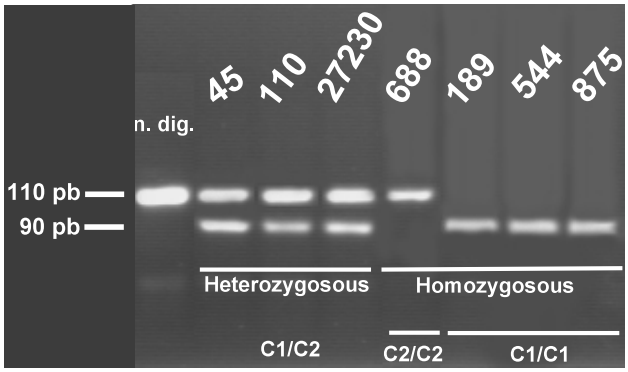

Figure 1B:
B. Polymorphism P570S in amino acid sequence leading variant C2 of transferrin.
Exon 15 was amplified by PCR with appropriated primers. PCR products were digested with *Bst E II* to investigate transition T1815C. The variant C2 of transferrin is characterized by the loss of *Bst E II* restriction site. Digested products were migrated in 2% agarose gel. Children 45, 110, and 27230 give a profile heterozygous C1/C2, 688 was unique homozygous C2/C2 and 189, 265, 544, and 875 give an example of profile of homozygous C1/C1, n.dig corresponds to non-digested PCR fragment of exon 15.

significantly different from the two other groups, where no C3 variants were found ($p<0.05$).

We also investigated the frequencies of variant C2 corresponding to C1815T transition in exon 15 of transferrin (mutation P570S), leading to the loss of a *Bst E II* restriction site [24]. The genotype profiles according to amino acid 570 are shown in Figure 1B. The frequency of this variant in the study population was 13.8%. Only one homozygote child C2C2 was observed in the NAM group, with 13g/dL of haemoglobin and 8500 parasites/µL. The C 2 heterozygote variant was present at 1.7% (n=1) for the SMA group, at 11.4% (n=20) for the MMA group and at 26.1% (n=24) for the NAM group. A significant difference was observed in the frequency of C2 between the SMA and NAM groups ($p<0.0005$ [OR (Odds Ratio) =0.05, $CI_{95\%}$ (Confidence Interval 95%) 0.00-0.05]). This difference was less pronounced between the SMA and MMA groups ($p= 0.03$ [OR =0.13, $CI_{95\%}$ 0.01-0.98]). Comparing SMA and MMA gathered (n= 21, 8.9%) versus NAM (n=24, 26.1%), we found that the C2 variant has a protector effect against anaemia during acute malaria ($p<0.00006$ [OR =0.28, $CI_{95\%}$ 0.01-0.36]). That is confirmed by comparison of the mean of haemoglobin level between heterozygous C1C2 (8.77g/dl $\pm$ 0.61) and homozygous C1C1 children (7.86 g/dl $\pm$ 0.39) by t-test, which showed a significant difference ($p= 0.04$).

In the study population, we have not detected polymorphisms in R300H (*Dchi* allele) and A477P, in exons 8 and 12, respectively.

The frequencies of TfR1 polymorphism S142G were 13.6%, 12.5%, 13.0% respectively for SMA, MMA, and NAM suggesting that it had no influence on the risk of malarial anaemia ($p=0.98$ [OR=1,04, $CI_{95\%}$ 0.66-1.62]).

DISCUSSION

SMA is the most widespread complication of childhood malaria in Gabon, and it is often associated with microcytosis reflected iron deficiency [4]. The prevalence found here is consistent with previous data showing that malaria accounts for 40% of febrile paediatric consultations. These data are consistent with previous findings from a larger sample population of 8,036 Gabonese children [4]. They also confirm that SMA is the most frequent complication of severe malaria in Gabon, and that it is often associated with iron deficiency characterized by the decrease of MCV.

Here, we investigated the influence of polymorphisms of transferrin and its receptor (TfR1) on the development of this disease.

The role of the variant C2 of transferrin in the pathogenesis of other diseases is controversial. It is associated with an increased risk of rheumatoid arthritis [13], while its influence in Alzheimer's disease is contentious. Certain reports show that this allele is associated with the risk of Alzheimer's disease, while others are contradictory [25, 26].

The frequency of transferrin variant C2 found here is consistent with previously described results for Black African populations [27]. We found that the frequency of this variant in NAM is 15 times greater than in SMA, suggesting that it is associated with decreased risk of SMA development.

Comparing SMA and MMA versus NAM, the C2 variant has a protector effect against anaemia during malaria. This suggests that this variant limits iron deficiency during malaria. The C2 variant may provide an improved iron uptake which compensates for the iron used by the parasite. However, it has been suggested that C2 variant retains the structure and iron-binding properties of the wild-type protein and that it does not have a different iron-binding capacity [28]. The mechanism of the implication of transferrin variant C2 in human pathogenesis therefore needs to be elucidated.

Only 4 children carried transferrin variant C3, a finding that is consistent with previous reports, showing that this allele is rare in black African people [27, 29]. These children have developed SMA; this is consistent with the increased risk of anaemia associated with C3 variant [14].

The prevalence of C3 variant found in the present study is higher than the frequency previously described due to the SMA and the smallness of our study population size. Because of the low frequency of transferrin variant C3, the number of patients needs to be increased to confirm the involvement of transferrin variant C3, despite the fact that the size of each group are sufficient for statistical analysis. Indeed, some studies on the involvement of genetic factors necessitate a greater number of patients, but in the context of Gabon, several years are needed to obtain a sufficient number of children to use in these studies.

Since C3 is one of the factors of the susceptibility facing SMA, it could result in a negative selection of subjects with C3 allele, which in turn may explain the rarity of this allele in Black African people.

Transferrin variant *Dchi* was not found in the present study. This variant is widely described among mongoloid and Asian populations [30-32], but it has not been associated with any pathogenesis nor described in African people. Also, the mutation A477P has not been found in the study population. This polymorphism is extremely rare and has been described only once in an atransferrinemia patient [16].

The present study is the first to report on S142G polymorphism of TfR1 in an African population, and the first to investigate the influence of S142G mutation of TfR1 on the pathogenesis of SMA. Our observations are consistent with previous reports showing that S142G mutation in TfR1 is not an independent factor in human pathogenesis [17].

Only polymorphisms which can be analysed using PCR-RFLP were investigated. Polymorphisms requiring a sequencing exploration, such as variant D1 which is present among 5% of the Black African people [33], should also be explored in future studies.

LIMITATION

The findings in this report are subject to at least three limitations. One is, the number of children included in each study group. Since international efforts and funds for malaria control have been stepped up, with a substantial increase from 2003 in Gabon, a decrease has been observed in severe malaria in children, particularly severe malarial anaemia. Secondly, with regard to some infectious diseases in the country, the haemoglobin rate is usually less than 11 g/dL and clinicians consider 10g/dL of haemoglobin to be non-anaemic malaria. Thirdly, the demonstration of mechanisms to explain the role of transferrin polymorphism on the development of SMA is extremely demanding.

CONCLUSION

The present study is the first to investigate the influence of transferrin on the pathogenesis of SMA, and it reports the association of transferrin polymorphism with the risk of SMA in Gabonese children. Data revealed that transferrin polymorphisms are a supplementary factor of risk in SMA. Further studies in a larger population and family-based analysis are required.

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REFERENCES

1. Hay SI, Guerra CA, Tatem AJ, Noor AM, Snow RW. The global distribution and population at risk of malaria: past, present, and future. Lancet Infectious diseases 2004; 4:

327-36.
2. Dondorp AM, BJ Angus, K Chotivanich, K Silamut, R Ruangveerayuth, MR Hardeman, et al. Red blood cell deformability as a predictor of anemia in severe falciparum malaria. Am J Trop Med Hyg 1999; 60: 733-737
3. Dondorp AM, Kager PA, Vreeken J, White NJ. Abnormal blood flow and red blood cell deformability in severe malaria. Parasitol Today. 2000; 16: 228-232.
4. Dzeing-Ella A, Nze Obiang PC, Tchoua R, Planche T, Mboza B, Mbounja M, et al. Severe falciparum malaria in Gabonese children: clinical and laboratory features. Malaria J. 2005; 4: 1-8.
5. Looareesuwan S, Merry AH, Phillips RE, et al. Reduced erythrocyte survival following clearance of malarial parasitaemia in Thai patients. Br J Haematol. 1987; 67: 473-478.
6. Bienzle U, Eggelte TA, Adjei LA, Dietz E, Ehrhardt S, Cramer JP, et al. Limited influence of haptoglobin genotypes on severe malaria in Ghanaian children. Tropical Medicine and International Health 2005; 10: 668-71.
7. Sun H, Li H, Sadler PJ, Transferrin as a metal ion mediator. Chem Rev 1999; 99: 2817-42.
8. Beckman G, Beckman L, Sikstrom C. Transferrin C subtypes and spontaneous abortion. Hum Hered 1980; 30: 316-9.
9. Kamboh MI, Ferrell RE. Human transferrin polymorphism. Hum Hered 1987; 37: 65-81.
10. Smithies O. An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. Biochem J 1959; 71: 585-7.
11. Giblett ER, Hickman CG, Smithies O. Serum transferrins. Nature 1959 Jun 6;183:1589-90.
12. de Jong G, van Dijk JP, van Eijk HG. The biology of transferrin. Clin Chim Acta. 1990; 190: 1-46.
13. El-Hazmi MA, al-Ballaa SR, Warsy AS, al-Arfaj H, al-Sughari S, al-Dalaan AN. Transferrin C subtypes in patients with rheumatoid arthritis. Br J Rheumatol 1991; 30: 21-23.
14. Lee PL, Halloran C, Trevino R, Felitti V, and Beutler E. Human transferrin G277S mutation: a risk factor for iron deficiency anaemia. Br J Haematol 2001; 115: 329-333.
15. Borie C, Gasparini F, Verpillat P, Bonnet AM, Agid Y, Hetet G, et al. Association study between iron-related genes polymorphisms and Parkinson's disease. J Neurol 2002; 249: 801-4.
16. Beutler E, Terri Gelbart, Pauline Lee, Renee´ Trevino, Mark A. Fernandez, and Virgil F. Fairbanks. Molecular characterization of a case of atransferrinemia. Blood 2000; 9613: 4071-4.
17. Robinson JP, Johnson VL, Rogers PA, et al. Evidence for an association between compound heterozygosity for germ line mutations in the hemochromatosis (HFE) gene and increased risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2005; 14: 1460-1463.
18. Hill AV, Allsopp CE, Kwiatkowski D, Anstey NM, Twumasi P, Rowe PA, et al. Common West African HLA antigens are associated with protection from severe malaria. Nature 1991; 352: 595-600.
19. Abel L, Cot M, Mulder L, Carnevale P, Feingold J. Segregation analysis detects a major gene controlling blood infection levels in human malaria. American Journal Human Genetics 1992; 50: 1308-17.
20. Rihet P, Traore Y, Abel L, Aucan C, Traore-Leroux T, Fumoux F. Malaria in humans: Plasmodium falciparum blood infection levels are linked to chromosome 5q31-q33. American Journal of Human Genetics 1998; 63: 498-505.
21. Planche T, Krishna S, Kombila M, Engel K, Faucher JF, Ngou-Milama E, Kremsner PG, et al. Comparison of methods for the rapid laboratory assessment of children with malaria. Am J Trop Med Hyg 2001; 65: 599-602.
22. Owusu-Agyei S, Fryauff DJ, Chandramohan D, Koram KA, Binka FN, Nkrumah FK, Utz GC, Hoffman SL. Characteristics of severe anemia and its association with malaria in young children of the Kassena-Nankana District of northern Ghana. Am J Trop Med Hyg. 2002; 67(4): 371-7.
23. Beckman LE, Van Landeghem GF, Sikstrom C, Beckman L. DNA polymorphisms and haplotypes in the human transferrin gene. Hum Genet 1998; 102: 141-4.
24. Namekata K, Oyama F, Imagawa M, Ihara Y. Human transferrin (Tf): a single mutation at codon 570 determines Tf C 1 or Tf C2 variant. Hum Genet 1997; 100: 457-8.
25. van Rensburg SJ, Carstens ME, Potocnik FC, Aucamp AK, Taljaard JJ. Increased frequency of the transferrin C2 subtype in Alzheimer's disease. Neuroreport 1993; 4: 1269-71.
26. Kim KW, Jhoo JH, Lee JH, Lee DY, Lee KU, Youn JY, Woo JI. Transferrin C2 variant does not confer a risk for Alzheimer's disease in Koreans. Neurosci Lett. 2001; 308: 45-8
27. Cleve H, Schwendner E, Rodewald A, Bidlingmaier F. Genetic transferrin types and iron-binding: a comparative study of a European and an African population sample. Hum Genet 1988; 78: 16-20.
28. Zatta P, Messori L, Mauri P, van Rensburg SJ, van Zyl J, Gabrielli S, Gabbiani C. The C2 variant of human serum transferrin retains the iron binding properties of the native protein. Biochim Biophys Acta 2005; 1741: 264-270.
29. Nabulsi A, Emmerich E, Cleve W, Gurtler LG, Cleve H. Haptoglobin (HP), transferrin (TF) and group-specific component (GC) subtype distribution in Bantu-speaking people from Malawi. Hum Hered 1993; 43: 323-5.
30. Gopalam KB, Rao PR. Genetic studies on Vysyas of Andhra Pradesh, S. India: A1A2BO, Rh (O) D, transferrin, group specific component, haptoglobin and pseudocholinesterase types. Acta Anthropogenet 1981; 5: 175-180.
31. Saha N, Tay JS, Murugasu B, Wong HB. Transferrin subtypes and spontaneous abortion in a Chinese population. Hum Hered 1990; 40: 141-4.
32. Shibata K. Haptoglobin, group-specific component, transferrin and alpha I-antitrypsin subtypes and new variants in Japanese. Jinrui Idengaku Zasshi 1983; 28: 17-27.
33. Kasvosve I, Delanghe JR, Gomo ZA, Gangaidzo IT, Khumalo H, Wuyts B, et al. Transferrin polymorphism influences iron status in blacks. Clin Chem 2000; 46: 1535-1539.