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Detection of a Rare Blood Group "Bombay (Oh) Phenotype" Among the Kutia Kondh Primitive Tribe of Orissa, India

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ABSTRACT Tribals constitute a major chunk of the total population of India. Out of a total 475 tribal groups in India, about 75 are primitive tribes. Kutia Kondhs of Orissa state are one of the most primitive tribal people of the country. A total of 2488 children aged 6-15 years belonging to 15 major tribes were screened for ABO and Rhesus (D) blood groups at random from various Ashram schools in eight districts of Orissa. Out of sixty five Kutia Kondh children studied from Belghar area of Tumudibundh block in Phulbani district of Orissa two cases showed a rare ABO blood group, Bombay (Oh) phenotype for the first time, giving an incidence of 1 in 33 among Kutia Kondh tribe, 1 in 127 in Kondh tribe and 1 in 1244 among the tribal populations of Orissa. This is the highest incidence of Bombay phenotype so far reported from India. These findings have been discussed in the light of relevant studies from India.

INTRODUCTION

Of the hereditary conditions of blood, the blood group serology plays an important role in the transfusion medicine. The discovery of a rare blood group, "Bombay phenotype" in three unrelated individuals in Mumbai (formerly Bombay) by Bhende et al. (1952) was important in the field of immunohematology. The discovery later helped Watkins and Morgan (1959) and Gerard et al. (1982) to elucidate biosynthetic pathway for ABH and Lewis (Le) antigens, suggesting that secretor (Se) and H are closely linked structural genes. Recently, molecular genetics studies were carried out to determine the role of the H, Se, and Le genes in the expression of H antigen in secretions and Lewis blood group antigen on erythrocytes (Kaneko et al. 1997; Oriol et al. 2000).

It is important to be cautious in predicting the ABO blood type of children based on the phenotypes of their parents. This is due to the fact that a third antigen (H) on the surface of red cells can prevent the expected ABO blood type from occurring. Normally, if an A blood type mother has an O type child, the father is expected to be type O or at least to carry the O allele (OO, AO, or BO genotype). The child has inherited an O allele from both parents. However, an O blood type child can also be born to parents who do not have the O allele if a recessive form of the allele for the H antigen also is inherited from both parents. The H antigen is a precursor to the A and B antigens. For instance, the B allele must be present to produce the B enzyme that modifies the H antigen to become the B antigen. It is also true for the A allele. However, if only recessive alleles for the H antigen are inherited (hh), the H antigen will not be produced. Subsequently, the A and B antigens also will not be produced. The result is an O phenotype by default since the lack of A and B antigens is the O type. This impossible phenotype has been referred to as a Bombay (Oh) phenotype.

The present study was designed with the following objectives: i). To detect and identify the rare blood groups like Bombay (Oh) phenotype, if any in the tribes of Orissa; ii).To discuss the data on Bombay phenotype in the light of earlier studies from India.

BOMBAY PHENOTYPE

The gene interactions come across instances where a novel phenotype does not appear but an effect caused by one gene pair interferes with or hides an effect caused by another gene pair. This type of interaction is called epistasis and may be considered the counterpart of dominance relations between alleles (when one allele modifies or hides the effect of another allele at the same gene pair). Epistasis may be caused by the presence of homozygous recessives at one gene pair. This pattern of epistatic interaction

can also be seen in humans, where the appearance of detectable ABO blood type antigens has been shown to depend upon the presence of H gene. The ABO gene is located on chromosome 9(9q34.1). The other traits associated with genes on chromosome 9 are galactosemia, nail-patella syndrome, and xeroderma pigmentosa. An individual who is homozygous for the very rare recessive h allele, shows no such antigens and is phenotypically of blood type O (Bombay phenotype). The H gene is responsible for the attachment of certain subterminal sugars to those polysaccharides upon which the terminal sugars attach themselves as specified by the ABO genes. Thus, homozygous (hh) individuals lack the polysaccharide organisation for terminal sugar attachment and, therefore, appear to lack ABO blood type antigens, although they do not lack ABO genes (Strickberger 1999).

The existence of a human H/h genetic polymorphism was first established by the discovery in India (Bombay) of an individual devoid of the H antigen on red cells, who had antibodies in plasma reacting with all the cells exhibiting the normal red cell ABO phenotypes (Bhende et al. 1952). However, this H deficient or Bombay phenotype was rare, since it occurred in about one in 10,000 individuals in India and one per one million individuals in Europe. More recently, a large series of H deficient individuals (~1:1000) was found in a small French Island 800km east of Madagascar, in the Indian Ocean, called Reunion Island (Le Pendu et al. 1983). Two distinct phenotypes were found, the classical Bombay phenotype among Tamoul Indian immigrant families and a new, partially deficient phenotype, called the "Reunion" phenotype. The two phenotypes resulted from products, or lack of products, of two different alleles of *FUT1* and *FUT2* genes (Le Pendu et al. 1983); the same and also additional alleles of both *FUT1* and *FUT2* were documented in other populations, particularly, in Japan, where the incidence of Bombay and para-Bombay individuals was shown to be 1-2 in 300,000 (Kaneko et al. 1997). In Taiwan, para-Bombay phenotype has a frequency of 1:8000 (Yu et al. 1997).

The mutational analysis has revealed that the Bombay phenotype fails to express the ABH antigens of ABO blood group system on red blood cells and in secretions because of a lack in activities of the H gene (FUT1)- and Secretor gene (FUT2)-encoded alpha (1,2) fucosyltransferases (Koda et al. 1997). In this study, they examined the FUT1 and the FUT2 from three unrelated Indian individuals with the Bombay phenotype and found to be homozygous for a T725G mutation in the coding region of the FUT1, which inactivated the enzyme activity. Further, they could not detect any hybridized band corresponding to the FUT2 by Southern blot analysis using the catalytic domain of the FUT2 as probe, indicating that the three individuals were homozygous for a gene deletion in the FUT2. These results suggested that the T725G mutation of FUT1 and the gene deletion of FUT2 are responsible for the classical Indian Bombay phenotype. Later studies (Fernandez-Mateos et al. 1998) have pointed out that the Indian red cell H null Bombay phenotype depends on a new mutation of the FUT1 gene, i.e. T725G changing Leu242→Arg. Their salivary nonsecretor phenotype is secondary to a complete deletion of the FUT2 gene. The red cell H weak Reunion phenotype depends on another new mutation of FUT1, C349→T, which induces a change of His117→Tyr. Their salivary nonsecretor phenotype is due to the known Caucasian inactivating mutation G428→A (Fernandez-Mateos et al. 1998).

There is the possibility of linkage disequilibrium of *FUT1* and *FUT2* genes. The two genes lie in close proximity on chromosome 19q13.3. There is co-existence of unique sets of mutations in *FUT1* and *FUT2*, each set occurring in individuals of a certain ethnic group. Thus in India, the *FUT1* mutation 725T>G travels almost always (one exception) with a total deletion of *FUT2*; in Reunion Island (Caucasian), the major inactivating mutation of *FUT1* or 349C>T, travels almost always with the inactivating mutation of *FUT2* or 428G>A; and the main Oriental inactivating mutations of *FUT1 t*ravel almost always with the wild type *FUT2 (*Fernandez-Mateos et al. 1998*)*.

After the first report of Oh phenotype from Mumbai (formerly Bombay) in 1952 by Bhende and coworkers, several other workers detected this peculiar phenotype in India (Simmons and D'senna 1955; Roy et al. 1957) and also in the European countries (Alosia et al. 1961; Aust et al. 1962). Later on, it was found that many of the European cases, which were initially labelled as typical Bombay phenotypes, turned out to be para Bombay phenotypes after absorption elution

studies (Levine et al. 1955; Lanset et al. 1966; Bhatia and Solomon 1967).

MATERIALS AND METHODS

Out of the 62 scheduled tribes in the state of Orissa, 15 major scheduled tribes each comprising more than 1 lakh individuals as per the 1991 Census were studied. These major scheduled tribes included Bathudi, Bhumiz, Kolha, Lodha and Santal from Mayurbhanj district, Bhuyan, Kharia, Kissan, Munda and Oraon from Sundargarh district, Bhatra from Nawarangpur district, Gond from Kalahandi district, Kondh from Phulbani district, Paraja from Koraput district and Saora from Ganjam and Gajapati districts in the Central-Eastern part of India.

The highest concentration districts were first identified for each tribe and then the Ashram schools were listed in that locality, of which 4-5 at random selected, representing different geographical locations in each district. A total of 2,488 blood samples were collected from eight districts of the state after taking informed consent from each individual during the period 1995-2000. It was also ensured to collect blood samples only from unrelated individuals belonging to either sex.

Kutia Kondhs are one of the most primitive tribal people of the country. They are one of the three endogamous subgroups of Kondh tribe, the other two being Dongria and Desia Kondhs (Basu et al. 1995). The marriage between three subgroups of Kondh tribe is strictly now prohibited and they remain completely isolated from each other (Balgir 1997). Kutia Kondhs live mainly in the interior hilly terrains of the thick forests of the Tumudibundh Block (Belghar area) of Balliguda Subdivision of Phulbani district in Orissa. As per the Kutia Kondh Development Agency (KKDA), the total population of Kutia Kondhs was 4,971 in Tumudibundh Block in the year 1999 (Unpublished data). This primitive tribe is reported to have a static growth rate due to a number of reasons (Balgir 1997). For Kutia Kondhs, Belghar Residential Sevashram, Belghar in Tumudibundh Block of Phulbani district of Orissa was studied during the month of November 1999. Out of a total 254 Kondh tribals screened for ABO and Rhesus blood groups, 65 Kutia Kondh children aged 6-15 years, were included in the study.

Blood and saliva samples from all the students present on the day of our visit in the school were collected after taking informed consent. Both sexes were equally represented in the samples taken for the study. About 1-2 ml. intravenous blood samples were collected from each student in Disodium salt of Ethylene Diamine Tetra Acetic Acid (EDTA) coated vials. The blood samples were transported under ice-cold conditions to the laboratory at Bhubaneswar within 24 hours of collection and were analysed using standard procedures as per the instructions of the manufacturer of anti-sera (Tulip Diagnostics Private Limited, Panaji, Goa, India). The red cells of these blood samples were tested with anti-A, anti-B and anti-H. To label a case as typical Bombay phenotype certain specialized tests like absorption-elution studies, titration of naturally occurring antibodies at different temperatures, inhibition of anti-H by O saliva secretor and secretor-status were performed as described by Bhatia (1977) and Balgir and Sharma (1988).

Data on ABO and Rhesus blood groups have been reported elsewhere (Balgir et al. 2004). This paper reports only on Bombay phenotype detected in two Kutia Kondh children.

RESULTS

Of the total 254 Kondh tribals screened for ABO and Rhesus (D) blood groups from Phulbani district, 65 Kutia Kondhs were studied from Belghar Residential Sevashram, Belghar in Tumudibundh Block of Phulbani district of Orissa. Out of 65 children, two unrelated cases of Bombay (Oh) phenotype were detected. These results were further confirmed by using another antisera of Ortho-Diagnostics Systems Limited, Mumbai. Both were the boys of 8 and 13 years old. Their hemoglobin levels were 11.8 and 13.6 g/dl, respectively.

DISCUSSION

The most striking finding of the present study was the detection of two unrelated cases of Bombay (Oh) phenotype in the primitive tribe, Kutia Kondh from the Belghar area of Phulbani district in Orissa. No case of Bombay phenotype has ever been reported among the primitive tribes from the state of Orissa and this is the first such report from Central East part of India. The difficulty in Bombay phenotype is that the individual having blood group of Bombay

phenotype (Oh) can only receive blood from an individual of Bombay phenotype and no other blood will match in case of an emergency for blood transfusion.

The present study shows an incidence of Bombay phenotype 1 in 33 among the Kutia Kondh tribe, 1 in 127 among Kondh tribe and 1 in 1,244 among the tribal populations of Orissa. Since the population size of Kutia Kondh tribe was relatively small (around 5000 individuals) and the practice of endogamy is strictly followed, therefore, the inbreeding and consanguinity amongst them is not ruled out which may be one of the major factors for the combination of recessive rare alleles like Bombay phenotype among the Kutia Kondh tribe. Bhatia and Sanghvi (1962) calculated the incidence of this phenotype as 1 in 13000 individuals in Mumbai. Later on, Bhatia and Sathe (1974) found its incidence as 1 in 7600 after screening a large number of samples in Mumbai. Gorakshakar et al. (1987) after systematic screening of the rural population from Ratnagiri and Sindhudurg districts of Maharashtra reported the incidence of Bombay phenotype as 1 in 4500 in that region, while Moores (1980) found its incidence as 1 in 18404 amongst Indians settled in South Africa.

Regarding the distribution and spread of Bombay phenotype in different states of India, the Oh phenotype is more common in state of Western and Southern parts of India as compared to other states (Fig. 1). Of the 179 cases reported by Sathe et al. (1988) from the Institute of Immunohematology (formerly Blood Group

Fig.1. Map of India showing different states

Reference Centre, Bombay), Mumbai, 112 (62.6%) cases belonged to the state of Maharashtra alone. The frequency was also high in Karnataka (14 cases), Andhra Pradesh (8 cases), Goa (6 cases), Gujarat (5 cases), Uttar Pradesh (5 cases) and so on in the decreasing order.The incidence of Bombay phenotype is high in those states of India where the consanguineous marriages are more prevalent, i.e. Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra, Gujarat, etc. than the other states (Table 1).

Table 1: Distribution of Bombay (Oh) phenotype cases reported from different states of India

S.No.	*State*	*No. of Bombay phenotype cases*
1.	Andhra Pradesh	8
2.	Bihar	2
3.	Goa	6
4.	Gujarat	5
5.	Karnataka	14
6.	Kerala	4
7.	Madhya Pradesh	4
8.	Maharashtra	112
9.	North India (unclassified)	2
10.	Orissa	1
11.	Pondichery	1
12.	Rajasthan	2
13.	South India (unclassified)	1
14.	Tamil Nadu	2
15.	Uttar Pradesh	5
16.	Not Known	10
	Total	179

Data from Sathe et al. 1988.

The Bombay phenotypes were also detected in Japan (Okubo 1980; Kaneko et al. 1997), Malaysia (Lopez, 1972), Thailand (Sringarm et al. 1977) and Sri Lanka (De Zoysa 1985). Yunis et al. (1969) found seven individuals of Oh phenotype in two generations in an Indian family settled in the USA. They were the natives of Orissa state. Similarly, Moores (1980) found 24 cases of Oh phenotypes in eleven unrelated Indian families settled in Natal, South Africa. Most of these families were either Tamil or Telugu speaking. Therefore, their origin presumed to be Andhra Pradesh or Tamil Nadu. This indicates that the Bombay phenotype is mostly confined to South-East Asian countries.

Further molecular and mutational research is required on Bombay phenotype regarding the evolutionary significance and the operation of natural selection among the Kutia Kondh primitive tribe of India.

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