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Mutational study and prevalence of Duchenne/Becker muscular dystrophy in different caste groups in Uttar Pradesh

S. Mishra*, V. Singh, L. S. Chaturvedi, R. D. Mittal† and B. Mittal

Departments of Genetics and Biochemistry†, Sanjay Gandhi Post-graduate Institute of Medical Sciences, Lucknow 226 014, India

*Present address: National Biotechnology Centre, Indian Veterinary Research Institute, Izatnagar 243 122, India

Social relationships have implication on the incidence of genetic diseases in the population. To analyse this aspect in Duchenne/Becker muscular dystrophy (DMD/BMD) patients, DNA profile of the dystrophin locus was carried out by PCR and Southern hybridization. The retrospective analysis of clinical and molecular data of the patients showed higher prevalence of the disease in brahmins and vaishyas as compared to the other caste groups in this region. However, the mutational pattern is not very different in various caste groups. The results have been explained by presence of repetitive dA-dT or transposon-like sequences in the introns of the dystrophin gene.

DUCHENNE/Becker muscular dystrophies are allelic X-linked recessive genetic disorders with a frequency of 1 in 3500 live male births¹. Duchenne type (DMD) is a severe form of the disease which causes loss of ambulation in early life, and death usually occurs by the end of the second decade. Becker type (BMD) disease follows a milder course and patients can remain ambulatory till 40-50 years of age².

Population-based variations in the incidence and pattern of gene mutations in DMD/BMD are well recognized. Higher prevalence of DMD has been reported in Indian migrants in UK as compared to local and other ethnic populations in that region³. In various European

populations, gene mutations in DMD/BMD patients show nonrandom distribution which changes with linguistic boundaries of the continent⁴. We have also reported higher proportion of dystrophin gene deletions in the patients from North India⁵. Moreover, analysis of the carrier status of mothers of these patients by quantitative polymerase chain reaction has yielded very high frequency of new mutations⁶. Further, the prevailing caste-based division in Indian society provides a unique opportunity to study genetic consequences of the disease in the population. The present study depicts the retrospective analysis of molecular data related to gene mutations in DMD/BMD patients belonging to various caste groups of Uttar Pradesh.

All patients were enrolled in the out-patient department. Most of the patients came from Eastern and Central part of UP, but some were also from the north-western part of the state (Figure 1). The clinical diagnosis of DMD/BMD was based on detailed proforma which included age of onset, relative strength of muscles, Gower's sign, Pradhan's sign^{7,8}, degree of ambulation and levels of creatine phosphokinase (CPK) in serum. Multiplex PCR and Southern hybridization were carried according to standard methods⁹. Translational reading frame of the putative mRNA in deletional cases was determined by border type analysis¹⁰. Caste information was voluntarily provided by the guardians. Patients having parents/grandparents with intercaste marriages were excluded from the present analysis.

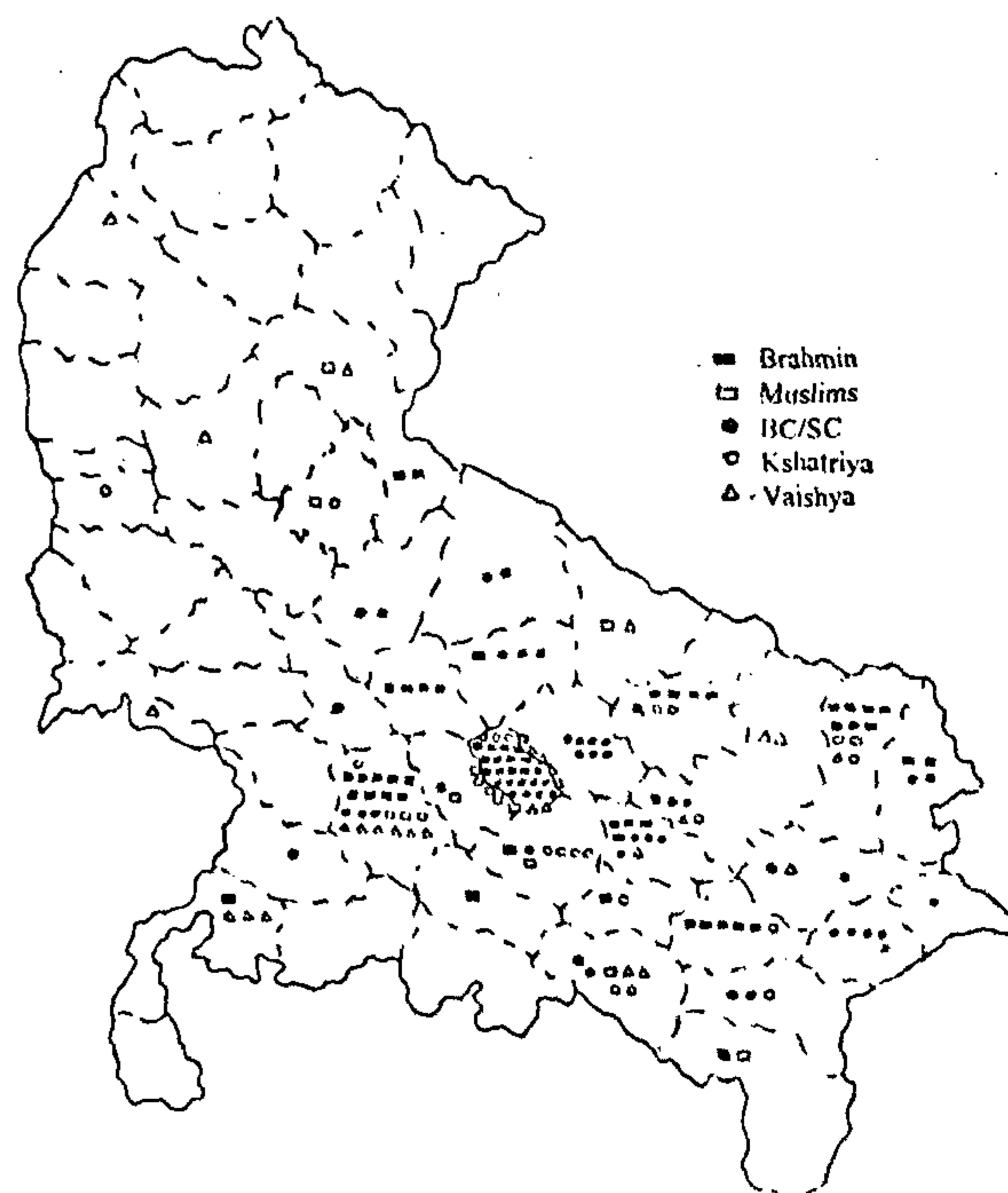


Figure 1. Regional distribution of cases of DMD/BMD among different caste groups in Uttar Pradesh.

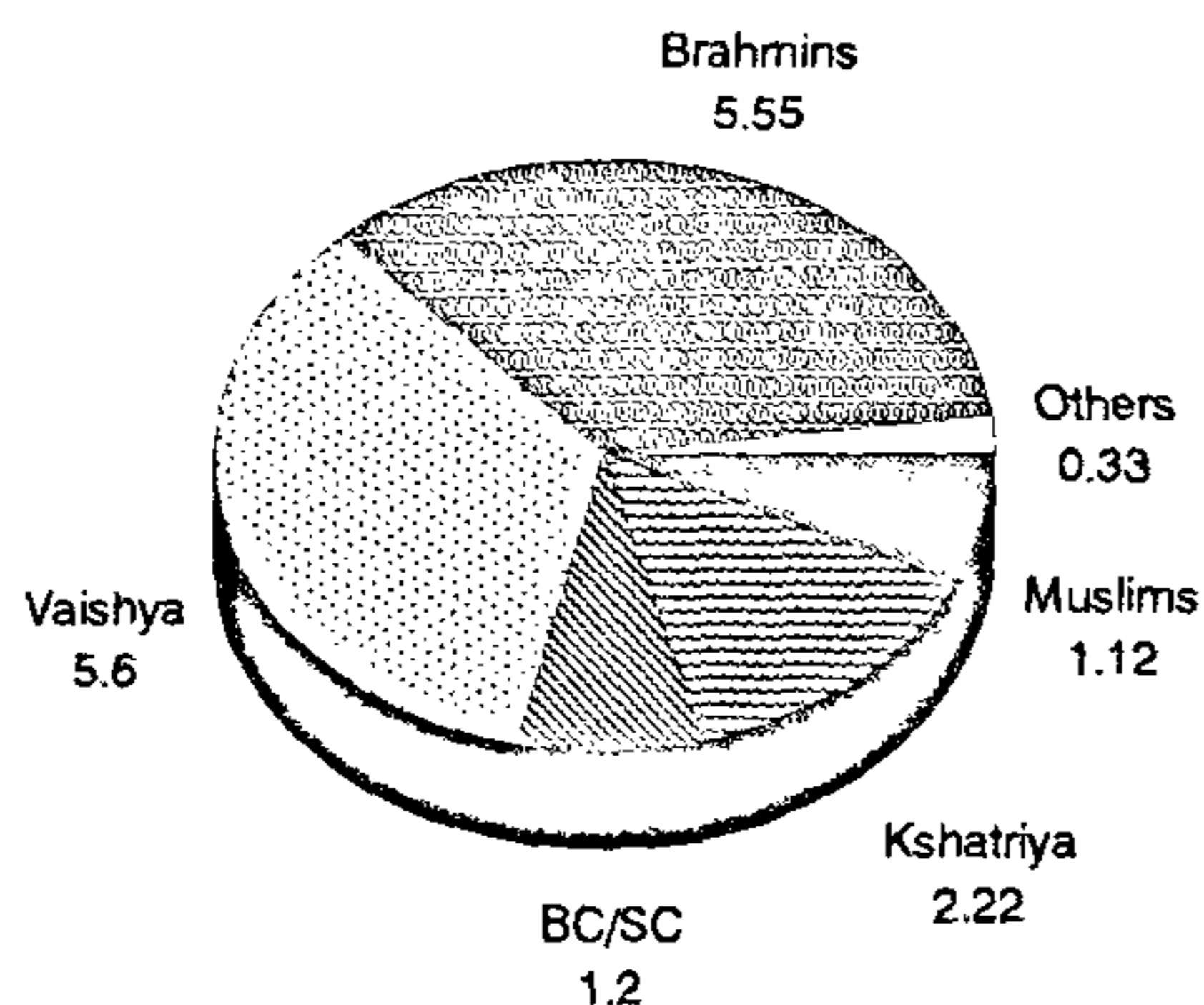


Figure 2. Comparative incidence of disease in different caste groups in UP. Relative figures are obtained by dividing number of patients with per cent population of particular caste.

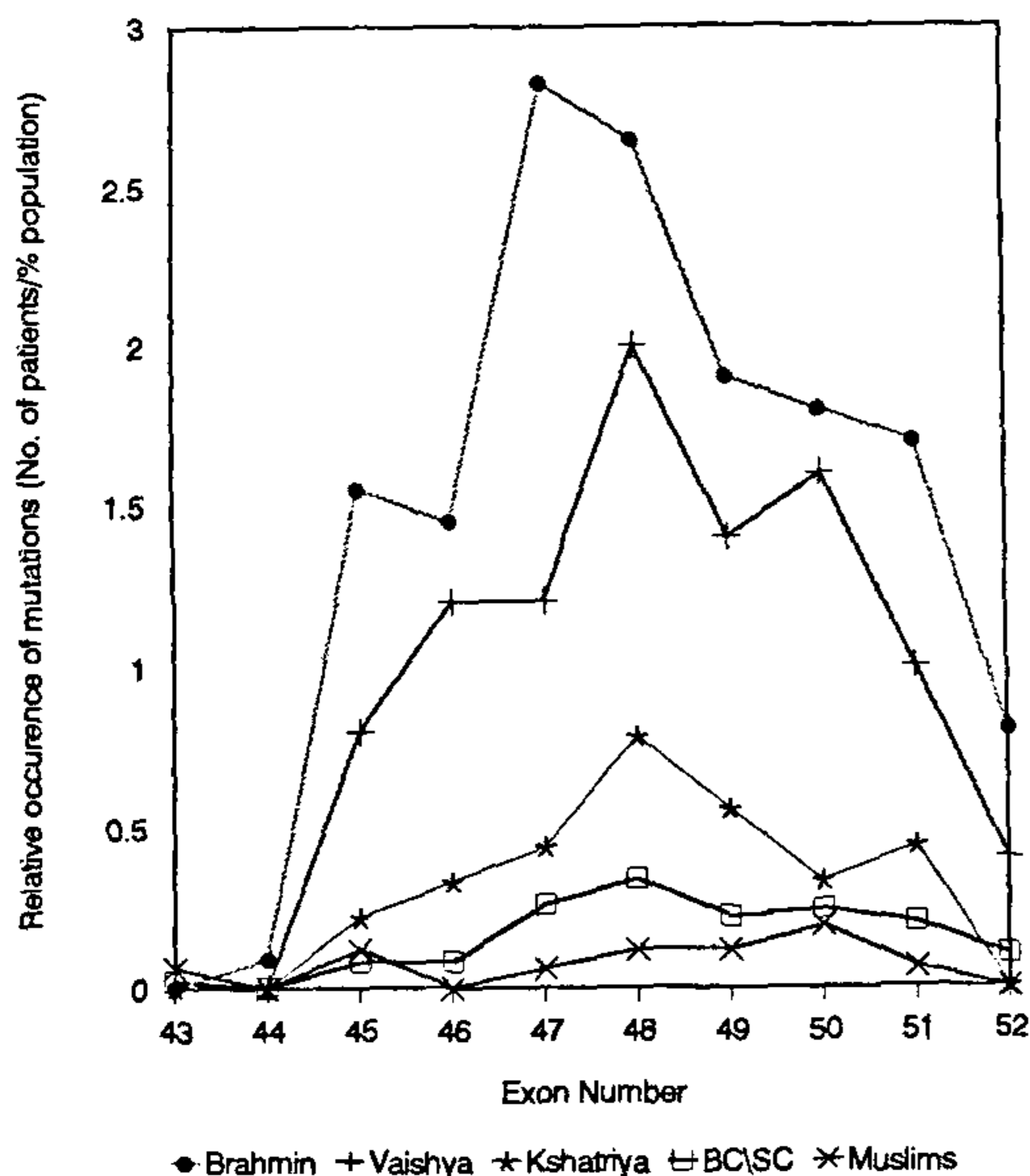


Figure 3. Relative involvement of central region exons of dystrophin gene mutations among different caste groups.

Retrospective analysis of clinical and molecular data on the basis of major caste groups of the region showed that more patients belong to *brahmins* as compared to other caste groups. Supposing that the number of patients registered in the present study is proportional to their number in general population of the region, relative incidence of disease was calculated by dividing number of patients to their percentage population in UP based on data from *India Today*, 15 May 1996). The relative incidence of DMD/BMD was found to be much higher in *brahmins* and *vaishyas* as compared to other caste groups (Figure 2). From combined results of PCR

and Southern hybridization, intragenic deletions could be detected in 70% of cases with DMD/BMD. The proportion and over-all pattern of deletions in different caste groups were similar, though minor variations in exonic involvements were observed at the central hot spot region of the gene (Figure 3).

The biology of social relation of humans is most interesting and debatable. The social structure of a community has its impact on the inheritance of genetic diseases. Communities with consanguineous marriages show higher incidence of some genetic diseases. It is impossible to plan selective breeding in human population, but in India, prevailing caste-based marriage systems provide some control over random breeding. The people generally prefer marriages in their own caste and the effect of this controlled breeding may be studied by analysing occurrence of genetic defects and polymorphism in different caste groups^{11,12}.

Higher relative incidence of DMD/BMD has been observed in *brahmins* and *vaishyas* as compared to other caste groups. It may be attributed to some degree of endogamous marriages followed in these caste groups. Both castes have various subgroups which prefer marriages in their own groups. Though other castes also have subgroups, the populations of *brahmins* and *vaishyas* are comparatively low in the region (*India Today*, May 15 1996), which limits their gene pool.

In DMD/BMD, about two-thirds of the patients show intragenic deletions ranging from one to several exons and the remaining one third patients are believed to have point mutations and other regulatory defects¹³. We had earlier observed relatively higher proportion of gene deletion in DMD/BMD patients from North India as compared to other populations particularly in Asia. Also, more than 80% of the mutations are clustered at the central hot spot region between exon 43 and 52 (ref. 5). This pattern holds true for all caste groups of UP despite the higher incidence of mutations responsible for causing the disease in *brahmins* and *vaishyas*. Exons(s) of the dystrophin gene involved in the patients from various caste groups are also not appreciably different (Figure 3). Moreover, no specific exon(s) deletion in the dystrophin gene could be correlated with particular caste groups analysed¹⁴.

Molecular basis for two phenotypes resulting from mutation in the same gene has been explained by reading frame hypothesis. According to it, deletional mutations changing the translational reading frame of dystrophin should cause severe form of the disease (DMD) but deletions still maintaining the frame result in milder form (BMD)¹⁵. We have determined the in-frame and frame-shift deletions in various caste groups. In *brahmins*, in-frame deletions account for about 42% of their deletional cases while in others, it ranges from 14% to 23% (Table 1). However, in spite of in-frame deletions, many *brahmin* patients have severe form of the disease (DMD).

Table 1. Incidence of DMD/BMD in different caste groups of Uttar Pradesh

| Caste | Population* per cent in UP | No. of affected families (patients) | Gene deletion observed in families | Gene deletion in-frame/out-frame |
|-----------|----------------------------------|--|--|-------------------------------------|
| Brahmin | 11 | 46 (60) | 33 | 14/19 |
| Kshatriya | 09 | 19 (20) | 10 | 2/8 |
| Vaishya | 05 | 21 (28) | 13 | 3/10 |
| BC/SC | 50 | 51 (60) | 26 | 5/21 |
| Muslims | 16 | 17 (18) | 07 | 1/6 |
| Others | 09 | 02 (03) | 00 | 0/0 |

*% Population of different castes was obtained from *India Today*, 15 May 1996.

Various factors have been attributed for population-based variations in the dystrophin gene mutations. Presence of repetitive elements like dA-dT stretches and transposon-like sequences have been implicated in the high frequency of deletional mutations at the central hot spot region of the dystrophin gene^{16,17}. It is possible that due to phenomena like genetic drift, local DNA environment might be different in various caste groups. Such differences can probably account for variations in occurrence of pathogenic mutations at the DMD locus. It would be of interest to further explore specific haplotypes for Xp²¹ locus (location of dystrophin gene) in different caste groups. These observations may have implications for genetic counselling programmes in our country.

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Studies on antianaphylactic activity of fractions of *Albizia lebeck*

C. C. Barua, P. P. Gupta, G. K. Patnaik,
D. K. Kulshrestha and B. N. Dhawan

ICMR Centre for Advanced Studies for Traditional Remedies,
Division of Pharmacology, Central Drug Research Institute,
Lucknow 226 001, India

Two fractions (F079 and F080) of the hot aqueous extract of stem bark of *Albizia lebeck* have been evaluated for antianaphylactic/antiallergic activity *in vitro* and *in vivo*. Both fractions inhibited antigen-induced contraction of the sensitized guinea pig ileum (Schultz-Dale phenomenon). The bronchoconstriction induced by the antigen egg albumin in presensitized guinea pig was also inhibited by these fractions in a dose-dependent manner. The fractions, however, did not possess any bronchodilatory effect *per se* in nonsensitized animals.

THE treatment of bronchial asthma is far from satisfactory. A potent drug against bronchial asthma is still wanting. However, there are claims in the traditional systems of medicine for the treatment of bronchial asthma. *Albizia lebeck* is a major constituent of the traditional medicines used against bronchial asthma¹. The decoction of the bark of *A. lebeck* was found to protect guinea pigs against antigen-induced challenge and there was a marked inhibition of Schultz-Dale phenomenon². Chronic treatment with the bark decoction also protected the sensitized guinea pigs against antigen challenge³. It has been observed that the aqueous extract of the plant possesses antiallergic activity. In a previous study we have observed that the hot aqueous extract of the plant showed promising antiallergic activity in PCA (passive cutaneous anaphylaxis) and mast cell stabilizing activity⁴. The most active fraction from this extract was further chromatographed and fractions F079 and F080 showed maximum activity. The antiasthmatic/