Short report

Haptoglobin groups in motor neuron disease

NILS FRÖHLANDER,* LARS FORSGREN
From the Departments of Medical Genetics* and Neurology, University of Umeå, Sweden

SUMMARY Haptoglobin (Hp) groups were investigated in 81 patients with motor neuron disease. A significant excess of heterozygotes was observed, accentuated among males and in the progressive spinal muscular atrophy subgroup. The results are discussed in terms of a possible influence of Hp in the immunological response.

Motor neuron disease is a progressive disorder of the upper and lower motor neurons. Affected individuals become severely disabled and many die within a few years. Most cases are sporadic but 5–10% occur in a familial pattern suggesting autosomal dominant inheritance. A slight male overrepresentation is often observed. Three clinical varieties are recognised, depending on involvement of upper or lower motor neurons: amytrophic lateral sclerosis (ALS) shows signs of upper and lower motor neuron disorder, whereas progressive bulbar paralysis (PBP) and progressive spinal muscular atrophy (PSMA) show only lower motor neuron signs. With time the symptomatologies of these variants often merge and in late stages of the disease subgroups are difficult to distinguish.

The cause and pathogenesis of motor neuron disease are unknown and various aetiologies, such as viral, immunological, genetic and environmental, have been proposed. On the island of Guam, with a focus of high incidence for motor neuron disease, extensive investigations of genetic markers have been performed without finding any striking associations. Apart from histocompatibility antigens genetic markers have not, to our knowledge, previously been investigated in cases of motor neuron disease outside the high incidence areas.

Haptoglobin (Hp), the major haemoglobin-binding serum protein, is an acute phase reactant with various properties of unknown biological significance. Hp seems to inhibit prostaglandin synthetase and some lysosomal enzymes such as cathepsin B. Furthermore, phytohaemagglutinin-stimulated lymphocytic blastogenesis and the chemotaxis of monocytes appears to be inhibited by Hp. Whether the different Hp phenotypes differ with respect to these properties is unknown, but individuals carrying the Hp 1–1 phenotype have been shown to yield a weaker humoral response to vaccination or viral infections.

With primary reference to immunological aspects of the disease, we have investigated the distribution of Hp groups in 81 patients with motor neuron disease.

Material and methods

Serum samples were collected from 64 patients with motor neuron disease, 36 males and 28 females, seen in the Department of Neurology, Umeå University Hospital, between 1981 and 1986. An additional 17 patients, 11 males and six females, attending the Department of Neurology, Karolinska Hospital, University of Stockholm, during 1984–1986 were included. The diagnosis of motor neuron disease was based upon clinical findings of a progressive pure motor disorder of spinal innervated musculature (progressive spinal muscle atrophy; PSMA), in bulbar innervated musculature excluding ocular muscles (progressive bulbar paralysis; PBP) and signs of central motor neuron involvement (amyotrophic lateral sclerosis; ALS). The diagnosis was supported by electromyogram in all cases.

Haptoglobin groups were determined by horizontal polyacrylamide gel electrophoresis using a discontinuous buffer system followed by staining with amido black. The statistical analysis was performed using the Chi-square test. There were no statistically significant differences between the Umeå and the Stockholm patients or controls. The patients were thus pooled and as controls served a large population sample from the county of Västerbotten.

Address for reprint requests: Dr Nils Fröhlander, Department of Medical Genetics, University of Umeå, S-90185 Umeå, Sweden.

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**PSMA and PBP.** As the disease proceeds these differences are usually diminished and in the terminal stage mostly all striated muscles, except ocular and sphincter, are paralysed. Motor neuron disease is rapidly progressive with a 5 year survival of about 25–30%, PSMA having a somewhat milder course.16

Throughout the years a vast variety of aetiological theories have been proposed, including metabolic disorders, cellular aging, virus, environmental factors such as heavy metal incorporation, genetic and immunological mechanisms.2 Clusters of the disease have been observed on southern New Guinea, the Japanese Kii peninsula and among the Chamorro population on Guam. The increased risk of developing the disease seems to accompany Chamarros emigrating to the US, suggesting a genetic predisposition.17

In a search for a genetic marker for the disease extensive studies of the HLA system, blood groups, red cell enzymes, serum proteins, immunoglobulin allotypes and dermatoglyphics have been performed on Guam without disclosing any major association.4–6

Investigations of HLA antigens in motor neuron disease from Europe and the US have presented diverging results and probably there is no association between the disease and the histocompatibility antigens.7

In the present investigation of 81 cases of motor neuron disease we observed an excess of the Hp 2–1 phenotype among patients, most pronounced among males and in the PSMA subgroup. The significance of this finding is unclear. Hp is known as an acute phase protein and its inhibitory activity on prostaglandin synthetase and cathepsin B has been proposed as a part of the regulation of the inflammatory reaction.18

Its inhibitory effects on lymphocyte blastogenesis and monocyte chemotaxis at concentrations corresponding to those in inflammatory states suggests an involvement in the immunological response.10–11 The Hp variants show great structural dissimilarities.19–20

Hp 1–1 is a simple tetramer composed of two $\alpha^1\beta$-dimers whereas Hp 2–1 is composed of some $\alpha^1\beta$-dimers but mainly of a series of linear polymers of $\alpha^1\beta$- and $\alpha^2\beta$-dimers. Thus Hp 2–1 occurs as hybrid molecules. Hp 2–2 results from circular polymerisation of $\alpha^2\beta$-dimers. With these structural differences it seems plausible that for any function or property the Hp variants could show considerable quantitative differences. Variations between phenotypes have not been investigated for all the properties mentioned above, but it has been shown that individuals of the Hp 2–1 or 2–2 phenotype yield a stronger humoral response to vaccination and viral infections than do Hp 1–1 individuals.12–13 This may not, however, be true for all infectious agents.21

A sensitisation of motor neuron disease lympho-

### Results

The distribution of Hp groups in patients, subgrouped according to sex and type of motor neuron disease, and in controls is shown in the table. Patients, particularly females, generally had higher Hp1 gene frequencies than controls, though the difference was not significant. Male patients deviated significantly from the expected Hardy-Weinberg equilibrium ($\chi^2 = 6.04, p < 0.02$) whereas the deviation for the whole patient material was less pronounced ($\chi^2 = 3.24, N.S.$). The male patient group also deviated significantly from controls with respect to phenotype distribution ($\chi^2 = 6.30, p < 0.05$). The skew distributions resulted from an excess of Hp 2–1, among male patients in particular ($\chi^2 = 6.29, p < 0.02$) but significant also in the whole patient group ($\chi^2 = 4.56, p < 0.05$). In the latter group the decrease in Hp 2–2 was significant as well ($\chi^2 = 5.62, p < 0.02$). In the ALS subgroup we observed a heterozygote excess of the same magnitude as for the whole patient group whereas in PSMA the Hp 2–1 frequency equalled that of the male patients. Due to the small numbers in each subgroup these differences were however not statistically signficant.

The rather pronounced decrease in Hp 2–2 is consistent throughout the material and one might therefore suggest that this is the primary deviation from normal and that the excess of heterozygotes is a secondary effect. However, breakdown of the difference from unity yields that the greatest contribution to the Chi-square value is generally an effect of the heterozygote deviation. Furthermore, the excess of Hp 2–1 among male patients and in PSMA results in a compensatory decrease in both homozygotes. In the further discussion we will therefore be referring to the excess of heterozygotes among patients as the primary effect.

### Discussion

Motor neuron disease is a disorder of the upper and lower motor neurons resulting in paralysis of striated musculature. Depending on the area primarily engaged the disease is clinically subdivided into ALS, PSMA and PBP.
cytes to neuronal membrane components\textsuperscript{22} and cytotoxic activities of ALS plasma against erythrocytes\textsuperscript{23} have been demonstrated, suggesting an immunological mechanism. In laboratory animal experiments a 56K sprouting factor released from denervated muscle has been identified. This factor reacted with antibodies present in ALS plasma capable of suppressing terminal axonal sprouting and subsequent reinnervation of skeletal muscle.\textsuperscript{24} If in fact Hp groups are involved in the regulation of any part of the immunological response one may interpret the observed association between HP 2–1 and motor neuron disease in these terms. That is, the quantitatively different functional properties of one particular type of Hp may, in combination with other factors, become relevant in the pathogenesis of a disease. From the results of the present investigation one may propose that Hp 2–1 exerts a quantitatively different influence on some step in the supposedly impaired immune control, and that other factors working in the same direction being present makes the Hp effect apparent. However, this is the first investigation of Hp groups in motor neuron disease outside of the high risk areas, and thus further discussions on the possible involvement of Hp in the pathogenesis of this disease should be postponed until these findings are confirmed.

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References