Immunoglobulin levels in dystrophia myotonica

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SUMMARY Levels of immunoglobulins IgG, IgA, and IgM were measured in 38 patients with myotonic dystrophy, in normal members of their families, and in matched controls. Log IgG was significantly reduced in the patients. IgG investigation provides a further parameter to appraise the status of apparently unaffected members of myotonic dystrophy families.

Dystrophia myotonica is inherited as a dominant disease but its clinical manifestation and age of onset are highly variable, which means that many patients reproduce before they themselves develop symptoms. Help and advice on the risks to their offspring should be available to them as early as possible. In a recent survey of patients in Newcastle, affected and unaffected members of their families were examined by clinical, electrophysiological, genetic, and other procedures (Polgar *et al.*, 1972). The object of the investigation was to assess the usefulness of these methods for detecting cases of dystrophia myotonica, while still in the presymptomatic stage, in other members of a family where one is already known to be suffering from the disease. It was concluded that in families in which there was no informative gene linkage clinical examination and electromyography were the two most useful screening procedures. One variable that may be relevant in early detection is the immunoglobulin levels (Bundey *et al.*, 1970), and these were not included in the previous report. The present study examines the immunoglobulin levels in the Newcastle series of patients and their families.

Material and methods

The details of the families studied together with the results of the clinical, electrophysiological, genetic, and other investigations have already been reported (Polgar *et al.*, 1972). The serum specimens obtained for examination of serum protein types and subsequently stored frozen at -20°C were utilized for the present part of the study.

Immunoglobulins A, G, and M were estimated by means of the single radial immunodiffusion technique (Mancini *et al.*, 1965) using commercially

available plates and standard antigen preparations (Behringwerke AG). Immunoglobulins A and M were measured on LC partigen plates and immunoglobulin G was measured on tripartigen plates.

Stabilized standard human serum was used in recommended dilutions to calibrate the curve for IgA and IgM estimations and an IgG standard was used for IgG estimation. Using a Hamilton microlitre syringe three wells of each plate were filled with 5 or 20 μl of the three dilutions of the standard and the remaining wells filled with 5 or 20 μl of test samples, and left at room temperature during the time recommended for the diffusion (3 days). After the diffusion was complete the squares of the precipitin ring diameters were plotted against the different concentrations of the standard in order to obtain a standard reference curve. The concentrations of immunoglobulins (mg/100 ml) in the test samples were then determined by taking intercepts on the reference curve. As the precipitin rings on the LC partigen plates were usually too weak to be read with accuracy the gel plates were treated with tannic acid. This procedure consisted of rinsing the plates with phosphate-buffered saline (pH 7.2) for 24 hours, immersing them in a 4% aqueous solution of tannic acid for 20 minutes, and finally transferring them into distilled water for one hour, after which the rings could be measured.

Duplicate tests were carried out at intervals of several days on all specimens, and in all cases duplicate readings were within acceptable limits. The mean of the two readings was taken to characterize each subject. To confirm that storage had not affected the Ig levels duplicate specimens were obtained from 20 of the subjects. In all three Ig's there was close correlation (0.7-0.8) with the level in the stored specimens.

Of the 125 specimens tested 87 were from

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Fig.

members of the myotonic dystrophy families. There were 38 affected individuals (Fig.), of whom 18 were known from the case records at the beginning of the study, and 20 others were found to have clinical signs during the course of the study. For comparison, there were 13 unaffected spouses of patients and 36 unaffected first- and second-degree relatives. In addition, specimens were obtained from 38 healthy control individuals matched for age, sex, and locality of residence to the patients. Against the results from these comparisons specimens were examined from a further 8 family members who could not be clearly identified as either affected or not affected.

Results

The distribution of IgG levels showed a pronounced skew both in the affected and the normal individuals. A logarithmic transformation was applied. The resulting distribution appeared normal (visually and by χ^2 comparison of observed with expected numbers in each quartile) and all calculation on IgG were therefore performed on the log of the reading. The same procedure was followed for IgM. The distribution of IgA, however, appeared sufficiently normal for no transformation to be necessary, and the calculations were based on the raw data.

Neither IgA nor log IgM showed any difference between normal and affected subjects, confirmed by analysis of variance (Table 1). The mean for IgA in normal family members was 250.7, in affecteds 249.6, and in population normals 249.2. The mean for log IgM in family normals was 2.077, in affecteds 2.127, and in population normals 2.099. However, the mean for log IgG in the 38 affected individuals (2.935) was clearly lower than in the unaffected relatives (mean 3.103), and this difference was highly significant ($t = 5.67$, with 72 degrees of freedom). It was clearly lower than the mean in the 38 control individuals ($t = 2.53$; DF 74; $P < 0.01$, one-tailed), and analysis of the differences in the matched pairs proved also significant ($t = 2.35$; $P < 0.02$, one-tailed). There was no difference between unaffected relatives (mean 3.103) and the spouses of patients (mean 3.054), and there was no difference of

Table 2 Probability that a clinically unaffected family member is affected

Log IgG	Probability of being affected
< 2.9	0.96
2.9-3.0	0.51
3.0-3.1	0.42
3.1-3.2	0.22
> 3.2	0.14

either or both from the sample of 38 controls (mean 3.005). These similarities suggest that the environments of the families do not contribute to the depressed levels in the patients.

To exclude the possibility that there is some intrinsic difference in Ig levels between families a similar analysis was carried out using deviations from the mean of each family instead of the actual Ig values. Again there was no significant difference between affected and unaffected in IgA and log IgM, but the highly significant difference between these again emerged for log IgG ($t = 5.05$; DF 69; $P < 0.001$).

Eight individuals who could not be pronounced definitely affected or clear in the earlier study were also tested. From the inheritance pattern each of these had a 50% chance of carrying the myotonic dystrophy gene and being in a presymptomatic state. From the distributions of levels in affected and unaffected may be calculated the relative odds that any individual of a known level falls into either category (Table 2). For example, the probability that B III₁, K I₂, or P II₃ is affected is only 0.22, and for B III₃ it is 0.42. These figures can be combined with the prior probabilities to calculate joint and posterior probabilities. For all of these eight individuals the IgG levels and the probabilities embodying them appear optimistic.

Discussion

Low serum levels of immunoglobulin G in patients with dystrophia myotonica were first pointed out by Zinneman and Rotstein (1956), while Wochner *et al.* (1966) showed that the deficiency of IgG was due to an abnormally fast catabolism of IgG. Bunday *et al.* (1970) found that while immunoglobulin levels showed much individual variability the means of IgG and IMg in patients and controls differed significantly. In first-degree relatives of affected patients the distribution of IgG and IgM, though lying between those of the control and index patients with an intermediate mean, were not bimodal but the clinically affected all lay within the patients' distribution. The level of the means suggested that immunoglobulin changes may precede clinical manifestation of the disease. Grove *et al.* (1973), however, failed to find any difference in IgG or IgM in a short series of 15 patients.

Table 1 Immunoglobulin levels (mg/100 ml)

	No.	IgA		Log IgM		Log IgG	
		Mean	SD	Mean	SD	Mean	SD
Affected	38	249.6	105.7	2.127	0.228	2.935	0.148
Normal family members	36	250.7	85.9	2.077	0.168	3.103	0.098
Spouses	13	258.2	85.4	1.998	0.175	3.054	0.112
Population normals	38	249.2	78.9	2.099	0.206	3.005	0.084

SD = standard deviation.

While the present results show once again the clear relations of IgG levels to dystrophia myotonica several features deserve comment. First, by contrast to the findings of Bunday *et al.* (1970), there is the lack of difference in IgM levels between patients and unaffected relatives, and we can offer no explanation for this. It is not due to the storage of specimens, as comparison with measurements carried out on 20 fresh repeat specimens showed. It is not due to age, for though each immunoglobulin has its own pattern of age variation no age effect emerges over the age range of the individuals in the present study. The IgM difference in the study of Bunday *et al.* was significantly only at 0.05 and the authors pointed out the need for further investigation. But the implication is important, for if, as the present results suggest, IgM does not differentiate dystrophia myotonica patients then there is no reason to discard the hypothesis that a class-specific catabolic mechanism is involved in dystrophia myotonica, which the apparent independence of the IgG levels from the other immunoglobulins earlier suggested, and this is at variance with the suggestion of Grove *et al.* (1973) that myotonica dystrophia may carry a wider derangement of immunological function.

It is interesting that in all eight of the individuals tested in the present series in whom a definitive diagnosis was not given, the IgG levels appear normal, and the probability therefrom that these subjects are affected is consistently low. The suggestion by Bunday *et al.* (1970) that changes in immunoglobulin levels may precede clinical manifestation of the disease was based on the observation that the distribution of log IgG values in total first-degree relatives occupied an intermediate position between those of index and controls and so, less strikingly, did the distribution of clinically normal first-degree relatives. While most of the clinically normal first-degree relatives in the lower part of the distribution were not examined by slitlamp or EMG those with evidence of myotonic dystrophy by slitlamp with or without EMG showed a wide range of readings—all within the normal range (some indeed in its upper half, like the two subclinical cases in our series) but some in the zone of overlap of affected and unaffected distributions.

On account of this wide range, as with other continuous variables discriminating between affected

and unaffected, one cannot be sure of the status of any given individual but only assign a probability that he belongs in one or other category—provided that the presence of any other condition affecting IgG levels is discounted. But it must be emphasized that the probabilities based on IgG levels are not to be transferred to data from other laboratories, which should devise their specific probability tables to avoid the possible effects of slight differences in technique. In the present series none of the pending cases showed any unequivocal evidence of myotonic lenticular opacity, though other types of lenticular defect were noted in three, and similarly none of them showed electromyographic evidence of myotonia. Perhaps the optimistic probabilities suggested for them by the IgG results are in fact correct.

The present work has reiterated the importance of immunoglobulin G levels in distinguishing those affected with myotonic dystrophy. It suggests that IgG investigation alone, though unlikely to provide a definitive indication, could profitably be included with other quantitative measures in any examination of members of families with this disorder.

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