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Carrier detection in Duchenne muscular dystrophy

Evidence from a study of obligatory carriers and mothers of isolated cases

J. R. SIBERT, P. S. HARPER, R. J. THOMPSON, AND R. G. NEWCOMBE

Departments of Child Health, Medical Genetics, Medical Biochemistry and Medical Statistics, Welsh National School of Medicine and University Hospital of Wales, Cardiff

SUMMARY The mean levels of serum creatinine phosphokinase (CPK) were studied in three groups of women: normal controls (57), obligate carriers for Duchenne muscular dystrophy (30), and mothers of isolated cases of this disease (35). The distribution of the levels in these groups was significantly different and was in keeping with the hypothesis that one-third of isolated cases result from new mutations. The control and carrier ranges overlapped considerably, with the level of CPK of 33% of obligate carriers coming within the 97½ centile of the normal range. Odds against an individual being a carrier were derived for specific mean values of CPK. They should be considered with genetic information using Bayes's theorem. The mean CPK levels in obligate carriers showed significant familial clustering. This may have implications in carrier detection.

The primary metabolic defect in Duchenne muscular dystrophy remains unknown, but a raised level of serum creatinine phosphokinase (CPK) has until now been the best available method for detecting the female carrier in this disease (Schapira *et al.*, 1960). The fact that carrier and normal ranges overlap makes the interpretation of values within the normal range difficult, even when biochemical and genetic information is integrated. The mothers of isolated cases are a particular problem, and it has been suggested (Roses *et al.*, 1976) that almost all such women are carriers, although genetic theory and the results of CPK studies do not confirm this.

The present work, based on a population study of Duchenne muscular dystrophy in Wales, assesses the efficacy of the serum CPK in carrier detection, and re-examines the problem of the proportion of isolated cases which represent new mutations. The evidence for familial correlation of CPK levels in carrier women, which is of considerable practical consequence in genetic counselling, is also investigated.

Welsh National School of Medicine and University Hospital of Wales, Cardiff
J. R. SIBERT, consultant paediatrician
P. S. HARPER, reader in medical genetics
R. G. NEWCOMBE, statistician
Department of Clinical Biochemistry, New Addenbrooke's Hospital, Cambridge
R. J. THOMPSON, lecturer in medical biochemistry

Methods

All families in Wales known to contain at least one member with Duchenne muscular dystrophy were investigated as part of a more general genetic study of the disorder in Wales in which complete ascertainment was attempted. 75 such families have so far been studied. Patients were diagnosed as having Duchenne muscular dystrophy if they had had a severe disability before age 12 years and had greatly increased serum CPK, in addition to the recognised clinical features of the disease. Great care was taken to differentiate families with the Becker form of X-linked dystrophy.

Three groups of women were studied: (1) obligate carriers, a woman with at least one son and one other male relative affected or with two or more affected sons; (2) mothers of isolated cases; (3) healthy nonpregnant female volunteers of comparable age range.

Three separate blood samples were taken from these women with at least 3 days' interval between each venepuncture. Only one sample could be taken from 3 elderly obligate carriers. We did not investigate girls under 15 years. All blood samples were taken under conditions of normal activity, and heavy exercise during the preceding 24 hours was avoided. All CPK levels were determined on an Unicam SP 1800 recording spectrophotometer by the Boehringer (Mannheim) UV-System 10 assay

method by one of us (R.J.T.). Blood samples were either separated within 2 hours and assayed directly or were stored at 4°C overnight. This made no significant difference to the recorded activity. Care was taken to protect sera from light.

Results

We studied the mean of the 3 serum CPK values for each woman. The distribution of CPK levels in normal controls (57), obligate carriers (30), and mothers of isolated cases (35) is shown in the Figure. From these distributions it can be seen that the normal range and the range for obligate carriers overlapped to a great extent. Approximately 33% of obligate carriers came within the 97½ centile for normal range. Three of our control women had mean CPK values of at least 100 mU/ml. The distributions are not gaussian but if logarithmic CPK values are used they approximate to a distribution of this form, with the means and SDs as shown in Table 1.

The odds against an individual being a carrier can be derived for specific values of serum CPK, and are shown in Table 2. They can be combined with the genetic odds and other information using Bayes's theorem, enabling precise odds to be given for values of CPK within the normal range. Details of the working of these calculations can be found in textbooks of medical genetics, such as Emery (1976).

Take for instance the case of a daughter of a known carrier. She has a prior probability of being a carrier herself of one in 2. However, information from CPK values, and any normal sons she has must also be considered. If she for instance had a mean CPK of 45 mU/ml, and a normal son, odds of approximately one in 8 for CPK value, and one in 2 for the normal son must be combined with the prior probability. Using Bayer's theorem this gives a final probability for this woman being a carrier of one in 17, significantly from the one in 2 original figure.

The CPK values for the three groups of women we studied differ significantly. Comparing normal controls with obligate carriers $t = 7{\cdot}20$ $P<0{\cdot}001$, and normal controls with mothers of isolated cases $t = 9{\cdot}09$ $P<0{\cdot}004$. If obligate carriers are compared with mothers of isolated cases $t = 3{\cdot}34$ $P<0{\cdot}002$.

The mean serum CPK values of the 30 obligate carriers is shown in Table 3 arranged within families. The ratio F of between family variance to within family variance based on logs of means of replicates is $3{\cdot}96$ $P<0{\cdot}05$. There is thus a significant familial clustering of CPK values.

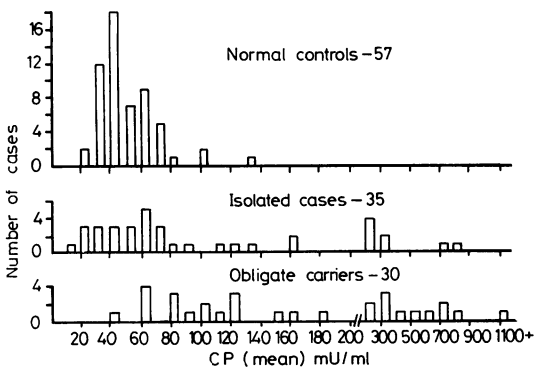

Figure *Distribution of serum CPK levels in normal controls, isolated cases, and obligated carriers.*

Table 1 *Means and standard deviations of cases*

	No. of cases	*Geometric mean*	*Mean log CPK*	*SD log CPK*
Normal controls	57	49·8	1·698	0·147
Obligate carriers	30	188·1	2·274	0·426
Mothers of isolated cases	35	83·9	1·924	0·417

Table 2 *CPK (mean of observed values) and likelihood ratio for carrier status*

Range of CPK	*LR*	*Range of CPK*	*LR*
<40	8·221	120–	0·0782
40–	8·033	130–	0·0398
50–	6·103	140–	0·0204
60–	3·831	150–	0·0106
70–	2·167	160–	0·00553
80–	1·157	170–	0·00292
90–	0·598	180–	0·00156
100–	0·305	190+	<0·001
110–	0·154		

LR = likelihood ratio.

Table 3 *Mean CPK levels in obligate carriers, arranged in families*

Family code	*Mean CPK (mU/ml)*	*Family code*	*Mean CPK (mU/ml)*
D2	187	D34	244
	122	D35	116
D3	60	D36	84
	48	D41	764
D5	438	D44	65
	159		61
D8	82		64
D9	519		162
D11	128	D45	123
	304	D46	100
	387	D47	246
D13	109	D48	90
	80	D49	627
D26	879		2300
D28	335	D60	773

Discussion

Several methods have been suggested for detecting carriers of Duchenne muscular dystrophy. Changes in muscle histology and histochemistry have been noted in some carriers, but many have given normal results. There is less discrimination between normal subjects and carriers than with serum CPK estimations. Qualitative electromyography is not of value in carrier detection; however quantitative work does show some discrimination but this method does not appear to improve the accuracy of detection based on serum CPK estimations. There is no suitable marker gene close enough to the Duchenne locus on the X chromosome to be of value in linkage work, and there is a greater overlap in other serum enzyme levels between normal subjects and carriers than with serum CPK estimations. Thus although this CPK method is far from ideal no better alternative is available. The subject has been reviewed by Emery (1969) and Gardner-Medwin *et al.* (1971).

Other workers as well as ourselves have found that control and carrier ranges of CPK values overlap greatly (Table 4). This overlap may represent variable X chromosome inactivation in the heterozygous females, as well as lack of relationship of the test to the primary biochemical defect of the disease.

We found the calculation of the odds of a woman being a carrier of Duchenne dystrophy at a certain CPK value within the normal range adds considerably to the precision of genetic counselling, as have the studies of Emery (1969) and Dennis *et al.* (1976). These odds must be combined with gentic information by the use of Bayes's theorem.

Roses *et al.* (1976) suggested, based on the study of phosphorylation of red-cell membrane proteins, that the mothers of almost all isolated cases are carriers, possibly as a consequence of unequal mutation rate between the sexes. The distribution of CPK values of the three groups of women we studied (obligate carriers, mothers of isolated cases, and normal controls) differs significantly, which argues strongly against this hypothesis. Our results are compatible with the classical genetic hypothesis of Haldane (1935) that one-third of isolated cases in a lethal X-linked recessive disorder are the results of new mutations. If this hypothesis is correct, mothers of isolated cases in our series should have a mean log CPK of 2·082 with a SD of 0·445. The values of CPK in these women are compatible with this, and give no grounds for rejecting the classical hypothesis. The implications for genetic counselling for families of isolated cases are considerable. Pickard and his colleagues (1978) recently presented data on lymphocyte capping on a variety of neuromuscular conditions, including mothers of boys with Duchenne dystrophy. They suggested that new mutations may be more common than Haldane thought. However they have not clearly differentiated obligate carriers on family history from mothers of isolated cases, nor have they analysed statistically the differences between these two groups of women and normal controls. Furthermore they did not state whether their normal controls are women of child-bearing age or not.

Our results show that correlation within families may exist for levels of CPK in carriers; this has important implications in carrier detection. Thus a relatively low CPK level in a potential carrier where obligate carriers in the family are also known to have a low CPK level may need to be interpreted with caution. More extensive data on families with multiple obligate carriers will be required to assess accurately the degree of such correlation and to allow it to be used with other information.

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Table 4 *Data on obligate carriers with CPK levels lying outside normal range in some previous series*

Series	*Normal controls (No.)*	*Obligate carriers (No.)*	*Obligate carriers with CPK levels outside normal range (%)*
Wilson *et al.* (1965) updated by Dennis *et al.* (1976)	61	32	78
Thompson *et al.* (1967)	90	37	65
Emery (1969)	107	34	53
Thomson (1969)	Not stated	11	81
Gardner-Medwin *et al.* (1971)	34 (including 9 males)	35	57
This series	57	30	67

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Correspondence to Dr J. R. Sibert, Department of Child Health, Llandough Hospital, Penarth, Glamorgan CF6 1XX.

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