The detection of carriers of benign (Becker-type) X-linked muscular dystrophy

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The use of serum creatine kinase (SCK) estimations in the detection of female carriers of the severe (Duchenne-type) X-linked muscular dystrophy was first introduced by Okinaka and his colleagues in 1959, and since then the value of this test has been confirmed by many others (Dreyfus and Schapira, 1961; Hughes, 1963; Richterich, *et al*, 1963; Emery, 1965; Rothauwe and Kowalewski, 1965; Wilson, Evans, and Carter, 1965; Dreyfus *et al*, 1966; Thompson, Murphy, and McAlpine, 1967). Approximately two-thirds of women who carry the gene for Duchenne muscular dystrophy have been found to have significantly elevated levels of SCK and this is now accepted as the most reliable single test for carrier detection (Emery, 1969b).

Becker was the first to report the existence of a benign form of X-linked muscular dystrophy which was distinct from the well-recognized Duchenne type (Becker and Kiener, 1955; Becker, 1957, 1962). This form of muscular dystrophy may be defined as a comparatively benign proximal myopathy affecting several males in at least two generations of a family, the pattern of inheritance being consistent with that of an X-linked recessive trait. Although clinically similar in presentation to the Duchenne type, with pseudohypertrophy of the calves and weakness affecting first the pelvic girdle and then the pectoral girdle musculature, the Becker type is of later onset and has a more benign course. Affected individuals usually become chair-ridden only after about 20 years and most survive at least into middle age.

As in the Duchenne type affected males with the Becker type of muscular dystrophy have grossly elevated levels of SCK in the preclinical and early stages of the disease (Emery, 1968). The test has also been used as a method for detecting female car-

riers of Becker muscular dystrophy by a number of investigators with varying degrees of success. From reports in the past it would appear that approximately 50% of known carriers of the gene for Becker muscular dystrophy have a raised level of SCK (Emery *et al*, 1967). The present communication is to report the results of a study of an extended series of Becker muscular dystrophy carriers, in which the SCK was estimated by the more recent and sensitive assay described by Rosalki (1967). How the SCK level and pedigree data may be used to calculate probabilities for genetic counselling purposes is also discussed.

Materials and methods

During a study of 10 large families in each of which there were several males with Becker muscular dystrophy, the opportunity arose to study 31 definite and 36 possible carriers of Becker muscular dystrophy. A *definite* carrier has been defined in genetic terms as a daughter of an affected man or a woman with an affected son and a family history of other male relatives having been similarly affected (Emery *et al*, 1967). A *possible* carrier is a woman who is a daughter or grand-daughter of a definite carrier. SCK estimations were also carried out on all sons of definite and possible carriers. Three preclinical cases of Becker muscular dystrophy were identified in this way. Two were the sons of known definite carriers and the third was the son of a possible carrier who was therefore included in the series of definite carriers.

Blood for enzyme determinations was obtained from 120 normal, healthy women of comparable age to the carriers. None of these women had any history of neuromuscular disease nor of any other condition known to affect the SCK level.

Blood specimens for enzyme determinations were taken during normal everyday activity. Whenever it was not possible to perform the assay on the same day that the blood was taken, the serum was frozen and kept at -20°C until assayed. The SCK levels were assayed in duplicate according to the method of Rosalki (1967). The results are expressed in international units per litre (IU).

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Results

The distribution of SCK levels in the controls and definite and possible carriers is shown in Fig. 1. A close approximation to a straight line was obtained when the cumulative distribution of SCK levels in the controls was plotted on a log probability plot (Fig. 2). From this graph the normal 95th centile was estimated to be 50 IU. Of the 31 definite carriers, 19 (61%) had SCK levels which exceeded the normal 95th centile and from Fig. 2, 60% of carriers

would be predicted to have values greater than the normal 95th centile.

Of the 36 possible carriers (at 50% risk of having inherited the gene) 13 (36%) had SCK levels above 50 IU (Table I), compared with an expected number of 11. Ten possible carriers who, being granddaughters of definite carriers, were at a 25% risk were also tested. Of these 10, three (30%) had abnormally high SCK levels whereas 1.5 would have been expected. The differences between the observed and the expected numbers of possible carriers who had raised SCK levels were not significant.

There was no significant regression on age of SCK levels in controls, but in carriers SCK levels decreased with age and the regression was highly significant ($p \leq 0.003$), as shown in Fig. 3. Thus for genetic counselling purposes, the age as well as the SCK level of a suspected carrier has to be considered.

TABLE I
PROPORTION OF CARRIERS WITH ABNORMAL
SERUM CREATINE KINASE LEVELS

	Total	Abnormal	
		Expected	Observed
Definite carriers	31	—	19
Possible carriers (50% risk)	36	11	13
Possible carriers (25% risk)	10	1.5	3

FIG. 1. Distribution of SCK levels in controls and definite and possible carriers.

FIG. 2. Cumulative percentage frequency of the logarithms of the SCK values.

FIG. 3. Regression of SCK on age in (A) the control women and (B) the definite carriers.

Genetic counselling

In a number of X-linked disorders tests are now available which will detect symptomless female carriers. As in Becker muscular dystrophy however, the proportion of carriers who can be detected is not 100%. Thus in genetic counselling the problem often arises of having to advise a suspected carrier who has a normal test result. Methods of calculating probabilities in such situations, taking

into account the results of specific tests, have been described in detail with particular reference to Duchenne muscular dystrophy (Emery, 1969a).

In relation to suspected carriers of Becker muscular dystrophy, the relative probabilities of normal homozygosity to heterozygosity (*h*) can be estimated from knowing the proportions of normal healthy women and known carriers with an SCK level the same as the suspected carrier. In the case of Duchenne muscular dystrophy, no significant correlation between age and SCK levels has been found in carriers (Emery, 1967) and '*h*' is therefore expressed as the ratio of observed frequencies for the two genotypes, within 10-unit ranges of SCK. A significant regression of SCK on age was found however, amongst the Becker muscular dystrophy carriers. Modifications must therefore be made in order to enable this age effect to be taken into account in the calculation of probabilities.

It can be supposed that the population frequency for a given level of SCK can be determined from a mathematical function of that level. If the SCK level is *s*, and $f_N(s)$ and $f_B(s)$ are the expected frequencies of normals and Becker carriers, then '*h*' can be computed as the ratio $f_N(s)/f_B(s)$. The commonly applied Gaussian relationship appears consistent with findings on SCK levels, so that we can suppose:

$$f(s) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(s-\mu)^2}{2\sigma^2}}$$

where μ is the mean SCK value in the population and σ^2 is the variation about that mean. These values will differ between normals and carriers, so defining f_N and f_B . Indeed we can permit μ to vary with some additional factor; we can suppose μ to vary linearly with age so that:

$$\mu = \alpha + \beta \times (\text{age})$$

where α is the mean SCK level at birth and β is the variation in level per year of life.

Thus it follows that '*h*' depends on the SCK level in a way controlled by the six parameters α_N , β_N , σ_N^2 , α_B , β_B , and σ_B^2 . These are estimated from the available data by the method of maximum likelihood to be:

$$\begin{aligned} \alpha_N &= 28.6 \pm 2.6 & \alpha_B &= 131.5 \pm 20.8 \\ \beta_N &= -0.027 \pm 0.056 & \beta_B &= -1.22 \pm 0.37 \\ \sigma_N^2 &= 141.2 & \sigma_B^2 &= 1352.7 \end{aligned}$$

Assuming the Gaussian distribution and the linear effect of age, we can compute '*h*' as the natural anti-logarithm of

$$1.29 + \frac{(\text{SCK} - 131.5 + 1.22 \times \text{age})^2}{2705.4} - \frac{(\text{SCK} - 28.6 + 0.027 \times \text{age})^2}{282.4}$$

TABLE II
CALCULATED MEAN VALUES OF '*h*' FOR VARIOUS AGE GROUPS AND SERUM CREATINE KINASE (SCK) LEVELS

SCK	Age						
	0-10	11-20	21-30	31-40	41-50	51-60	61-70
3-7	92.96	34.71	14.46	6.72	3.48	2.01	1.30
8-12	126.42	48.92	21.11	10.16	5.46	3.27	2.18
13-17	146.73	58.83	26.31	13.12	7.30	4.53	3.14
18-22	145.33	60.38	27.98	14.46	8.34	5.36	3.85
23-27	122.84	52.88	25.39	13.60	8.12	5.41	4.02
28-32	88.61	39.53	19.67	10.92	6.76	4.67	3.59
33-37	54.55	25.22	13.00	7.48	4.80	3.43	2.74
38-42	28.66	13.73	7.33	4.37	2.91	2.15	1.78
43-47	12.85	6.38	3.53	2.18	1.50	1.15	0.99
48-52	4.92	2.53	1.45	0.93	0.66	0.53	0.47
53-57	1.61	0.86	0.51	0.34	0.25	0.21	0.19
58-62	0.45	0.25	0.15	0.10	0.08	0.07	0.07
63-67	0.11	0.06	0.04	0.03	0.02	0.02	0.02
68-72	0.02	0.01	0.01	0.01	0.01	0.00	0.00

The values of '*h*' for various levels of SCK in different age groups are given in Table II.

Applying Bayes' theorem (see Emery, 1969a), the probability of a daughter of a known carrier being a carrier is:

$$\frac{1}{1 + 2^n h_m}$$

where '*n*' is the number of her normal sons and ' h_m ' is the relative probability of her being a carrier based on her SCK level and her age (see Table II).

The probability of a grand-daughter of a known carrier being a carrier is:

$$\frac{1}{1 + h_c + 2^{n+1} h_c h_m}$$

where ' h_c ' is her relative probability of being a carrier based on her SCK level and her age, and '*n*' is the number of her normal brothers. If the SCK level is not known then '*h*' is unity.

For example the probability of a grand-daughter of a definite carrier being herself a carrier, if her

TABLE III
PROBABILITY OF BEING A CARRIER BASED ON BOTH THE SERUM CREATINE KINASE LEVEL AND FAMILY HISTORY

Pedigree Status	Probability of Being a Carrier
Daughter (m) of a known carrier	$\frac{1}{1 + 2^n h_m}$
Grand-daughter (c) of a known carrier	$\frac{1}{1 + h_c + 2^{n+1} h_m h_c}$

Where '*n*' = number of normal sons (for m).

number of normal brothers (for c).

'*h*' = relative probability of being a carrier based on the serum level of creatine kinase (Table II).

If the serum level of creatine kinase is not known then '*h*' is unity.

mother has an SCK level of 40 IU at 50 years and she has two normal brothers and an SCK level of 50 IU at the age of 25 years, is (see Table III):

$$\frac{1}{1 + 1.45 + 2^3(1.45)(2.91)} \\ = \frac{1}{36.20} \text{ or } 2.8\%.$$

Summary and conclusions

Approximately two-thirds of women who carry the gene for Duchenne muscular dystrophy have significantly elevated levels of SCK. The results of the present study indicate that about 60% of women who carry the gene for the benign (Becker-type) X-linked muscular dystrophy may also be detected by estimation of their SCK level. Using the more sensitive assay of Rosalki (1967) has enabled identification of a higher proportion of Becker carriers than was possible in a previously reported series (Emery *et al.*, 1967).

In Becker carriers, unlike Duchenne carriers, it has also been shown that there is a highly significant regression of SCK on age. The wider age range of the carriers (6–79 years) in this series may have enabled the demonstration of this positive correlation between the two parameters, which has not been found in a previous series. This decrease in SCK with age must be taken into account when calculating probabilities for genetic counselling purposes. Methods have been described by which both pedigree data and age corrected SCK values may be used in the calculation of such probabilities.

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