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CARRIER TESTING IN FAMILIES OF ISOLATED CASES OF DUCHENNE MUSCULAR DYSTROPHY

Creatine Kinase Activities in Female Relatives of Mothers with Normal CK Activity

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SUMMARY

A study of the serum creatine kinase (CK) activity in the female relatives of mothers of isolated cases of Duchenne muscular dystrophy (DMD) was undertaken. It was restricted to the relatives of mothers with normal serum CK values. Ninety-eight females in 19 families were studied; none was found to have a serum CK activity more than 3 SD above the normal mean. This evidence, derived from a study in which the measurement of serum CK activity was used as the sole means of detecting carriers, suggests that very few distant female relatives in such families are carriers of the gene and provides some evidence against the hypothesis that mutation is a rare cause of isolated cases of Duchenne muscular dystrophy.

INTRODUCTION

Among the families of boys with Duchenne muscular dystrophy (DMD) approximately 35% have more than 1 case and 65% contain a single affected individual. The proportion of isolated cases may be increasing as a result of genetic counselling.

Serum creatine kinase estimation is the most reliable single test of carrier status (Walton and Gardner-Medwin 1974) and detects between 50% and 80% of known carriers, depending on the method used, the laboratory in which the tests

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are performed, the confidence limits applied and the age of the carriers tested (Nicholson et al. 1979).

Haldane (1935) estimated that one-third of all cases of an X-linked lethal disorder must arise as a result of new mutation in order to maintain the disease in genetic equilibrium. He assumed that other possible mechanisms which could maintain the disease in genetic equilibrium, such as heterozygote advantage or differential fertility, were not operating. The results of CK testing in mothers of isolated cases of DMD appear to confirm that about half of such mothers are carriers and that about one-third of cases of DMD arise as a consequence of new mutation, as estimated by Haldane (Gardner-Medwin 1970).

Some recent reports have suggested that estimates of mutation rates in DMD are too high as many mothers and more distant female relatives of isolated cases appeared to be carriers when a number of different tests were combined to determine carrier status (Roses et al. 1977). Similar results have been reported using single biochemical tests for some other X-linked diseases, e.g. haemophilia (Biggs and Rizza 1976; Ratnoff and Jones 1977) and the Lesch–Nyhan syndrome (Franke et al. 1976). Various explanations have been offered for these results, including biased ascertainment (Morton and Lalouel 1977), heterozygote advantage, reproductive compensation (Lange et al. 1978) and a higher mutation rate in males (Biggs and Rizza 1976; Franke et al. 1976).

We elected to study the frequency of raised CK levels in the female relatives of mothers with normal CK levels who had had isolated sons with DMD. No attempt was made to survey all such families in the region served by our Centre but we were able to examine all families and relatives within commuting distance of Newcastle upon Tyne. If mutation rates in DMD are much lower than previously estimated, then most families should contain carriers and using our CK assay we should detect at least 50% of them.

METHODS

Mothers of isolated boys with DMD attending the Newcastle General Hospital over the 1976–77 period were identified. The female relatives of mothers with normal CK activities who lived in the Newcastle General Hospital area were studied. Pedigrees were compiled by interviewing these relatives. Relatives who had not been seen were contacted via those relatives who had already been interviewed. Distant relatives and relatives living some distance from Newcastle were encouraged to attend or were seen on home visits where this was more practical. Blood samples were obtained from all female relatives interviewed, after excluding any form of unusual exercise in the preceding 48 h and any recent illness. Serum was separated within 4 h of collection and stored at $-20\,^{\circ}C$.

The pedigrees were analysed by computer using a modification of the PEDIG programme (Heuch and Li 1972) to obtain the probability of each female tested being a carrier.

Serum CK activity was assayed by a modification of the method of Pearce

et al. (1964). Where repeat samples were obtained the mean value of all estimations was used. Normal ranges and means were obtained from 80 adult female volunteer control subjects and similarly from 58 girls under the age of 16 years as previously reported (Nicholson et al. 1979). The upper limit was obtained from 95% confidence limits of the log CK values (60 IU/l for adult females and 73 IU/l for girls). This procedure was employed since the log CK values were symmetrically distributed about their median value and a cumulative frequency plot was linear except for extreme values.

RESULTS

During the course of the study 2 families who were initially included in the study group were subsequently excluded. Investigation of one family revealed 2 other affected boys. The mother was re-classified as a definite carrier. A second family was excluded as repeat CK values on the mother were consistently elevated.

The pedigrees of the remaining 19 families of isolated cases are shown in Fig. 1. Individual results for serum CK activities in individuals in the pedigrees are given in the Appendix. Where possible, the age of each subject is given but in some instances where the exact ages of the subjects were not known, we indicate whether the subject was an adult or a child. Ninety-eight female relatives were tested. Four females were found to have a mean serum CK activity exceeding 2 SD above the normal mean value for the subject's age; these were 1 grandmother, 1 sister and 2 other more distant relatives (Table 1). This result was not significantly different from that expected from false positive results above the 95% confidence limits. No CK activity exceeded 3 SD above the normal mean; thus, no female tested had an unequivocally abnormal serum CK activity.

The number of carriers expected in these families was determined by analysing the pedigree information using the PEDIG computer programme (Heuch and Li 1972). For calculation, it was assumed that the disease was maintained in genetic equilibrium by new mutation and that male and female mutation rates were equal.

TABLE 1

SUMMARY OF SCK RESULTS FOR FEMALE MEMBERS OF FAMILIES CONTAINING ISOLATED DMD CASES WHOSE MOTHERS HAD NORMAL SCK ACTIVITIES

Relative of affected boy	Number present in family	Number tested	Proportion of available subjects tested (%)	Number with raised SCK activities
Sisters	21	17	81	0
Female cousins	34	15	44	0
Aunts	38	21	55	0
Grandmothers	16	8	50	1
Others	—	37	—	3

Results for mothers were excluded as this group was selected to have normal CK levels. Under these conditions, 10 carriers would be expected among these families. We might have hoped to detect 5 of these by serum CK estimation, assuming a 50% detection rate, as previously determined in this clinic (Nicholson et al. 1979). Our results showed that there were 4 females with raised CK activities; this finding was in agreement with the computer prediction derived from the pedigree data. Alternatively, if we assumed that no new mutant cases occurred in these families and that all mothers were carriers, it was possible to calculate that 67.75 carriers would be expected amongst the 98 females tested. Using the same 50% carrier detection rate when serum CK activity was the sole method of detection employed, 34 carriers should have been found by CK testing.

DISCUSSION

A few female relatives of isolated DMD patients had minimally raised serum CK activities but the number found was not greater than that expected from the 95% confidence limits for the normal range, indicating that these could be false positive results. However, one would expect some mothers in our families to be undetected DMD carriers. Serum CK measurement detects 50–60% of known DMD carriers in our laboratory but it is not possible to determine from our results which families contain carriers. Family 17, in which the grandmother of the affected boy had a slightly elevated serum CK activity, might contain carriers since there is some evidence to indicate that CK activities in carriers may fall with increasing age (Moser and Vogt 1974; Nicholson et al. 1979). If this is correct, a slightly raised serum CK activity in a grandmother may be more significant than a similar slight elevation in a young female. Previous evidence published from this clinic suggests that serum CK assay may detect almost all carriers if carried out in early life (Nicholson et al. 1979). Most of the sisters of cases in this series were older than 16 years at the time of testing. Since only half the daughters even of definite carriers would be expected to be carriers, no more than 3 or 4 of the sisters in this series would be expected to have raised serum CK activities. Only one was found (family 12); but this result could have occurred by chance.

The proportion of families in which we found a relative with raised serum CK activity was much less than that reported by Roses et al. (1977). This might be due in part to differences in the "detection rate" by the CK methods used (80% of known carriers detected at Duke University using the method of Rosalki (1967) and 55% at Newcastle using the method of Pearce et al. (1964)). Another important difference is that the mothers of isolated cases in our study (but not in that of Roses et al.) were selected for normal CK activities. Sibert et al. (1979) recently found a significant correlation between serum CK activities in members of the same family, so that there may be "high CK" families and "low CK" families. Indeed, independent genetic control of serum CK activities has been suggested (Meltzer et al. 1978). One must consider therefore whether serum CK activities in the female relatives of mothers in our series might be normal irrespective of their carrier status.

A number of studies have suggested that most mothers of DMD patients are carriers (Radu et al. 1967; Hausmanowa-Petrusewicz et al. 1977; Roses et al. 1977). In these studies a combination of several different carrier detection tests was used; and the reliability of apparently increased detection rates obtained by using a combination of tests is suspect unless some form of weighting by discriminant function analysis is employed (Tukey 1977), and the results are expressed in terms of the confidence limits for the test battery as a whole.

Although this study reveals few, if any, carriers in the distant relatives of the families tested, it cannot altogether exclude the possibility that all mothers of affected males are carriers. Thus, for example, a mutation rate much higher in girls than boys might result in a series of mothers, all of them carriers, having no carrier relatives. The low proportion of mothers of isolated cases who have a high serum CK activity (Gardner-Medwin 1970) would, however, require an additional explanation. Genetic heterogeneity in the biochemical manifestation of the carrier state is not a sufficient explanation, since if the families in this series simply contain non-manifesting carriers the absence of other affected boys requires explanation. However, the results of this study, in which serum CK activity was used as the sole method of carrier detection, are more in favour of the traditional view that many isolated cases of DMD represent new mutants.

APPENDIX

The ages (years) and SCK activities (IU/l) in subjects tested in the families whose pedigrees are illustrated in Fig. 1.

Family 1 III-5 adult 42.4, 29.8, 37.1; IV-1 age 15, 59.0, age 19, 31.4, 25.5

Family 2 II-1 adult, 12.5, 36.3; II-2 age 38, 30.5, 18.3; II-3 age 26, 26.1, 20.5; III-2 age 11, 41.1; III-3 age 10, 40.6

Family 3 III-3 age 65, 24.5, 22.7, 53.4; IV-6 adult 17.5; IV-7 age 21, 39.2, 33.8, age 24, 32.0, 29.3; IV-11 adult 32.3, 20.7; IV-12 adult 35.5; IV-13 37.0; V-8 age 11, 25.0, age 13, 30.0, age 14, 25.6; V-11 age 12, 32.3, 24.9, 20.7; V-12 age 19, 46.1

Family 4 III-4 age 74, 23.7, 35.7; III-5 age 80, 25.3; III-7 age 71, 25.3; III-8 age 78, 29.7; IV-1 age 40, 24.6, 23.8; IV-2 age 40, 41.9, 38.7; IV-5 age 46, 75.6; IV-10 adult 40.5; IV-13 adult 26.7; V-2 age 13, 58.5, 27.7; V-3 age 20, 27.7; V-9 adult 38.8; V-11 adult 35.0; V-15 age 10, 64.4

Family 5 II-4 age 81, 27.0, 22.5; III-1 adult 47.4, 35.9, 98.8, 32.9, 35.5; III-2 age 54, 25.3, 18.0; IV-1 adult 31.1, 38.4, 29.3; IV-2 adult 27.0, 28.5; IV-3 adult 27.5

Family 6 III-6 age 40, 17.0, 30.4, 34.5, 43.4; III-7 age 47, 29.7; III-8 age 43, 24.2; III-10 age 60, 40.2; III-11 age 64, 21.0; III-13 age 50, 57.5; III-16 age 55, 27.6, 30.9; III-17 age 50, 50.3, 37.3, 34.4; III-18 age 38, 24.7, 31.1, 26.4; IV-4 age 17, 69.5, 23.7, 22.7, 18.9; IV-7 age 17, 19.8; IV-8 age 19, 80.0; IV-9 age 17, 23.1; IV-10 age 10, 37.6; IV-14 age 30, 24.8; IV-22 age 32, 40.1; IV-27 age 28, 29.3, 27.9; IV-28 age 13, 46.9, 56.7, 76.4; IV-29 age 16, 62.0, 50.9, 42.4; IV-30 age 30, 50.4; IV-31 age 28, 45.8, 37.0, 37.1; V-7 age 7, 59.1; V-8 age 6, 65.5

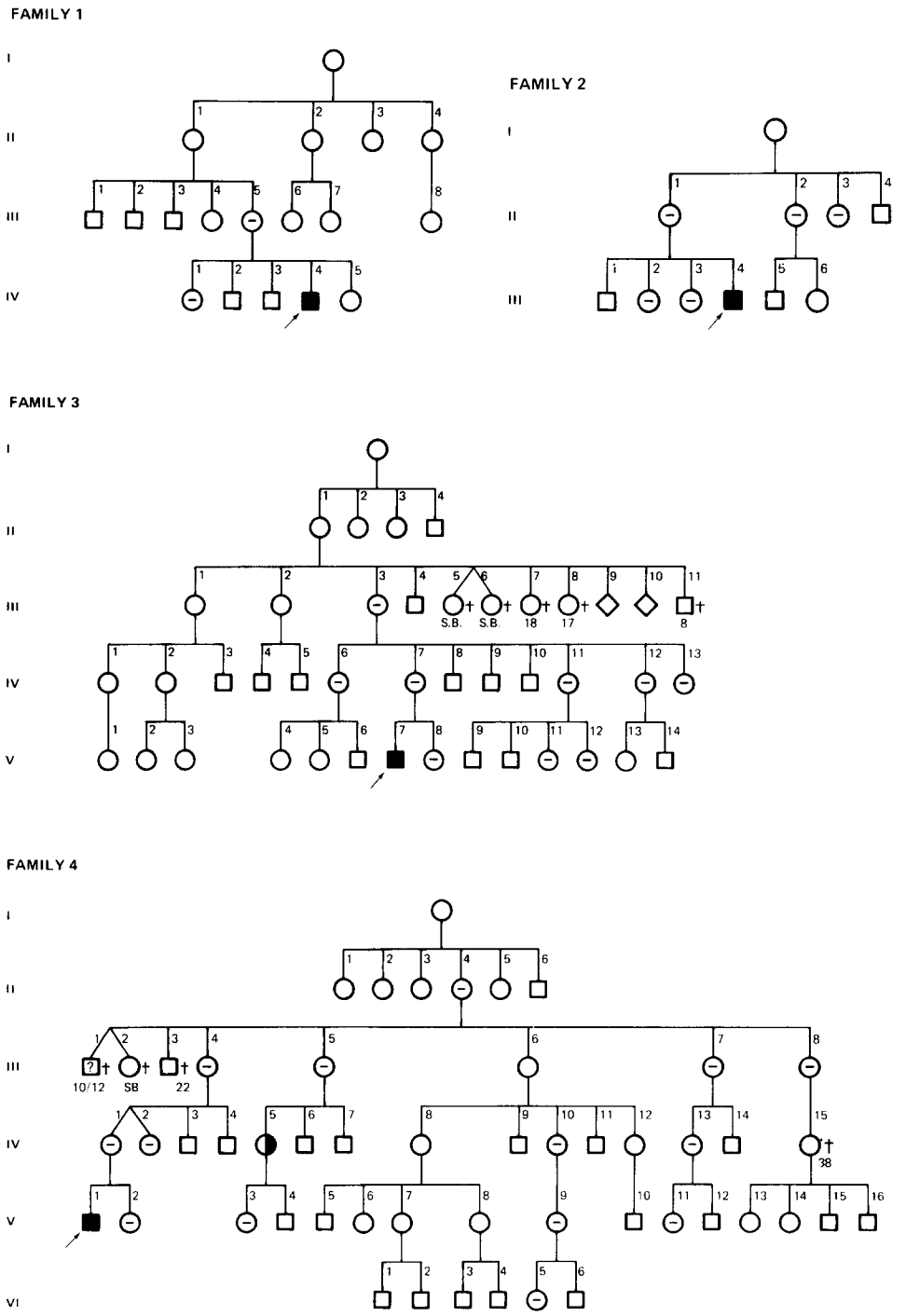

Fig. 1. Pedigrees of 19 families with single affected DMD boys shown as ■ born to mothers with normal SCK activities. Females in the pedigrees with raised SCK activities are shown as ◑. Tested females with normal SCK activities are shown as ⊖. Full details of SCK activities are given in the Appendix.

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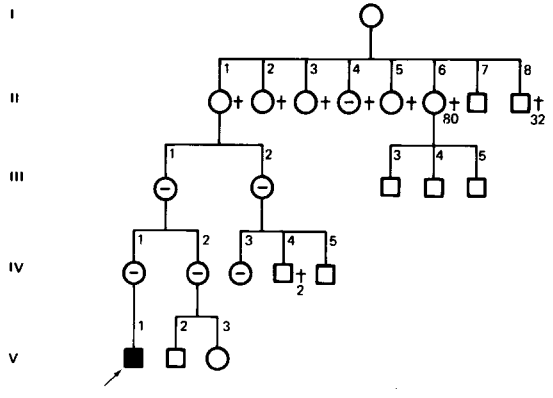

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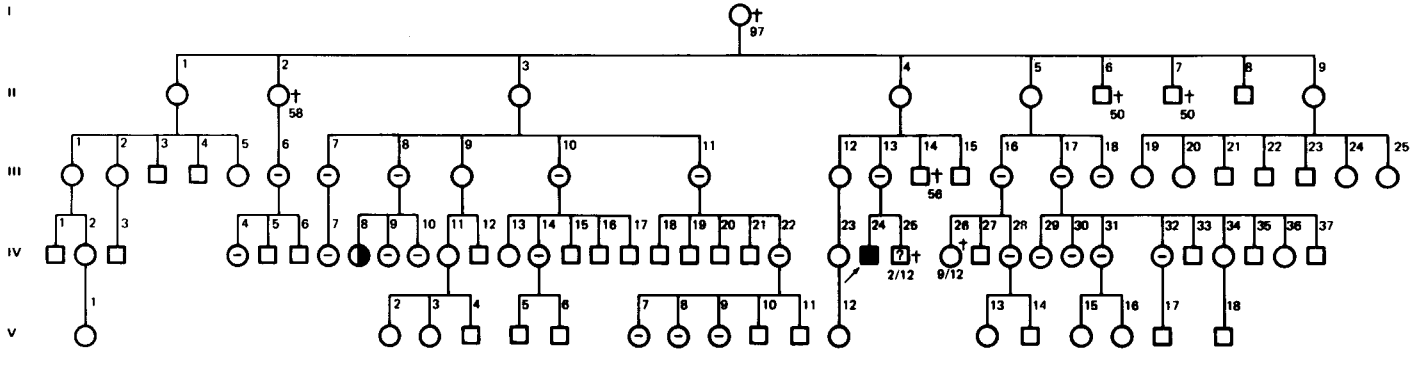

Family 7 III-17 age 68, 24.5; III-18 age 62, 18.5; III-19 adult 17.7; IV-11 age 27, 48.3, age 36, 43.4, 43.7; IV-12 adult 23.1; IV-13 age 31, 21.6; V-1 age 10, 22.4; V-2 age 19, 24.8; V-4 age 14, 19.4; V-6 age 5, 58.9

Family 8 III-14 age 61, 33.0, 39.5; III-15 age 58, 38.3; III-16 adult 22.4; III-19 adult 25.1, 37.0; III-20 adult 24.6, 23.4; IV-10 adult 32.0, 27.1; IV-12 age 26, 22.6, 18.3, 17.3; IV-13 adult 23.4; IV-17 age 10, 58.2, 59.2; IV-21 age 14, 16.9, 12.5; V-6 age 8, 26.7

Family 9 II-3 adult 26.8, 26.1, 15.0; III-5 age 1, 90.2; refused further investigation

Family 10 III-1 age 51, 27.9, 43.0; IV-7 adult, 19.0

Family 11 IV-4 adult 23.1, 17.2, 17.4, 21.1; IV-5 adult 30.0, 18.7, 25.2, 30.5; IV-6 adult 24.4; IV-7 adult 19.7, 20.5, 18.3, 25.7; IV-22 adult 23.7; IV-23 adult 27.8; V-6 age 14, 16.4, 20.2; V-7 age 9, 34.4; V-8 age 12, 28.0, 22.4, 33.1, age 13, 20.1; V-21 age 6, 26.7; V-24 age 8, 50.1

Family 12 III-5 age 32, 47.5, age 35, 45.5, age 38, 29.8; IV-5 age 8, 90.3, 73.8; IV-6 age 6, 62.7, 54.4

Family 13 II-1 age 70, 16.6; III-1 age 31 15.5, 17.4, 14.9; III-6 age 32, 20.3, 10.7; III-7 age 36, 14.8; IV-3 age 6, 17.4, 14.4; IV-6 age 7, 45.7, 35.4; IV-9 age 10, 20.5; IV-10 age 15, 17.5

Family 14 II-2 age 64, 32.6; III-1 adult 25.9; III-2 adult 53.4; IV-1 adult 36.0; IV-3 age 16, 24.8

Family 15 III-1 age 30, 56.0, 51.5, age 33, 24.2, 26.0, 30.1; III-3 adult 26.0; IV-5 age 12, 35.5; IV-7 adult 18.0; IV-8 adult 26.0; V-1 age 6, 35.0

Family 16 II-1 adult 17.0; refused further investigation

Family 17 III-1 adult 69.0; IV-1 adult 15.4; IV-4 adult 34.0; V-4 age 20, 34.0

Family 18 II-4 age 54, 42.2, 48.3; III-9 age 23, 28.3; III-11 age 28, 26.1; III-12 age 30, 27.6; III-13 age 6, 55.7; IV-5 age 8, 57.1; IV-7 age 9, 57.1; IV-8 age 1, 53.7

Family 19 III-4 age 70, 23.4; IV-6 age 38, 31.1; IV-8 age 43, 32.1, 18.4, 17.4; IV-9 age 54, 43.1, 24.0; IV-10 age 47, 29.0, 23.4; IV-11 age 43, 35.9, 32.3; V-4 age 12, 19.6; V-8 age 28, 31.3, 31.3; V-9 age 18, 21.5, 20.3; V-12 age 13, 45.9, 48.7; V-13 age 8, 31.0, 50.7

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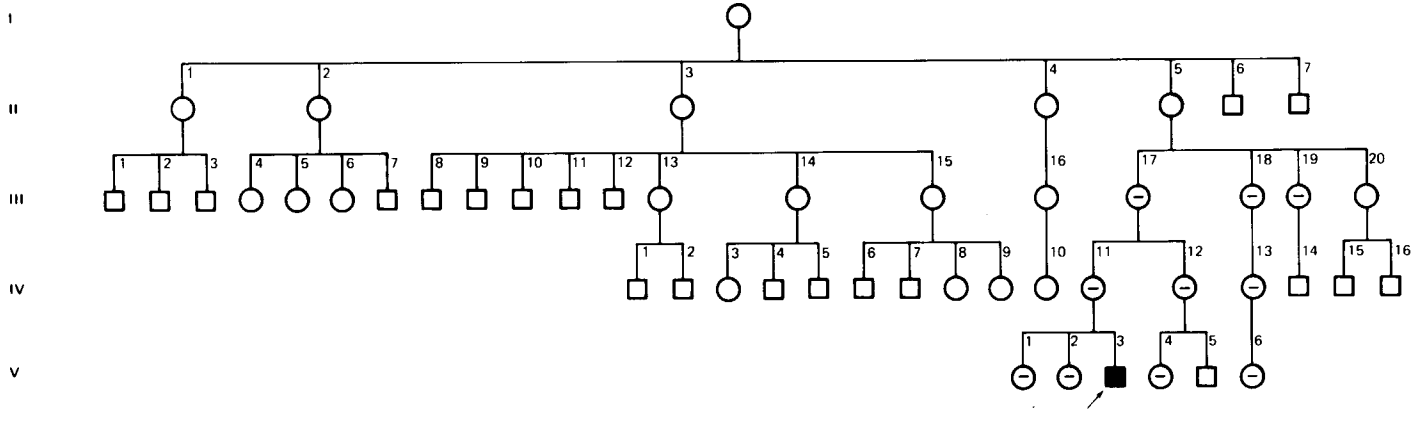

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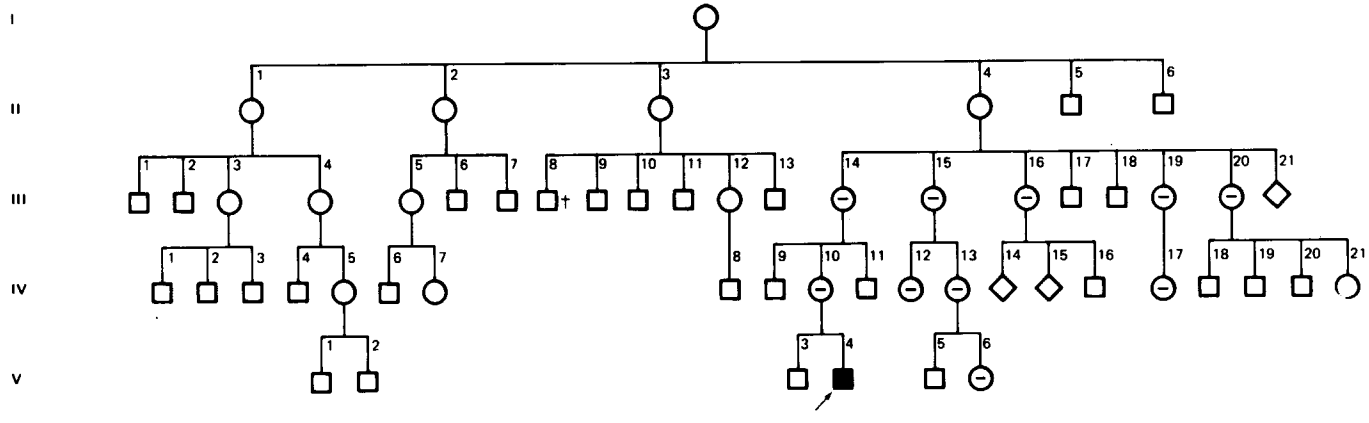

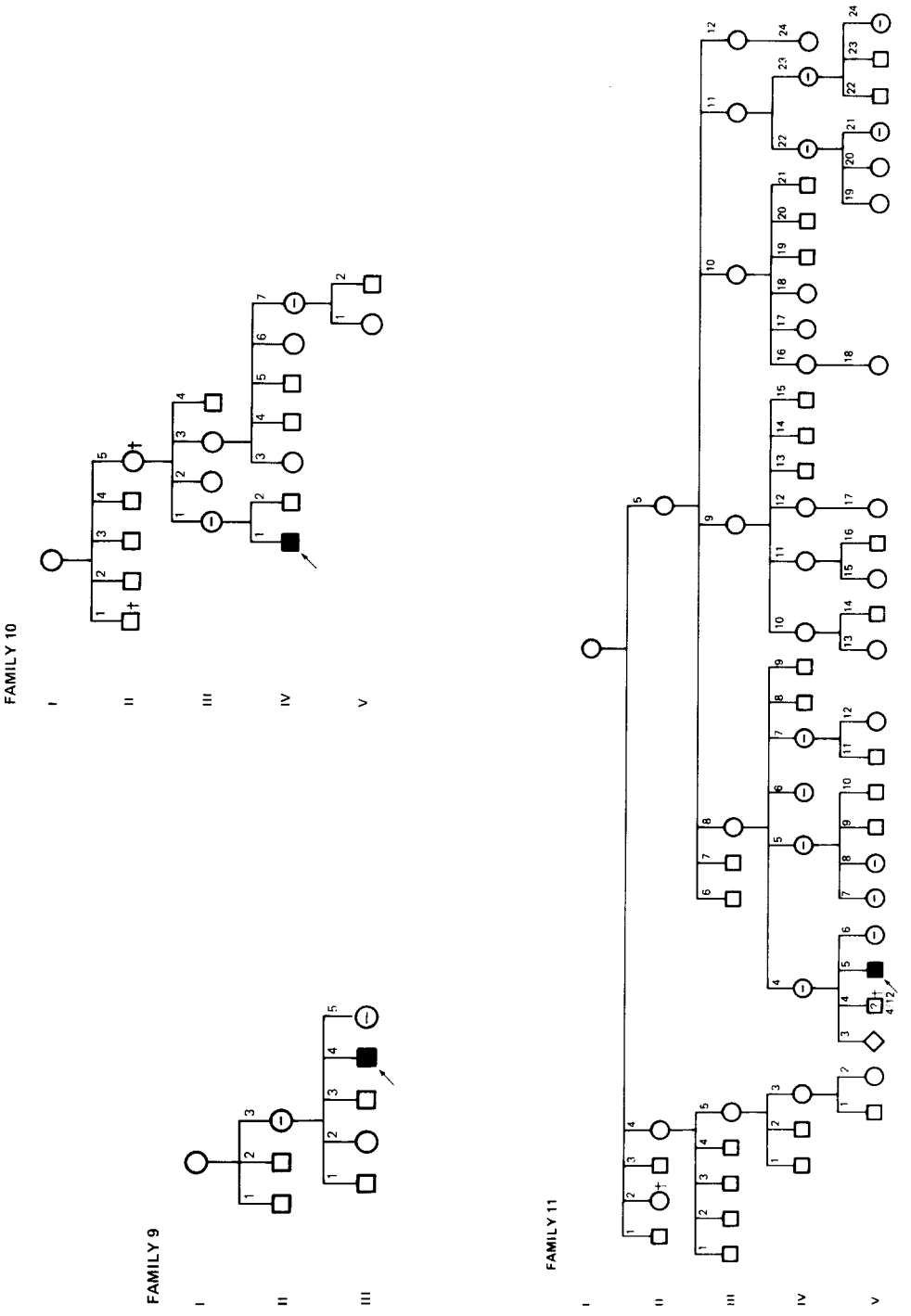
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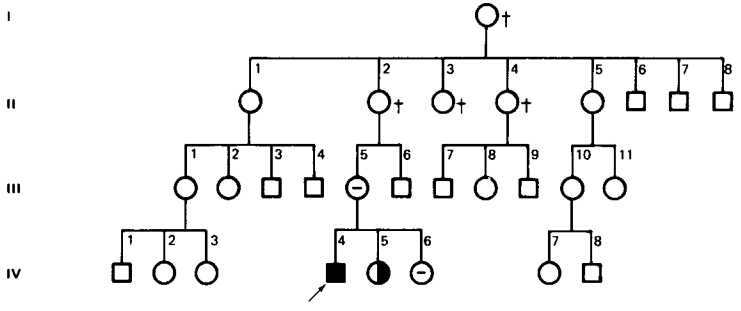

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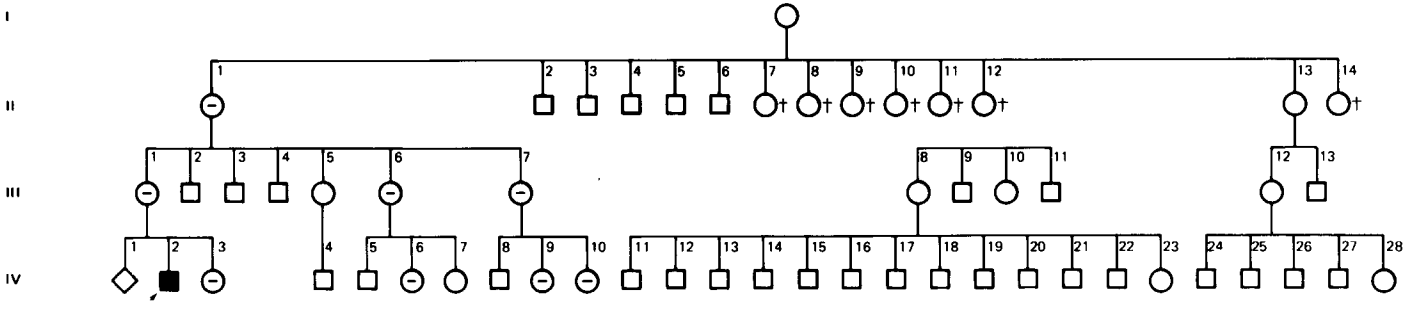

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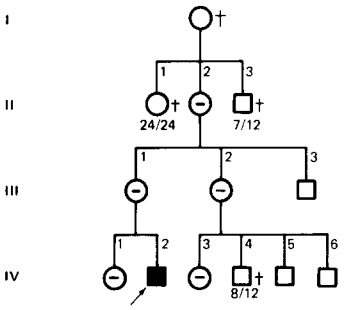

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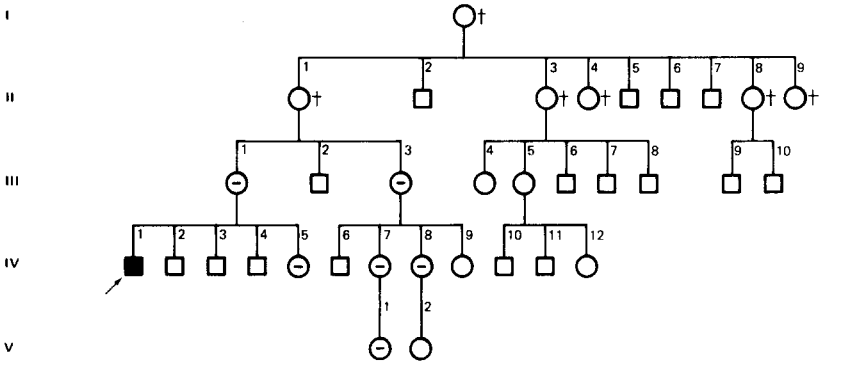

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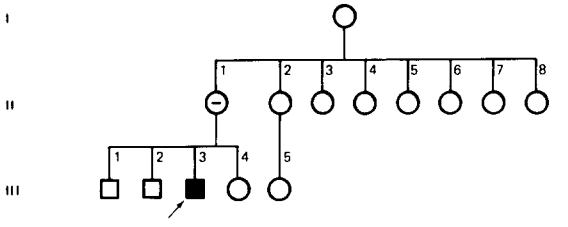

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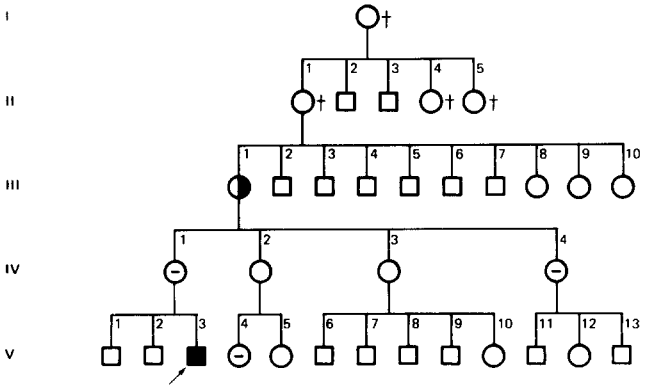

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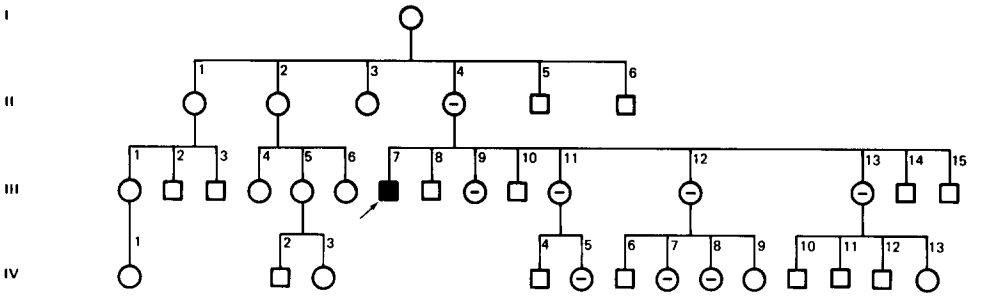

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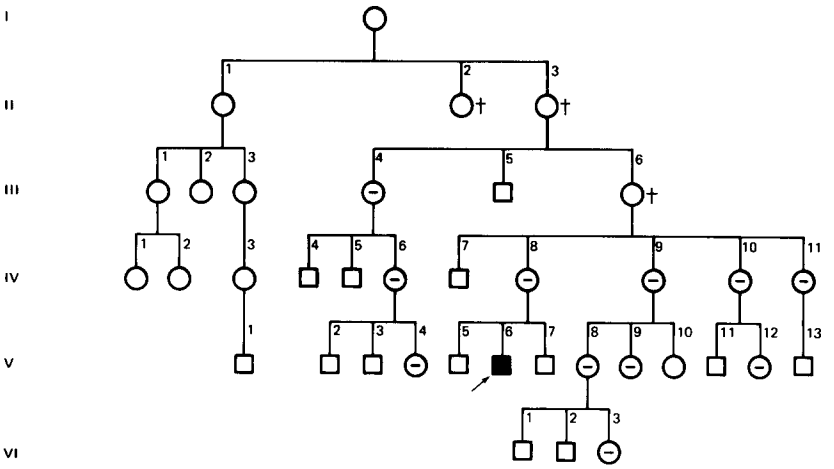

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