Menkes’ X-linked Disease: Prenatal Diagnosis and Carrier Detection

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Increased $^{64}$Cu uptake into cultured cells is a biochemical marker for mutant cells in Menkes’ disease (McKusick 30940). Using this marker selective prenatal diagnosis has been carried out in more than 80 at-risk pregnancies. The $^{64}$Cu uptake into cultures from affected male fetuses is, however, negatively correlated to the fetal age at amniocentesis. After the 18th week of gestation the risk of false negatives is significant.

Using copper uptake into uncloned cultures, a number of obligate and possible carriers showed significantly increased values, but the range of values of obligate carriers considerably overlapped those of the normal controls. All values of normal controls were within a limited range and values above the upper limit in females at risk must, therefore, be caused by mutant cells and establish the carrier diagnosis. However, the extreme skewing of the distribution towards normal values in obligate carriers indicates a strong selection against the mutant cell type and this will hamper the detection of all female carriers in risk families.

C-banding heteromorphism of the X-chromosome provides a supplementary carrier detection method. Linkage analysis in five Danish families demonstrated a close physical relationship between the gene for Menkes’ disease and the centromere region. By comparative gene mapping (mouse/man) the most likely localization of the gene for Menkes’ disease can be suggested to be in band q13 on the long arm of the human X-chromosome. This regional assignment facilitates the choice of appropriate X-specific DNA probes in search for linkage at the DNA level.

INTRODUCTION

Menkes’ disease (McKusick 30940) is an inborn error of copper metabolism (Danks et al., 1972) with a progressive clinical course, and patients with the classical form of the disease rarely survive more than 3 years. The clinical picture is characterized by severe psychomotor retardation, seizures, temperature instability, vascular and bony changes, and strikingly peculiar hair (French, 1977). Prevention of the disease is, therefore, a desirable objective.

The copper disturbances associated with the disease include low serum copper and caeruloplasmin levels, and abnormal tissue binding of copper in vitro as well as in vivo (Heydorn et al., 1975; Horn, 1976; Horn et al., 1978). Copper binding in cultured cells provides a genetic marker which can be used for diagnostic purposes.

Menkes’ disease is due to a recessive gene, which is located on the X-chromosome and will be expressed only in the absence of the normal allele. Accordingly, all males who carry the mutant gene will be affected and can be identified by means of the genetic marker. As a result of Lyonization female carriers will, however, rarely show obvious manifestations of the disease. Identification of female carriers is therefore an important preventive measure. It is the aim of the present paper to summarize the use of copper studies for prenatal diagnosis of hemizygous males and for identification of female heterozygotes. The survey will be confined to the experience obtained at the John F. Kennedy Institute in Denmark. In addition, our present knowledge about the position of the Menkes’ locus on the human X-chromosome will be summarized with a view to the diagnostic use of other X-linked markers.

HEMIZYGOTES

The discovery of specific copper disturbances in a male fetus at risk established that Menkes’ disease also manifests itself in utero. The disturbances included accumulation of copper in several extrahepatic tissues and in cultured amniotic fluid cells (Heydorn et al., 1975; Horn, 1976). Increased copper binding is, therefore, a genetic marker for affected males both in prenatal and in postnatal life. In our laboratory the diagnosis of hemizygous males is based on the uptake of labelled copper into cultured cells, and investigation of the index patient usually precedes examination of other family members. The average copper uptake into fibroblasts from Menkes’ disease patients is about three times higher than that of normal controls (Horn, 1980). There is no overlap between the two genotypes, and consequently the test is clearly discriminatory for affected males.

A similar accumulation was observed when amniotic fluid cell cultures from affected male fetuses were compared to those of normal males at risk (Horn, 1981). The two genotypes were, however, not so clearly separated due to a lower average copper uptake into cultures from affected males. A few cultures gave even borderline normal values. Cultures initiated after abortion also tended to have lower copper incorporation than those used to establish the diagnosis. It was, therefore, suspected that the gestational age might influence the copper uptake. Incorporation values of a number of cultures from affected male fetuses can be described by a linear regression function of the gestational age in terms of weeks ($t$):

$$y(t) = 95.355 - 2.477t$$

The negative slope of the regression line is highly significantly different from zero \((p = 0.0007)\), confirming the suspicion of a negative correlation between fetal age at amniocentesis and the copper uptake. No significant age dependence was observed in the group of unaffected males. Incorporation values into independent cultures gave a mean copper uptake of \(15.43 \pm 3.89 (SD) \text{ng}^{64}\text{Cu} \text{per mg protein per 20h}\). One sided 99 \% and 95 \% confidence limits were estimated for each genotype (Figure 1). The upper 99 \% confidence limit of normal males at risk and the lower 99 \% confidence limit of affected males intersect close to the 18th week of gestation. This gives an estimate on the gestational age below which the test is clearly discriminatory. Thus, in order to avoid misclassification, prenatal diagnosis should not be carried out later than the beginning of the 17th week of gestation. Usually amniocentesis is carried out at 15–16 weeks of gestation, but a substantial number of cases (approximately 20 \%) are postponed until the 18th week of gestation or later and the risk of false negatives may, therefore, be of significance.

In order to minimize the risk of false negatives the prenatal diagnosis is based on the highest value for the male fetus under study (Horn, 1981). This may increase the risk of false positives, but identification of all affected male fetuses is considered to be the main objective.

The increased tissue binding of copper in affected males can be used to confirm the prenatal diagnosis. In addition to an accumulation in various extrahepatic tissues, copper is also increased several-fold in placental tissue (Horn, 1981; Damsgaard \textit{et al}., 1982). Placental tissue is easy to obtain, and placenta copper determination has the advantage of being independent of the copper incorporation assay used to establish the diagnosis. Furthermore, the method is not susceptible to growth failure of fetal cells as is observed after prostaglandin induced abortion.

**HETEROZYGOTES**

In order to prevent the birth of affected males identification of female carriers becomes important. As mentioned briefly above, the problem inherent in carrier detection of an X-linked disorder is mainly due to Lyonization. Lyonization is the hypothesis put forward to explain the dosage compensation in a female mammalian cell (Lyon, 1972). The hypothesis states that in any one cell only one X-chromosome is active, the other being inactivated early in embryonic life, and the inactivation pattern is maintained in all descendants of the cell. Female heterozygotes are, therefore, natural mosaics of two cell populations; one expressing the mutant phenotype because of inactivation of the normal allele and the other expressing the wild type allele. Inactivation occurs at random and great phenotypic variability may exist in heterozygotes. Some female carriers may, by chance, have the abnormal X-chromosome inactivated in most of their cells and thus be phenotypically normal. Occasionally, affected females may be observed as an extreme result of the random X-inactivation and two possible cases have been recorded in medical literature (Grover and Scrutton, 1975; Matsubara \textit{et al}., 1978).

Mosaicism was demonstrated in skin fibroblasts from heterozygous females by means of cell cloning techniques and hence provided conclusive evidence on Lyonization of the Menkes' locus (Horn \textit{et al}., 1980). The cloning technique is, however, too complicated and time consuming to be used routinely in carrier diagnosis.

Studies on mixed cell populations from Menkes' disease patients and control subjects demonstrated that no metabolic co-operation existed between the two cell types. A clear linear relationship between copper uptake and the proportion of mutant to normal cells was found (Horn, 1980). High copper uptake into uncloned fibroblasts from a heterozygote would, therefore, indicate a high proportion of mutant cells. Because of the random X-activation the majority of heterozygotes would be expected to show intermediate copper uptake values.

The distribution of copper uptake values from the control group and for that of Menkes' disease patients are symmetrical curves (Figure 2). In case of no preferential cell growth of either cell type, the distribution of the mosaic cell population of heterozygotes would also be expected to be a symmetrical one with its mean intermediate between the means of the two other distributions. An extreme skewing of the distribution towards normal values was, however, observed in a group of heterozygotes (Figure 2). This indicates a strong selection against the mutant cell type. The copper uptake values for carriers considerably overlap those of normal homozygotes, and several females will remain undetected by the test. The overlap is so pronounced that it will not be possible to define any limit below which a woman may safely be classified as a non-carrier.

In the control group of normal homozygotes all values observed were within a limited range. Values above the