X chromosome inactivation and the diagnosis of X linked disease in females

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Abstract

In studies of female patients with suspected deficiency of the Elα subunit of the pyruvate dehydrogenase complex, we have found that X inactivation ratios of 80:20 or greater occur at sufficient frequency in cultured fibroblasts to make exclusion of the diagnosis impossible in about 25% of cases. Pyruvate dehydrogenase Elα subunit deficiency is an X linked inborn error of metabolism which is well defined biochemically and is unusual in that most heterozygous females manifest the condition. The diagnosis is usually established by measurement of enzyme activity and the level of immunoreactive protein and these analyses are most commonly performed on cultured fibroblasts from the patients. Skewed patterns of X chromosome inactivation make it impossible to exclude the diagnosis if the normal X chromosome is expressed in the majority of cells. While most of the observed variation appears to be the expected consequence of random X inactivation, it may be further exaggerated by sampling and subsequent expansion of the cells for analysis.

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There are relatively few X linked diseases which are expressed in most heterozygous females and for which the responsible gene and its product have been defined. As a consequence, relatively little attention has been given to problems which might arise in this situation when diagnosis is based on measurement of the function or structure of the gene product. One example of an X linked disease which does behave in this way is pyruvate dehydrogenase (PDH) Elα subunit deficiency. This is a relatively common inborn error of metabolism with a broad spectrum of clinical presentation ranging from severe lactic acidosis in the newborn to a progressive neurodegenerative disease with prolonged survival.1,4

The PDH complex is an important and widely distributed housekeeping enzyme which catalyses the conversion of pyruvate to acetyl coenzyme A within the mitochondrion. This is a key regulatory step in the central pathways of energy production in the cell and the enzyme operates at close to its maximal activity in a number of tissues. This is particularly important in the brain which has an obligatory requirement for aerobic glucose oxidation under normal circumstances. Relatively modest reduction in PDH activity therefore has significant consequences, especially for central nervous system function.

Within the PDH complex, the Elα subunit contains the pyruvate binding site and the phosphorylation sites by which the activity of the whole complex is controlled.5 The gene for the Elα subunit is located on the short arm of the X chromosome in the region Xp22.1.6 However, in all reported series of patients with PDH Elα deficiency, there are approximately equal numbers of males and females.2,4 The clinical presentation does differ between the sexes with the acute metabolic form more common in males and neurological presentations predominating in females. The disorder in females, although generally less severe, still usually results in death before adulthood and only a few subjects with mutations in the Elα gene are known to have had children. PDH Elα deficiency therefore represents an unusual case of an X linked disease involving a housekeeping gene product, in which almost all cases are sporadic and where the threshold for developing symptoms is so low that it is usually exceeded in heterozygous females.

The diagnosis of PDH Elα deficiency is usually established in the first instance by measurement of enzyme activity in samples from the patients. Most commonly, cultured fibroblasts from a skin biopsy are used, although results obtained with lymphoid cells or tissue biopsy samples are occasionally reported. In conjunction with these enzyme assays, most laboratories also determine the levels of immunoreactive protein corresponding to the different subunits of the complex. Subsequently, the underlying mutation may be defined but, at present, direct analysis of the basic gene defect is not feasible as a primary screening procedure.

For reliable diagnosis of PDH Elα deficiency in females, it is essential that each X chromosome is represented in the active form in a significant proportion of the cells in the sample. Increasing deviation away from approximately equal representation towards a situation in which the normal X chromosome is expressed in the majority of cells will progressively make it more difficult to identify the

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presence of an E1α mutation against the background of considerable normal variation in enzyme activity. In practice, less than 20% representation of an X chromosome with a null E1α mutation would not be detectable.

In this paper, we compare methods for X inactivation analysis in cultured human fibroblasts and assess the significance of the observed variation in the pattern of X inactivation for the diagnosis of PDH E1α deficiency in females based on experience with 48 patients. We also present data on X chromosome inactivation patterns in chorionic villus cell cultures which indicate that these are often unsuitable for the diagnosis of X linked disease based on measurements of gene expression.

Materials and methods

CELL CULTURE

Fibroblasts from forearm skin biopsies were cultured in Basal Medium (Eagle) with 10% fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cultures were always split at high density to minimise the possibility of expanding small cell populations and introducing a sampling bias. The cultures were regularly screened for mycoplasma contamination using the method of Chen.7 Chorionic villus cell cultures were established in Chang medium from cleanly dissected villi. They were subsequently cultured in Ham’s F10 medium containing 20% fetal calf serum, 2% Ultraser G (Gibco), 100 units/ml penicillin, and 100 μg/ml streptomycin. The possibility of clonal expansion during subsequent subculture was again reduced by ensuring that the cultures were never allowed to grow up from low density.

ENZYME ASSAY

Overall PDH activity was measured after maximal activation with dichloroacetate, using [1-14C]-pyruvate as substrate as described previously.8 In this assay, the activity of normal control fibroblasts ranges from 0.7 to 1.1 nmol 14CO2 produced/mg protein/min.

X INACTIVATION ANALYSIS

DNA was prepared from fibroblast cell pellets using a standard proteinase K-SDS method. Restriction enzymes were purchased from Boehringer Mannheim and digestion was carried out using a 3 to 5 fold excess of enzyme in the manufacturer’s recommended buffer system. For analysis of X inactivation patterns using the M27β probe, MspI/BamHI and HpaII/BamHI double digests were performed as described previously.9 Briefly, the MspI and HpaII digests were carried out overnight. The buffer was adjusted by addition of Boehringer buffer B and the second digest with BamHI was carried out for four to six hours. PstI, MspI and PstI/HpaII digests were also performed on some samples. These digests were carried out either by first digesting with MspI or HpaII overnight followed by a second digestion with PstI for six hours after adjusting the presence of an X chromosome with a null E1α mutation would not be detectable.

For analysis of X inactivation patterns using the PGK probe, a double digest with PstI and BstXI was performed, followed by digestion with HpaII10; 12 μg samples were digested at 37°C overnight (~15 hours) with PstI. BstXI enzyme was added and the second incubation was carried out for eight hours at 55°C. Samples were then ethanol precipitated and resuspended in Boehringer buffer L and divided into two aliquots. One aliquot was subsequently digested with HpaII and the other was left undigested. Samples were separated by running in 1:1% agarose for ~22 hours at 1.5 V/cm.

Initial densitometric measurements of relative band intensities indicated that the X inactivation ratio could be reliably determined to within 10%. Comparison of densitometric tracings with ratios estimated by eye indicated that visual classification was equally accurate as a semiquantitative measure and this was subsequently used to group ratios into centile bands. At least two different times of exposure of the x ray film were used in estimating the ratios and in many cases several different analyses of the patient samples were performed with the various methods.

PROBES

The M27β probe used to detect the DXS255 locus was a 2.3 kb EcoRI insert prepared from the subclone M27β.11 The PGK probe, pSPT/PGK, was an 800 bp BamHI/EcoRI fragment from the 5’ end of the PGK gene12 and was kindly supplied by Dr B Vogelstein.

Results

METHYLATION PATTERN ANALYSIS

All samples were analysed for methylation at the DXS255 locus using the M27β probe. Double digests were performed with MspI or HpaII and either BamHI or PstI. The DXS255 locus is contained within a 30 to 35 kb BamHI fragment and within this MspI/ HpaII sites immediately flanking the VNTR region of the locus are separated by 6 to 12 kb. Methylated DNA which is not digested by HpaII remains in the high molecular weight (30 to 35 kb) band and different alleles are not usually resolved. Unmethylated DNA (on inactive X chromosomes) is cut to generate a two band pattern (figs 1 to 3A).13 The methylation pattern was analysed by calculating the proportion of total digested DNA contributed by each band and is expressed as a ratio with the larger allele first.

Double digestion with PstI/HpaII generates a more complex pattern. After digestion with PstI alone, fragments in the range 4 to 10 kb are generated. After additional digestion with MspI, the PstI fragments are reduced by...
Figure 1  X inactivation analysis of fibroblasts from a patient with a defined E1α subunit mutation. Fibroblast DNA from the patient was analysed with both M27β and PGK probes and, in addition, fibroblast DNA from both parents was analysed at the PGK1 locus. In all cases, the size of the major fragments in kb is indicated on the left. (A) Analysis of the DXS255 locus. MspI/BamHI double digestion (track 1) generates fragments of 9 and 8 kb. With HpaII/BamHI digestion (track 2), the ratio of the 9 kb band to the 8 kb band was determined by densitometry to be 20:80. (B) Analysis of the 5’ MspI site of the DXS255 locus using PstI digestion shows fragments of 6 and 5 kb (track 1). Double digestion with MspI (track 2) reduces the fragments to 5-5 and 4-5 kb. After double digestion with HpaII, a four band pattern is generated (track 3). Methylated DNA remains undigested in the PstI/BstXI band position and unmethylated DNA moves to the MspI/PstI position. The ratio of the larger to smaller allele at the MspI/PstI position after HpaII digestion (track 3) was estimated visually as approximately 20:80. (C) Analysis at the PGK1 locus. PstI/BstXI digestion shows that the father of the patient has the 1/05 kb allele (track 1). Tracks 2 and 3 show the PstI/BstXI pattern in mother and daughter respectively. In track 4, the pattern after additional HpaII digestion of DNA from the daughter shows that the relative band intensity is approximately 80:20 with respect to the 1/05 kb allele.

X INACTIVATION PATTERNS IN CULTURED FIBROBLASTS

Fibroblasts from 20 normal subjects and 48 patients were analysed for X inactivation using the M27β probe. All patients were referred for investigation of suspected PDH deficiency. Sixty-two out of the total of 68 samples analysed were informative with the M27β probe (91%) while the remaining six (9%) had alleles that were too similar in size to be resolved by agarose gel electrophoresis. Within both the control and patient groups, the patterns of X inactivation varied considerably. While the majority had ratios which were approximately 50:50 (within the range 30:70 to 70:30), many had more extreme patterns with one X expressed predominantly and 24% had ratios that were more extreme than 80:20 (table, fig 4).

Forty-six samples were screened for polymorphism at the PGK1 locus and 13 (28%) were found to be informative. Results of the patient samples are presented in the table. Out of 34 patient samples screened, 10 were informative. One sample which was informative at the PGK1 locus was uninformative at the DXS255 locus, but nine samples were informative at both loci and in each case the proportion of each X chromosome which was active as determined by the PGK probe correlated closely with the figure obtained from analysis with the M27β probe. An additional three out of 12 normal controls were informative with PGK and the patterns were also the same as those obtained with M27β (results not shown). Even within the small number of samples studied by this method, it is clear that there is a large variation in the X inactivation pattern between different females.

Figure 2  X inactivation analysis of fibroblasts from a patient in whom diagnosis of PDH E1α deficiency cannot be excluded. (A) Analysis at the DXS255 locus. MspI/BamHI digestion generates fragments of 12 and 94 kb (track 1) and HpaII/BamHI digestion (track 2) shows that the 12 kb allele is almost fully methylated and therefore active in most cells. (B) Analysis of the DXS255 5’ MspI site using PstI digestion shows fragments of 9 and 6-4 kb (track 1). Double digestion with MspI results in fragments of 85 and 5-9 kb (track 2) and double digestion with HpaII (track 3) again shows that the larger allele is almost fully methylated with most of the DNA remaining in the PstI/PstI position. The smaller allele is almost completely digested to the MspI/PstI position. (C) Analysis at the PGK1 locus confirms that one X chromosome (represented by the 1/05 kb band) is active in the majority of cells.

SPECIFIC PATIENT EXAMPLES

Three examples of patient analyses have been chosen to illustrate typical results and their interpretation. Fig 1 shows X inactivation patterns at the DXS255 and PGK1 loci for a patient in whom the diagnosis of PDH E1α deficiency is established (patient 4, table). At the DXS255 locus, the alleles give rise to fragments of 9 kb and 8 kb when digested at the 5’ and 3’ MspI sites immediately flanking ~500 bp and after digestion with HpaII four fragments result with undigested methylated DNA remaining in the PstI/PstI band position and digested unmethylated DNA moving to the PstI/MspI fragment position (figs 1 to 3B). Analysis of methylation at the PGK1 locus was performed using a double digest with PstI and BstXI to show the polymorphism, followed by digestion with HpaII. The fragment sizes generated are 1-05 and 0-90 kb (figs 1 to 3C). The active X chromosome is unmethylated in the region detected by the probe and the inactive X is methylated. The bands remaining after digestion are therefore a measure of the inactive X chromosome unlike the M27β system in which bands generated after HpaII digestion, rather than residual band intensity, are a measure of the inactive X chromosome.
the VNTR region (fig 1A, track 1). In the HpaII/BamHI lane (track 2), the relative intensity of the bands, determined by densitometry, was 20:80. Similar results were seen following digestion with PstI and either MspI or HpaII (fig 1B). In this case, the two alleles generate bands of 5-5 kb and 4-5 kb (track 2) and the relative intensity after HpaII digestion is approximately 20:80 (track 3). In fact, both alleles generate a faint doublet pattern with a second minor band visible just above the major band at the MspI/HpaII position.

Extra bands appearing as doublets located between the expected PstI and MspI fragments occur quite commonly and are the result of a cluster of three MspI sites very close together. The site nearest the VNTR is always methylated whereas the other two are usually unmethylated on the inactive X chromosome. The major fragment generated by HpaII digestion extends to the middle site; however, in some subjects this is methylated in a proportion of cells resulting in an extra intermediate sized fragment defined by the distal MspI site. Mostly these intermediate bands are faint and do not contribute significantly to the major band ratio. Occasionally, however, they are of similar intensity to the expected fragments and in such cases Gale et al. have shown that it is appropriate to include them in the analysis.

Fig 1C shows the X inactivation pattern obtained with the PGK probe. Lanes 1 and 2 illustrate the paternal and maternal alleles while lanes 3 and 4 show the patient alleles and the X inactivation pattern. The 0.9 kb allele is the maternal one and, as it is approximately 80% digested with HpaII, it is active in that proportion of cells. In this patient, the residual enzyme activity in cultured fibroblasts was ~25% of normal and the mutation has been defined as a deletion of a 7 bp sequence.43 It has previously been established that the larger allele at the DXS255 locus is the maternal one44 and, as it is only 20% digested with HpaII, it represents the more active allele in the fibroblasts. In this particular case it was possible to establish not only that the overall pattern of X inactivation is the same with both probes but that both methods identify the same allele as the more active one.

Fig 2 shows X inactivation patterns obtained from a second patient who was investigated for suspected PDH E1α deficiency (patient 42, table). Figs 2A and B show HpaII/BamHI and PstI/HpaII digests respectively, probed with the M27β probe. Fig 2A shows that the 12 kb allele is only digested to a small extent while the 9.4 kb allele is almost fully digested with the ratio between them being approximately 10:90. Half of the total material remains undigested in the 30 to 35 kb band. Fig 2B again shows that the upper allele is only slightly digested, with most of the DNA remaining in the position of the PstI band while the lower allele is almost totally digested. The ratio of
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X inactivation analysis of chorionic villus cells

A total of 23 chorionic villus cell cultures were analysed with the M27β probe and 22 were informative. MspI/BamHI and HpaII/BamHI digests were carried out for all samples, and PstI/MspI and PstI/HpaII digests were also performed on 10. A significant feature was the high proportion of cultures which had extreme patterns of X inactivation. Twelve of the 22 cultures had X inactivation patterns in the range 90:10 to 100:0. Seven of the PstI double digests gave results that were very similar to the results from the BamHI double digests. Three cultures showed some differences which appear to be the result of hypomethylation on the active X chromosome as >50% of the total DNA in the sample was digested by HpaII. Of 22 samples screened for polymorphism at the PGK locus, six were informative. When PstI/BstXI/HpaII digests of these six samples, plus another three samples which were not polymorphic, were analysed only one sample gave a signal after HpaII digestion and in this case the pattern was very similar to that obtained with M27β (50:50). The remaining eight samples were almost completely digested by HpaII indicating hypomethylation of both X chromosomes.

Four of the chorionic villus samples studied were received for prenatal diagnosis of PDH Elα deficiency and the remaining 19 samples were studied as controls. Two of the samples for diagnosis had normal levels of enzyme activity and had X inactivation patterns in the range 30:70 and 60:40 respectively and they were considered to be normal. The other two also had normal enzyme activity but had almost unilateral patterns of X inactivation (90:10) and so the diagnosis could not be excluded.

Discussion

The high frequency of manifestation of X linked PDH Elα deficiency in heterozygous females, combined with the fact that the majority of cases are sporadic, can lead to considerable diagnostic problems. Diagnosis based on gene expression demands that both X chromosomes are active in the sample at a sufficient level to ensure that their products, or lack thereof, are readily detectable. In this study, we show that this cannot be assumed in fibroblast cultures from human females and that the diagnosis cannot be excluded in a significant number of patients in whom there is a high index of clinical suspicion.

There are a number of different methods currently in use for the analysis of X inactivation patterns, none of which is entirely satisfactory for all situations. All are based on different patterns of methylation on active and inactive X chromosomes. Most commonly, use is made of methylation of cytosine residues within the 5' promoter regions of housekeeping genes such as PGK on the inactive X chromosome. Although the methylation patterns at these loci are closely correlated with X inactivation, the loci themselves tend to be

Figure 4 Distribution of X inactivation ratios in control and patient fibroblasts. The X inactivation pattern was determined from the methylation status at the DXS525 locus. Control samples are shown in black, patient samples in grey. Samples were divided into centile bands with the exception of those with approximately equal representation of each X chromosome which were pooled into one group. The distribution is shown on the right as there is no basis for expressing the ratios in either direction.
conserved and relatively few females are heterozygous for different alleles. The analysis is therefore informative in only a minority of cases (28% with PGK in the present study). Another approach is based on the hypervariable locus, DXS255, which although not expressed, is differentially methylated on active and inactive X chromosomes. Because this locus contains a VNTR sequence, it is highly polymorphic and virtually all females are informative (90% in this study). However, questions have been raised concerning the general use of DXS255 for X inactivation analysis. Firstly, methylation at this locus is associated with the inactive X chromosome, the opposite to the situation with expressed genes. Secondly, hypermethylation of this locus has been observed in some cell types, in particular peripheral blood leucocytes and some malignant cells. In this case, more than half of the DNA in the sample remains undigested with HpaII and interpretation of the results relies on the assumption that there is no allele specific difference in the hypermethylation to distort the intensity of the bands representing digested DNA. Hypermethylation may also result in very faint fragment patterns in some samples which are difficult to analyse. An additional problem may arise with the use of the DXS255 locus owing to differences in methylation at sites on either side of the VNTR sequence. This has been shown in white blood cells where it appears that only methylation at the 5' sites correlates well with the active X chromosome. Digestion with PstI/HpaII removes the 3' methylation site from the analysis and could potentially provide clearer results. As differences in methylation on either side of the VNTR had not been studied previously in cultured fibroblasts, additional analysis was performed in a number of cases. However, no significant difference was observed in patterns generated by either BamHI/HpaII or PstI/HpaII (table), and the complexity of the PstI/HpaII fragment patterns often made interpretation difficult (three of 25 patient samples could not be analysed because of overlapping bands).

The general complexity of methylation analysis at the DXS255 locus has been considered a major factor restricting the widespread use of this locus for X inactivation studies. Recently, however, much of the complexity has been removed by detailed restriction mapping and sequencing. These studies have provided an explanation for different fragment patterns and led to more rational experimental design, based on specific methylated sites. The MspI sites used for methylation analysis are located within the CpG island of a LINE-1 repetitive element which is extensively methylated on the inactive X chromosome.

In the present study, a close correlation was found between X inactivation patterns determined with either PGK or M27β in nine patient samples and three normal control samples where both were informative. This is in agreement with the study of Gale et al in which a similar correlation was shown between the two methods in blood and bone marrow samples from 37 females. We have also previously shown a good correlation between the X inactivation pattern determined with M27β and expression of the PDH E1α gene and these observations have been further confirmed and extended in the present study. The conclusion is that this system provides a reliable guide to X inactivation status in cultured fibroblasts.

The results show wide variation in the pattern of X inactivation in fibroblast cultures from different females (fig 4). To a large extent, this reflects variations in the original skin biopsies as there is no demonstrable difference between cells in culture expressing the normal versus a mutant allele for the PDH E1α subunit. This is in contrast to the situation in other X linked diseases such as adrenoleukodystrophy, in which a clear selective difference has been shown in cultured fibroblasts expressing the mutation.

Although it is widely held that there is a narrow distribution of X inactivation ratios in human females around the mean of 50:50, considerably greater variation has been reported in a number of recent studies. In clonality studies for identification of carriers of severe X linked immunodeficiency, Puck et al determined X inactivation ratios in T cells from normal controls which showed an average of 53% but a range of 20 to 86%. Extensive results have been obtained with peripheral blood leucocytes by Gale et al. In 65 normal females analysed by PGK or HPRT, 15 (23%) had an X inactivation ratio >3/1 and, of 23 post-chemotherapy patients, 26% had ratios >3/1. In a second study using DXS255, results were obtained from 41 females, including both normal controls and haematologically normal patients post-chemotherapy. Nine of these (22%) had X inactivation ratios which were highly skewed. In these studies, the frequency of extreme X inactivation ratios is very similar to the present findings in cultured fibroblasts, where 24% had ratios of 80:20 or greater. In addition to the variation between different females defined in these studies, there appear to be considerable differences in X inactivation patterns in different tissues of the same subject.

In the absence of biological or in vitro selection for particular cell populations, random X inactivation should result in proportions which follow a binomial distribution; however, the results in fig 4 show a bias towards extreme outliers. This may simply represent statistical fluctuation owing to the relatively small number of samples or may indicate that some additional selective process(es) are operating. A tendency for fibroblast cultures to be clonal could result from sampling only a small population of cells in the original biopsy. However, when multiple biopsies have been obtained from widely spaced sites, the X inactivation patterns are quite consistent (unpublished observations). Another possibility is clonal expansion as a consequence of growing up the cells from low density in establishing the cultures and, while great care was taken to avoid
this, the possibility cannot be formally excluded. Regardless of the mechanism, the high frequency of extremely skewed X inactivation patterns in cultured fibroblasts remains a significant factor in many cases.

When X inactivation analysis was coupled with PDH enzyme assay, the patient samples could be divided into a number of groups. In the first group (patients 1 to 14 in the table, representing 29%), the diagnosis was indicated immediately by abnormally low enzyme activity. Of these patients, an underlying mutation has subsequently been defined in six and the others are currently being analysed. In the second group, the diagnosis was suspected because PDH activity was significantly reduced below the normal range. Of the three patients in this group (15 to 17 in the table), the residual activity correlates well with the X inactivation pattern, assuming a normal X chromosome is being expressed in the majority of cells. The diagnosis in these patients can only be established by screening the Elα genes for mutations.

Two groups of patients had enzyme activity within the normal range. In one group of 17 patients (numbers 18 to 34 in the table, 35% of the total), the X inactivation pattern was sufficiently close to 50:50 to exclude the presence of a PDH Elα mutation on one of the X chromosomes. In the other group of 12 patients (numbers 35 to 46, 25%), the diagnosis of Elα deficiency could not be excluded as the X inactivation pattern tended towards expression of only one of the X chromosomes (ratio 80:20 or greater). As most cases of PDH Elα deficiency are sporadic, there is no possibility in this situation of determining which of the X chromosomes might be carrying a mutant Elα gene. In one of these patients (number 35 in the table), a mutation in the Elα coding sequence has subsequently been defined.23 The diagnosis has not been excluded in an additional two patients who had normal enzyme activity and were uninformative at the DXS255 locus (numbers 47 and 48).

In this large group of female patients with clinical features highly suggestive of PDH Elα deficiency, determination of the X inactivation pattern was required in 60% because of normal results with enzyme assay and immunohistochemical analysis. The additional investigation failed to exclude the diagnosis in 25%, leaving direct gene analysis as the only definitive method. This represents a significant burden on laboratories providing a diagnostic service for X linked diseases which behave in this manner and is a direct consequence of highly variable patterns of X inactivation in different human females. This variability has not been widely considered in the past as most defined X linked diseases rarely manifest in heterozygous females. This is because there is a considerable functional reserve of most known X linked gene products and this sets a threshold for development of symptoms which is only exceeded if there is extreme bias in the X inactivation pattern. Diagnostic difficulties owing to extreme X inactivation patterns have also become apparent in clonality studies for assessment of haematological malignancies and identification of carriers of X linked immunodeficiencies.

While exclusion of the diagnosis of PDH Elα deficiency in cultured fibroblasts may be impossible in up to 25% of cases, greater problems can arise with prenatal diagnosis based on measurements of PDH activity in chorionic villus cell cultures as these are likely to be clonal. Although chorionic villi contain a mixed population of cells, the established cultures are composed of fibroblast cells derived from the mesodermal core and may represent expansion of only a few clones. Coupled with this is the observed hypomethylation in some of these cultures which may make direct analysis of the X inactivation pattern difficult. Although hypomethylation was observed at both the DXS255 and PGK1 loci, it appears to be more extreme at the PGK locus as, in seven out of eight samples, the inactive X chromosome was unmethylated in the 5' region of this gene. Hypomethylation of the inactive X chromosome in chorionic villus cells has been reported previously and methylation status in the fetus may also vary during development. For these reasons, methylation may differ with the timing of the chorionic villus biopsy and may alter further during successive passages in culture. It is at present uncertain if methylation status in chorionic villus cells can be reliably correlated with X inactivation.

The spectrum of PDH Elα deficiency in females has not been fully defined as there is a bias towards ascertainment of more severely affected patients. Recognition of milder cases or asymptomatic carriers of this and other X linked diseases may be even more problematic as an X inactivation pattern skewed towards expression of the normal X chromosome may be the major determinant of their phenotype. In the case of PDH Elα deficiency, we believe that patients with a convincing clinical presentation (including typical cerebral pathology and raised blood or cerebrospinal fluid lactate concentration), normal enzyme activity, and a skewed X inactivation pattern should be screened directly for a mutation in the PDH Elα gene. Similarly, demonstration of the mutation in an affected subject is a desirable precondition for prenatal diagnosis so that the presence or absence of the same mutation in the fetus can be determined directly. This avoids the problem of non-representation of the mutant gene product which would give a false negative result.

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14 Hendriks RW, Hinds H, Chen ZY, Craig IW. The hypervariable DXS255 locus contains a LINE-1 repetitive element with a CpG island which is extensively methylated only on the active X chromosome. Genomics 1992;14:598-603.


17 Hendriks RW, Kraakman MEM, Mensink RJG, Schuurman RKB. Differential methylation at the 5' and 3' CCGG sites flanking the X chromosomal hypervariable DXS255 locus. Hum Genet 1991;88:105-11.


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