Is skewed X inactivation responsible for symptoms in female carriers for adrenoleucodystrophy?

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Abstract
A study of X inactivation in 12 female carriers for adrenoleucodystrophy showed no evidence that skewed patterns are related to clinical manifestation. Other possible mechanisms to explain manifestation in females are considered.

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The X linked condition of adrenoleucodystrophy/adrenomyeloneuropathy (ALD) is characterised by marked variability in clinical manifestation. The condition in hemizygous males ranges from an acute lethal degenerative disorder of the first decade, to spastic paraplegia and adrenocortical failure in adult life, and possibly to no manifestation in adults.1 There is also variability in female carriers, some of whom develop progressive adult onset spastic paraplegia, accompanied by sensory symptoms, but rarely by adrenal hypofunction.1 The clinical variability in both males and females does not correlate with the extent to which very long chain fatty acids (VLCFA) are raised. Why do some carriers develop clinical problems and why do others escape?

There are several possible explanations. Firstly, is adrenoleucodystrophy an X linked dominant, so that all, or nearly all, obligatory heterozygotes will develop a spastic paraplegia in time? Secondly, does clinical manifestation depend upon whether the carrier has inherited the mutant gene from her mother or from her father? In investigating this possibility it is important to know whether a symptom free but dead father could have been a gene carrier. Thirdly, could manifestation depend upon whether it is the mutant or wild type gene that is predominantly inactivated? In this respect it is interesting to note the in vitro studies of Migeon et al2 in which clones from cultured fibroblasts of carrier mothers showed a preponderance with the mutant gene active.

Fourthly, could secondary phenomena, such as an autoimmune response, account for the variability in manifestation, as suggested by Moser et al?5 Fifthly, could autosomal modifier genes influence the expression of the X linked mutant gene? Finally, could it be that the ALD gene is associated with an insert, so that, like the fragile X syndrome and myotonic dystrophy, the size of the insert correlates with the severity of the clinical picture?

In order to consider the third of these possibilities, namely the role played by X inactivation patterns, we carried out an investigation into X inactivation in manifesting and non-manifesting female carriers.

The probe M27β detects locus DXS225 which contains a VNTR sequence within it.6 The degree of polymorphism shown by the sequence indicates that there is > 90% heterozygosity in females. In addition the locus also contains Mspl sites which are methylated on the active X chromosome but are unmethylated on the inactive X chromosome. Such sites are vulnerable to digestion by the isoschizomer HpaII only when they are unmethylated, that is, when they lie on the inactive X chromosome. Therefore, M27β can be used to differentiate between the active and inactive X chromosomes, and to analyse the pattern of X inactivation in any particular female. Correlations between skewed X inactivation and phenotypic expression of X linked disease have been documented using both PGK and HPRT probes.5,6 The PGK and HPRT probes are more limited in their application, however, owing to the low level of heterozygosity shown by females.

Methods

Patients
We studied 12 women who were carriers for ALD; their genetic and clinical features are...
listed in the table. They belonged to five families, of whom four were described by Del Mastro et al.\(^8\) (families A, B, D, and E); the pedigree of the fifth family (family F) is presented in fig 1. All women in these families were examined; three had a progressive spastic paraplegia, which was accompanied by sensory symptoms in one (FIII-4), thus raising the diagnostic suspicion in her of multiple sclerosis.

LABORATORY METHODS

Blood samples of 20 ml were collected in potassium EDTA tubes from each female under study. DNA was extracted from lymphocyte pellets using a 340A Nucleic Acid Extractor (Applied Biosystems). Parallel DNA samples (5 μg) were restricted with PstI/MspI and PstI/HpaII or, if uninformative, AvaI/ MspI and AvaI/HpaII following the method of Boyd and Fraser.\(^11\) The samples were then electrophoresed on 0.7% agarose at 65 V for 24 to 36 hours. Gels were Southern blotted under standard conditions using Hybond N+ (Amersham International Ltd). The probe M27β was labelled with [32P]dCTP using a Random Primed DNA labelling kit (Boehringer Mannheim Diagnostics Ltd). Filters were hybridised overnight in a hybridisation oven (Techne Hybridiser HB-2). Washes were performed under standard conditions.\(^12\) The Southern blots were first looked at by two independent observers and then subjected to densitometry to determine whether or not there was a skewing of the X inactivation process in these subjects. The relative band intensities were measured using a densitometer (LKB Ultrascan XL Laser densitometer).

Owing to the presence of extraneous bands which sometimes appear after digestion with HpaII, more detailed studies have recently been made of the DXS255 locus.\(^13\) The 5′CpG island on the active X chromosome was found to be invariably fully methylated, but variable methylation patterns could sometimes be detected on the inactive X. This was found to be because of additional MspI sites located in the region.\(^13\) In all samples a majority of cells had site 2 unmethylated on the inactive X. Although patterns of methylation were heterogeneous between subjects and even between different tissues from the same subject, at least one of the MspI sites was found to be unmethylated on the inactive X chromosome.\(^11\)\(^12\) For this reason, in this study comparisons were made between the residual PstI bands, that is, material uncut by HpaII, as this represented the percentage of fully methylated or active X chromosome. If any or all of the MspI sites remained unmethylated on the inactive X chromosome, then the Cpg island would be cut by HpaII and the band would alter in size and change position in the gel. Comparison of intensities of the bands corresponding to the original PstI alleles thus represented the more accurate determination of the percentages of active and inactive X present (fig 2).

We considered how to define 'skewedness' using three approaches. Firstly, there is the statistical approach, based on the premise that there are 20 primordial stem cells that contribute to the bone marrow,\(^14\) and that initially these should be randomly allocated to one of two groups (maternal X active, or paternal X active) with a 50% probability for both. On this basis, a distribution of 75:25 would be significantly different from that expected at the 5% level, and one of 80:20 would be significantly different from that expected at the 1% level. Secondly, results from a control series of women can indicate a 'normal distribution'. In the series of Harris et al.,\(^15\) four of 42 women (9.5%) displayed skewing of 80:20 or greater. This is a higher proportion than would be expected on theoretical grounds and indicates that any pattern between 80:20 and 50:50 should not be considered to be abnormal. Lastly we could ignore the degree of skewedness and just compare any change from 50:50 with a clinical manifestation; thus a ratio of 65:35 might well be significant when related to clinical symptoms. However, as our experiments could not distinguish between the mu-

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**Figure 1** Pedigree of family F.

**Figure 2** Methylation analysis of three carriers of ALD using the probe M27β.\(^P\) = PstI digest, \(M\) = MspI + PstI double digest, \(H\) = HpaII + PstI double digest. DI-2 and FIII-4 show random X inactivation with ratios of 60:40 and 55:45 respectively. Bands used for densitometric studies were those uncut by HpaII.
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12 Hendriks RW, Kraakman MEM, Mensink RGJ, Schuurman RKJ. Differential methylation at the 5' and the 3'CCGG sites flanking the X chromosomal hypervariable DXS255 locus. Hum Genet 1991;88:105-11.

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


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