SHORT COMMUNICATIONS

X inactivation patterns in females with Alport's syndrome: a means of selecting against a deleterious gene?

David Vetrie, Frances Flinter, Martin Bobrow, Ann Harris

Abstract

The patterns of X chromosome inactivation in 43 females from families segregating classic Alport's syndrome (AS) (X linked hereditary nephritis with deafness) have been analysed. AS carrier females have a most variable clinical course. The aim of the study was to establish whether there was any correlation between the X inactivation pattern of a carrier female and the severity of her disease. No correlation was found in DNA derived from peripheral blood lymphocytes. However, it remains possible that differential patterns of X inactivation may occur in the tissues affected by AS, namely the basement membrane of the kidney, eye, and ear.

(J Med Genet 1992;29:663-6)

Classical X linked Alport's syndrome (AS)^{1,2} which maps to Xq21-22³⁻⁵ is caused by mutations in the gene encoding the $\alpha 5$ chain of basement membrane collagen type IV (COL4A5).⁶⁻⁸ Female carriers show a wide spectrum of severity of expression; some show all the characteristic signs of the disease seen in male patients, namely chronic renal failure, high tone sensorineural deafness, and specific eye defects (lenticonus and macular flecks), others may only be minimally affected, and the majority remain healthy throughout a normal life span, the only sign of AS being microscopic haematuria.⁹

It has been suggested that the variable phenotype in carrier females might be related to X inactivation patterns. Selection against cells expressing the mutant AS allele might result in a less severe disease. Alternatively, inactivation of a high proportion of normal X chromosomes in the critical tissues could lead to a more severe clinical manifestation.

It is known that at the DNA level the phenomenon of X inactivation can be assessed in terms of methylation of certain base sequences. Polymorphisms in the housekeeping genes hypoxanthine phosphoribosyl transferase (HPRT)¹⁰ and phosphoglycerate kinase (PGK)¹¹ have been used to distinguish maternal and paternal X chromosomes. The methylation state of the two polymorphic DNA fragments is then investigated using methylation

sensitive enzymes which will not cleave methylated DNA.

In order to establish whether skewed inactivation possibly resulting from selection against cells expressing a mutant AS gene has any effect on disease severity in Alport's syndrome we have now investigated X inactivation patterns in a group of 22 families segregating for AS. These families were selected from 30 that were previously subjected to extensive linkage analysis³ because one or more female members was heterozygous for polymorphisms in either of the two X linked genes for HPRT or PGK. X inactivation patterns were established in both AS carrier and non-carrier females by the analysis of methylation patterns of one of these two genes.

Forty-four females from 22 AS families were studied. Thirty subjects were classified as AS carriers based on clinical and genetic evidence. This group included 25 AS carriers with normal renal function and five subjects with impaired renal function (IRF). The remaining 14 females were clinically normal and did not carry a mutant AS gene (table 1).

Details of the HPRT and PGK polymorphisms are described elsewhere.¹⁰⁻¹⁴ Briefly, the HPRT probe detects a polymorphism after simultaneous cleavage with *Bam*HI and *Pvu*II giving band sizes of 18 and 12 kb. The PGK probe detects a polymorphism after simultaneous cleavage with *Eco*RI, *Bgl*II, and *Bgl*III showing polymorphic fragments of 1.7 and 1.3 kb. The methylated polymorphic allele (that is not cleaved by *Hpa*II) has been shown to be on the active chromosome for the HPRT probe^{12,15} and on the inactive chromosome for the PGK probe.¹³

Total genomic DNA was extracted from peripheral blood cells as described previously.³ Digests, Southern blotting, and probing for HPRT and PGK were carried out as described in the accompanying paper.¹⁶

Table 1 X inactivation patterns in AS females and their normal relatives

	AS females	Normal relatives
Symmetrical	16	7
Moderately skewed	13	6
Extremely skewed	1	1
Total	30	14

Paediatric Research Unit, Division of Medical and Molecular Genetics, United Medical and Dental Schools of Guy's and St Thomas's Hospitals, 8th floor, Guy's Tower, London SE1 9RT.
D Vetrie
F Flinter
M Bobrow
A Harris

Correspondence to Dr Harris, Paediatric Molecular Genetics, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU.

Received 7 March 1992.
Revised version accepted 8 May 1992.

X inactivation patterns in subjects were classified as being symmetrical, moderately skewed (that is, an inactivation pattern that deviates noticeably from a 50:50 maternal:paternal ratio), or extremely skewed (that is, one parental allele being virtually completely inactive). Autoradiographs were scanned on a Biorad Model 620 video densitometer and the symmetrical pattern included subjects with a ratio of fragment intensities after *HpaII* digestion of 50:50 to 65:35, the moderately skewed group included ratios of 65:35 to 80:20, and a ratio of more than 80:20 was defined as extremely skewed.

The X inactivation patterns in Alport's syndrome females and their non-AS relatives are summarised in table 1. Data for HPRT and PGK have been combined. It is clear that about the same proportion of AS (16/30) and non-AS (7/14) women show completely symmetrical patterns of X inactivation. Of those showing skewed patterns, 13/14 (93%) of AS women and 6/7 (86%) of non-AS females have moderately skewed patterns. The remainder show inactivation of the same parental X chromosome in all their peripheral blood cells.

Data relating X inactivation patterns in AS females to the severity of their renal disease are shown in table 2. Subjects with impaired renal function (IRF) are compared with those who merely have microscopic haematuria. In the former group, one of five (20%) shows a symmetrical pattern while the remaining four show a moderately skewed pattern. In the latter group, 15 of 25 (60%) show a symmetrical pattern, 9 of 25 (36%) show a moderately skewed pattern, and one of 25 (4%) are extremely skewed.

The X inactivation patterns in AS females with or without impaired renal function have been further analysed with respect to whether the inactivated HPRT or PGK locus cosegregates with the Alport lesion. This correlation was possible in 14 families and the data are shown in table 3, and illustrated in the figure. AS females without IRF are seen to inactivate the HPRT or PGK locus cosegregating with the normal allele or the mutant allele at approximately equal frequencies (two and three

respectively). All four AS females with IRF who show skewed patterns of X inactivation have preferentially inactivated the HPRT or PGK locus cosegregating with the mutant allele. Cosegregation of the Alport locus with the HPRT or PGK loci for representative families is illustrated in the figure. The polymorphic band sizes are shown for the HPRT or PGK markers in the informative family members. The maternal (M) and paternal (P) X chromosomes are shown as having the mutant (AS) or normal (N) COL4A5 allele. In pedigree A, subject 5 has IRF and has preferentially inactivated the X chromosome carrying both the 12 kb HPRT allele and the mutant AS gene. Pedigree B shows two subjects (3 and 4) with IRF who both show moderately skewed inactivation of the X chromosome bearing the 1.7 kb PGK and mutant AS alleles. Pedigree C shows subjects without IRF (3 and 6) who have preferential inactivation of the X chromosome carrying the 1.7 kb PGK and mutant AS alleles, while pedigree D shows two family members without IRF who have symmetrical X inactivation (2) or extremely skewed inactivation (1) of the X chromosome carrying the 1.7 kb PGK fragment and the normal AS gene.

In an analysis of 44 females from kindreds segregating for Alport's syndrome we have not observed evidence for selection against cells expressing the mutant Alport's gene as determined by patterns of X inactivation in peripheral blood cells. The ratios of symmetrical, moderately skewed, or extremely skewed inactivation patterns among AS females are broadly similar to those seen in their female relatives without AS (table 1). Furthermore, they are not substantially different from the ratios we have observed in a large group of control females, either normal or carrying non-X linked diseases.¹⁶

There does not seem to be any correlation of X inactivation patterns with disease severity in AS females (table 2), and since we could only examine five AS females with impaired renal function (IRF) the small numbers are likely to account for the differences between IRF and non-IRF groups (specifically the low percentage of females with IRF who show symmetrical inactivation patterns). This explanation is supported by the data in table 3 where X inactivation patterns in AS females have been investigated further to determine whether the inactive HPRT or PGK locus segregated with the AS mutation or the normal allele. In all four AS females with IRF who showed skewed inactivation patterns the inactive locus was segregating with the AS mutation. If X inactivation were playing a role in selecting against cells expressing the mutant allele so resulting in milder disease, then AS females with IRF would be expected to show more cells expressing the mutant gene. The data also fail to support a hypothesis that chance inactivation of a high proportion of normal alleles could be a mechanism for increasing disease severity. It is of course possible that by analysing methylation at a locus linked to the AS gene rather than at the locus itself we are not

Table 2 X inactivation patterns in AS carrier females with or without impaired renal function (IRF).

	AS without IRF	AS with IRF
Symmetrical	15	1
Moderately skewed	9	4
Extremely skewed	1	0
Total	25	5

Table 3 Inactivation patterns of X chromosomes carrying the normal and mutant AS allele in female carriers with and without impaired renal function (IRF).

	AS without IRF	AS with IRF
Symmetrical	8	1
Inactivation of chromosome carrying normal allele	2	0
Inactivation of chromosome carrying mutant allele	3	4

Figure 1 RFLP methylation analysis in selected subjects of four AS kindreds. The polymorphic band sizes are shown for the HPRT or PGK markers in the informative family members. The maternal (M) and paternal (P) X chromosomes are shown as having the mutant (AS) or normal (N) Alport's allele. In all cases (–) denotes before HpaII cleavage and (+) after HpaII cleavage. In pedigree A, subject 5 has impaired renal function (IRF) and is preferentially inactivating the X chromosome carrying both the 12 kb HPRT allele and the mutant AS gene. Pedigree B shows two subjects (3 and 4) with IRF who show skewed inactivation of the X chromosome bearing the 1.7 kb PGK and mutant AS alleles. Pedigree C shows subjects without IRF (3 and 6) who have preferential inactivation of the X chromosome carrying the 1.7 kb PGK and mutant AS alleles, while pedigree D shows two family members without IRF who have symmetrical X inactivation (2) or skewed inactivation (1) of the X chromosome carrying the 1.7 kb PGK fragment and the normal AS gene.

generating an accurate picture of AS gene specific inactivation patterns.

In conclusion, our studies have not observed unusual frequencies of skewed X inactivation patterns in the peripheral blood cells of affected AS females. Further, we have seen no evidence for X inactivation patterns affecting the disease severity. However, it remains possible that these mechanisms are operating in the tissues where AS has its pathological effects, namely in the basement membranes of the kidney and ear and in specific cells within the eye.

This work was supported by The Generation Trust and The Spastics Society. DV is a United Kingdom Commonwealth scholar. We thank Drs D Jolly and J Singer-Sam for probes.

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