Electronic letter

Multiple metachromatic leucodystrophy alleles in an unaffected subject: a case of dispermic chimaerism

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Metachromatic leucodystrophy (MLD) is a neurodegenerative lysosomal storage disease resulting from a deficiency of arylsulphatase A (EC 3.1.6.1), an enzyme involved in the normal catabolism of cerebroside sulphatide.¹ The arylsulphatase A gene, located on chromosome 22, has a few common mutations that occur in a significant proportion of cases. Among these is P426L, sometimes known as the "A" or adult onset allele,² which is associated with juvenile or adult onset MLD. Another less frequent mutation, A212V,³ is responsible for a more severe deficiency and has been identified in subjects of British, French, and Acadian descent.³⁻⁴ A pseudodeficiency allele also occurs in the arylsulphatase A gene, consisting of a glycosylation site mutation (N350S) and an A→G in the first poly A addition signal (AATAAC→AGTAAC). This pair of mutations has an allele frequency of up to 20% in the general population.⁵⁻⁶ The N350S mutation occurs alone in about 5% depending on ethnicity. Only one case of the poly A site mutation occurring alone has been documented.⁷

Subjects may have apparent mixtures of independent cell types at the level of a tissue or the whole body and may be described as mosaic or chimaeric. The distinction between mosaics and chimaeras has been recognised for some time.⁸ Cell lines of mosaics will have an underlying genetic identity and arise from events in chromosome duplication and cell division. In contrast, chimaeric cell lines are genetically distinct, arising from different zygotic lineages. We recently reported a family in Nova Scotia, Canada where a child with juvenile onset MLD carried A212V and

P426L.³ We describe here a brother of the affected child, who is clinically well but apparently carries both A212V and P426L. The presence of additional genetic markers in the arylsulphatase A gene and elsewhere on chromosome 22 suggests that he is a chimaera carrying two arylsulphatase A alleles from each parent.

Material, methods, and results

The patient, "T", is a healthy 16 year old boy whose older brother was diagnosed at the age of 10 with juvenile onset MLD. This family has been described previously.³ The affected sib is a compound molecular heterozygote for the P426L allele inherited from the father and the A212V allele inherited from the mother. All the sibs were tested biochemically for arylsulphatase A activity (table 1). Arylsulphatase A activity in leucocyte or cultured fibroblast extracts was determined using a p-nitrocatechol sulphate substrate.⁹ "T" (II.2) appeared to be a carrier on the basis of his level of arylsulphatase A activity, which was lower than that of either parent in the same assay. Similarly, on the same basis, all his unaffected sibs except for II.1 also appeared to be carriers.

At the family's request, the parents and all the children were tested for the A212V and P426L mutations. DNA was extracted from leucocytes by standard methods. PCR amplification followed by restriction enzyme digestion was used to test for the two pseudodeficiency mutations, the P426L late onset mutation and A212V infantile onset mutation as described previously.³ The results are shown in fig 1. Clearly, the allele status of "T" (II.2) did not fit with his healthy condition so a second DNA leucocyte sample was requested from "T" and DNA was isolated independently of any other family members to rule out possible sample contamination. The same result was obtained. Further testing of family members for other markers indicated the mother was a carrier of the N350S polymorphism, usually associated with pseudodeficiency, but occasionally occurring on its own. N350S was also detected in "T" although it segregated independently of A212V in other family members, suggesting the two mutations were carried on separate alleles in the mother.

Table 1 Arylsulphatase A activity in family members

Case and relationship to "T"	Arylsulphatase A (nmol nitrocatechol released/h/mg protein†)	Percent normal mean
I.1 (father)	10.3	130
I.2 (mother)	6.4	81
II.1 (sib)	16.1	203
II.2 ("T")	4.0	50.6
II.3 (affected sib)*	0 (fibroblast activity)	0
II.4 (sib)	4.9	62.0
II.5 (sib)	5.8	73.4
II.6 (sib)	3.7	46.8

*Arylsulphatase A activity for the affected child was measured in cultured fibroblasts. All other data were determined for leucocytes.

†Normal ranges: leucocytes 2.8–17.9, mean 7.9; fibroblasts 22.3–49.6, mean 37.9.

Figure 1 Family pedigree with arylsulphatase A mutations. The MLD affected child is II.3. II.2 is "T". The alleles are shown as black (A212V), diagonally striped (P426L), horizontally striped (N350S), or white (normal). The birth order on the pedigree has been altered to protect confidentiality.

The absence of MLD alleles in II.1 was consistent with her non-carrier status determined biochemically (table 1). Subjects II.4, 5, and 6 all carried one MLD allele (P426L) consistent with their biochemically determined carrier status.

Crossing over during PCR priming was ruled out because the mutations were detected originally on separate PCR products. The possibility that there had been an intragenic recombination event between the mother's two alleles in "T" was addressed by amplifying a 1257 bp DNA product spanning the A212V and N350S mutation sites. This PCR product was digested with *AatII*, specific for the A212V site, generating 335 bp and 922 bp fragments where A212V is present. The 922 bp fragment carries the N350 glycosylation site. The residual uncut 1257 bp segment and the 922 bp segment were separated by agarose gel electrophoresis and tested for the N350S mutation separately. N350S was found in the 1257 bp segment but not in the 922 bp fragment in both mother and son, suggesting that the two markers are indeed on separate alleles.

A series of microsatellite markers specific for chromosome 22 (Research Genetics, Bethesda, MD) was amplified and examined by electrophoresis and autoradiography as described earlier.¹⁰ Fig 2A shows results for D22S684 and TOPIP2 indicating that "T" inherited four different chromosome 22 alleles from his parents, whereas his sibs inherited the usual two. Addi-

Figure 2 Microsatellite analysis in leucocyte DNA. Amplification and analysis were as described in Methods. a→d indicates decreasing product size. Quantitation of the alleles is not indicated in their designation. (A) Chromosome 22 markers. Markers D22S684 (upper panel) and TOPIP2 (lower panel) were used. Two separate DNA samples from "T" were tested and both are shown in the figure. Subjects are shown in the same order as in fig 1 and table 1. (B) Chromosome 12 marker. Inheritance of D12S77 was analysed in leucocyte DNA from the parents and "T".

tional informative chromosome 22 markers summarised in table 2 also indicated more than two alleles. These results were consistent with the intragenic marker findings.

Microsatellite analysis was extended to other chromosomes. Many of those surveyed were not informative; however, selected markers on chromosomes 3, 7, 12, and 16 indicated the presence of at least three alleles in "T". The results for D12S77 are shown in fig 2B. The intensity of the single "b" band for the paternal marker suggested that the father carried two copies of the "b" allele. Results for other markers are summarised in table 2. The X chromosome marker, DXS987, showed only one of the two maternal alleles and not the paternal allele.

Cultured fibroblasts from "T" were later obtained and tested for arylsulphatase A in a different laboratory and yielded 33% as much activity as a parallel normal control. An MLD control yielded no detectable activity. Fibroblast

Table 2 Summary of results with informative microsatellites

Marker	Chromosome	DNA source	Alleles present*		
			Father	"T"	Mother
D22684	22	Leucocyte	cd	abcd	ab
		Fibroblast	cd	abcd	ab
TOPIP2	22	Leucocyte	bc	abcd	ad
		Fibroblast	bc	abcd	ad
D22S283	22	Leucocyte	bd	abcd	ac
D22S284	22	Leucocyte	bc	abc	ac
D16S398	16	Leucocyte	bc	abc	ab
D7S669	7	Leucocyte	bc	abc	ab
D12S77	12	Leucocyte	b	abc	ac
D3S1581	3	Leucocyte	bc	acd	ad
D3S1261	3	Leucocyte	bc	acd	ad
DXS987	X	Leucocyte	a	c	bc

*Denotes only the presence of the marker alleles, not the quantity. a→d indicates decreasing product size.

DNA was isolated from two different culture samples in different laboratories. The same mutations and polymorphisms were found as in leucocyte DNA. The microsatellite marker, D22S684, was also used to analyse the fibroblast DNA with an outcome identical to the leucocyte DNA results that were shown in fig 2A.

Karyotype analysis showed a normal 46,XY pattern. Urinary sulphatide fell in the normal range. Blood group analysis for ABO/Rh and Jk^b (Kidd antigen) showed mixed field reactivity consistent with chimaerism.

Discussion

In summary, we have investigated a child who carried two allelic MLD mutations and had an affected brother but who was, himself, clinically well. Through the use of informative intragenic polymorphic markers, we have shown that he carried an additional maternal allele. Intragenic recombination events have been ruled out. Microsatellite analysis indicated that he carried two chromosome 22 markers from each parent. Several microsatellites on other chromosomes were also informative for the presence of at least three markers. Cultured fibroblasts obtained from this child yielded the same intragenic mutation and polymorphism results. The D22S684 microsatellite result was also confirmed for the fibroblast DNA. These results taken together with the blood group analysis are suggestive of chimaerism.

The original arylsulphatase A enzyme assays performed on leucocytes suggested arylsulphatase A activity in the carrier range. Assays of fibroblast extracts were also in the carrier range. Since the leucocytes or fibroblasts represent a mixture of two cell lines of different genotypes, the level of assayable arylsulphatase A activity will depend on the relative proportion and the specific allele combination of each. Also the N350S mutation may mean that protein produced from this maternal allele has a somewhat compromised activity.¹¹

Phelan¹² described two types of human genetic chimaeras: dispermic or tetragametic chimaeras resulting from fusion of two genetically distinct zygotes and blood group or haematological twin chimaeras resulting from exchange of haematological precursors via vascular anastomoses in the placenta. Haematological chimaerism in dizygotic twins has been well documented.¹²⁻¹⁶ This combined with the "lost twin" phenomenon¹⁷ might have accounted for the apparent combination of alleles observed with "T". This was indeed what we supposed had happened until we obtained skin fibroblasts. Fibroblast DNA was tested to determine the true genotype of "T", but instead the results indicated a different kind of chimaerism, the dispermic type.

Descriptions of chimaeras and how they are generated have been reported over many years.^{8 18-22} In the more recent reports, the use of DNA polymorphisms to distinguish haematological from dispermic chimaerism is particularly emphasised.^{21 22} The most likely events appear to be fusion of a fertilised ovum with a fertilised polar body, either the one arising

from meiosis II and directly associated with the secondary oocyte or one of those arising from meiosis I. The former would be expected to result in the presence of one maternal allele for all markers tested.^{19 22} The latter would yield both maternal alleles for all markers barring recombination. Fusion of two distinct embryos would be expected to yield representation of both alleles of some markers and not others.^{18 20} In the case presented here, all the maternal alleles were represented for all markers examined except for DXS987 (Xp22) where only one was found. Since we cannot rule out recombination in this case, the chimaera may have been generated by fusion of two distinct zygotes as in two earlier cases^{18 19} or by fertilisation of a meiosis I polar body.

The incidence of such events is impossible to determine because they are so rare. Those that do not involve XX/XY combinations lack any obvious phenotypic indicators and so would only be picked up by chance through blood group or transplant antigen analysis. Such is the case described by Watkins *et al*²³ where analysis of glycosyl transferases suggested chimaerism. Even then they might be mistaken for the more common haematological chimaeras unless testing of other tissues is undertaken.

Chimaerism, of either sort, could be a complication in prenatal diagnosis if DNA testing is performed in the absence of enzymological assay. Haematological chimaerism is sufficiently common that an occasional fetus will be designated affected when in fact it is not. In the present case, the chimaerism was detected during testing for carrier status in the family of a known MLD patient whose mutations had been determined. The presence of the two mutations in "T" did not fit with his enzyme level and his healthy clinical condition. It was the simultaneous presence of the N350S polymorphism from the mother that was the clue to the chimaerism.

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