Key words: lipid peroxidation, docosahexenoic acid, posterior subcapsular cataract, tapetoretinal degeneration

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References

Aniridia: Enzyme Studies in an 11p— Chromosomal Deletion

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A patient with aniridia and an interstitial deletion of the bands p13–p14 of the short arm of chromosome 11 was studied to determine the relative locations of the gene(s) encoding for the aniridia-Wilms' tumor association with other genes on the same chromosome. Quantitative analysis was performed on the red blood cell enzymes lactic acid dehydrogenase-A (LDH-A) and catalase, the genes for which are located on the short arm of chromosome 11. The activity of LDH-A was normal; the activity of catalase was reduced to approximately half normal. This evidence supports loci for the genes encoding for both catalase and the aniridia-Wilms' tumor association within the bands p13–p14 of the short arm of chromosome 11; the normal activity of LDH-A supports a locus outside this region. Invest Ophthalmol Vis Sci 25:612–616, 1984

The association of aniridia, genitourinary malformations, developmental delay, and Wilms' tumor with interstitial deletions of the short arm of chromosome 11 initially was recognized by Smith and co-workers. Although neither the smallest possible deletion resulting in this association nor the number of genes involved is established, a patient with aniridia, Wilms' tumor, and no demonstrable chromosomal deletion was reported recently. As Wilms' tumor is life-threatening, methods for identifying those patients with aniridia who are at higher risk for this malignancy would be clinically useful. Regional gene mapping by correlating cytogenetic break points with enzymatic activities of genes known to be located on a given chromosome may provide such clues. We selected two enzymes, the genes for which are known to be on the short arm of chromosome 11, for study in a patient with aniridia and an interstitial deletion of the same chromosome; quantitative analyses of the red cell enzymes catalase and lactic acid dehydrogenase-A (LDH-A) were performed to determine activity; both of these enzymes have been shown to exhibit a gene–dose relationship.

Materials and Methods. A Caucasian girl was identified as having absent pupils at 6 months of age; a diagnosis of aniridia was made, and she was referred to the UCLA Genetics Clinic. The full clinical features will be described in a separate report. Informed human consent was obtained prior to undertaking the study. Giemsa and reverse (R) banding of cultured, synchronized lymphocytes of the patient and of her parents in late prophase or early metaphase were performed; three full karyotypes and six partial karyotypes of pair 11 of the patient were reviewed.

Quantitative analysis of red cell catalase was performed twice on the proband and three unrelated controls by the technique of Beutler; for each analysis, the degradation of H2O2 was monitored spectrophotometrically twice on the Gilford 2000 (Gilford Instrument Laboratories; Oberlin, OH).

Red cell LDH-A was assayed twice on the proband and three unrelated controls by electrophoresing a sa-
Fig. 1. Early metaphase karyotypes (lymphocytes) of chromosome 11 of patient. Large arrows on schematic chromosome 11 indicate the break points of our best estimate of the interstitial deletion; the possibility that the break points are within the p12 and/or p14 bands cannot be excluded. Small arrows identify the margins of the deletion on the normal chromosome 11; the abnormal chromosome with the deletion of the bands p13–p14 is shown on the right of each pair.

ponin-hemolysate of washed red cells on a starch gel over 18 hr (4 V/cm) with a phosphate buffer of pH 7.0. The gel was photographed with black and white Polaroid film (55); both the photograph and the gel were scanned in a densitometer equipped with a chart recorder. The peaks of the five isoenzyme patterns of LDH-A and -B polypeptides were compared. The five LDH isoenzymes migrate anodally, 1 faster than 5; as erythrocytes preferentially synthesize LDH-B, isoenzymes 1 through 3 normally have higher activity than 4 and 5. In this system, superoxide dismutase migrates within the isoenzyme 2 band, which results in the appearance of six bands.

Results. An interstitial deletion of the short arm of one chromosome 11 from band p13–p14 was present in the patient’s lymphocytes (Fig. 1) and skin fibroblasts; bands 11p12 and p15 appeared to be intact. Prophase banding of the karyotypes of both parents were normal.

Quantitative assay of catalase in the simultaneously run control groups ranged from 137,267 to 249,251
International Units of catalase per gram of hemoglobin (IU per gram of Hb); the patient's activities ranged from 65,513 to 72,065 IU per gram of Hb. The catalase activity of the first five normals studied in our laboratory ranged from 133,250 to 188,180 IU per gram of Hb with a mean of 162,091.

The LDH isoenzyme patterns and the recordings of the densitometer scans are shown in Figures 2 and 3. There are no observable differences in the intensities of the isoenzyme bands 4 and 5 between the patient and controls.

Discussion. Gene mapping on the basis of interstitial chromosomal deletions is useful, but may be imprecise, as the identification of the break sites and of the missing material may be difficult; cumulative evidence, however, may permit the localization of a gene(s) to a

![Fig. 3. Densitometer chart profiles obtained by scanning LDH starch gel of control samples (upper) and of patient (lower). Peaks are labeled with LDH isoenzymes. No relative deficiency of LDH-A (isoenzymes 4 and 5) is evident. Arrows indicate superoxide dismutase band.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933119/)
specific band. Smith and colleagues originally suggested that a gene for aniridia was located on the short arm of chromosome 11 when they described two patients with aniridia, mental retardation, and genital abnormalities and a partial deletion of this chromosome.\textsuperscript{1} Since then, 29 additional aniridia patients with deletion of the short arm of chromosome 11 have been added to the literature; in those reports with detailed information, all deletions have included the p13 band of the short arm.

The genes for two enzymes, catalase and LDH-A, also are located on the short arm of chromosome 11. LDH-A initially was localized to this chromosome by somatic cell hybridization studies. Francke and her colleagues\textsuperscript{2} studied patients with different but overlapping deletions of the short arm of chromosome 11; they established a gene–dose relationship for LDH-A and suggested an assignment to the 11p12 band. Further evidence that the gene for LDH-A is not in the p13 band was presented by Rethoré and her colleagues,\textsuperscript{6} who noted an increase in LDH-A activity in a patient trisomic for 11p with the exception of band 13. LDH-A activity has been studied in a total of 12 aniridia patients with interstitial deletions of the short arm of chromosome 11\textsuperscript{7,11}; the break points of our case and all others have included at least a portion of the 11p13 region. Two of the patients with aniridia and a deletion of the short arm of chromosome 11 had decreased LDH-A activity\textsuperscript{5,7}; both had deletions of chromosome 11 from p11.3–p14 and neither had a Wilms' tumor nor a gonadoblastoma. Six patients with aniridia and deletions of chromosome 11 have had normal LDH-A activities\textsuperscript{5,8–10}, all deletions excluded the 11p12 region. However, three patients with aniridia and deletions of the short arm of chromosome 11, including a portion of the p12 region, have had normal LDH-A activity.\textsuperscript{5,11,12} One patient without aniridia but with a deletion including the 11p12 band had reduced LDH-A activity.\textsuperscript{5} The deletion in our patient does not involve the p12 band; the normal LDH-A activity in her red cells supports localization of this gene outside the bands 11p13–11p14.

Catalase initially was assigned to the short arm of chromosome 11 by somatic cell hybridization.\textsuperscript{13–15} Recently, an aniridia patient with a gonadoblastoma and a deletion of the p13 band of the short arm of chromosome 11 was found to have reduced catalase activity, which suggested a gene–dose relationship.\textsuperscript{9} As further support for the localization of this gene, a patient who was trisomic for the 11p13 band had elevated catalase activity; a third patient who was trisomic for the entire short arm of chromosome 11, except band p13, had normal catalase activity.\textsuperscript{9} Six patients with aniridia and deletions of the short arm of chromosome 11 have been reported by abstract, and, although the specific deletions were not described, all included the p13 band; two had reduced catalase activity.\textsuperscript{16} An additional patient with aniridia and a deletion of the short arm of chromosome 11 extending from the p12 through the p15.1 regions has been found to have reduced catalase activity.\textsuperscript{12} Our case, with less than 50% catalase activity, supports the localization of the catalase gene within the bands 11p13–p14.

The compilation of available evidence strongly supports the localization of the gene(s) for the aniridia/Wilms' tumor association and the catalase gene to the 11p13 band. It appears that the LDH-A gene is proximal to the genes for the aniridia/Wilms' tumor complex and catalase. Gene mapping on the basis of chromosomal rearrangements is vulnerable to the subjective element in interpreting break sites. As the bands of the short arm of chromosome 11 are of similar width, the interpretations are particularly difficult. Further reports of enzymatic activity in aniridia patients with chromosomal deletions are necessary to establish the relative locations of these genes.

Key words: aniridia, Wilms’-aniridia, chromosomal deletion, 11p–, catalase, lactic acid dehydrogenase A, chromosome 11, gene mapping

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References

Effects of Subretinal and Systemic Osmolality on the Rate of Subretinal Fluid Resorption

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Small nonrhegmatogenous retinal detachments (blebs) were made in Dutch rabbit eyes by injecting solution into the subretinal space. There was no difference in resorption time between blebs made with isotonic, hypertonic or hypotonic sodium chloride. However, blebs made with sucrose solution took longer to resorb than those made with Hanks' solution, regardless of whether the sucrose was hypotonic or hypertonic. Intravenous injection of hyperosmotic solution (mannitol) accelerated the resorption of Hanks'-filled blebs but the injection of hypoosmotic solution (water) had no clear effect. The authors conclude that osmotic differences between solutions were made by producing two blebs of the same or different animals but varies between different retinal loci; comparisons between solutions were made by producing two blebs within the same eye, each containing a different solution.


To change the rate at which subretinal fluid is resorbed across the RPE, one may either alter metabolic parameters that affect transport by the RPE, or alter the physical factors that affect resorption such as osmotic or hydrostatic pressure. We have shown previously that metabolic inhibitors such as ouabain, acetazolamide, or cyanide can accelerate or slow down subretinal fluid resorption. In the present study, we examined the effects of osmolality both within the subretinal space and systemically. Systemic hyperosmotic solutions have been shown previously to open the tight junctions of the RPE reversibly, but little is known about the effects of osmotic changes on the resorption of subretinal fluid.

Materials and Methods. The experiments were performed on Dutch rabbits weighing 1.3 to 1.8 kg. Small retinal detachments were made in the avascular posterior pole, free from the medullary rays, by passing a micropipette through a limbal incision and across the vitreous to penetrate the subretinal space, into which an experimental fluid was injected. The methods of anesthesia and techniques of bleb formation and measurement have been described previously. We observed the blebs until 50% of their diameter had become reattached, and used this endpoint for comparative analysis. Bleb resorption time may vary between eyes of the same or different animals but varies less between different retinal loci; comparisons between solutions were made by producing two blebs within the same eye, each containing a different solution.