*Am. J. Hum. Genet. 61:765–768, 1997*

## Autosomal Dominant Axenfeld-Rieger Anomaly Maps to 6p25

*To the Editor:*

After the recent genetic localization of the ocular genetic disorder iridogoniodysgenesis anomaly to 6p25 (Mears et al. 1996), we now provide evidence that a second disorder of the anterior chamber of the eye, Axenfeld-Rieger anomaly (ARA), maps to the same chromosomal location. ARA is a neurocristopathy that presents with ocular features including a prominent, anteriorly displaced Schwalbe's line (posterior embryotoxon) attached to strands of peripheral iris bridging the iridocorneal angle, displaced pupils (corectopia), and iris hypoplasia (Shields 1983). Axenfeld-Rieger syndrome (ARS) has the same ocular features as ARA, in addition to nonocular findings that include a dysmorphic midface, redundant periumbilical skin, and dental anomalies including oligodontia and microdontia (Alward and Murray 1995).

Iridogoniodysgenesis anomaly (IGDA) and iridogoniodysgenesis syndrome (IGDS) are two related ocular disorders. Similar to ARA, patients with IDGA present with ocular features alone, including iridocorneal angle-differentiation abnormalities, hypoplasia of the anterior stromal layer of the iris, and increased intraocular pressure leading to juvenile glaucoma (Pearce et al. 1983; Mears et al. 1996). IGDS has the same ocular features as IGDA, with the additional nonocular features of ARS, including dental and umbilical abnormalities. All four clinically related anterior-segment iris-hypoplasia disorders (ARA, ARS, IGDA, and IGDS) are autosomal dominant disorders resulting in glaucoma in 50%–75% of cases.

Linkage analysis has mapped the ARS gene to 4q25 (Murray et al. 1992). Recently, mutations in the gene, RIEG, have been shown to underlie the ARS phenotype in ARS families in which the disease is linked to 4q25 (Semina et al. 1996). RIEG is a developmental control gene of the bicoid homeobox gene family. The mapping of IGDS to 4q25 raises the possibility that ARS and IGDS are allelic variants of the same disorder (Héon et al. 1995; Walter et al. 1996).

However, in addition to marked variable expressivity, Axenfeld-Rieger/iridogoniodysgenesis eye formations are genetically heterogeneous. Legius et al. (1994) described a family with ARA that did not map to 4q25, consistent with genetic heterogeneity in Axenfeld-Rieger eye malformations. A second locus for ARS has been recently mapped to 13q14 (Phillips et al. 1996); however, the sensory hearing loss reported in this ARS family suggests that the ARS loci at 4q25 and 13q14 can be distinguished clinically. IGDA has recently been mapped to 6p25 (Mears et al. 1996), further indicating that these four iris-hypoplasia disorders do not represent variable expression of the same locus. We have now tested a family with ARA to determine whether the disease phenotype is linked to either of the previously described critical regions, at 4q25 or 6p25.

Clinical examinations of 13 members of a family revealed 7 to be affected with ARA (fig. 1). Individuals diagnosed with ARA had a prominent and anteriorly displaced Schwalbe's line, iris stromal hypoplasia, and corectopia (fig. 2). Nonocular features of ARS (including jaw, dental, and umbilical anomalies) were not present. The study and collection of blood samples from all individuals included in this report were approved by the Research Ethics Board of the Faculty of Medicine of the University of Alberta. Microsatellite analysis was performed by use of oligonucleotide primers from Research Genetics. Microsatellite markers had $^{35}S$ dATP directly incorporated into PCR products, as described elsewhere (Mirzayans et al. 1995). PCR products were separated on 6% polyacrylamide gels. Linkage analysis was conducted as described elsewhere (Mears et al. 1996). Analysis was conducted under the conservative assumption that ARA is an autosomal dominant disorder with 95% penetrance, although there is no evidence of reduced penetrance. Linkage to the ARS/IGDS region at 4q25 was excluded by examination of polymorphic

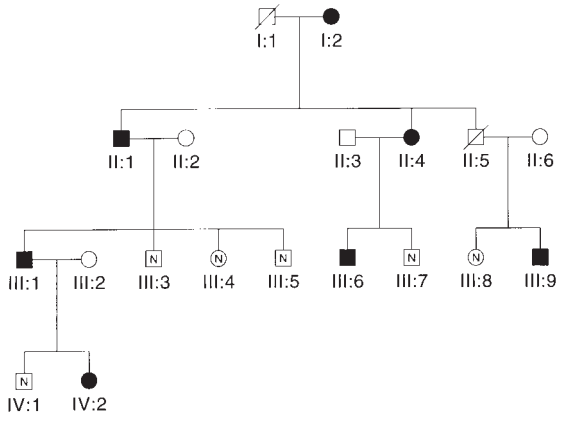


**Figure 1** Family demonstrating autosomal dominant ARA, from western Canada. Males are denoted by squares, and females are denoted by circles; unblackened symbols denote individuals who were not examined, blackened symbols denote individuals who were examined and found to be affected with ARA, and a symbols containing an "N" denote individuals who were examined and found to be unaffected. A diagonal line through a symbol indicates that the individual is deceased. Individual II:5 is deceased and was not examined but is presumed to have been affected, because he has an affected son (III:9) and ARA has not been reported with nonpenetrance. Linkage analysis was conducted, however, under the conservative assumption of 95% penetrance.

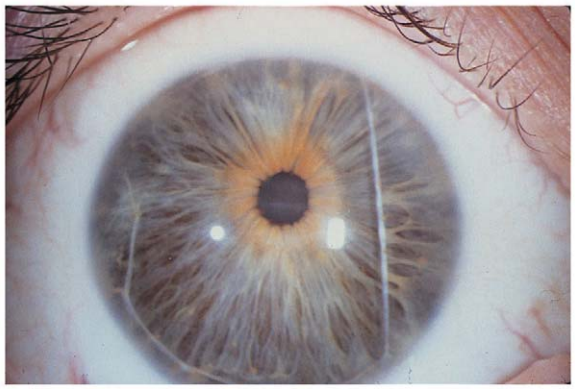

**Figure 2** Photograph of the right eye of affected member III:1 of the ARA pedigree. The photograph shows a prominent, centrally displaced Schwalbe's line that is detached from the cornea and crosses the anterior chamber (*white line across iris*), in addition to marked iris stromal hypoplasia and a superiorly displaced pupil.

markers in the region (table 1), by use of the criterion of a LOD score $\leq -2$ as evidence of exclusion of linkage (Morton 1955). For ARA, significant linkage was demonstrated to the IGDA critical region at 6p25. A maximum LOD score ($Z_{max}$) of 3.31 was obtained with marker D6S344 at a maximum recombination fraction ($\theta_{max}$) of .00 (table 1), indicating that ARA is significantly linked to markers on the distal short arm of chromosome 6 (6p25). These results are consistent with the likely hypothesis that ARA and IGDA are allelic; however, it remains a possibility that two or more genes involved in the formation of the anterior segment of the eye are located at 6p25 and that mutations of different genes underlie the related ARA and IGDA phenotypes. A key recombinant in this ARA pedigree (fig. 1, individual III:1) reduces the ARA critical region to a 6.4-cM interval between markers D6S1600 and D6S1617 (fig. 3).

These results represent the first localization of a locus for ARA, distinct from the localizations of ARS at 4q25 and 13q14. Our results are consistent with the observations of genetic heterogeneity among the four iris-hypoplasia phenotypes (Legius et al. 1994; Mears et al. 1996). In addition to ARA/IGDA, individuals with 6p deletions have also been identified who have aniridia (Levin et al. 1986) and Peters anomaly (Reid et al. 1983). We suggest that this region at the distal end of the short arm of chromosome 6 contains a locus or loci of importance for development of the anterior segment of the eye. Efforts are currently underway to reduce the 6.4-cM minimum critical region(s) for ARA and IGDA prior to conducting gene-isolation experiments to identify and clone the gene(s) responsible for anterior-segment phenotypes.

ARA maps to 6p25 and thus colocalizes with IGDA. Interestingly, the two iris-hypoplasia anomalies (ARA and IGDA) in which the phenotype is confined to the anterior segment of the eye both map to 6p25. The two related syndromes (ARS and IGDS) that present with the typical nonocular features both map to 4q25, and the ARS with sensory hearing loss in addition to the typical ARS features maps to 13q14. The clinical diagnostic criteria currently used to distinguish Axenfeld-Rieger and iridogoniodysgenesis eye malformations apparently do not reflect the genetic basis of the disorders. Instead, our results suggest that the presence or absence of nonocular features appears to be a fundamental diagnostic criterion for determining which genetic locus underlies these iris-hypoplasia phenotypes.

ARA, IGDA, aniridia, and Peter anomaly all appear to fall into an overlapping spectrum of anterior-segment disorders. Genes responsible for anterior-segment malformations have now been mapped to a variety of chromosomes, including 4, 6, 11, and 13 (Jordan et al. 1992;

**Table 1**

**Linkage to 4q25 and 6p25**

| | LOD SCORE AT RECOMBINATION FRACTION OF | | | | | | |
|---|---|---|---|---|---|---|---|
| LOCUS | .00 | .05 | .10 | .20 | .40 | $Z_{MAX}$ | $\theta_{MAX}$ |
| ARA vs. 4q25 loci: | | | | | | | |
| D4S3256 | −7.68 | −2.93 | −2.07 | −1.12 | −.22 | ... | ... |
| D4S2623 | −8.48 | −1.70 | −1.03 | −.44 | −.05 | ... | ... |
| ARA vs. 6p25 loci: | | | | | | | |
| D6S1600 | 1.51 | 1.39 | 1.25 | .96 | .32 | 1.51 | .00 |
| D6S942 | 2.60 | 2.35 | 2.08 | 1.51 | .34 | 2.60 | .00 |
| D6S344 | 3.31 | 3.03 | 2.74 | 2.13 | .74 | 3.31 | .00 |
| D6S967 | 2.88 | 2.62 | 2.35 | 1.76 | .49 | 2.88 | .00 |
| D6S1617 | −7.68 | .57 | .72 | .69 | .29 | .74 | .13 |
| D6S1713 | −5.59 | 1.38 | 1.48 | 1.34 | .57 | 1.48 | .10 |
| D6S477 | −6.45 | 1.05 | 1.20 | 1.14 | .46 | 1.22 | .13 |

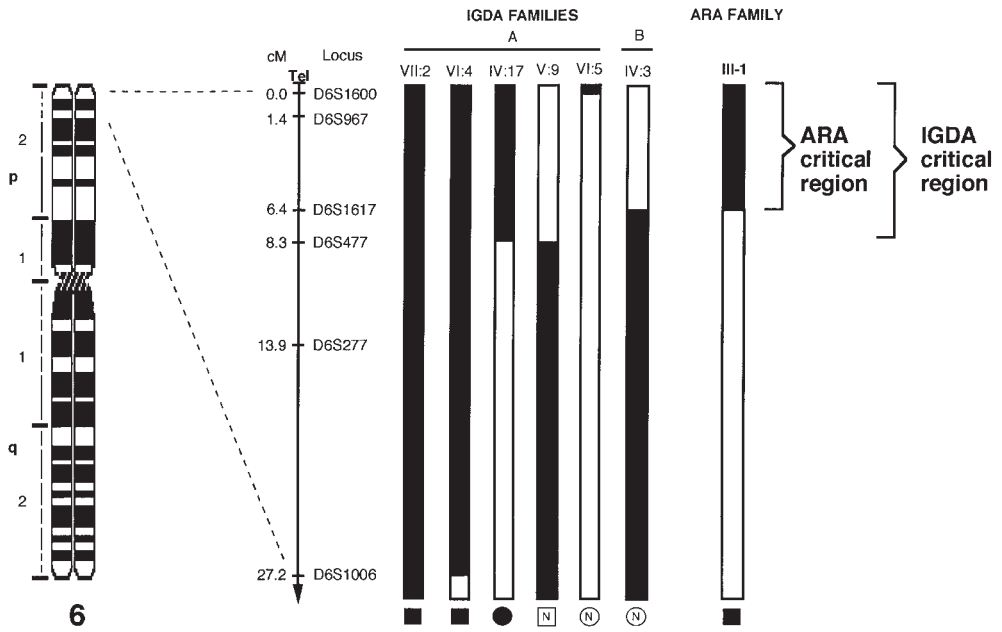


**Figure 3** Schematic presentation of chromosome 6, modified from the study by Mears et al. (1996), illustrating the location of the 6.4-cM ARA/IGDA critical interval. Cumulative genetic distances (in cM; obtained from Genome Database) from the telomere are indicated to the left of the idiogram. Key individuals from the study by Mears et al. (1996) (IGDA families A and B) and this study are indicated at the top, and disease status is indicated at the bottom. Blackened rectangles denote the region cosegregating with the disease in the respective families.

Murray et al. 1992; Mears et al. 1996; Phillips et al. 1996). The future cloning and mutational characterization of these genes will, it is hoped, elucidate the molecular mechanisms underlying anterior-chamber dysgenesis and lead to a better understanding of the clinical consequences of mutations of these loci.

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## The Genetic Clock and the Age of the Founder Effect in Growing Populations: A Lesson from French Canadians and Ashkenazim

*To the Editor:*

Use of the genetic clock with molecular data allows analysis of the occurrence of genetic events in the context of population histories. These analyses suggest that the majority of disease mutations present at variable frequencies among human populations have been spread by neutral mechanisms related to migration and demographic expansion. In human genetics, "founder effect" refers to the presence of genetic disorders that are either endemic to an isolated population or very rare elsewhere (Diamond and Rotter 1987); it is observed in small human isolates such as Tristan da Cunha (Roberts 1968) and in populations as large as that of Europe (Kerem et al. 1989). New reports rekindle interest in the origin of founder effects: Do they involve neutral mechanisms (migration and drift)? Are they due to a selection in response to the environmental challenge or to other causes? Molecular approaches provide new insights into the underlying mechanisms. Here we discuss the use of linkage-disequilibrium data to estimate the age of founder effects in Ashkenazi Jews from eastern Europe (Motulsky 1995) and in French Canadians from the Charlevoix-Saguenay region (De Braekeleer 1991), populations in which a number of rare genetic disorders are found at particularly elevated frequencies. When their time of appearance is known, founder effects can be better understood in the context of the social and demographic history of the populations (Roberts 1968; Motulsky 1995; Risch et al. 1995*a,* 1995*b;* Zoosman-Diskin 1995; Labuda et al. 1996).

A founder chromosome carrying a new disease allele is introduced into a population by migration or by a de novo mutation. The mutation is then in extreme linkage disequilibrium with the adjacent polymorphisms. A set of alleles at these polymorphic sites represents the founder haplotype, a "genetic signature" of the founder chromosome. Because of recombinations, a fraction of the carrier chromosomes with the founder haplotype decrease over the time (Jennings 1917; Robbins 1917). If polymorphic loci in the haplotype recombine at a rate of $\theta$/generation, then a proportion $(1 - \theta)$ will cosegregate; after $g$ generations the expected proportion of the nonrecombined carrier chromosomes is $P = (1 - \theta)^g$. At a small $\theta$, $(1 - \theta)^g \approx e^{-\theta g}$, and

$$\ln P = -\theta g \, . \qquad (1)$$

In this equation, relating time, recombination rate, and the divergence ($\ln P$) of haplotypes, we recognize the *genetic clock,* by analogy to the molecular clock (Zuckerkandl and Pauling 1965) that relates time, mutation rate, and the divergence of genes.

In a recent study of idiopathic torsion dystonia (ITD), an autosomal dominant disease, equation (1) was applied to estimate the age of the founder effect in Ashkenazi Jews from eastern Europe (Risch et al. 1995*b*). Strong linkage disequilibrium over a considerable genetic distance around the ITD locus indicated a founder effect whose origin was estimated by the authors as being at the middle of the 17th century (time range 1400–1750). At that time however, the Jewish population of eastern Europe was already reaching hundreds of thousands, and numbered $\geqslant$10,000 individuals in 1400 (Barnavi 1992; Beinart 1992; Motulsky 1995; Risch et al. 1995*a,* 1995*b;* Zoosman-Diskin 1995). This would put the initial ITD mutation frequency at $10^{-4}$–$10^{-5}$, too low to explain, on the ground of demographic growth alone, its current frequency of $2–6 \times 10^{-3}$. To resolve the discrepancy between the demographic and the genetic data in the case of ITD, social selection was proposed, whereby the present-day Ashkenazim descended from a smaller, wealthier fraction of the original population, a fraction with the higher survival rate (Motulsky 1995; Risch et al. 1995*b*).

Social selection could have influenced the genetic profile of the present-day Ashkenazi population and seems to provide a good collective explanation for an elevated frequency of a number of unrelated recessive disorders