Chromosomal Studies in Choroideremia

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Since the first reports of choroideremia in 1871,1 most authors have concerned themselves with elucidating the pattern of inheritance of this disorder.2 They have found the disease to be a distinct genetic entity which is a sex-linked recessive and to date has been reported in over 100 individuals.3 While these careful studies have elucidated the pattern of inheritance, relatively few investigators have dealt with chromosomal analysis and genetic mapping of the disease.4

One investigator correlated the presence and absence of the Xg blood group in one family and concluded that since recombinations did occur, the locus for the defect in choroideremia must be relatively distant from that of the Xg blood group.5

Recombination was noted between the locus for protanopia and choroideremia in the series of Kurstjens, although he did not calculate the frequency of crossing over.6

According to the results of a pedigree analysis of over 600 people, choroideremia is rarely seen in association with other defects.1 If this is true, it would be surprising to find abnormal karyotypes in either affected individuals or carriers. In two prior studies, it was reported that the karyotypes were normal in two carriers and one affected individual.4,6 However, other investigators have reported the association of choroideremia with a number of other findings which include other tapetoretinal degenerations, central choroidal sclerosis, familial dwarfishism, myopia, posterior polar cataracts, mental deficiency, nystagmus, verruciform-like acrokeratosis, skeletal and dental deformations, and general retardation of growth.3

Since only three individuals' karyotypes have been reported, with less refined techniques that are now available, it seemed reasonable to us to do karyotype analysis of affected individuals and carriers by the most modern methods. Fluorescent labeling of chromosomes makes it possible to demonstrate that, despite the presence of an apparently normal karyotype, as indicated by measurement of chromosome length and centromeric index, there may be present abnormalities such as equal reciprocal translocations, paracentric inversions, and small deletions and additions.5,8 In addition, in such subjects, the abnormal chromosomes can now be identified with a degree of certainty not possible when utilizing ordinary Giemsa staining.9

MATERIALS AND METHODS

Ten milliliters of venous blood was withdrawn from each patient with a heparinized syringe. The blood was allowed to sediment at room temperature and the top 1 ml of supernatant leukocyte suspension
Fig. 1: Pedigree showing three and possibly four generations of choroideremia examined in this study. Subject No. 23 was affected. His mother (subject No. 12) and two daughters (subjects No. 27 and 28) were carriers. His maternal grandfather (subject No. 5) may have been affected.

removed and placed in a culture flask together with 7.0 ml S-MEM with HEPES buffer, 0.2 ml Pen-Strep, 0.3 ml phytohemagglutinin, and 1.0 ml fetal calf serum. The pH was adjusted to 7.2 and the culture incubated at 37°C for 72 hours. One half hour before harvesting, 0.1 ml Velban (Grand Island) was added. Cultures were harvested by means of hypotonic treatment with 0.7 per cent sodium citrate, fixation with 3:1 methanol-acetic acid and subsequent exposure to 45 per cent acetic acid. The final suspension was placed on clean cold slides, and flame-dried. Some slides were stained with Giemsa and others were prepared for fluorescent microscopy in the following manner: three grains of quinacrine hydrochloride were dissolved in 40 ml distilled water; slides were stained for six minutes at room temperature, washed in running tap water for three minutes, rinsed first in distilled water and then in Macilvaine's buffer (pH:5.5) for 30 seconds, and mounted in 60 per cent sucrose solution in buffer.10 * Well-spread metaphase chromosomes were photographed with a Zeiss Photomicroscope II, equipped with an HBO 200 W mercury lamp, BG12 excitor filter, K530 barrier filter, and a 100X objective with iris diaphragm. Pan-Atomic X film was used and prints were made on Ilford R4-1P paper. Karyotypes were performed on two generations of a family, including one affected male, one normal male, one normal female and two carrier females.

RESULTS

Only the clinical summary of the three carriers and one affected individual will be presented since the ophthalmological examination of the son (subject No. 26) and mother (subject No. 12) were entirely within normal limits.

Clinical Findings

Subject No. 23, 32 years old, had first noted symptoms of night blindness before age 7 and by age 11 was declared legally blind. A physical examination revealed corrected visual acuity: OD, count fingers; OS, 20/50. Refractive error was −8.00. Slit lamp examination revealed band keratopathy OD from an old corneal ulcer. OS was within normal limits. The lenses were clear and there was nonspecific vitreous debris. The fundus was similar OU with marked choroid and retinal atrophy with bare sclerae throughout the posterior pole except for a small amount of retina and choroid noted at the macula. There

*Dev VG: personal communication.
was a searching, pendular nystagmus. Visual fields were contracted to 5 degrees with a 60/1000 white test object. The electroretinogram was flat, and the patient failed all color plates of the AO-HRR series.

Subject No. 27, 7 years old, denied night blindness but stated that she had difficulty seeing the blackboard in school. Visual acuity was 20/20 OU. Cycloplegic refraction revealed a small amount of hyperopic astigmatism. Pupillary responses were brisk. Findings from an electroretinogram and AO-HRR color plates were normal. Examination of the visual fields revealed hysterical tunnel vision. There was no nystagmus. Indirect ophthalmoscopy revealed fine peripheral pigmentation and mottling in the macula.

Subject No. 12, 53 years old, denied any symptoms. Vision was 20/20 OU with a small amount of hyperopic astigmatism. Pupillary responses were brisk; findings from examination of the visual fields and AO-HRR color plates were normal. There was no nystagmus. Funduscopcy revealed many areas of deep choroidal pigmentation with choroidal and retinal atrophy.

Subject No. 28, nine years old, was asymptomatic. Her visual acuity was 20/20 OU without correction. Funduscopcy revealed many areas of choroidal and retinal atrophy with some fine pigment mottling at the macula. Findings from visual fields examination, an electroretinogram, and AO-HRR color plates were all normal.

**Pedigree Analysis**

The family history involved a kindred of 29 persons, of whom 15 were male and 14 female. The affected male (subject No. 23) had one normal son (subject No. 26) and two carrier daughters (subjects No. 27 and 28). The affected male's mother (subject No. 12) was also a carrier and his maternal grandfather (deceased) was described as having an eye disease and being blind. There was no other eye disease known in the kindred. Thus, the inheritance of choroideremia in this pedigree is a classical one of a sex-linked recessive
gene, with a carrier female, affected male, carrier female pattern in at least three and probably four successive generations.

**Karyotype Analysis**

Karyotypes of the two carriers, affected male, unaffected mother, and unaffected son were 46XX for the females and 46XY for the males. All of the karyotypes were within the normal morphological range (Fig. 2).

Fluorescent staining of the chromosomes in the same subjects revealed a normal banding pattern of the chromosomes (Fig. 3). There was no evidence of translocations, additions, or deletions of the genetic material totaling more than five per cent. Furthermore, the banding patterns did not reveal any indications of inversions or duplications.

**COMMENT**

The present analysis of three generations of a family with choroideremia confirms many of

the findings previously reported for this disease. The pattern of inheritance is clearly sex-linked recessive. Carrier females pass the disease to affected males who then transmit the trait to their female children. This mode of inheritance has been carefully studied by McCulloch and McCulloch.²

The affected males, such as subject No. 23, demonstrate striking funduscopic changes, may have nystagmus, and have high myopia, abnormal visual fields, loss of all color discrimination, and flat electroretinograms.¹

Carrier females, on the other hand, usually have good visual acuity, normal color vision, and normal visual fields, usually do not have myopia, and have normal electroretinograms.¹ Until recently, it has been emphasized that the carrier females may have abnormal findings on fundus examination but normal visual function.¹ However, case reports of severely affected carriers have been published in recent years and it has been shown that
females may rarely have abnormal vision, visual fields, color plates and electroretinograms. It is recognized that the degree to which function is impaired in females is unrelated to the appearance of the fundus. The finding of affected females has been explained by Krill using the Lyon hypothesis which states that, in female mammals, one X-chromosome becomes genetically inactivated some time during development, and it is a random matter whether the inactive chromosome in a particular cell is of paternal or maternal origin. Furthermore, once the "time of decision" has passed, all descendants of a particular cell have the same X-chromosome inactivated. If a female is a carrier with one of her X-chromosomes carrying the defect, one would expect that 50 per cent of the cells would possess normal function and 50 per cent abnormal function. However, since random inactivation does occur in the early embryo, it can be expected that the percentage of cells carrying the normal X-chromosome will vary according to a Gaussian curve and therefore, in a few subjects, most of the normal chromosomes will be inactivated and the majority of the functioning X-chromosomes will be abnormal. In these individuals, the disease would be evident. Because of psychological factors involved, we do not know the true extent of the visual defect in our patient (subject No. 27). Findings from her electroretinogram, color plates, and pupillary responses were normal, and she appeared to get around quite well. The true extent of her defect, if any, is unknown at present.

Karyotype analysis of diseases involving the X-chromosomes can be fruitful because in the carriers one of the two X-chromosomes in each cell is genetically abnormal. If the two X-chromosomes, as in this individual, are morphologically identical, then the total amount of genetic material is unchanged. Various chromosome abnormalities can be masked when the criteria used are merely chromosome length and centromere position. The total genetic material appears normal but its distribution is not. It is these cases that are aided in detection by the fluorescent banding technique. In this study of choroideremia, the fluorescent banding pattern was also normal. Thus the genetic defect for choroideremia must be a small one, most probably a point or gene mutation or perhaps a small deletion or chromosomal rearrangement involving less than five per cent of the genetic material of the X-chromosome.

SUMMARY

Karyotype analysis was performed on two generations of a family affected with choroideremia. Karyotypes were normal in both carriers and affected individuals. By use of the fluorescent banding technique it was further demonstrated that the defect of choroideremia involves changes in less than five per cent of the genetic material of the X-chromosome. The combination of these techniques indicates that the defect in choroideremia is not a translocation, deletion, large inversion, or reduplication. The defect of choroideremia probably represents a point mutation on the X-chromosome.

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REFERENCES