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High Incidence of Autosomal Recessive Nonsyndromal Congenital Retinal Nonattachment (NCRNA) in an Iranian Founding Population

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In an isolated and founding Iranian population the prevalence of congenital total blindness is 1.1%. Clinical findings such as lack of perception of light, massive retrolental mass, shallow anterior chamber and nystagmus, in otherwise normal individuals, correspond to nonsyndromal congenital retinal nonattachment. To determine the inheritance of this disease we constructed an extensive nine-generation pedigree of the affected kindred living in the Iranian founding population. The pedigree, which includes 42 patients from 25 sibships, clearly suggests autosomal recessive inheritance. To verify the inheritance, we compared the average coefficient of inbreeding (F) of the affected sibships with that of the control sibships, calculated the patients' sex ratio, and also compared the observed relative frequency of the disease with its expected relative frequencies for different modes of inheritance. The patients' average F value is significantly greater than that of the controls ($P < 0.001$). The sex ratio of the patients is close to unity and the observed relative frequency of the disease is close to that of an autosomal recessive trait. All these findings strongly support autosomal recessive transmission of this disease. Am. J. Med. Genet. 78:226–232, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: nonsyndromal congenital retinal nonattachment; detachment of retina; congenital blindness; autosomal recessive; Iran; consanguinity; coefficient of inbreeding; founding population; founder effect; genetic drift; inbred population; isolated population; relative frequency

INTRODUCTION

Hereditary retinal nonattachment can be divided into syndromal and nonsyndromal types. In the former, the defect is part of a syndrome and is accompanied by other abnormalities, while in the later the affected individuals are otherwise normal [Warburg, 1979]. In the literature, only a few small pedigrees convincingly segregating autosomal recessive or dominant types for each of these categories have been reported [Weve, 1938; Phillips et al., 1973; Warburg, 1976, 1979; McKusick, 1994].

We have identified an Iranian founding population with 32 living individuals affected with a form of nonsyndromal congenital retinal nonattachment (NCRNA). Suspecting that a mutant gene was responsible for the disease in this population, we undertook the present study, with the goals of determining the mode of inheritance of the disease and providing genetic counseling to the affected families.

MATERIALS AND METHODS

Study Population

The study population for pedigree construction was from four neighboring villages and includes 43 congenitally blind individuals. Thirty of them were living

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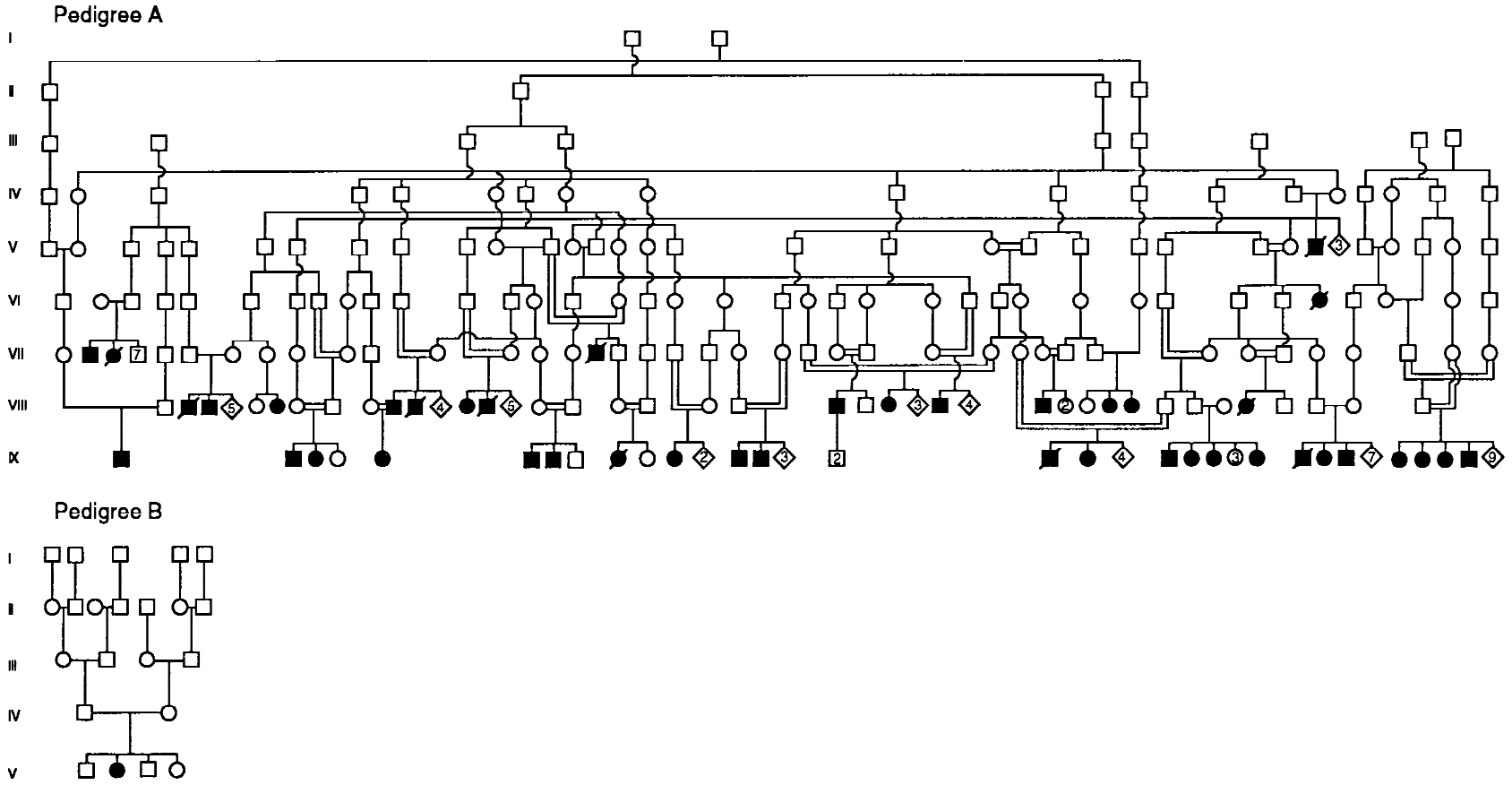

Fig. 1. Two pedigrees segregating autosomal recessive nonsyndromal congenital retinal nonattachment. Both pedigrees are part of the same isolated and founding Iranian population. Twenty-six of the living 32 affected individuals from both pedigrees (not including individuals VII-2, VIII-6, IX-29, IX-30, IX-31, and IX-32 from pedigree A) have been examined and all have shown NCRNA ocular abnormalities. Squares represent males, circles represent females, and diamonds represent individuals without regard to their sex. Filled symbols represent affected individuals, double-line marriages indicate consanguineous unions, and a line through the symbols represent deceased individuals.

at the time of this study, 11 in different generations had died of other causes, and 2 were born after our study was done and were not included in the statistical studies (Fig. 1, pedigree A, individuals VIII-6 and IX-24).

The inhabitants of these villages are descendants of a small founding population of Kurds who were moved from the western Iranian province of Kurdistan to the eastern province of Khorasan some centuries ago. The religion of the study population is Islam. The present population, like the founding population, speaks Kurdish, which is different from Persian, the language of the surrounding population. Also, being too distant from Kurdistan, the study population has lost contact with the mother Kurdish-speaking population. Therefore, because of a language barrier, this founding population was also genetically isolated from the neighboring population. This situation confined the disease allele to this founding population until recent years. However, due to availability of schools in Iranian villages for more than three decades, the inhabitants of these villages have increasingly learned to speak Persian, thereby weakening the language barrier. A factor that in the last few years has strongly encouraged the inhabitants of these villages to favor outbreeding and intercommunity marriages has been their realization (through our local informal classes for the high-risk families) that the offspring of native couples, especially those of related parents, have a greater chance of acquiring this devastating disease.

After having been informed about the etiology of the disease, and especially because of the devastating effects of the disease on the life and well being of the patients and their families, all the affected families and many of the unaffected couples of this traditional farming community now appreciate the importance of genetic counseling and predictive tests, including carrier detection and prenatal diagnosis. While arranged marriages are still common in this community, to reduce the chance of having blind grandchildren the parents of young adults have increasingly become more interested in intercommunity arranged marriages.

Pedigree Construction

Pedigrees for 43 affected individuals from 26 families were constructed in group discussion sessions held in the local health center. In each session, in addition to the affected families, the available grandparents and great-grandparents of the patients also participated. Moreover, in all these sessions four to six elders from the main affected kinship, who were very knowledgeable about the history and genealogy of their community, also participated.

Frequency and Relative Frequencies of the Disease

To calculate the frequency and relative frequencies of the disease the population of the central village, for which we had reliable census information, was considered. This population, which in this article will be referred to as the "main population," included 1,857 normal and 21 affected individuals at the time of this study. The frequency of the disease in the main population (P), was calculated by dividing the number of living patients in the main population (21) by its population size (1,878).

To verify the mode of inheritance of NCRNA, we compared the observed relative frequency of the disease with its expected relative frequencies for different modes of inheritance [Emery, 1986]. The observed relative frequency of the disease was calculated by dividing the frequency of the disease in the subpopulation of living affected sibships (S) by the frequency of the disease in the main population (P). The expected relative frequencies of the disease, if it was transmitted in autosomal dominant, autosomal recessive, or polygenic modes of inheritance, are 1/2P, 1/4P or $1/\sqrt{P}$, respectively [Emery, 1986].

Clinical Examination

Twenty two affected individuals from the main population and four affected individuals from two of the adjacent villages were examined. The examinations were done by direct and indirect ophthalmoscopy without anesthesia. At the time of clinical examination, only one of the patients was of neonatal age and the age of the other 25 participants ranged from 5 months to 28 years. The youngest patient was first examined when 3 days old and had regular monthly examinations until the age of 4 months, when, as with the older patients, further direct examination of her eyes was no longer possible because of massive retrolental mass.

Calculation of the Average Inbreeding Coefficient (F)

The relationships between parents of the 25 affected sibships (the affected group) and also the relationship between parents of the unaffected sibships from the main population (the control group) were determined by using the information collected on a previously prepared questionnaire. The parents of individual VII-6, who was born after this study was carried out, are not included in the affected group. The control group included 311 available couples from the main population who, along with the affected group, comprised more than 95% of the couples of the main population. The questionnaires were completed by each person before interviewing the affected group for pedigree construction. Each questionnaire elicited information about the history of congenital blindness in the sibship and relatives, the age of the children and their parents, the relationship between the couples, and, if they were related, a pedigree showing all their relationships and their common ancestor(s).

The inbreeding coefficient (F_i) of each affected and also of each control sibship was calculated by path analysis [Emery, 1986] as follows: The average inbreeding coefficient (F) for each group is equal to

$$\Sigma p_i F_i$$

where p_i is the proportion of marriages with inbreeding coefficient F_i [Emery, 1986]. Frequency estimation for the disease allele and normal carriers was calculated in

an inbred population where the incidence of a certain autosomal recessive disease is equal to

$$Fq + q^2(1\text{-}F)$$

where F is the average coefficient of inbreeding and q is the frequency of the disease allele [Emery, 1986]. The proportion of heterozygotes for an autosomal recessive disease in an inbred population is equal to $(1–F)H_O$, where F is the average of inbreeding coefficient for the inbred population and H_O is the proportion of heterozygotes if the population was not inbred [Emery, 1986]. In this study, these two relationships were used to estimate the frequencies of the NCRNA allele and normal carriers in the inbred population.

RESULTS

Clinical Findings

Clinical examination of the patients older than 5 months showed bilateral total insensitivity to light, shallow anterior chambers (ACs), massive retrolental masses (which made further direct examination impossible), microphthalmia, and nystagmus in otherwise normal individuals. In some patients corneal opacities were also seen. In all of the patients the presence of retinal tissue could be verified by ultrasound examination. The youngest patient was a 3-day-old affected girl, born at term with normal birthweight and with no prenatal, perinatal, or neonatal problems (no oxygen was given). This individual was the only patient in whom detailed direct retinal examination was possible. From her eye examination without anesthesia, the following findings were noticeable: no perception of light, no external ocular anomaly, clear cornea, round and regular pupils, retinal nonattachment, clear lenses except for some arteries emerging from the posterior pole and extending to the anterior surface, substantial vitreous hemorrhage in both eyes, and normal ACs.

Subsequent follow-ups of this patient showed a progressive worsening of ocular abnormalities. At one month, lens opacities had become apparent, and ACs had become shallower. At two months, posterior synechiae (PS) had formed, and the pupils had become irregular. At three months more retinal detachment was seen, PS was apparent at 360 degrees, ACs were very shallow, and retrolental masses (and vitreous organization) were prominent. At four months, due to massive retrolental masses, funduscopy was impossible. The presence of vitreous and retinal tissues could only be confirmed by ultrasound examination. At this age nystagmus and phthisis bulbi had become apparent. No sign of corneal edema due to intraocular pressure could be seen at any stage of the disease.

Since many of the manifestations of this disease are similar to those seen in retinopathy of prematurity, we also reviewed the neonatal history of most of the NCRNA patients of the study population and found no history of oxygen therapy, low birthweight, or prematurity.

Pedigrees

All 43 affected NCRNA individuals in the study population aggregate in two pedigrees: an extensive nine-generation pedigree, including 42 patients from 25 affected and related families in five generations, and a small pedigree with only one affected individual (Fig. 1). Most of the affected individuals are offspring of known consanguineous marriages and the large pedigree clearly shows the characteristics of an autosomal recessive mode of transmission for the disease.

Average Inbreeding Coefficient (F)

Sixty-eight percent of the couples with affected children and 39% of the couples of the control group had consanguineous marriages. The average F values for the affected group and the control group were 0.0162 and 0.0113, respectively, and their difference was significant ($P < 0.001$). The average inbreeding coefficient of the main population (F) was estimated to be 0.0112, which was not significantly different from the average F of the control group.

Frequency and Relative Frequencies of the Disease

In the main population, with 21 living congenitally blind individuals and 1,857 unaffected individuals, the frequency of NCRNA was calculated to be 1.1%. The observed relative frequency and expected relative frequencies of the disease in polygenic, autosomal recessive, and autosomal dominant modes of inheritance are shown in Table I.

Disease Allele Frequency and Number of Carriers in the Main Population

Since the incidence of the disease, 0.011, is equal to $Fq + q2(1–F)$, the frequency of the disease allele was estimated to be ~0.1. The proportion of heterozygotes if the main population had been a Mendelian population would have been $2pq = 0.18$. In this inbred population the proportion of heterozygotes for this autosomal recessive disease is estimated to be

$$0.9888 \times 0.18 = 0.1779$$

Hence, the number of carriers in this population was estimated to be 334.

Sex Ratio

Of the 43 NCRNA patients of the pedigree, 22 were male and 21 were female. The sex ratio is close to unity

TABLE I. Frequency and Relative Frequencies of NCRNA in an Iranian Founding Population

Frequency		Relative frequencies			
			Expected if inherited as		
Affected sibships (S)[a]	Main population (P)	Observed (S/P)	Polygenic ($1/\sqrt{P}$)	AR (1/4P)	AD (1/2P)
0.328	0.011	29.818	9.542	22.727	45.455

[a]S and P are the frequencies of the disease in the affected sibships and the main population respectively; S/P is the observed relative frequency of the disease in the main population; $1/\sqrt{P}$, 1/4P, and 1/2P are the expected relative frequencies of the disease in the main population if it were inherited as a polygenic, autosomal recessive (AR), or autosomal dominant (AD) trait, respectively.

and sex differences among the patients are statistically insignificant.

DISCUSSION

Prior to this study, only a few small pedigrees convincingly segregating NCRNA (also called monosyptomatic congenital retinal nonattachment) had been reported [Phillips et al., 1973; Warburg, 1976, 1979; Weve, 1938]. This isolated founding population, with a high incidence of NCRNA, has provided an exceptional opportunity to study some aspects of this rare disease. The high incidence of NCRNA in this population is not simply due to the high rate of inbreeding; it is also an outcome of the high frequency of the disease allele. The high frequency of the NCRNA allele in this founding population ($q = 0.1$) could have been the result of genetic drift at the time of the formation of this founding population by a number of related individuals a few centuries ago. It is also possible that the disease allele originated through a de novo mutation in one of the small number of founding individuals of this isolated population.

The founder effect in this genetically isolated population has resulted in the appearance of a very large kindred with many affected families segregating NCRNA. Our findings, which include horizontal transmission of the disease in the pedigree (characteristic to autosomal recessive inheritance), significantly greater average coefficient of inbreeding among the patients compared to their controls, sex ratio close to unity, and the closest agreement between the observed relative frequency of the disease with its expected relative frequency if it had an autosomal recessive mode of inheritance (Table I), strongly suggests that the disease is transmitted as an autosomal recessive trait. This population has also provided appropriate human resources to enable mapping and identification of the gene responsible for NCRNA. Chromosomal localization of the NCRNA locus would soon facilitate the development of a linkage-based predictive test for this disease in the study population, therefore making reliable genetic counseling and prevention of the disease based on carrier detection and prenatal diagnosis possible. Application of accurate predictive testing coupled with genetic counseling is required for successful containment of the disease allele and to prevent its spread into a larger portion of the Iranian population.

The clinical ocular findings in the otherwise normal individuals of the pedigree are in agreement with those reported for congenital retinal nonattachment [Phillips et al., 1973; Warburg, 1976, 1979; Weve, 1938]. However, definite and more detailed diagnosis of the disease in this population (which we have called NCRNA) awaits histological and developmental studies of a number of affected eyes, especially those of the affected fetuses in different developmental stages. These specimens only could become available after the availability of reliable prenatal tests for this disease to the affected population.

The finding that in the study population 32% of the obligate carriers for NCRNA are considered unrelated indicates that these and many other apparently unrelated individuals in the main population are in fact genetically related and have had the same common ancestor carrying a rare NCRNA allele. This fact has two different impacts on the accuracy of risk estimates for the disease in this population. Many individuals with remote consanguinity, some of whom are heterozygous for the very same disease allele, are considered "unrelated." This could make genetic counseling and risk estimates for this disease based on the limited knowledge of concerned couples about their consanguineous relationships very unreliable. On the other hand, since many generations have passed between the time the disease allele was introduced into this founding population and the present population, there could have been opportunities for many crossovers to occur in the vicinity of the disease gene. This in turn would make it possible to map or delimit the NCRNA gene to a short segment of the human genome, therefore making the development of a reliable linkage-based predictive testing for this disease possible.

Differential Diagnosis

Retinal detachment and retrolental masses, which are the main ocular findings in NCRNA, are also seen in a number of other eye diseases. However, the extent of these lesions, the degree of visual acuity, the mode of inheritance, and also the existence of other clinical findings in other diseases make the differential diagnosis of NCRNA from similar eye diseases possible.

Familial Exudative Vitreoretinopathy (FEVR)

This disease is usually observed as an autosomal dominant trait, but its X-linked and autosomal recessive forms have also been reported [Shastry, 1997]. While the mode of inheritance alone distinguishes autosomal recessive NCRNA from the autosomal dominant and the X-linked forms of FEVR, differentiation between autosomal recessive FEVR and autosomal recessive NCRNA is also possible from comparison of the severity of ocular findings and visual impairment in the affected individuals. In contrast to autosomal recessive FEVR, in which affected individuals enjoy some visual acuity [Shastry, 1997], NCRNA patients suffer from congenital bilateral lack of light perception. Moreover, while retinal detachment is the rule in all the NCRNA patients, it is only seen in some of the patients with FEVR [van Nouhuys, 1991]. However, in a few cases where due to extreme similarities one could not differentiate between these two conditions differential diagnosis of these ocular abnormalities awaits mapping or identification of the gene for at least one of these diseases.

Norrie's Disease

Norrie's disease is an X-linked ocular disease with bilateral congenital retinal nonattachment and retrolental masses [Anderson and Warburg, 1961; Warburg, 1975], which in some patients may be accompanied by hearing impairment and mental retardation [Warburg, 1975]. While differential diagnosis between NCRNA and Norrie's disease with hearing loss and/or mental retardation is easy on the basis of these clinical find-

ings, these diseases can also be distinguished on the basis of their modes of inheritance. NCRNA is not accompanied by hearing and mental deficiency or any other abnormality and is transmitted as an autosomal recessive trait.

Retinal Dysplasia

Retinal dysplasia, in its severe form, manifests bilateral retrolental masses associated with multiple systemic abnormalities [Hunter and Zimmerman, 1965], whereas in NCRNA no other abnormality is associated with bilateral retrolental masses. In mild forms of retinal dysplasia, the vision is severely impaired but still there are some degrees of visual acuity [Theodore and Ziporkes, 1940]. Therefore, insensitivity to light in NCRNA can help to differentiate this disease from mild forms of retinal dysplasia.

X-Linked Juvenile Retinoschisis

Usually the clinical findings in juvenile retinoschisis are distinctly different from those of NCRNA. For example, unlike NCRNA, the visual acuity in patients with juvenile retinoschisis is usually good in early childhood and average visual acuity in adult patients is reported to be 0.34 [Forsius et al., 1973]. Another difference that can help to differentiate NCRNA from X-linked juvenile retinoschisis is the fact that the latter is an X-linked recessive disease and is usually seen in males [Chew and Pinckers, 1983], while the former is an autosomal recessive disease and affects both sexes equally. Meanwhile, unlike NCRNA, in which all patients manifest retinal detachment, only a fraction of patients with X-linked juvenile retinoschisis manifest retinal detachment [Chew and Pinckers, 1983].

Incontinentia Pigmenti

This X-linked dominant disease is recognized by certain characteristic cutaneous lesions [Carney,1976]. In some incontinentia pigmenti patients severe retinal detachment is seen [Goldberg and Custis, 1993]. Absence of dermatological lesions in NCRNA and also the autosomal recessive inheritance of this disease make the differential diagnosis of NCRNA from incontinentia pigmenti with characteristic dermatological lesions and X-linked dominant inheritance very easy.

Persistent Hyperplastic Primary Vitreous

In this disease, ocular anomalies such as retinal detachment are not hereditary and are nearly always unilateral [Reese, 1955]. Moreover, in contrast to NCRNA patients, who have no perception of light, those suffering from persistent hyperplastic primary vitreous have substantial visual acuity [Pruett and Schepens, 1970]. Therefore, this disease is easily distinguished from NCRNA in which congenital insensitivity to light is seen in all affected.

Detachment of the Retina Due to Traumata

Traumatic retinal detachments are fairly common [Cheng-Jong et al., 1995]. Most traumatic retinal detachments are due to blunt trauma [Malbran et al., 1972]. A positive history of trauma in this condition helps to differentiate it from NCRNA, which has no association with trauma.

Retinopathy of Prematurity

Retinopathy of prematurity (retrolental fibroplasia) is an important cause of blindness in infants [deJuan and Machemer, 1987]. This condition is more often seen in premature infants with low birthweight who have received intensive oxygen therapy [Kinsey, 1956]. Differential diagnosis of this condition from NCRNA is easy. In contrast to NCRNA, which occurs in full-term infants who have not had any oxygen supplement, oxygen has always been administered to patients affected with retinopathy of prematurity.

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