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Congenital Aniridia*

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INTRODUCTION

CONGENITAL ANIRIDIA is a condition characterized by bilateral absence of the iris. It is accompanied by ocular nystagmus and deficient macular vision, and frequently complicated by cataracts and glaucoma, leading to very marked impairment of sight at an early age. The condition has been reported by many investigators to follow the pattern of autosomal dominant inheritance (reviews in Bell, 1932, Møllenbach, 1947, and Sorsby, 1951). Since the phenotype is recognizable at birth and presents no diagnostic difficulties, it lends itself well to studies concerned with such aspects of human genetics as the estimation of mutation rates and selection coefficients.

The present study was undertaken as one of a series designed to determine the impact and population dynamics of selected inherited diseases in the state of Michigan. In addition to providing data concerning the frequency and relative fertility of the aniridia phenotype and an estimate of the rate with which this phenotype appears as a result of mutation, we shall present evidence that in families in which the aniridia gene is segregating, there is a significant departure from the expected 1:1 ratio.

THE COLLECTION OF MATERIAL

In order to determine the frequency of aniridia, a roster of affected individuals residing in the lower peninsula of the state of Michigan was compiled. (Hereinafter, whenever "Michigan" is used it is understood to refer to the lower peninsula only.) The aniridia cases in the present study were ascertained from the following sources, given in approximate chronological order as the study progressed:

1. Referrals to the Department of Human Genetics from the Department of Ophthalmology at the University of Michigan Medical Center, from June, 1942 to January, 1960.
2. Survey of University of Michigan Hospital records with a diagnosis of congenital aniridia for patients seen between July, 1928 and March, 1956.
3. Survey of approximately 3,600 diagnostic cards at the Michigan School for the Blind, Lansing, of all children enrolled between 1925 and 1956.

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4. Survey of approximately 3,000 current and old records filed in the State Department of Social Welfare, Division of Services for the Blind, Lansing.
5. Survey of approximately 7,000 current and old records filed in the State Department of Social Welfare, Aid to the Blind, Lansing. (Although a few names were duplicated in the Aid to the Blind and Division of Services for the Blind files, they were treated as separate ascertainments since the amount of overlapping between these two sources was small.)
6. Survey of 1,805 current and old files in the Sight Saving and Braille classes of the Department of Special Education, Detroit Public Schools.
7. Follow-up of 11 records of children reported to have congenital defects of the iris and listed with the Vision Section, Division of Maternal and Child Health, Michigan Department of Health, Lansing.
8. Survey of 209 cases of congenital eye defects and diseases of the orbit listed in the files of the Office of Vocational Rehabilitation, State Department of Public Instruction, Lansing.
9. Replies from inquiries to Jackson, Dearborn, Kalamazoo, and Grand Rapids Boards of Education, Sight Saving and Braille classes, regarding possible aniridic children listed in their diagnostic files.
10. Correspondence with the National Society for the Prevention of Blindness, New York City. (The reply stated that the only Michigan cases in the Society records were referred through the State School for the Blind and the special sight saving classes which we had already contacted.)
11. Letters to records librarians of 165 hospitals in Michigan, requesting names of individuals listed as in-patients or out-patients, with a diagnosis of congenital aniridia. (One hundred and fifty librarians replied to our letter, a response rate of 0.91.)
12. Correspondence with 122 board-certified ophthalmologists in Michigan, requesting names of aniridia cases seen in their private practices, of whom 61 (50 per cent) replied to our query. (We did not send letters to 41 ophthalmologists to whom we had previously written concerning patients in our study, thus decreasing our chances for reascertainment.)
13. Information volunteered by two individuals who were aware of our study but were not contacted by us.

Table 1 summarizes the number of cases obtained from each of the sources listed above. Through these various channels, 109 aniridics became known to us through 171 "separate" ascertainments. Subsequent family studies led to information on 67 additional affected individuals, giving a total number of 176 cases, the material on which this study is based. The sex ratio of the entire group was 82 males to 94 females ($\chi^2 = 0.82$; $.40 > P > .30$).

The treatment of certain types of population data is greatly simplified if, where some affected individuals are repeatedly ascertained, each ascertainment is independent of the other. Unfortunately, in studies such as the present, where the need is to build up as complete a roster as possible, repeated ascertainments are seldom entirely independent of one another. We have used the

TABLE 1. NUMBER OF PROBANDS AND REASCERTAINMENTS OBTAINED FROM VARIOUS SOURCES

Source of ascertainment	Probands	Reascertainments	Total ascertainments
1. Ophthalmology Department, University Hospital	20	2	22
2. University Hospital Records Survey	15	12	27
3. Michigan School for the Blind	18	8	26
4. Division of Services for the Blind	8	10	18
5. Aid to the Blind	14	8	22
6. Detroit Sight Saving and Braille Classes	3	1	4
7. Vision Section, Michigan Department of Health	2	3	5
8. Office of Vocational Rehabilitation	0	0	0
9. Sight Saving Classes of four large cities	4	0	4
10. National Society for the Prevention of Blindness	0	0	0
11. Letters to hospital records librarians	14	11	25
12. Letters to ophthalmologists	9	7	16
13. Non-professional individuals aware of the study	2	0	2
Totals	109	62	171

number of reascertainments at each source as a rough guide of the progress of the study and the thoroughness of coverage of the survey. Each time a name appeared from a different source it was listed as a separate ascertainment although we are quite aware that sources (1) and (2) are not independent, and sources (11) and (12) are also overlapping. In fact, it is possible to interrelate most of the above sources with each other. Certainly no single source would be expected to reveal all cases of aniridia in the state, and any given aniridic would not have an equal probability of being ascertained from all given sources. As an example, one would not expect to find a 65-year-old aniridic's name in the School for the Blind files (source 3) nor would one expect to locate a 5-year-old individual's record in the files of the Aid to the Blind Division (source 5) since blind pensions are not available to children. It may be theoretically possible to assign each aniridic individual in the state of Michigan a separate probability value for being located at each of the 13 sources, based on sex, age, severity of disease, socio-economic status, place of residence, etc., to arrive at an overall estimate of the "probability of ascertainment." In practice we feel that such calculations are not justified because they are based on arbitrary assumptions.

Several definitions of terms used in this paper are necessary at this juncture. A "familial" case refers to an individual with aniridia who has an affected parent, while an "isolated" aniridic is one whose parents have normal eyes. An "isolated" case, then, may be the starting point for a "familial kindred" and if the isolated aniridic has affected offspring, these children, by the definition above, are "familial" cases.

Of the above mentioned 67 non-ascertained familial cases discovered only through family studies, 44 were deceased or living out of state (table 2) and were thus not expected to appear in the rosters of most sources listed above. In the majority of the remaining 23 "non-ascertained" Michigan familial cases

TABLE 2. STATUS OF ANIRIDIA POPULATION ON JANUARY 1, 1959

Classification of aniridics	Probands ascertained by sources listed in Table 1	Non-probands "discovered" only through family history	Total
Living in Michigan on January 1, 1959			
"Familial" cases	53	23	76
"Isolated" cases	40	0	40
"Illegitimate" cases	2	0	2
Sub-Total	95	23	118
Living out of state on January 1, 1959	6	22	28
Dead on January 1, 1959	5	22	27
Born in Michigan after January 1, 1959	3	0	3
Total	109	67	176

the relatives were able to supply a reason why such individuals were not located by our methods of ascertainment. Such reasons included living out of state during school years although born in Michigan, females married to "good providers" who did not require state aid, mild cases who have not presented complications requiring an ophthalmologist's care, clinical consultation outside Michigan or eye surgery in a non-Michigan hospital, etc.

For the frequency estimates essential to the derivation of mutation rates and selection constants, one must select a reference point in time. As of January 1, 1959, 118 of the total 176 were alive and residents of the state of Michigan. The status of the remaining 58 cases is revealed in table 2. Although the "out-of-state" and "deceased" patients do not enter into the calculation of the incidence of the disease, they are of value in the derivation of segregation ratios, fertility estimates, etc.

The family material is summarized in four figures. Fig. 1 contains 12 kindreds in which each affected person had one affected parent, or in the cases of older generations of deceased individuals, one parent was presumably affected. In other words, all aniridics in Fig. 1 would be classified as "familial" cases. Fig. 2 summarizes the histories of 17 "isolated" cases, all of whom were probands, and all of whom reproduced. Eleven of these 17 isolated aniridics had at least one aniridic descendent. These affected children (and grandchildren) in the generations following the original isolated aniridic were classified as "familial" cases according to the definition given above. Fig. 3 is composed of 30 abstracted pedigrees of isolated probands who did not reproduce. It should be pointed out that whenever an "isolated" case was found, as much information as possible was obtained on all the direct ancestors whom the informants could recall, as well as specific information pertaining to the eyes of living and deceased collateral relatives. It was felt that if an informant could recall the color of the eyes of the individual in question, that individual most probably did not have aniridia. Often photographs from the family album would give evidence of the presence of irides, particularly in deceased individuals and those living too far away to be examined. With one exception, in the histories of every living isolated aniridic in our study, surnames of the four grandparents and some infor-

mation pertaining to their eyes were obtained, and in many cases information pertaining to the eyes of the great-grandparents seemed reliable. There were two cases of illegitimate aniridics with unknown paternity who were left unclassified, as shown in Fig. 4. It is perhaps significant that both of these cases were born in the same village in which the largest aniridic family in the study resided. In one instance the proband's mother lived only a few blocks from the affected family. In the second case the mother is deceased, but her twin brother stated that she had known one of the affected males in Kindred 1699. Blood and saliva tests were carried out on the probands, the possible aniridic father, the mother in Kindred 5400, and the father named on the birth certificate in Kindred 4027. This latter man was excluded on the basis of the Rh tests. The possible aniridic father was not excluded in either case.

The total population of 176 aniridics was distributed among 61 kindreds, of which 54 had at least one affected member living in Michigan on January 1, 1959. The remaining seven kindreds contained single, isolated cases. Three of these probands were living in Ohio (1671, 5090, 6610), the fourth was a 43-year-old single female born in Michigan but maintaining residence in Chicago for many years (4813), the fifth and sixth were males born to normal parents during 1959 (7016, 7084), while the seventh was an aniridic male who died in October, 1958 at two years of age (7110).

Three of the kindreds (4816, 4952, 4955) were Negro, with five aniridics residing in Michigan. The percentage of Michigan Negro aniridics was 4.24, compared to a "non-white" population percentage in Michigan, as given in the 1950 census data, of 7.12. The remaining cases were Caucasian of European ancestry, including English, Irish, Scotch, Dutch, German, Polish, Lithuanian, Hungarian, Italian, French, Danish, Swedish, and Norwegian.

THE FREQUENCY OF ANIRIDIA

The civilian population of the lower peninsula of Michigan for January 1, 1959 (estimated from data supplied by the State Bureau of Vital Statistics) was 7,604,811, and the known living Michigan aniridia population on the same date was 118. This gives a *minimum* incidence figure of 1:64,448, which is higher than that reported for Denmark by Møllenbach (1947). He found that in 1944 there were 40 aniridics living in Denmark, with a total population of 3,844,000, or an incidence of 1:96,100. If a Poisson distribution is assumed, the standard errors may be calculated, giving comparative frequency estimates of $(1.55 \pm 0.14) \times 10^{-5}$ for Michigan, and $(1.04 \pm 0.16) \times 10^{-5}$ for Denmark. These frequencies differ significantly at the 5 per cent level by the chi-square test.

As shown in table 3, 41/118 or 35 percent of the Michigan aniridics living on January 1, 1959 were ascertained two or more times, while 23/118 or 19 percent were missed by our methods of locating cases and discovered only in the course of family studies. In this effort to compile a complete roster of aniridia for Michigan, the number of familial cases missed should be negligible, since it is only necessary to ascertain one proband per familial kindred to "discover" the remaining cases by family history. This may not be said of the isolated cases of

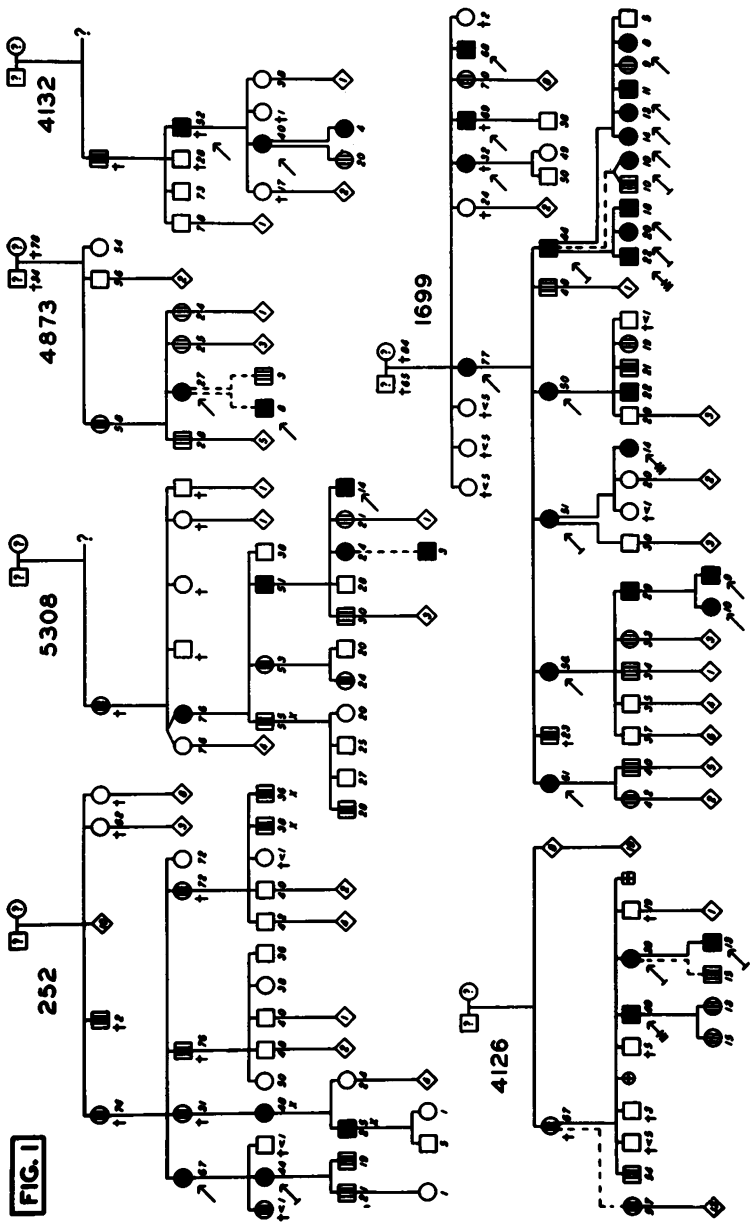
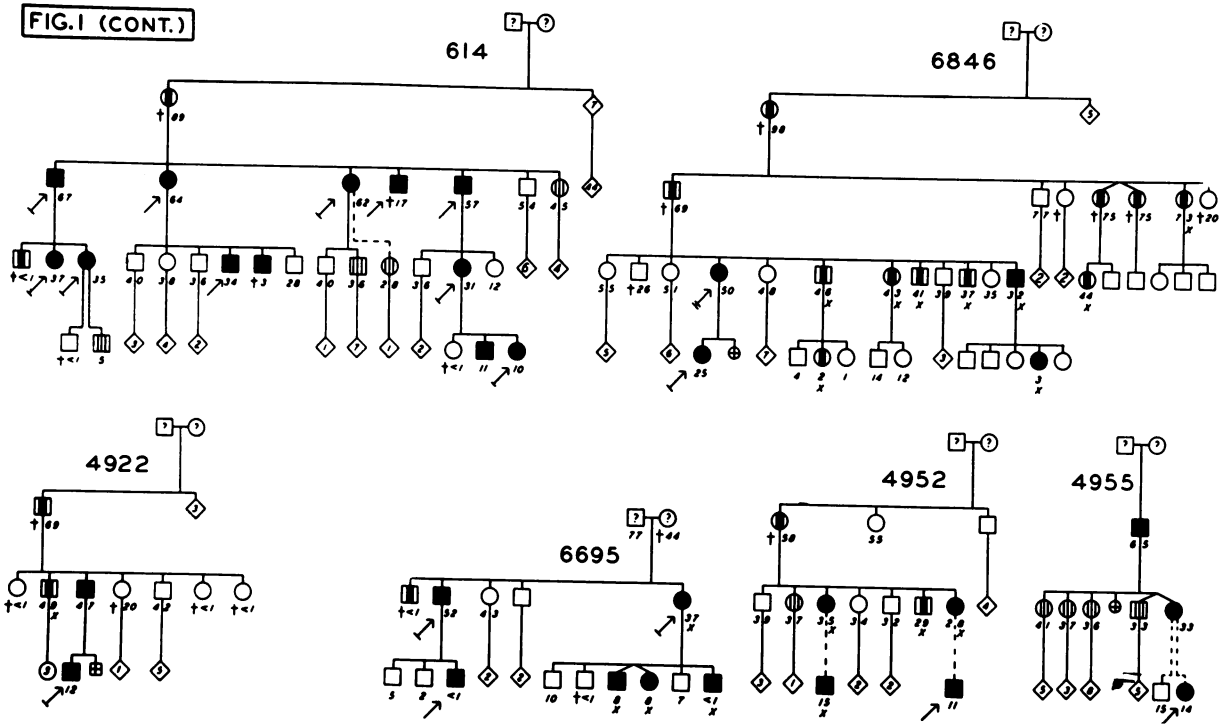


FIG. 1

FIG. 1 (CONT.)



One ascertainment
Two ascertainments
Three ascertainments
Four ascertainments

Male, reported normal
Female, reported normal
Examined, normal
Reported affected

Examined, affected
Sex not designated
Miscarriage
Stillbirth

† Dead on January 1, 1959
x Out of state on January 1, 1959
- Born after January 1, 1959

Digits inside symbols refer to number of individuals. Digits below symbols refer to age on January 1, 1959 or age at death. Digits above first sibship line refer to kindred number in files of Department of Human Genetics, University of Michigan, Ann Arbor.

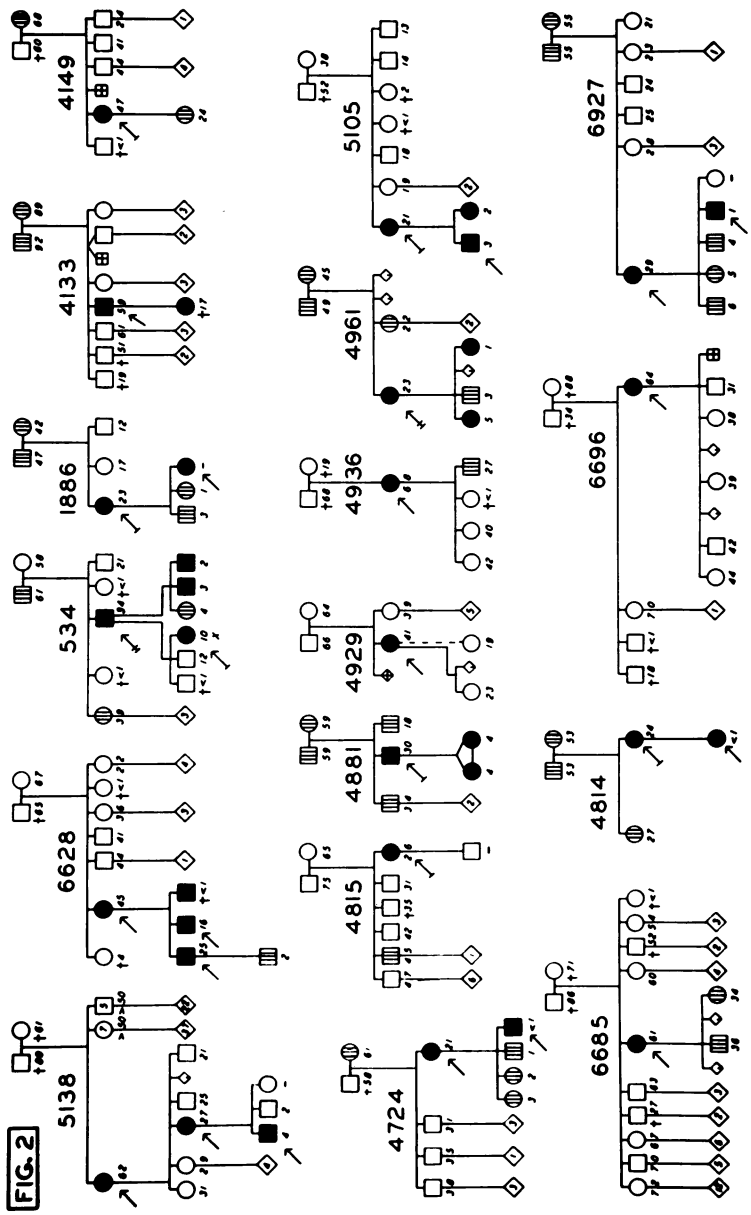

5138
6628
534
1886
4133
4149
4724
4815
4881
4929
4936
4961
5105
6685
4814
6696
6927

FIG. 2

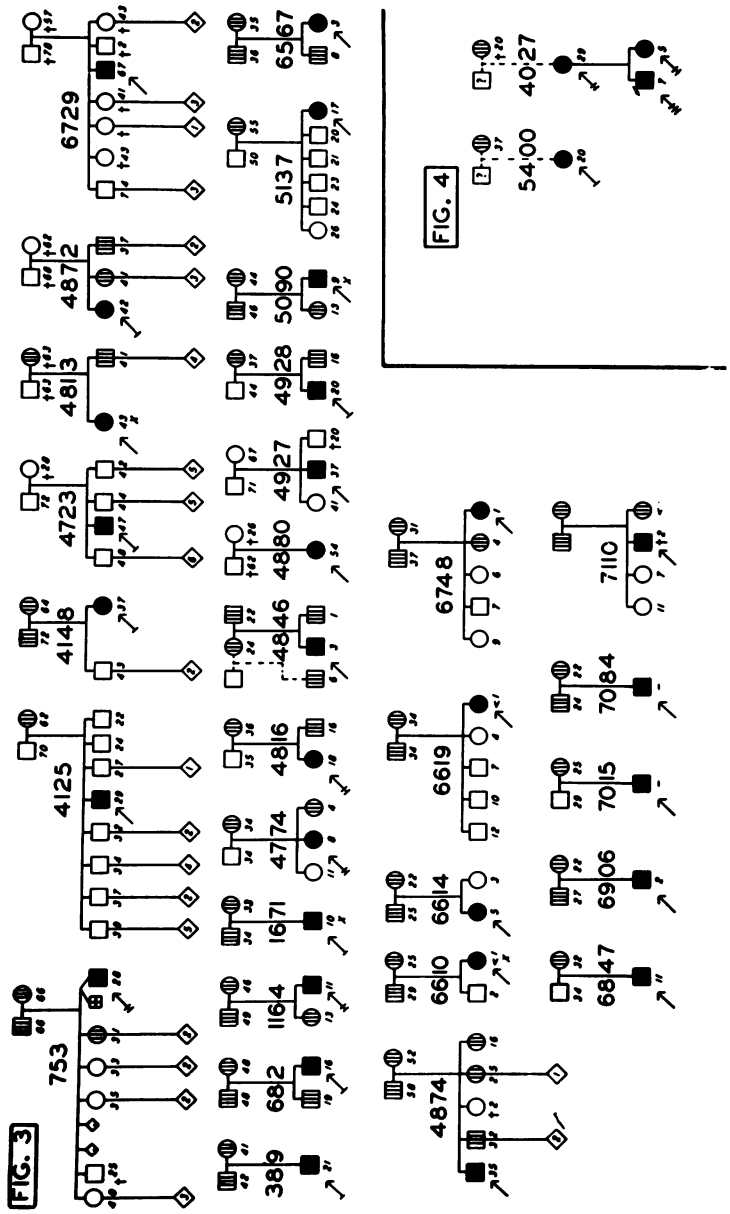


FIG. 3
753
4125
4148
4723
4813
4872
6729
389
682
1164
1671
4774
4816
4846
4880
4927
4928
5090
5137
6567
4874
6610
6614
6619
6748
6847
6906
7015
7084
7110
FIG. 4
5400
4027

TABLE 3. FREQUENCY OF REASCERTAINMENT OF LIVING MICHIGAN ANIRIDICS

Ascertained from four sources	4		
Ascertained from three sources	8		
Ascertained from two sources	29		
Ascertained from two or more sources		41	35%
Ascertained from one source		54	46%
Ascertained only through family history		23	19%
Total		118	100%

aniridia where all relatives are normal. From the knowledge of the non-ascertained familial cases (23/76), and with the broad assumption that, by the methods used, the probability of ascertaining familial and isolated cases is approximately equal, then by simple proportionality, it is estimated that approximately 18 isolated cases have remained undiscovered. The roughness of this figure is obvious. Utilizing this estimate of 18 "missed" cases, the figure of 1:55,918, or $(1.79 \pm 0.15) \times 10^{-5}$, may be closer to the true value of the frequency of aniridia in Michigan than the value of $(1.55 \pm 0.14) \times 10^{-5}$ given above. The gene frequency would be just one-half of this value, since 1) there are few or no "carriers" of the gene who do not exhibit the aniridia phenotype, 2) we will present evidence that there are few, if any, phenocopies, and 3) the aniridic has a normal life expectancy.

CLINICAL DESCRIPTION

Following ascertainment of cases, field work was carried out to obtain detailed family histories, vital statistics, medical information, and fertility data. During these interviews arrangements were made for examinations of the aniridics, their parents, siblings, and offspring. A total of 122 eye examinations were performed on aniridics in the present study, of which 75 were made in the Department of Ophthalmology at the University of Michigan Medical Center and an additional 47 were reported by 24 ophthalmologists. Other cases of aniridia discovered by family histories, were confirmed by hospital records, by eye reports, or by physicians' letters.

The degree of manifestation of aniridia was very similar in the two eyes of the same individual. There were no cases in the present study where the condition was unilateral, with normal iris tissue in the opposite eye. When tags of iris tissue were found at the angle of the anterior chamber there was still no doubt that a gross iris defect was present.

Although not easily mistaken for aniridia, a few conditions should be mentioned in the differential diagnosis. These include coloboma, ectopia pupillae, primary atrophy of the iris, and Axenfeld's syndrome (see Duke-Elder, 1941; Falls, 1949; Heath, 1953). Coloboma iridis has been reported to occur in several aniridia pedigrees (e.g., Bell, 1932; Beattie, 1947), but this association was not found in Møllenbach's (1947) Danish survey, nor in the present Michigan study.

TABLE 4. COMPLICATIONS OF ANIRIDIA FOUND IN 122 EXAMINED CASES

Type of complication	Present	Absent	Not stated
Nystagmus	99	7	16
Cataracts	90	16	16
Glaucoma	54	36	32
Ectopia lentis	17	31	74

TABLE 5. VISUAL ACUITY OF ANIRIDICS IN THE BETTER EYE, CORRECTED VISION

Acuity as defined in text	Under 40 when tested	40 or over when tested
Fair, better eye	38	1
Poor, better eye	43	6
Blind, both eyes	7	9
Not stated	16	2

The frequency of certain common ocular complications found in our examinations are lasted in table 4. The visual acuity, as determined by our examinations, is summarized in table 5. Total blindness in both eyes, due to enucleation of the globe, degenerative changes of the cornea, mature cataracts, glaucoma, or phthisis bulbi, occurred in 16 of the 104 aniridics for whom there is accurate information. Seven of 88 individuals less than 40 years of age were blind, but 9 of 16 age 40 or over had no vision. The threat of total blindness thus increases with age. With reference to table 5, fair vision was defined as "between 20/60 and 20/200 corrected in the better eye" while poor vision referred to "light perception," "sees moving objects," "counts fingers," or "corrected vision of 20/200 or less in the better eye." It is apparent that almost all aniridics over the age of 40 have a severe visual handicap.

The "ultimate lesion" in aniridia is unknown. The iris develops relatively late in the embryonic period, and is not identified as a discrete organ on tissue sections until the 11th or 12th week of gestation, at the 70 or 80 mm. stage (Mann, 1937). It is composed of two layers. The superficial anterior layer is formed from mesoderm while the deeper posterior layer is derived from neurectodermal tissue at the rim of the optic cup. Two suggestions have been advanced concerning the mode of action of the gene. Some authors (e.g., Waardenburg, 1932; Sorsby, 1951) believe that defective neurectodermal tissue is the basis of aniridia and cite examples of aplasia of the macula associated with aniridia as evidence for this theory. As an alternative explanation, Mann (quoted in Bell, 1932) has suggested that at a critical stage in the development of the eye, the network of collagen fibers and embryonic blood vessels which surround the lens and attach to the filtering angle of the eye is not properly absorbed, thereby preventing forward and inward growth of the neurectodermal leaf of the iris. If the rim of the optic cup is held back by these structures, then the scaffolding on which the mesodermal leaf normally makes its attachment is lacking and the latter may fail to develop. Thus, the posterior layer of the iris lies curled back upon itself and is not stabilized in its natural position. Certain observations in

individuals with aniridia tend to lend credence to this second theory. For example, we have observed that when goniotomy is performed to correct glaucoma (a surgical procedure which entails a sweeping motion of the knife around the angle of the anterior chamber) occasionally pieces of iris tissue may be freed by this maneuver and fall into place. Also, the extent of the iris defect is somewhat variable and it is not unusual to observe tags of iris tissue beneath the corneoscleral junction, particularly if the angle is viewed through a gonioscope (François, 1955). In addition, Pincus (1948) observed that in all reported histopathologic examinations of aniridic eyes a small rim of iris tissue encircles the periphery of the anterior chamber and may be adherent to the cornea, obliterating the angle. It seems reasonable, then, to suggest that a certain amount of iris tissue is formed in aniridia and the absence of visible iris is a secondary manifestation of the action of the gene.

During the course of investigation, no systematic attempt was made to evaluate the physical and mental status of the individuals affected. However, certain congenital defects were reported during the collection of the family histories, in hospital records, by physicians' letters, or noted during the ocular examinations. Møllenbach (1947) observed "a strikingly great number of [aniridic] individuals of low intelligence. Only about one-third of the children were able to get along with ordinary school work. About one-third of the adult patients are inmates of institutions for public care while the remainder are occupied with occupations for the blind and the women are barely able to perform elementary housework." We have been unable to confirm Møllenbach's impression. Three aniridics were definitely mentally retarded but one had a history of meningitis at the age of three with residual deafness. The other two had intelligence quotients of 46 and 72. Five others appeared dull but psychometric evaluations were not obtained and the problem of evaluating intelligence in the visually handicapped is well known. Of these five, two were reported by pediatricians to have possible mental retardation; one had a history of convulsions and was in the third grade at age ten; another had a speech defect and one examiner believed the mentality of the patient at age 15 was not beyond the fourth grade level; and the last, an adult female, was reported by the family members and the social worker to be mentally retarded. Not included in the enumerations above were a Mongolian idiot (6906) and a male who died at age two with hydrocephaly and associated retarded development (7110). Two cases of hypospadius and three instances of bilateral cryptorchidism were confirmed by examination. It should be emphasized that these findings are incidental to our eye examinations and we are unable to evaluate the true frequency of associated congenital malformations. However, these male genital anomalies may be related to our findings of decreased male fertility to be reported below.

The individual with aniridia finds himself at a social as well as a physical disadvantage. This is reflected in his need for special schooling and special employment. Twenty-seven of the 95 Michigan aniridics age 6 and over were known to have attended the State School for the Blind, while eight more have attended sight saving classes in public schools. More than one-half of the

aniridics over age 20 have received financial assistance or special services from the state. A more important social disadvantage which has biological implications is the aniridic's decreased success in securing a spouse. This will be discussed more fully under relative fertility.

SURVEY OF THE "FAMILIAL" CASES OF ANIRIDIA

There were 118 aniridics living in Michigan on January 1, 1959, and 76 of these individuals had an affected parent. These 76 "familial" cases fall into 24 kindreds (Figs. 1, 2, and 4). There were 35 males and 41 females, which is not a significant departure from a 1:1 sex ratio ($\chi^2 = 0.47$; $0.50 > P > 0.40$). In two kindreds there was a fairly reliable history of parents with normal appearing eyes and no visual problems, who reproduced more than one affected offspring. One of these (1699) involved a sibship of four affected and six normal offspring and has been discussed in detail in a previous publication by Reed and Falls (1955). Gonadal mosaicism has been offered as one explanation for this pedigree. Nonpenetrance seems to be an unlikely explanation since there is no evidence for it elsewhere, either in the present study or in reports by other investigators. In the second family (6695), the father is age 74, living in the state of New York, and unavailable for examination, whereas the mother died in 1928, but was reported by her husband and several other members of the family to have normal blue irides and normal visual acuity. This couple was naturally concerned when three of their five children were born with severe visual handicaps. They corresponded with relatives in Denmark, but could find no cases of aniridia in past or contemporary generations. One of the affected children died in infancy, but the other two have reproduced aniridic offspring. This situation has not arisen in any of our "younger" kindreds where both parents are living and available for examination.

There were no pedigrees in our series which revealed "skipping a generation." Reed and Falls (1955) tabulated 63 cases of individuals with an aniridic parent and aniridic offspring previously reported in the literature. (From the present series, 27 more cases can be added to their list.) In only five instances was there a question of a non-aniridic intervening between affected parent and affected child. In two of the reports there were iris irregularities of sufficient degree to presume the person in question actually carried the gene. In the other three cases the descriptions were inadequate to draw conclusions as to lack of penetrance. In none of these five unusual cases were all three generations ophthalmologically examined.

There were three individuals in three separate kindreds in the present study in which the usual phenotype was incompletely manifested. In one case (1699), examination revealed hypoplasia of the iris stroma, irregular pupillary margins, nystagmus, photophobia, and decreased visual acuity. In another case (614), the pupils were described as enlarged and egg-shaped or ovoid with definite iris tissue visible. Neither of these two individuals has reached reproductive age. The third person who demonstrated "incomplete aniridia" (5308) had an

aniridic mother, two aniridic sibs and two aniridic offspring. His eyes were carefully examined and the following findings recorded:

Right eye: anterior chamber of normal depth, defective mesodermal leaf of iris from 6:30 to 10 o'clock, clockwise, with baring of underlying neurectoderm; iridoschisis at 8 o'clock.

Left eye: iris pupillary border incompletely developed, temporal iridoschisis from 2 to 9:30 o'clock, clockwise; neural ectoderm seen through the atrophic dehiscent mesodermal leaf of iris.

With a moderate amount of pigmented neurectodermal iris tissue visible to the examiner, it is perhaps significant that this man considered himself to have aniridia like his affected relatives; for this reason he was eager to cooperate in our ophthalmological examinations. It is worth reiterating here that the degree of incompleteness of expression was approximately equal bilaterally in each of these three cases and the phenomenon of unilateral aniridia with normal iris tissue in the opposite eye has not been observed.

In summary, it may be concluded that there were no cases of aniridia in our familial groups which did not behave according to expectations for a condition caused by an autosomal dominant gene with complete penetrance and little variability in expression.

SURVEY OF THE "ISOLATED" CASES OF ANIRIDIA

There were 40 aniridic probands living in Michigan on January 1, 1959, who were found to have normal parents with respect to the aniridia trait. Seventeen of these 40 isolated cases have reproduced (Fig. 2). Of the remaining 23 (Fig. 3), 12 were under age 18 and 11 were age 18 or over and single. There were 16 males and 24 females, a non-significant excess of females. Detailed pedigrees were assembled on these 40 cases following careful histories obtained on as many individuals as feasible and in not a single instance was an affected parent, grandparent, great-grandparent, sib, or collateral relative found. Eleven of the 17 isolated cases who reproduced had at least one affected child. The isolated parent in these 11 kindreds almost certainly owes his disease to a dominant mutation. Among the remaining six individuals, two had a single child, two others had two children each, one had four offspring, and one had five. In addition to these 15 livebirths, these latter six women reported five miscarriages and one stillbirth (table 6).

Consanguinity between the parents of the isolated cases was not discovered. This fact plus the lack of clear demonstration of normal parents with two or more affected children rules against autosomal recessive inheritance. Sex-linked recessive inheritance is excluded by the sex ratio. This observation of six isolated cases who reproduced only normal children may thus most likely be attributed either to the fact that the isolated aniridic parents were phenocopies or to the vagaries of genetic segregation. (Only two reproductive histories among these six fertile isolated aniridics deviate to a degree that the possibility of non-genetic aniridia arises as a reasonable explanation.) By extrapolation, on the exaggerated assumption that six of 17 fertile isolated cases, or 35.3

TABLE 6. OFFSPRING OF 17 FERTILE ISOLATED ANIRIDICS

	Kindred no.	Sex of parent	Normal male	Normal female	Aniridic male	Aniridic female	Remarks
1	534	♂	2	1	2	1	One normal male died age 1 mo. of "malnutrition."
2	1886	♀	1	1		1	
3	4133	♂				1	Affected female died age 17 following thyroid surgery.
4	4149	♀		1			
5	4724	♀	2	1	1		
6	4814	♀				1	
7	4815	♀	1				
8	4881	♂				1	Identical twins.
9	4929	♀		2			One miscarriage.
10	4936	♀	1	3			One normal female died age 10 mos. of "meningitis."
11	4961	♀	1			2	One miscarriage.
12	5015	♀			1	1	
13	5138	♀	2	2		1	One miscarriage.
14	6628	♀			3		One affected male died age 5 days of hydrocephalus.
15	6685	♀	1	1			Two miscarriages.
16	6696	♀	2	3			Two miscarriages; one stillbirth.
17	6927	♀	2	2	1		
Totals			15	17	8	9	

percent, are phenocopies, the estimated maximum number of phenocopies among the 40 isolated cases is approximately 14. Reasons for doubting that there actually are phenocopies will be developed later. Accordingly, it will be assumed that at least 65 percent—and more likely close to 100 percent—of isolated cases of aniridia are due to dominant mutation.

Several environmental factors have been investigated for a possible relationship to the appearance of isolated cases, i.e., to the mutational process. These include season of conception, effect of birth rank, parental age effect, and history of maternal illness during early pregnancy.

1) The months of conception of isolated cases were fairly evenly distributed in the 36 cases for which the birthdates were known, from January to December, as follows: 2, 2, 3, 5, 2, 7, 3, 2, 1, 5, 1, and 3. Grouping these cases by three-month intervals, there is no significant departure from a random distribution, although it should be remarked that the second quarter of the year contributes 14 of the 36 cases.

2) The effect of birth rank on the appearance of isolated cases was tested by the method of Haldane and Smith (1948) and is presented in table 7. The difference between observed and expected (558–525) is less than one standard error.

3) Since the mean birth year of the isolated cases was 1928 and the median year was 1932, the census year 1930 was chosen to compare the age of mother in the general population with age of mother at birth of isolated cases. Penrose

TABLE 7. EFFECT OF BIRTH RANK AMONG ISOLATED CASES
(Method of Haldane and Smith, 1948)

Sibship size	Birth rank 1	2	3	4	5	6	7	8	9	10	Total
2	6	5									11
3	2	4									6
4		1		2							3
5	1	1	1		2						5
6						3					3
7	1	1		1	1						4
8					1						1
9											0
10						1					1
Total	10	12	1	3	4	4					34*

6A = 558
m = 525
V = 2115
σ = 45.88

* Birth rank unknown in one sibship and single offspring in four sibships. One individual (6927) was not included; she was first-born in a sibship of six.

TABLE 8. MATERNAL AGE EFFECT

Mother's age at birth of isolated case	Observed number of isolated cases	Expected number of isolated cases*
15–19	7	4.7
20–24	7	11.5
25–29	7	9.3
30–34	9	6.2
35–39	6	3.8
40–44	1	1.3
45+	0	0.1
Total	37**	
$\bar{x}$	27.3 years	26.6 years

* Expected values are derived from maternal age data given by the Bureau of Vital Statistics for United States, 1930, assuming no maternal age effect.

** Age of mother unknown in two cases; kindred 6927 not included.

(1955), in a review of data from several mutation rate studies, has called attention to a "parental age effect" amounting, on the average, to just over one year. As shown in table 8, the observed mean age of the mother of isolated aniridics is 0.7 years greater than expected, which is in keeping with the results of similar studies. The average difference in husband-wife age in 1930 was 4.6 years (Glick, 1957). The mean difference in fathers' and mothers' ages in the isolated cases was 3.2 $\pm$ 0.6 years. Therefore, we are unable to show a paternal age effect.

4) An abnormal pregnancy history was a very unusual feature in the general information provided by the mothers of the isolated cases. In two instances

there was a history of uterine bleeding during the first trimester. No mothers reported unusual complications of pregnancy such as toxemia, hydramnios, or placenta praevia while carrying the aniridic fetus and there were no histories of unusual parental radiation exposure.

MUTATION RATE

Mutation rate estimates can only be as accurate as the completeness of ascertainment. For this reason, it was felt worthwhile to compare the age spread of the aniridia population with that of the general population of Michigan to determine whether there were any glaring deficiencies in ascertainment for any particular age group. In the case of aniridia, since older individuals tend to come to medical attention because of the complications of their disease, or to state attention because of benefits to the blind, then young affected individuals should be missed more frequently. Conversely, if the life expectancy of affected individuals is less than that of unaffected persons, then a decrease in the proportion of aniridics in the older age group would be expected. Fig. 5 compares the age distribution of the general population of Michigan with that of our known aniridia population. There is no evidence of a deficiency in any particular age group. While it seems unlikely that all cases of this disease in the state have been located, there is, on the other hand, no hint that a disproportionate number of young affected individuals has been missed.

A direct estimate of the rate with which mutation results in the aniridia phenotype may be obtained in several ways. One approach utilizes the number of known isolated aniridics born in a specified time interval compared to the total number of livebirths in the state during the same time span. For the 40-year period from January 1, 1919 to January 1, 1959, the estimate obtained is

$$\frac{28}{4{,}664{,}799} \times \frac{1}{2} = (3.0 \pm 0.8) \times 10^{-6} \text{ mutations/locus/generation} \qquad (1)$$

Another estimate can be obtained from the number of isolated aniridics alive on a certain date and the total population. This, of course, assumes that there is no selective loss of aniridics due to a relatively decreased life ex-

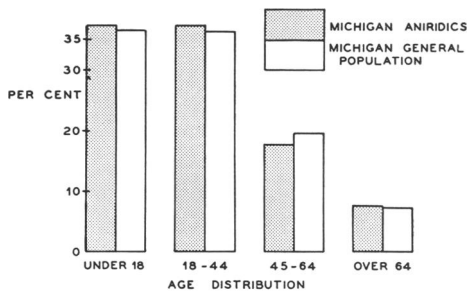


FIG. 5

pectancy. This is felt to be a permissible inference in view of the findings charted in Fig. 5. For the date January 1, 1959, a rate of

$$\frac{40}{7{,}604{,}811} \times \frac{1}{2} = (2.6 \pm 0.6) \times 10^{-6} \qquad (2)$$

is obtained, which agrees, within error due to chance, with the estimate given in equation (1).

If allowance is made for the possibility of non-genetic aniridia, then somewhat lower estimates result. In accord with the discussion of a *maximum* proportion of phenocopies in the previous section of 35.3 per cent, a reduction in the mutation rates given in equations (1) and (2) will yield lowered estimates of 1.9×10^{-6} and 1.7×10^{-6} respectively. We regard this as an overcorrection.

If isolated cases were missed by our methods of locating patients, then the above estimates are too low. By definition, all of the isolated cases are probands. However, as mentioned previously, 23 of the 76 familial cases, or 30.3 percent, were discovered by family history alone, giving a rough estimate of 18 isolated cases missed. If we apportion 13.3 of these 18 "missed" isolated cases to the group born in the 40-year period under consideration for the mutation rate estimate in equation (1) (on the basis of the birth dates of the "missed" or non-proband familial cases), then corrected values of $(4.4 \pm 0.7) \times 10^{-6}$ and $(3.8 \pm 0.5) \times 10^{-6}$, respectively, are obtained. We regard these values as the best approximation to the "true" rate. Again, it should be emphasized that this is an accurate locus rate only if mutations resulting in the aniridia phenotype are restricted to a single locus and all mutations at this locus result in that phenotype.

The only other available estimate for the mutation rate of the aniridia gene is given by Møllenbach's data for Denmark (1947). From his survey of records of seven hospitals, eye clinics, and blind schools, as well as through correspondence with Danish physicians, he located 28 "primary cases" born in the 70-year period from 1875 through 1944, in a total of 4,809,746 livebirths during the same period. Calculating a mutation rate directly from his data, $(2.9 \pm 0.8) \times 10^{-6}$ is obtained, which is in complete agreement with the Michigan rate derived by the same method and given in equation (1) above. However, Møllenbach emphasizes that he believes cases were missed because of high infant mortality rate, lack of proper medical care, and failure to keep complete records in some of the outlying districts of Denmark during the latter part of the last century and for this reason he does not believe the mutation rate should be estimated directly from his raw data. Instead, he assumes that the estimate derived from the 28 primary cases would be approximately 33 to 50 percent too low, so that the frequency at which new mutants occur should "more likely be estimated to be 1:100,000, reckoning with a suitable margin of safety." This would give an adjusted estimate of 5.0×10^{-6} mutations/gene/generation, a figure which has often been quoted in human genetic literature (Neel and Schull, 1954; Reed and Falls, 1955; Penrose, 1955).

This estimated mutation rate of the locus for congenital aniridia of ap-

proximately four per million gametes is one of the lowest reported for human genes. Considering the ease of identification of the phenotype for the aniridia gene and the fact that it is present at birth, fairly constant in expression, and exhibits high penetrance, it is one of the most satisfying conditions which could have been chosen for a genetic study. The issues at hand were not confused and complicated by incomplete pentrance, late onset, early death, and difficulties in diagnosis which have harrassed investigators dealing with other human traits.

RELATIVE FITNESS

The fertility of aniridics was estimated by comparing the number of their offspring with that of two control groups. These groups were (1) siblings of isolated aniridics, and (2) females in the general population of Michigan. It was found that the fitness of the aniridics did not differ significantly from either of these two groups. However, the control groups did differ significantly from each other. The control sibs were more fertile than the general population controls, while the aniridics occupied an intermediate position. The data on

TABLE 9. RELATIVE FITNESS OF ANIRIDICS BORN BEFORE JAN. 1, 1919 COMPARED TO SIBS OF ISOLATED CASES

	No. of aniridics born before 1-1-1919	No. of aniridics who reproduced	Total number of livebirths	Mean number of livebirths	Relative fitness of the aniridic individual	No. of aniridic livebirths	Per cent of aniridic livebirths	Mean number of aniridic livebirths	Relative fitness of the aniridia gene
	a	b	c	d	e	f	g	h	i
Male familials	18	12	43	2.39	0.81	15	34.9	0.83	0.56
Female familials	22	20	68	3.09	1.05	29	42.6	1.32	0.90
Male isolated cases	3	1	1	0.33	0.11	1	(100.0)	0.33	0.22
Female isolated cases	9	7	26	2.89	0.98	5	19.2	0.56	0.38
All males	21	13	44	2.10 ±0.52	0.71 ±0.20	16	36.4	0.76 ±0.38	0.52 ±0.26
All females	31	27	94	3.03 ±0.35	1.03 ±0.17	34	36.2	1.10 ±0.25	0.75 ±0.17
All familials	40	32	111	2.78 ±0.35	0.95 ±0.17	44	40.0	1.10 ±0.27	0.75 ±0.18
All isolated cases	12	8	27	2.25 ±0.58	0.77 ±0.22	6	22.2	0.50 ±0.25	0.34 ±0.17*
All Aniridics	52	40	138	2.65 ±0.30	0.90 ±0.15	50	36.2	0.96 ±0.22	0.65 ±0.17*

* Deviation from 1 > 2 S.E.

relative fitness of aniridics when compared to siblings of isolated cases are summarized in table 9. Several limitations should be noted here:

(1) Only aniridics born before January 1, 1919 were counted. These included both familial and isolated cases and included those who died and those who remained single as well as those who reached reproductive age, married, and proved themselves fertile. The year 1919 was chosen because these individuals would have reached their fortieth birthday or would have died prior to age 40 and their reproduction could be considered essentially complete.

(2) Those aniridics were excluded who were "discovered" because of their fertility, i.e., ascertained only through a child.

(3) All siblings of isolated cases born before 1919 were included regardless of viability, marital status, or fertility.

(4) Only liveborn offspring were considered.

Unaffected siblings of familial aniridics were not used for control purposes because it was considered possible that they might have deliberately limited their reproductivity. It has been shown by Reed and Neel (1959) in a study of Huntington's chorea that normal siblings of familial choreics had a lower reproductive performance than the choreics themselves, but that the latter in turn were less fertile than the general population.

There were 159 livebirths to 54 normal sibs of isolated cases, or 2.94 ± 0.36 mean livebirths per sib. As shown in table 9, column e, the overall relative fertility of aniridics when compared to these sibs is 0.90 ± 0.15. The fitness of the aniridic female is 1.03 ± 0.17 and of the male, 0.71 ± 0.20. This difference between male and female reproductive performance is not statistically significant. However, a difference in the same direction and of approximately the same magnitude has been noted in at least two other dominantly inherited diseases, neurofibromatosis (Crowe, Schull, and Neel, 1956) and Huntington's chorea (Reed and Neel, 1959), suggesting the generalization that in our present culture the male with a dominantly inherited disease of any severity is at a greater reproductive disadvantage than the female. In the present instance it is not clear to what extent the reduced fertility is biologically determined, as suggested earlier by the occasional findings of associated hypospadius and cryptorchidism, and to what extent sociologically determined, as reflected in the lower marriage rate of aniridic males.

In this latter connection, the overall marriage rate of Michigan aniridics living on January 1, 1959 who had reached age 20 is 50/70 or 71.4 percent. Table 10 reveals that a decreased marriage rate occurs in both sexes but is much more marked in the male, when compared to the general population. It

TABLE 10. COMPARATIVE MARRIAGE RATES

Sex	All persons over age 14 (Michigan census data, 1950)	Aniridics over age 14 (living in Michigan in 1959)	Persons blind from any cause (Franceschetti, 1935) (German statistics data)
Males	0.75	0.50	0.47
Females	0.81	0.72	0.72

TABLE 11. RELATIVE FITNESS OF ANIRIDICS AGE 40 AND OVER COMPARED TO GENERAL POPULATION†

	Number of aniridics who reached age 40	Number of aniridics who reproduced	Total number of live births	Mean number of livebirths	Relative fitness of the aniridic individual	Number of aniridic livebirths	Per cent of aniridic livebirths	Mean number of aniridic livebirths	Relative fitness of the aniridia gene
	a	b	c	d	e	f	g	h	i
Male familials	14	12	43	3.07	1.20	15	34.9	1.07	0.84
Female familials	19	18	65	3.42	1.34	28	43.1	1.47	1.15
Male isolated cases	3	1	1	0.33	0.13	1	(100.0)	0.33	0.26
Female isolated cases	9	7	26	2.89	1.13	5	19.2	0.56	0.44
All males	17	13	44	2.59 ±0.58	1.01 ±0.23	16	36.4	0.94 ±0.45	0.73 ±0.35
All females	28	25	91	3.25 ±0.36	1.27 ±0.14	33	36.3	1.18 ±0.28	0.92 ±0.22
All familials	33	30	108	3.27 ±0.36	1.28 ±0.14	43	39.8	1.30 ±0.31	1.02 ±0.24
All isolated cases	12	8	27	2.25 ±0.58	0.88 ±0.23	6	22.2	0.50 ±0.25	0.39 ±0.20*
All aniridics	45	38	135	3.00 ±0.32	1.17 ±0.13	49	36.3	1.09 ±0.24	0.85 ±0.19

† General population fertility taken from data of U.S. Census, 1950.
* Deviation from 1 > 2 S.E.

is interesting to note that the marriage rate of aniridics is almost identical with that reported by Franceschetti (1935) for all blind individuals residing in Germany. This would suggest that the decreased marriage rate is related to loss of vision per se, and not a unique reduction in fitness attributable to the aniridia gene. Severe visual handicap from any cause will hamper the male's success more than the female's in finding a spouse.

Utilizing the second control series, an estimate was obtained of the relative fertility of aniridics as compared to Michigan females. These data are presented in table 11. Again, it is necessary to note the following restrictions applied to the data:

(1) Only aniridics who reached their fortieth birthday were included. This was necessary because their fertility is compared to that of females in the general population who attain a given age as reported in the census data. From a biological point of view, this is a severe limitation because any reduced fertility due to reduced viability will not be apparent.

(2) All females in the general population of Michigan who reached age 40 were included, regardless of their marital status.

(3) Census fertility reports contain data for females only. For any large interbreeding population with a 1:1 sex ratio the fertility of all males compared to all females must be equal. However, for small subgroups where mating can occur outside the group, this truism does not necessarily apply.

(4) The standard errors in the census data are negligible compared with the magnitude of variance in our sample and are not considered when making comparisons.

The 1950 census returns yield a value of 2.56 mean livebirths for Michigan females age 40–59. The aniridics who reached age 40 have a fitness of 1.17 ± 0.13 when compared to these controls (table 11, column e). Again, the sex difference is noted, with a female fitness of 1.27 ± 0.14 compared to that of the male of 1.01 ± 0.23.

Finally, a comparison should be made between the two controls groups. It is not possible to compare them directly unless only the sib controls who reached age 40 are considered. There were 157 live babies born to 45 sibs who reached age 40, giving a mean number of livebirths per sib of 3.49 ± 0.34. Compared to the census figures of 2.56 mean livebirths per Michigan female as given above, the sibs of isolated cases have a relative fitness of 1.36 ± 0.13. This fitness value is a departure from unity by nearly three standard errors and raises the issue of whether even siblings of isolated cases may be used as "controls" since they do not reflect the fertility of the general population, at least in the present study.

GENETIC RATIOS

Although there is no doubt that aniridia is due to a dominant gene whose penetrance is close to 100 per cent, a variety of approaches suggest a significant departure from a 1:1 ratio in segregating sibships. If we restrict our attention to those kindreds which contain familial cases only (Fig. 1), then in 59 sibships in 12 kindreds there are 95 aniridic and 132 normal individuals (0.42:0.58), a departure from a 1:1 ratio which is significant at the .05 level. This treatment makes no allowance for the fact that in each kindred, ascertainment has been through one *or more* affected individuals. Accordingly, any correction for ascertainment can only increase the departure from a 1:1 ratio. Now, the statistical problems involved in correcting for the bias introduced by multiple ascertainments in multiple generations, the situation which exists in the present case, are of a rare degree of complexity and entail some rather arbitrary decisions concerning ascertainment probability which we are unwilling to make in view of the actual situation which obtains for these data, as discussed in the opening section. There are, however, some approximation procedures which entail minimum assumptions and corrections.

The simplest of these is to omit from any calculation of ratios the index case in each kindred. If one proband is deleted from each of the 12 familial kindreds in Fig. 1, then a ratio of 83 affected to 132 normal is obtained (0.39:0.61). This simple, but straightforward, correction for ascertainment further distorts the ratio, to a significance level of .001.

A second approximation to defining the true genetic ratio is to base the calculation of ratios on the offspring of each individual who served as a proband. This approach is perhaps the least biased of any method but sacrifices more than one-half of the data. If only offspring of probands in Fig. 1 are taken into consideration, there are 37 affected children and 47 normal children in 24 sibships (0.44:0.56). This is not a significant deviation from the 1:1 ratio ($P > .20$).

The treatment of the kindreds summarized in Fig. 2 presents a problem. As noted above, these 17 kindreds include 6 isolated cases of aniridia who were not proved by the progeny test to be mutants. Each of the remaining 11 kindreds was ascertained through the isolated affected parent and in addition, sometimes through an affected child as well. Since for all 17 kindreds ascertainment is through an affected parent, it seems legitimate to consider the ratio of normal to affected among their offspring with no further correction of the ratio for the additional ascertainments. There were 17 aniridic and 32 normal offspring of all 17 isolated cases who reproduced (Fig. 2), or a ratio of 0.35:0.65, the probability of this departure from a 1:1 ratio having a P value below 0.05. However, if we assume an expected proportion of 0.39:0.61, on the basis of the data derived from the familial cases above, it can be shown that this distribution of 17:32 fits quite well ($\chi^2 = 0.31$; $.60 > P > .50$). There are only 6 individuals in the present study on which an argument for the occurrence of phenocopies may be based. In view of the evidence that the ratio of normal to affected, when the offspring of these 6 are included with the offspring of proven genetic isolated cases, does not differ significantly from the ratio observed in the offspring of familial cases, we feel that the burden of proof is on him who maintains that any of these 6 individuals represents a phenocopy.

When all familial and isolated cases are considered together, with the *minimum* correction for ascertainment introduced by subtracting one propositus from each familial kindred, then a ratio of 100 affected to 164 normal is obtained (0.38:0.62), a departure from equality which is significant at the fiducial limit of 0.0001. We consider this value the best working estimate of the at-birth ratio to be derived from the present data.

Similar abnormal ratios have been obtained in other studies of aniridia. Beattie (1947) reported a large English pedigree with 29 affected and 44 normal (0.40:0.60). Paganelli (1951) found 41.8 per cent affected offspring in 74 sibships in 28 kindreds in a literature survey. In neither case was the information submitted sufficient to permit allowance for ascertainment. Grove, Shaw, and Bourque (1960) reported a large French Canadian kindred with 76 aniridics and 88 normals (0.46:0.54). This kindred was subject to multiple ascertainments and the best method of allowing for ascertainment bias is not clear. The number of affected individuals required to stimulate a geneticist's interest in a single pedigree depends on numerous, complex, known and unknown factors. In the Canadian kindred there were 21 cases reported in a single listing supplied by the Canadian National Institute for the Blind before the survey was undertaken, but subtracting 21 probands would be an obvious overcorrection. The data from

the Canadian and English pedigrees together with Møllenbach's (1947) eight Danish familial kindreds in which propositi are not designated are combined with the Michigan data in table 12. With *no* correction for ascertainment whatsoever, the segregation ratio in the combined studies stands at 239 affected to 317 normal (0.43:0.57), ($\chi^2 = 10.94$; $P < .001$).

Several causes for the abnormal ratios have been excluded. The deficiency of affected offspring is not related to the sex of the parent. In the Michigan survey, there were 21 aniridic males who produced 36 affected and 59 normal offspring (0.38:0.62), while 38 affected mothers had 59 affected and 73 normal children (0.45:0.55). The heterogeneity chi-square value for these two ratios is equal to 1.02, with a P value greater than .30. The explanation does not lie in the fact that one or two large kindreds are contributing to the abnormal ratio while the segregation in the other kindreds is normal. It is shown in table 13 that among 12 familial kindreds in Fig. 1 the genetic ratios were found to deviate in

TABLE 12. COMPARISON OF SEGREGATION RATIOS IN ANIRIDIA SURVEYS, WITH NO CORRECTIONS FOR ASCERTAINMENT

Locality	Number of kindreds	Number of sibships	Normal offspring	Affected offspring	Per cent affected	χ^2	P	Source
Michigan	24	76	164	112	40.6	9.80	<.005	Shaw, Falls, & Neel, 1960
England	1	11	44	29	39.7	3.08	.10–.05	Beattie, 1947
Canada	1	28	88	76	46.3	0.88	.40–.30	Grove, Shaw, & Bourque, 1960
Denmark	8	10	21	22	53.7	0.02	.90–.80	Møllenbach, 1947
Total	34	125	317	239	43.0	10.94	<.001	
(Survey of literature)	28	74	?	?	41.8			Paganelli, 1951

TABLE 13. GENETIC RATIOS IN SEGREGATING SIBSHIPS IN MICHIGAN "FAMILIAL" KINDREDS IN FIGURE 1*

Kindred number	Normal	Aniridic
252	15	9
614	14	12
1699	25	23
4126	8	2
4132	7	2
4873	4	1
4922	8	2
4952	4	4
4955	5	1
5308	13	8
6695	7	6
6846	22	13

* One proband deleted from each kindred.

TABLE 14. GENETIC RATIOS BY SIBSHIP SIZE

Size of sibship†	Number of sibships	Affected	Normal	Proportion affected	χ^2 (Yates' correction)
1	8	6	2	0.75	1.13
2	15	9	21	0.30	4.03*
3	9	10	17	0.37	1.33
4	6	8	16	0.33	1.96
5	9	16	29	0.36	3.20
6	3	6	12	0.33	1.39
7	6	22	20	0.52	0.02
8	0				
9	0				
10	1	4	6	0.40	0.10
11	1	8	3	0.73	1.45
12	1	6	6	0.50	0
Total	59	95	132	0.42	6.03*

* Significant at 0.05 level.

† Half-sibs were included in total sibship count.

the same direction in each case. Genetic ratios enumerated by sibship size may be found in table 14. There is no consistent trend toward the expected 1:1 ratio as the size of the sibship changes. As stated above, the ratio in the offspring of 24 probands was 37:47 (0.44:0.56). This may be compared to 35 non-probands in whom the offspring ratio was found to be 58:85 (0.41:0.59). These two ratios do not differ significantly from each other, suggesting that non-proband histories were probably as reliable as those of probands. Finally, in searching for clues as to the cause of these aberrant ratios, the information on individuals who died in infancy was examined to determine if inaccurate reporting of the condition of the eyes could account for the findings. Twelve infants who died under one year of age were reported to have normal eyes compared to four deceased infants reported to be aniridic. It seems unlikely, then, that errors in memory of presence or absence of the trait in deceased infants could account for more than about four cases.

If the departure from a 1:1 ratio is accepted as significant, several possible explanations must be considered. The first is "prezygotic" selection, encompassing such diverse situations as abnormal meiotic ratios, differential gametic survival, differential sperm motility, or sperm selection at the time of insemination or fertilization. Since the aberrant ratios appear in the offspring of both sexes, the latter two possibilities cannot be the sole factors. A second explanation is "postzygotic" selection. The developing zygote heterozygous for the aniridia gene could have a decreased viability. In the latter case, it might be possible to detect this by a history of excessive miscarriages. A tabulation of the miscarriages reported by isolated cases may be found in table 6; the frequency does not seem to be unusually high. Miscarriage data were obtained on these cases as carefully as possible whereas such detailed abortion histories in the familial kindreds were not so carefully sought and are not presented here because of incomplete information. Non-penetrance and incomplete expression as a cause for the abnormal ratios have already been dismissed.

Since the data in tables 9 and 11 suggest that aniridic individuals produce as many live offspring as normal individuals (column e), but that somewhat less than half of the offspring are affected (column g), it is not sufficient, in considerations of population dynamics, to confine comparison of the affected and normal individuals in the population to relative reproductive fitness, per se. In addition, the fitness of the *aniridia gene* in the present instance should be compared with that of its normal allele. This "gene fitness" may be derived directly from the data, and will include both "prenatal fitness" as well as "phenotypic fitness" or biologic fitness of the individual who appears with the trait.

Turning to table 9 (columns a and f), 52 aniridics produced 50 affected children. The mean number of affected offspring per affected parent is 0.96 $\pm$ 0.22 (column h). The control sib population produced 159 offspring, giving a mean number of normal offspring per normal parent of $159/54/2 = 1.47 \pm 0.18$. Assigning a relative fitness of one to the normal allele, the direct relative fitness of the aniridia gene when compared to this normal allele found in the sibs of isolated cases is estimated to be $(0.96 \pm 0.22)/(1.47 \pm 0.18)$, or 0.65 ± 0.17 (column i). By the same token, the fitness of the gene when compared to the normal allele in the general population is 0.85 ± 0.19 (table 11, column i).

It may be concluded, then, that the proportion of affected offspring of aniridic individuals, regardless of the sex of the parent, is about 0.38 instead of the expected Mendelian proportion of 0.50, and although persons with aniridia may expect to enjoy essentially normal longevity and fertility, their "gene fitness" is reduced and they will not maintain a constant gene frequency in the population from generation to generation unless new mutants replace those lost through whatever mechanism is responsible for the departure at birth from a 1:1 ratio, or unless reproductivity is increased to compensate for this loss.

SUMMARY

Congenital aniridia, or bilateral absence of the iris, is caused by an autosomal dominant gene with high penetrance and constant expression. An attempt has been made to survey all individuals with this trait living in the lower peninsula of the state of Michigan as of January 1, 1959. Through various channels, a roster of 176 aniridics distributed in 61 kindreds has been assembled. Of these, 118 cases were living in Michigan on January 1, 1959; 40 of these individuals were isolated cases and are considered to be mutants. The incidence of aniridia in Michigan is about 1.8×10^{-5}, while the mutation rate is approximately 4.0×10^{-6}/locus/generation.

Ocular examinations were conducted on 122 aniridics. The affected individual is visually handicapped, with the threat of total blindness increasing with age. Nystagmus, cataracts, and glaucoma frequently accompany the condition. The affected male is less apt to marry than the female; there is a slight decrease in male fertility.

The most striking finding to emerge from the present study is a 38:62 ratio of affected to normal children in segregating sibships instead of the expected

Mendelian 50:50 ratio. This deviation is not a function of decreased penetrance, sibship size, sex of parent, infant mortality, or heterogeneity among kindreds. Possible explanations for the deficiency of affected children are considered.

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